

Synthesis of a New Turn-On Fluorescent Dyes –Terephthalate Derivatives for Staining Proteins

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Diaminoterephthalate derivatives with maleimide moieties were synthesized and used as fluorescence dyes for sensing thiols. Whereas these “NiWa Blue” dyes showed no emission, the conjugate addition of a thiol to the maleimide group turned on a fluorescence at about 391 nm when irradiating the dye at 333 nm.

We prepared for example the dye **1** from dimethyl succinyl succinate and NH_4OAc while water was removed with a Dean-Stark trap. Under these reactions conditions, enough air was available to directly oxidize the double enamine to the aromatic compound. Conversion with one equivalent of maleic anhydride followed by cyclization of the intermediate monoamide with NaOAc and Ac_2O gave the *N*-acetyl monomaleimide in 39% yield.

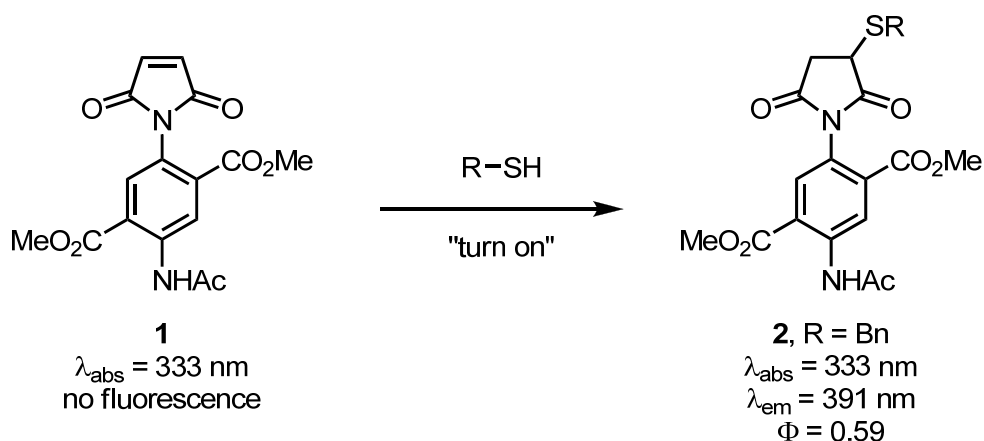


Figure 1: “Turn-on” probe **1** and its thiol addition product **2**.

The neuronal-calcium sensor protein recoverin possesses a single cysteine residue at position 39, which reacts with NiWa Blue, and is therefore labeled by a fluorophore with an emission at about 440 nm. In the absence of Ca^{2+} , irradiation at 280 nm of a tryptophan residue in close proximity to Cys-bound NiWa Blue lead to strong FRET, which was detected by emission of the dye at 440 nm. In the presence of Ca^{2+} , the protein holds a conformation with distal Trp and Cys residues, thus FRET of irradiated Trp to Cys-bound NiWa Blue was significantly weakened.