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Synthesis and spectroscopic characterization of red-shifted spironaphthoxazine based optical switch probes

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ABSTRACT

Spironaphthoxazine (NISO) is an efficient optical switch probe that has applications in high contrast detection of Förster resonance energy transfer (FRET) using optical lock-in detection (OLID). NISO exists in two distinct states, spiro (SP) and merocyanine (MC), that can be independently controlled by using alternate irradiation with near ultraviolet and visible light. Unfortunately, the SP-state of NISO has an absorption centered at 350 nm, which may lead to phototoxic effects when manipulating the probe within a living cell. To overcome this problem we introduce new, red-shifted amino-substituted NISO probes compared to NISO that undergo an efficient SP to MC transition in response to irradiation by using 405-nm light, which is less damaging to living cells. This study details the synthesis of amino-substituted NISO and their *N*-hydroxysuccinimide ester and maleimide derivatives and their use in generating covalent attached protein conjugates. This study also presents a characterization of the spectroscopic and optical switching properties of these red-shifted NISO probe in solution.

Spironaphthoxazine (NISO), a well known photochromic molecule, undergoes rapid and reversible, high fidelity, optically driven transitions between a colorless spiro (SP)-state and colored merocyanine (MC)-state (Fig. 1).[1–4] Near ultraviolet (365 nm) irradiation of the SP-state of NISO leads to an excited state reaction that generates the MC-state, which has a strong absorption band beyond 600 nm. The MC-state can be converted back to the SP-state upon illumination with visible light (>500 nm).[1–7] Previous studies have introduced NISO reagents that can react with amino- or thiol groups on proteins including chloroacetyl, isothiocyanate, halide, and succinimide ester.[5–7] However, the application of NISO and its conjugates as an optical switch for studies in living cells is limited by the need to irradiate the SP-state with 365 nm light. In principle, one could avoid any phototoxic response associated with near ultraviolet irradiation of cells, by shifting the SP-absorption band to beyond 400 nm. This change would also allow for optical manipulation of the NISO optical switch using new types of confocal microscopes that include a 405 nm laser as a standard feature.

We have shown that optical lock-in detection (OLID) of Förster resonance energy transfer (OLID-FRET) using an optical switchable acceptor probe such as spirobenzopyran (NitroBIPS) and NISO can greatly improve image contrast of the donor probe and thereby improve the detection of FRET complexes.[8–10] In these studies the donor probe may be GFP or a small molecule fluorophore such as tetramethylrhodamine (TMR) while only the MC-state of NitroBIPS

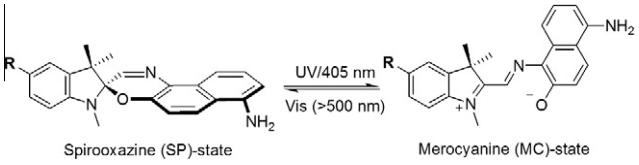

Figure 1. Optical switching between the SP- and MC-states of **NISO**.

or NISO can serve as an acceptor in FRET, effectively quenching the donor fluorescence.[8–10] On the other hand, the SP-state of these optical switches has no visible absorption and so no FRET occurs, that is, the donor emission is at a maximum. Repeated optical manipulation of the SP- and MC-states of the NISO that are in close proximity to the donor probe thereby allows the user to rapidly change FRET efficiency in the probe pair with concomitant modulation of the quantum yield of the donor probe. In our previous studies, we have shown that the emission from the donor probe can be modulated over many cycles of optical switching; moreover the modulated TMR fluorescence in a TMR-NISO probe is unique to the system and is easily isolated from background (un-modulated) signals in the sample by using a digital correlation technique. An image composed of the values of the resultant correlation coefficient exhibit far higher contrast compared to the original intensity image.[8,9]

The application of OLID-FRET to living systems would greatly benefit from efforts to shift the absorption maximum of the SP-state to more cell-tolerant wavelengths that lie beyond 400 nm. The goal for this study was to synthesize NISO probes having

