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Synthesis and spectroscopy of near infrared fluorescent dyes for investigating dichromic fluorescence

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ABSTRACT

We developed a series of near infrared (NIR) cyanine dyes to study dichromic fluorescence phenomenon, which provides new protocols for in vivo optical imaging. Preliminary spectroscopic studies show that dichromic fluorescence correlates with structural symmetry. This feature suggests the potential use of dichromic fluorescent molecules to study biological processes that can alter the structural symmetry of the molecular probes.

The development of near-infrared (NIR) dyes as fluorescence labels and sensors has gained increasing interest over the last decade because of their potential to advance and facilitate the translation of optical imaging to humans. Compared to blue-shifted dyes, NIR fluorescent probes offer several advantages: (a) NIR light is poorly absorbed by hemoglobin, water and lipids, resulting in deep tissue penetration;[1,2] (b) background autofluorescence is negligible; (c) light scattering in tissue is relatively low because scattering decreases as wavelength increases. Overall, NIR fluorescent dyes provide enormous opportunities for non-invasive in vivo applications.

Most NIR dyes used for in vivo optical imaging belong to the carbocyanine dye family. These dyes represent one of the most prominent classes of optical imaging agents with adjustable optical properties and high extinction coefficients. In addition, carbocyanine dyes can be conjugated with antibodies, peptides, or small targeting ligands to impart molecular specificity.[3–12] Using low quantities of these NIR agents, high specificity and good tissue penetration can be achieved. However, relatively low signal-to-background ratio is sometimes observed due to the signal from unbound NIR agents.

Quenched NIR agents that become detectable upon enzyme activation can increase signal-to-background ratios up to several hundred folds.[13] The quenching can be either based on self-quenching or fluorescence resonance energy transfer (FRET).[14,15] These so called 'smart NIR agents' have been developed to target diagnostic enzymes such as cathepsin B, K, D and H; caspase 1 and 3; matrix metalloproteinases (MMP) 2, 9, and 13; urokinase and other proteases for cancer imaging.[16–18] Optical imaging based on fluorescence lifetime is also made possible to image biological activities.[19] This technique is unique because it allows monitoring target site with less dependence on variations in fluorescence intensity or probe concentration. For example, a NIR dye-labeled hexapeptide, Cyp-GRD, was developed to image lung cancer.[20] In another study, small level of protein phosphorylation was monitored by combining fluorescence lifetime imaging and FRET.[21]

Despite the recent advancement of NIR optical imaging, NIR probes and signaling protocols that are suitable for in vivo biological studies are still limited. Recently, dichromic fluorescence phenomenon in NIR cyanine dyes was discovered.[22] It was reported that upon excitation, some carbocyanine dyes exhibit a second emission band at 700 nm, other than the commonly observed emission peak at 800 nm. Interestingly, dichromic fluorescence seems to be favored by structurally nonsymmetrical dyes (Fig. 1). Therefore, this technique can be potentially used to monitor enzyme activities by transforming a probe from a symmetrical to a nonsymmetrical form.

Unfortunately, dichromic fluorescence was studied on only a few molecules and the correlation between dichromic fluorescence and molecule symmetry needs to be further verified. Accordingly, we have developed a number of NIR dyes to study dichromic fluorescence at a larger scope using the method shown in Scheme 1.

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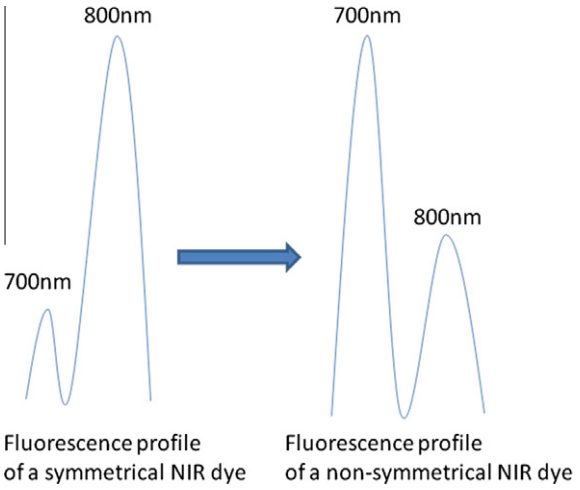
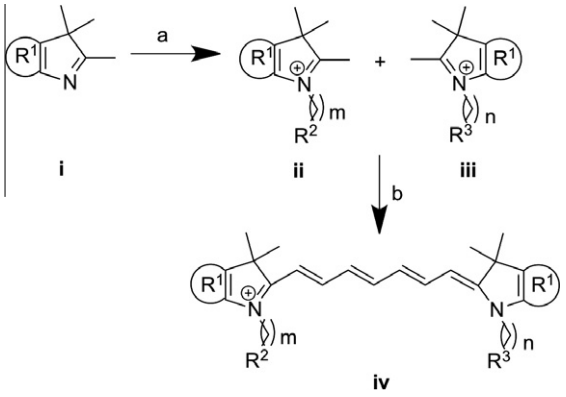


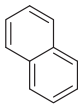
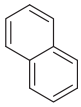
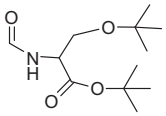
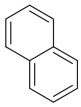
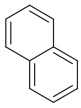
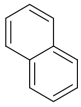
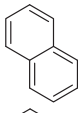
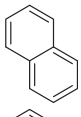
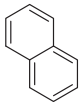
Figure 1. Illustration of fluorescence profiles of symmetrical and non-symmetrical NIR dyes. Non-symmetrical NIR dyes appear to have relatively higher emission at 700 nm channel but lower emission at 800 nm channel than symmetrical NIR dyes.



Scheme 1. Reagents and conditions: (a) dichlorobenzene, 110 °C; (b) *N*-(5-anilino-2,4-pentadienylidene)anilinehydrochloride, acetic anhydride, DCM, MeOH, sodium acetate.

As illustrated in Scheme 1, compounds with structures ii and iii were synthesized from indolium or benzoindolium compound i by refluxing in dichlorobenzene with bromo-alkyl molecules. Subsequent condensation of ii and iii in the presence of sodium acetate and *N*-(5-anilino-2,4-pentadienylidene)anilinehydrochloride yielded the dichromic fluorescent dyes. The products were purified by semi-preparative HPLC and characterized by LC–MS

Table 1
Dichromic fluorescent molecules

#	R^1	R^2	R^3	*m*	*n*	Abs (nm)	Emi 1 (nm)	Emi 2 (nm)	700/800 nm
1		CO_2H	SO_3H	2	4	782	698	809	0.67
2		CO_2H		2	2	782	698	809	1.71
3		CO_2H	SO_3H	5	4	783	699	808	2.82
4		CO_2H	CO_2H	5	5	784	698	808	0.25
5		CH_3	CH_3	0	0	777	694	800	7.04
6		CH_3	CH_3	0	5	781	697	799	11.9
7		CO_2H	SO_3H	2	4	745	661	770	3.78
8		CO_2H	CO_2H	2	2	745	661	770	0.85
9		CO_2H	SO_3H	2	3	783	695	809	1.34
10		SO_3H	SO_3H	3	3	783	695	810	1.51

Abs: maximum absorption, Emi 1: maximum emission at short wavelength channel, Emi 2: maximum emission at long wavelength channel.