Fluorescent Probes

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Rhodamine-Inspired Far-Red to Near-Infrared Dyes and Their Application as Fluorescence Probes**

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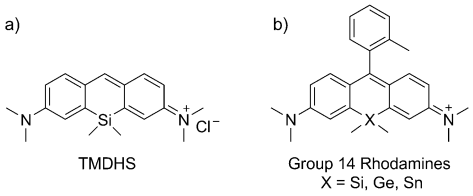
fluorescent dyes · fluorescent probes · rhodamine

Dedicated to Shanxi University on the occasion of its 110th anniversary

Fluorescent probes that have excitation wavelengths in the far-red to near-infrared (NIR) (>600 nm) region are attractive for biological applications because of minimum photodamage to biological samples, deep tissue penetration, and minimum interference from the background autofluorescence of biomolecules in the living systems.[1] Currently significant efforts are being made towards the use of NIR probes for bioanalytical applications.[1b–d] Unfortunately, few fluorophores show sufficient fluorescence quantum yields in the NIR region, especially in aqueous surroundings. Indeed, the only widely used NIR fluorophores are cyanine dyes, for example Cy5 and Cy7, which have been extensively employed in the design of NIR fluorescent probes.[2] However, even cyanine dyes have some deficiencies: for example, in many cases they suffer photobleaching; moreover, cyanine dyes have relatively high-lying occupied molecular orbital (HOMO) energy levels, so that the off–on switching of cyanine fluorescence by photoinduced electron transfer (PET) is less efficient.[3] Therefore, the development of new far-red to NIR fluorophores with high fluorescence quantum yields and tolerance to photobleaching, and whose fluorescence can be easily controlled, is highly attractive.

Among the various fluorophores, the rhodamine dyes have attracted considerable interest on account of their excellent photophysical properties, such as high molar extinction coefficients, excellent fluorescence quantum yields, and great photostability. As a result, rhodamines are widely used as fluorescent probes and molecular markers in chemistry and biochemistry.[4] However, the absorption and emission wavelengths of most rhodamine derivatives are below 600 nm, which is sometimes unsuitable for biological applications. In order to get longer-wavelength rhodamine derivatives, many efforts have been made, including extending the conjugation of the xanthene ring, replacing the central carbon with a nitrogen atom or introducing a cyano group on the central carbon atom, as well as replacing the oxygen bridge atom by other elements, such as N, C, S, and Se.[5] However, rhodamine analogues developed by these strategies have some disadvantages. They are difficult to synthesize and have decreased absorption or fluorescence intensity, and thereby are far from meeting current requirements. Recently, the research groups of Qian, Nagano, and Lin disclosed their studies on Si-pyronine, Si-rhodamine, Te-rhodamine, and Changsha NIR dyes, independently. These dyes show excellent properties and great potential for biological applications. In this Highlight, these important discoveries are summarized.

Inspired by the significant achievements with siloles and silaanthracenes,[6] Qian et al. reported the first red-emission Si-pyronine fluorophore (TMDHS) in 2008 (Scheme 1a).[7]



Scheme 1. The molecular structure of a) TMDHS (a) and b) Group 14 rhodamines.

The emission wavelength of TMDHS is strongly shifted to more than 650 nm with a high fluorescence quantum yield (0.39 in CH_2Cl_2, and 0.18 in water). The electrochemical measurement indicates that the replacement of oxygen by silicon affects the HOMO and LUMO energy levels simultaneously, and, as a result, a smaller energy gap is obtained, thereby leading to the large red shift. As in the siloles, the low-lying LUMO energy level of TMDHS is caused by the special σ*–π* conjugation, with the contribution of the σ* orbitals of the silicon–C(methyl) bonds and π* orbital of the adjacent carbons. The HOMO energy increase of TMDHS is ascribed to the inductive electron-donating effect of the dimethylsilyl group relative to oxygen.

In 2011, Nagano et al. extended this concept to the design of the Group 14 rhodamines Si-rhodamine (SiR), Ge-rhodamine (GeR), and Sn-rhodamine (SnR) (Scheme 1b).[8] SiR and GeR are stable under ambient conditions, while SnR decomposes readily. Similar to TMDHS, the absorption and emission of SiR and GeR are greatly red-shifted to the far-red to NIR region. Moreover, SiR and GeR retain the advantages

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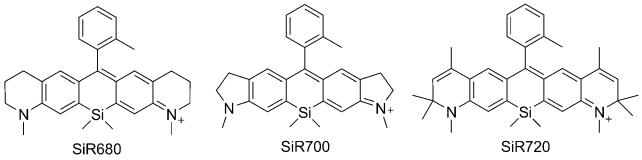
of the original rhodamines, including high quantum efficiency in aqueous media ($\Phi_{fl} = 0.31$ for SiR and 0.34 for GeR), tolerance to photobleaching, and good water solubility. Importantly, in contrast to most far-red to NIR fluorescence dyes, the fluorescence of SiR and GeR can be controlled by the photoinduced electron transfer (PET) mechanism. The authors have successfully employed this mechanism to design SiR-based NIR probes to test for differences in pH[8] and Zn^{2+}[8] and Ca^{2+} levels.[9]

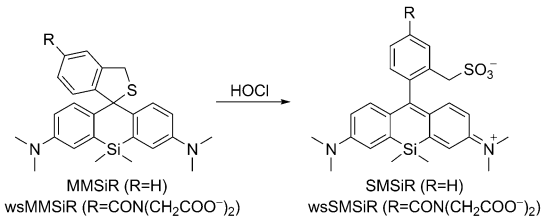
For practical in vivo imaging, two wavelength windows at 700 nm and 800 nm are often used as emission channels.[10] By expansion of the silaanthracene ring, Nagano et al. recently reported three new NIR fluorescent dyes, named SiR680, SiR700, and SiR720 (Scheme 2).[11] All three dyes exhibit



Scheme 2. The molecular structures of SiR680, SiR700, and SiR720.

absorption and emission maxima in the NIR region (670–740 nm). Moreover, SiR680 and SiR700 exhibit sufficiently high fluorescence quantum efficiency ($\Phi_{fl} = 0.35$ and 0.12) for application. The authors also prepared amine-reactive succinimidyl ester bearing SiR700, and applied it for in vivo tumor imaging.

