Energy transfer dyads based on Nile Red

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Abstract

This project was initiated to develop energy transfer dyads emitting in the 600 – 700 nm region by using Nile Red acceptors. Thus fluorescein- and BODIPY-based donors were linked to these acceptors via alkynes or triazoles. The product dyads (**1 – 5**) have energy transfer efficiencies of 77 – 97% in organic media.

Keywords

fluorophores; microwave reactions; Sonogashira coupling

Through-bond energy transfer is possible in molecules containing two fluorophores connected in such a way that they would be electronically conjugated if they were planar.[1−6] Our group is interested in the applications of such *dyads* in biotechnology,[7,8] especially for multiplexing experiments in which several outputs are to be observed from a single excitation source.[9,10] Nile Red is a solvatochromic dye with solvent dependent emissions. In polar media Nile Red derivatives have emissions in the 630 – 650 nm region. Outputs at such wavelengths, *ie* longer than cellular autofluorescence,[11] are highly desirable for cellular probes, so dyads containing Nile Red are logical targets, but they have not been described in the literature. Here we report the syntheses and photophysical properties of dyads **1 – 5**.
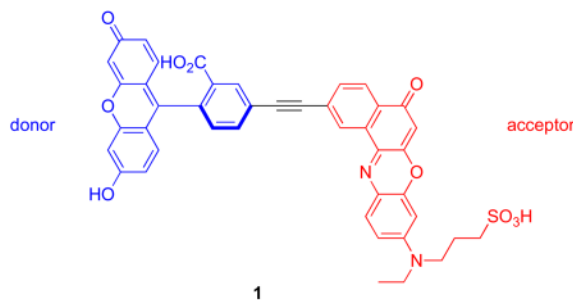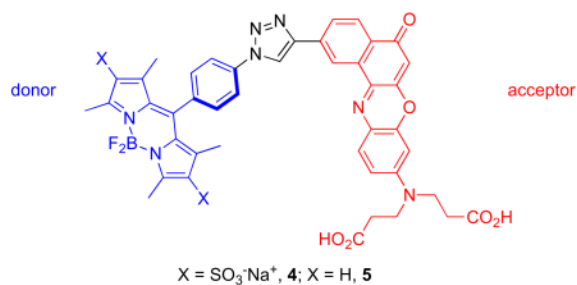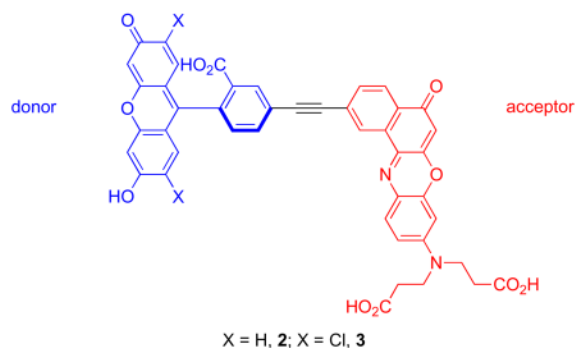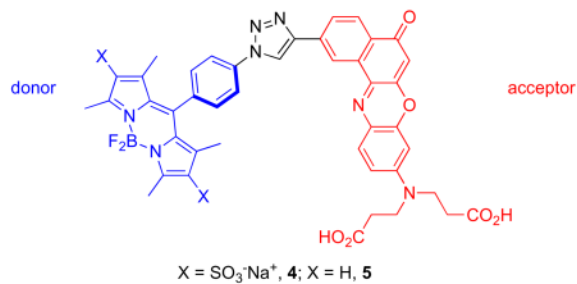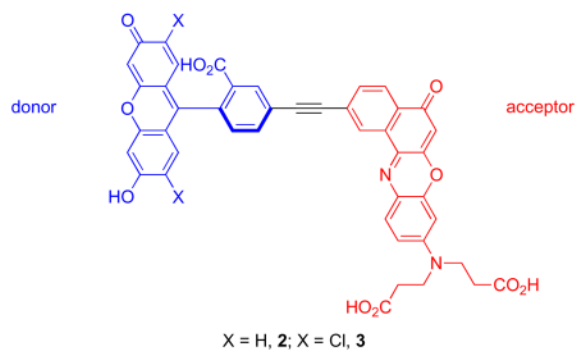
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Supplementary data
Synthetic procedures for all compounds, copies of ^{1}H, ^{13}C NMR spectra.