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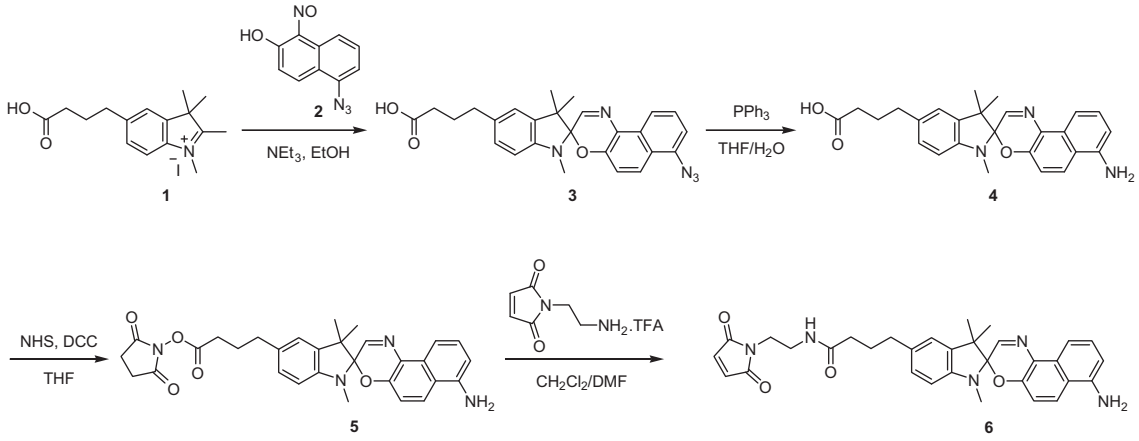
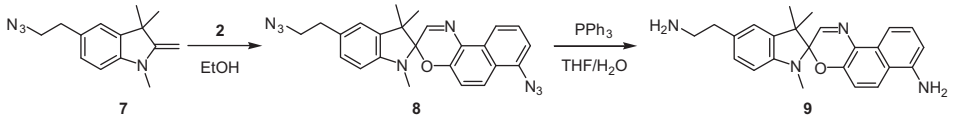
absorption spectra for their SP-over the 400–450 nm region. This spectral shift would allow the user to bring about the SP to MC transition by using 405 nm light, thereby minimizing photo-damage to living cells. A literature search of NISO related molecules suggested that introduction of certain functionalities, such as aldehyde, amino, and nitro onto the naphthalene side of NISO can bring about a red-shift in the SP-state absorption compared to NISO alone.[11] This knowledge was employed for the design and synthesis of amino-substituted NISO probes bearing reactive functionalities for coupling to proteins and to commercially available fluorescent dyes. These probes were further characterized to evaluate their spectroscopic and optical switching properties in solution.

The synthetic routes for the reactive NISO probes are presented in Schemes 1 and 2. 5-Azido-2-nitroso-1-naphthol (**2**) was synthesized in two steps from 5-amino-2-naphthol by conversion of amine to azide followed by nitrosation. These two-step reactions gave **2** in a good yield. Condensation of 1,2,3,3-tetramethyl-5-(carboxypropyl)-3*H*-indoleninium iodide (**1**)[10] and **2** in the presence of triethylamine (NEt_3) in ethanol gave NISO **3** in 59% yield. NISO **3** was further reduced with triphenylphosphine (PPh_3) in a 10:1 mixture of tetrahydrofuran (THF) and water to yield NISO **4** in 81% yield. Treatment of NISO **4** with *N*-hydroxysuccinimide (NHS) in the presence of *N*,*N*′ -dicyclohexylcarbodiimide (DCC) gave *N*-hydroxysuccinimide ester derivative **5**. Moreover, the maleimide was introduced into the substituted NISO by a coupling reaction between **5** and *N*-(2-aminoethyl)maleimide trifluoroacetate salt to give the maleimide derivative of NISO **6** with a yield of 72%. The amino derivative of NISO **9** also synthesized as shown in Scheme 2. The reaction of 5-(azidoethyl)-1,3,3-trimethyl-2-methyleneindoline (**7**)[10] with **2** gave the corresponding NISO **8** in 7% yield. Reduction of NISO **8** with PPh_3 in a mixture of THF and water gave the amino derivative of NISO **9**.

The photochromic behavior of the reactive NISO probes **5**, **6,** and **9** was studied by UV–vis absorption spectroscopy. For all experiments used in this study, these compounds were found to exist exclusively in their SP-state in solution at room temperature. The UV–vis absorption spectra of all NISO compounds were recorded



Scheme 1. Synthesis of *N*-hydroxysuccinimide ester and maleimide derivatives of 7′-amino NISO.



Scheme 2. Synthesis of amino derivative of 7′-amino NISO.