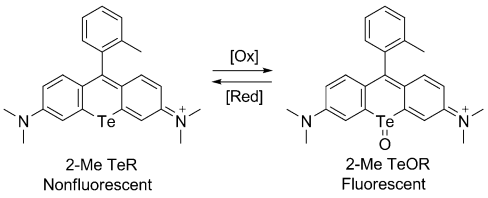
Besides the PET strategy, Nagano et al. also developed a SiR-based fluorescence probe (MMSiR) for hypochlorous acid based on the fluorescence on–off switching mechanism



Scheme 3. Si-rhodamine-based NIR probe for HOCl.

associated with spirocyclization (Scheme 3).[12] In the presence of HOCl, the thioether group in MMSiR is oxidized to sulfonate, with concomitant ring opening of the spirocycle to form the highly fluorescent SMSiR. Furthermore, with MMSiR, they conducted real-time imaging of phagocytosis by means of fluorescence microscopy, and with the more hydrophilic derivative wsMMSiR, they achieved the non-invasive in vivo imaging of HOCl generation in a mouse peritonitis model.

Very recently, Nagano et al. reported on a Te-rhodamine derivative (2-Me TeR) as a reversible NIR fluorescence probe for reactive oxygen species (ROS) whose action is based on the heavy-atom effect and the redox properties of the Te atom (Scheme 4).[13] As a result of the heavy-atom effect, 2-Me TeR

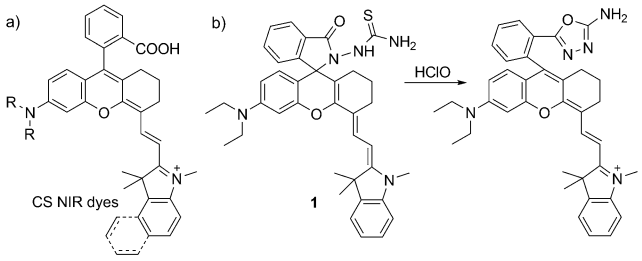


Scheme 4. Te-rhodamine (2-Me TeR)-based fluorescent probe for reactive oxygen species.

shows very low fluorescence ($\Phi < 0.001$); in contrast, its oxidized product, 2-Me TeOR, in which the heavy-atom effect of the Te atom is weakened by the binding of an oxygen atom, exhibits strong fluorescence at 690 nm ($\Phi_{fl} = 0.18$). In the presence of various ROS, 2-Me TeR is oxidized to the fluorescent 2-Me TeOR, while the generated 2-Me TeOR is quickly reduced by glutathione (GSH) to generate 2-Me TeR. Thus, 2-Me TeR shows great potential for monitoring the dynamics of ROS production continuously in vivo.

In fact, the rhodamine spirolactone/spirolactam platform has been regarded as one of the most suitable for the development of chemosensors. However, this attractive sensing platform has not been employed for the design of sensors based on Group 14 rhodamines. This leaves plenty of room for further development of this type of dyes.

By combining the spirocyclization-based fluorescence on–off switching mechanism of rhodamine with the NIR optical profiles of merocyanine, Lin et al. recently reported a series of NIR fluorescent dyes, Changsha NIRs (CS NIRs) (Scheme 5).[14] These dyes display both absorption (688–



Scheme 5. The molecular structures of a) CS NIR dyes and b) a CS NIR-based probe for HClO.

728 nm) and emission (721–763 nm) into the NIR region, with high fluorescence quantum yields ($\Phi_f = 0.29$–0.56) in EtOH. Like the classic rhodamines, the CS NIR dyes could be utilized as platforms for the design of NIR fluorescent probes. For example, using CS2 (one of the CS NIR dyes) as a platform, they constructed the NIR fluorescent turn-on sensor **1**, which is capable of imaging endogenously produced HClO in living animals, demonstrating the value of CS NIR functional fluorescent dyes.

Besides the above-mentioned rhodamine-inspired NIR dyes, many excellent NIR-emitting boron dipyrromethene (Bodipy) dyes, such as styryl-Bodipys and Azo-Bodipys, have been actively developed in recent years. The related informa-

tion can be obtained from several recent reviews reported independently by Ziessel,[15a] Burgess,[15b] and Dehaen.[15c]

In this Highlight, we focused on the recent noteworthy reports on rhodamine-inspired NIR dyes: Si-pyronine, Si-rhodamine, Te-rhodamine, and Changsha NIR dyes. It is expected that the design strategies presented here will contribute to the development of novel and improved NIR dyes. Also, it is expected that these dyes may serve as molecular tools for numerous biological applications, such as labeling artificial amino acids in proteins, probing various biologically important species, and acting as generators of singlet oxygen for photodynamic therapy. Furthermore, fluorescence resonance energy transfer (FRET)-based NIR ratiometric fluorescence versions of these dyes are also expected.

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