X = H, **2**; X = Cl, **3**

X = SO$_3^-$Na$^+$, **4**; X = H, **5**

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X = SO$_3^-$Na$^+$, **4**; X = H, **5**

Dyads **1** – **3** have fluorescein-based donors, while BODIPY derivatives perform this role in **4** and **5**. Three of the donor fragments **A** – **C** were prepared via methods already developed in this laboratory (Figure 2).[12–14] The other donor, the BODIPY derivative **6**, was synthesized from the nitro compound **D**15 via reduction with hydrazine.[13] The amine product is unstable hence it was immediately diazotized then treated with azide anion (reaction 1).

A X = H
B X = Cl

C

(i) $NH_2NH_2.H_2O$, 10 % Pd/C
EtOH, reflux, 30 min.

(ii) $NaNO_2$, HCl/MeOH, 0 °C, 1 h
(iii) NaN_3, H_2O, 25 °C, 1 h

D

6 38 %
reaction 1
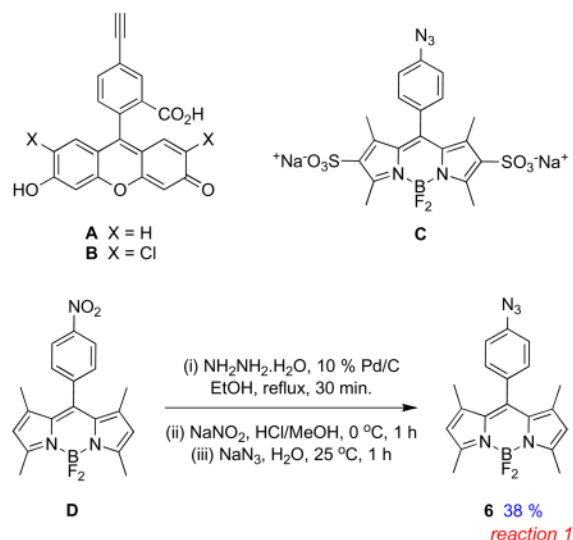
Water-solubilities are important since the long-term goals of this research are to prepare probes that can be used in biological environments. Consistent with this idea, donors **A** **C** are very hydrophilic. Nile Red acceptors that also tend to improve water solubilities were available from previous work by others[16] and by us.[17] Compounds in this series typically fluoresce with quantum yields around 0.4 in EtOH and 0.3 in pH 7.4 phosphate buffer.[17,18] Thus, the sulfonate **7** was made via triflation {$PhN(Tf)_2$, NEt_3, THF, 25 °C, 24 h}19 of the corresponding 2-hydroxy Nile Red derivative,16 and a similar reaction followed by Sonogashira coupling[20] (of trimethylsilylethyne, then TBAF deprotection) gave derivatives **8** and **9**.