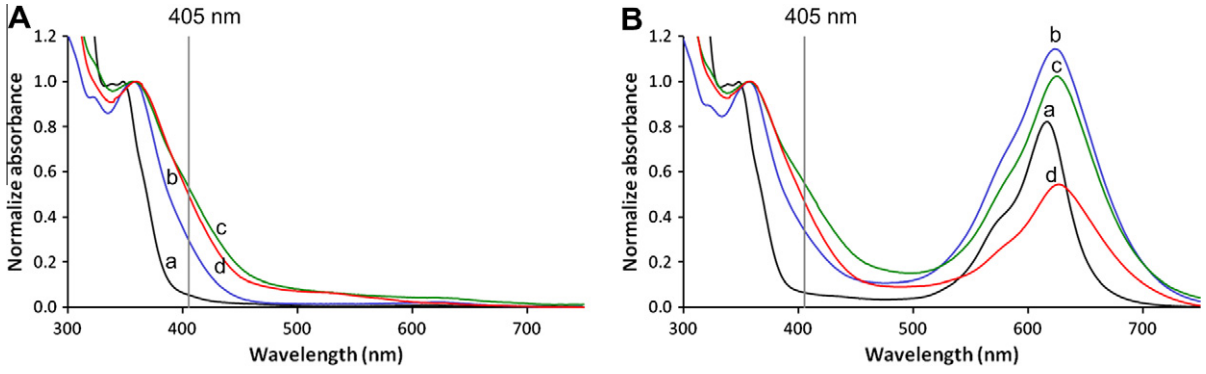


Figure 2. UV–vis absorption spectra of unsubstituted NISO, **5**, **6**, and **9** in glycerol (a–d, respectively) in the SP- (A) and MC-states (B). The SP-state was generated from the MC-state by irradiation of a 120 μl sample of NISO in a cuvette with 530-nm light for 1 min. The MC-state was generated from the SP-state by irradiating the SP-state with 365-nm light (a), or 405-nm light (b–d) for 1 min.

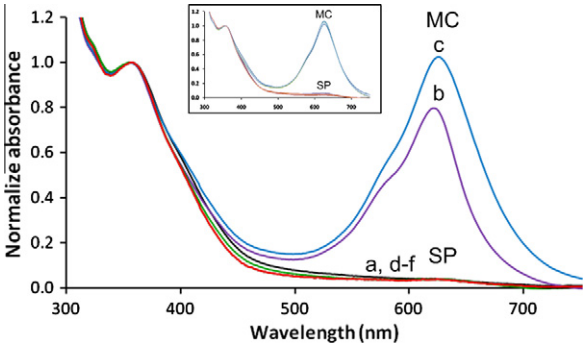


Figure 3. UV–vis absorption spectra of NISO **6** in glycerol. The MC-state was generated from the SP-state by irradiating the SP-state with 365-nm light (b), or 405-nm light (c) for 1 min. The MC-state absorption spectra of NISO generated by 365-nm light (b) and 405-nm light (c) have similar spectral shape. The SP-state was generated following a 1 min period of illumination with 530-nm light (d), 546-nm light (e), or 627-nm light (f). The SP-state absorption spectra (d–f) of NISO generated by visible lights have similar spectral shape as a beginning SP-state absorption spectrum (a). Inset: transitions between the SP- and MC-states of **6** using 365-nm and 530-nm lights were repeated for four cycles.

in glycerol, a viscous solvent, to reduce the thermally mediated transition of the colored MC-state to the colorless SP-state, as this is known to be rapid for NISO in methanol. The UV–vis absorption spectra of the 7′-amino-substituted NISO probes, **5**, **6,** and **9** in glycerol exhibit absorption red-shifts in their SP- and MC-states (of 356 nm and 625 nm, respectively) compared to unsubstituted NISO, which has a maximum SP- and MC-absorption values at 350 nm and 620 nm, respectively (Fig. 2). Additionally, the absorption spectra of 7′-amino-substituted NISO probes showed an absorbance value at 405 nm that was 35–50% of the maximum value at 356 nm. For comparison the 405 nm absorption of NISO is 7–10 times lower. Irradiation of the SP-state of NISO **6** with either UV (365 nm) or violet (405 nm) light was performed to investigate properties of the SP-to MC transition. Irradiation of a solution of SP-state of NISO **6** using the 405-nm output of a hand-held laser pointer (60 mW, Dragon Laser) generated the MC-state, as did excitation with the 365-nm output of a hand-held lamp employed for inspection of TLC plates. In both cases, the MC-absorption spectra were found to be very similar with an absorption maximum centered at 624–626 nm, suggesting that the same reaction occured regardless of the irradiation wavelength (Fig. 3). The MC-state was shown to convert back to the SP-state upon illumination of MC with either the 530-nm or 627-nm output from LEDs, or the 546-nm light from mercury arc lamp (Fig. 3). Optically driven transitions between the SP- and MC-states of **6** were realized for over four cycles by using alternate irradiation with 405-nm and 530-nm light—significantly, these photochemical reactions generated SP- and MC-absorption spectra that were almost identical between each cycle of optical switching as shown in Figure 3 inset. These data suggest that amino-NISO (**6**) undergoes multiple cycles of high fidelity optical switching between the SP- and MC-states with few signs of fatigue or secondary photochemistry. These features demonstrate the suitability of amino-NISO as a optical switch acceptor in TMR-NISO related FRET probes.[8,9]

In summary, we synthesized new amino-substituted NISO probes having red-shifted SP-absorption spectra. The optical switching cycle between SP- and MC-states in solution was successfully carried out by irradiating the SP-state with violet (405 nm) and the MC-state with visible (530 nm, 546 nm, and 627 nm). Investigations are underway to exploit these new probes within TMR-(amino)NISO derivatives for high contrast OLID-FRET imaging of specific proteins in living cells.[8]

Acknowledgement

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.10.084.

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