7

8 X = OTf

9 X = -⌇≡
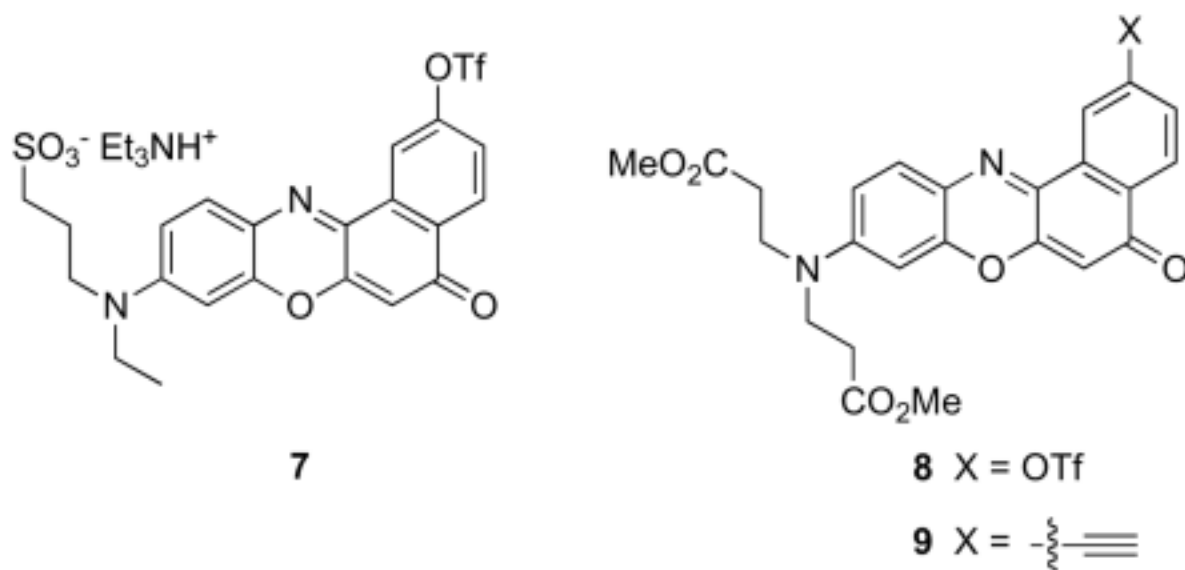
Scheme 1 outlines syntheses of the alkyne-linked dyads **1** – **3**. The coupling reactions proceeded only above 100 °C, and 130 °C was preferred. The dichlorofluorescein alkyne **B** is not very stable at 130 °C hence the coupling reaction using this was low yielding. Throughout, Pd$(PPh_3)_4$ was an effective catalyst while $Cl_2Pd(PPh_3)_2$ did not give any significant amount of product.

Dyads **4** and **5** were synthesized via copper catalyzed 1,3-dipolar cycloadditions using tris(1 - benzyl - 1H - 1,2,3 - triazol - 4 - yl)methyl amine (TBTA) ligand[21] as a copper stabilizer (Scheme 2) without which the yields were poor. Reaction of the water-soluble BODIPY-azide **C** with the Nile Red alkyne **9**, followed by hydrolysis in aqueous methanolic K_2CO_3 afforded cassette **4** as a dark purple material. The low yields obtained for these two dyads are probably due to the apparent instability of BODIPY fragments in basic aqueous media.

Absorption and emission profiles of dyads **1** – **5** in EtOH are shown in Figure 1. As expected, two distinct absorption maxima were observed in each case corresponding to the donor and acceptor fragments. Their fluorescence spectra show efficient energy transfer without significant —leakage of emission from the donor fragment. The prominent emissions from all the dyads derive from the acceptor fragment with comparatively less emission from the donor parts. Their quantum efficiencies when excited at the donor absorption maxima, were determined to be in the range of 0.31 – 0.41. The energy transfer efficiency (ETE %, quantum yield of cassette when excited at the donor divided by that when excited at the acceptor)[10] was calculated to be 77 – 97 % (Table 1). The later parameter is less informative, because it does not take into account energy lost in other processes. The apparent Stokes' shifts for the dyads ranged from 120 to 150 nm. Much less efficient energy transfer was observed for the dyads in aqueous buffer, but the large Stokes' shifts persisted (see supplementary).

In conclusion we have prepared five dyads based on Nile Red acceptors and fluorescein or BODIPY fragments as donors. These show efficient energy transfer in ethanol and fluoresce with a quantum yield of 0.31–0.41. Their long wavelength emission and large Stokes' shifts are useful for fluorescence studies in polar, hydrogen-bonding, organic solvents. Our current efforts are focused on making similar dyads with improved photophysical properties for use in biological media.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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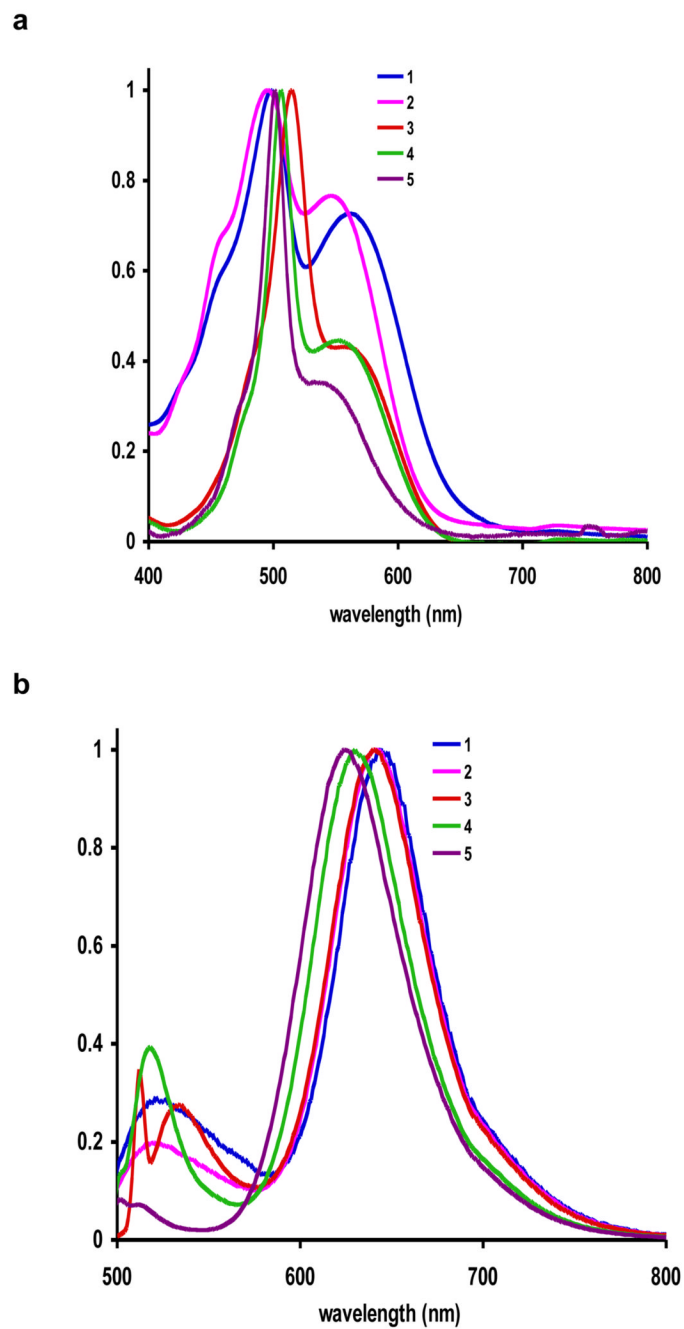
Figure 1.
Normalized absorbance (**a**) and fluorescence (**b**) spectra of dyads **1** – **5** in EtOH (at 10^{-6} and 10^{-7} M for absorbance and fluorescence measurements, respectively). All dyads excited at their respective donor absorption maxima.
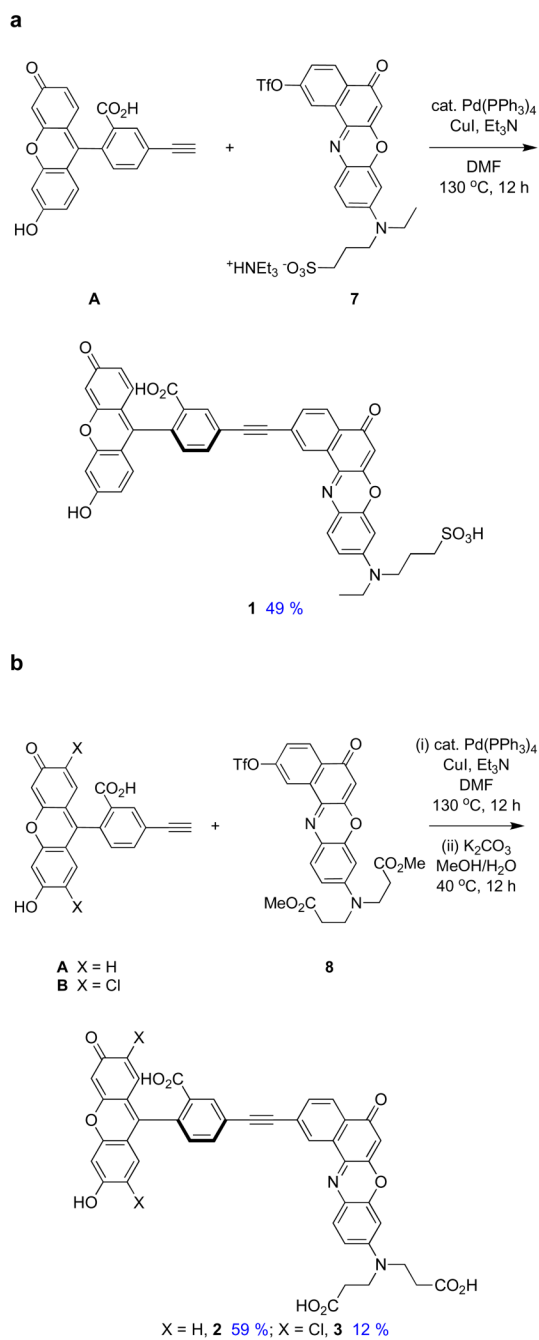
Scheme 1.
Syntheses of the alkyne linked dyads **1** – **3**.
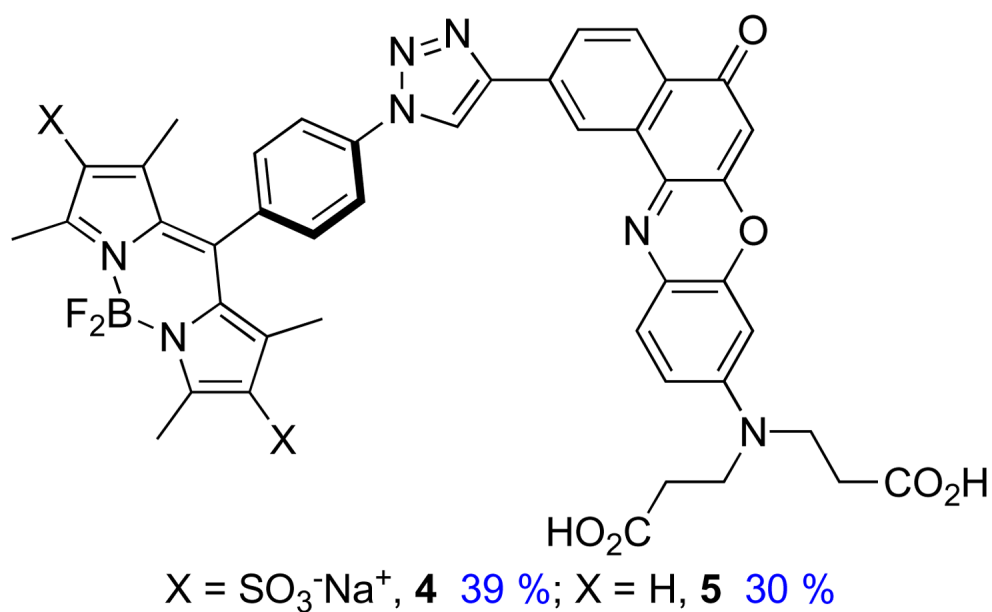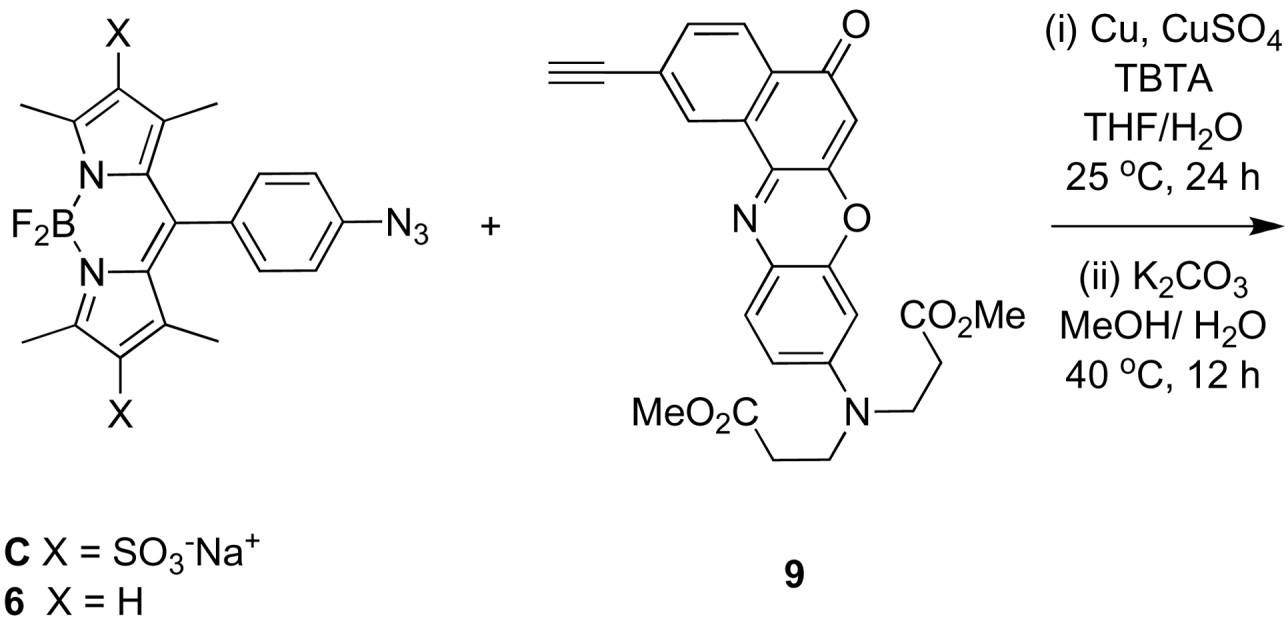
Scheme 2.
Synthesis of dyads **4** and **5**.

Table 1

Photophysical properties of dyads **1 – 5** in EtOH.

dye	λ_{abs}(nm)	$\lambda_{em.}$(nm)	Stokes Shift (nm)	Φ_D	$\Phi_A{}^c$	ETE % (Φ_D/Φ_A)
1	498, 561	648	150	0.31+/− 0.02[a]	0.40+/− 0.03	77
2	495, 546	644	149	0.34+/− 0.01[a]	0.38+/− 0.01	89
3	515, 555	645	130	0.36+/− 0.03[b]	0.43+/− 0.02	83
4	506, 553	632	126	0.41+/− 0.02[b]	0.49+/− 0.02	81
5	501, 541	624	123	0.36+/− 0.02[b]	0.37+/− 0.01	97

Φ_D values correspond to excitation of dyads at the donor absorption maxima; Φ_A relates to excitation of dyads at the acceptor absorption maxima. Standards used for the quantum yield measurements:

[a] fluorescein in 0.1 M NaOH (Φ0.92),

[b] rhodamine 6G in EtOH (Φ0.94),

[c] rhodamine 101 in EtOH (Φ1.0). Quantum yield measurements were repeated three times and averaged.