ChemComm

Energy transfer cassettes based on BODIPY® dyes†

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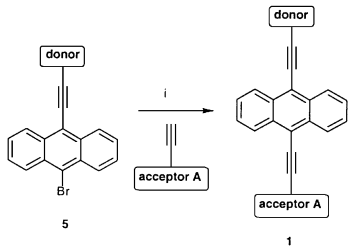
Received (in Corvallis, OR) 16th August 2000, Accepted 8th September 2000
First published as an Advance Article on the web 31st October 2000

The donor–acceptor dye cassettes 1–4 were designed to capture energy at a single wavelength and to convert it to well-resolved, intense fluorescence emissions; in practice, Stokes' shifts of 40–148 nm, quantum yields of 0.12–0.60, and efficient energy transfer was demonstrated.

Many biological experiments involve irradiating sets of different fluorescent labels with a single excitation source.[1,2] To be effective in such multiplexing experiments, dyes must give fluorescence emission peaks that are both well resolved and intense. These requirements place conflicting demands on the labels. Having sets of dyes that emit close to the excitation wavelength causes resolution problems, while including dyes that emit far from the excitation wavelength gives loss of intensity. In response to this problem, radiationless electronic energy transfer has been used to improve resolution and/or intensities in multiplexing.[3] Systems which exploit this characteristic relay energy from a donor-dye (that absorbs at relatively short wavelengths) to an acceptor-dye that fluoresces at longer wavelengths. Experimentally, it is most convenient if the donor and acceptor components of such systems are introduced simultaneously as a single unit, *i.e.* in an "energy transfer cassette". A challenge in this emerging field[3] is to form cassettes that circumvent the limitations of the classical fluorescein–rhodamine systems (*e.g.* resolution, pH dependence, gel mobility shifts). To address this issue, our group is preparing cassettes wherein the donors and acceptors are linked *via* rigid conjugated linkers that allow radiationless electronic energy transfer through bonds (Dexter and superexchange mechanisms) and/or through-space (Förster mechanism).[4] This communication describes synthesis and spectroscopic studies of new cassettes based on the 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY®) dyes shown below.

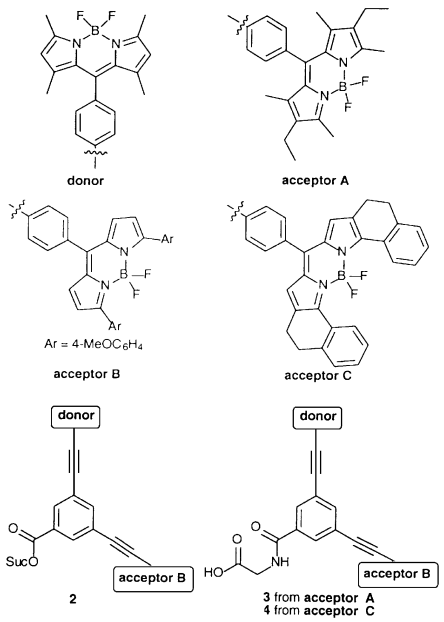
To access the cassettes, iodoaryl-substituted BODIPY®s were prepared[5,6] then transformed into the corresponding alkynes by Sonogashira couplings (trimethylsilylethyne, cat. $Pd(PPh_3)_4$, CuI, NEt_3, THF; then removal of the silane, Bu_4NF, THF, −78 °C, 10 min). Invariably, the yields for these two reactions were very high (up to 99%). Sonogashira reactions were also used to assemble the cassettes from the BODIPY® starting materials. Scheme 1 outlines a synthesis of a linear cassette **1**. The branched derivatives **2**–**4** were obtained *via* similar sequences featuring Sonogashira couplings.



Scheme 1 *Reagents and conditions*: i, cat. $Pd(PPh_3)_4$, CuI, DABCO, toluene, 80 °C, 26 h (51%).

Cassette **1** was prepared mainly to assess its spectroscopic properties; it does not have a functional group that could be conveniently used to link to a biomolecule. A succinimide ester was incorporated into cassette **2** so that, in subsequent work, this dye could be used as a label. Activated esters of aromatic acids, however, are less active than their aliphatic analogs, hence cassettes **3** and **4** were functionalized with glycine attachment points. These variations have no effect on the spectroscopic properties of the dyes reported in this communication; this discussion is only included here to explain how these differences between the structures are consistent with the overall direction of this project.

Table 1 summarizes some light spectroscopic properties of the cassettes dissolved in $CHCl_3$. Using EtOH as the solvent had little effect (a few nm blue-shift relative to $CHCl_3$) on the position and band shape of the fluorescence and absorption spectra (data not shown). The UV absorption spectra of the cassettes resemble those that would be obtained by superimposing the donor and acceptor spectra, indicative of two independent dye fragments. However, when irradiated at the absorption maxima of the donor, the donor fluorescence is almost completely quenched. Thus energy is absorbed at wavelengths corresponding to the donor fragment and emitted at wavelengths governed by the acceptor part, hence large *pseudo*-Stokes' shifts result. Förster radii of the cassettes were determined and found to be significantly greater than the calculated donor–acceptor distances, hence the calculated energy transfer efficiencies were high.[7,8] Experimentally, it was shown that the energy transfer efficiencies (E_{expt}) were very high, meaning the cassettes are very effective. These are slightly



† Electronic supplementary information (ESI) available: absorption and emission spectra of donor and acceptor A and of **1** and experimental details for the spectroscopic measurements. See http:www.rsc.org/suppdata/cc/b0/b006769p/

DOI: 10.1039/b006769p

Table 1 Selected light spectroscopic data for compounds **1**–**4**

Parameter[a]	Compound (in $CHCl_3$) **1**	**2**	**3**	**4**
$\lambda_{max\ abs}$ of donor in cassette/nm	504	504	504	504
$\varepsilon_{max} \times 10^{-3}$ ($mol^{-1}\ dm^3\ cm^{-1}$)	75.8 (donor) 46.3 (acc.)	75.8 (donor) 54.1 (acc.)	75.8 (donor) 46.3 (acc.)	75.8 (donor) 115 (acc.)
$\lambda_{max\ em}$ of acceptor in cassette/nm	544	632	545	652
Pseudo-Stokes' shift/nm	40	128	41	148
Förster radii/Å[b]	50.0 ± 1.0	47.2 ± 1.0	50.0 ± 1.0	39.4 ± 1.0
Calc. donor–acceptor distance/Å[c]	24.5	19.9	19.9	19.9
Calc. energy transfer efficiency[d]	0.982	0.994	0.996	0.983
Expt. energy transfer efficiency[e]	0.968	0.936	0.941	0.946
Fluorescence quantum yield[f]	0.54	0.15	0.58	0.42
Fluorescence quantum yield[g]	0.60 ± 0.03	0.12 ± 0.02	0.56 ± 0.03	0.41 ± 0.03

[a] Additional data (not shown here) was obtained on the donor and acceptor fragments A–C as the corresponding aryl iodides to calculate some of the parameters listed. [b] Calculated from molar absorptivity of the acceptor and corrected fluorescence spectrum of the donor *via* a literature procedure;[8] the κ^2-values (average angular dependence of dipole–dipole coupling in the dynamic limit) used were 0.5 for **1** and 0.665 for **2**–**4**.[10] [c] Estimated using standard bond lengths. [d] From Förster theory as described; uncertainties of the values are within 0.010.[11] [e] From steady-state fluorescence and absorption measurements; uncertainties of the values are within 0.010.[11] [f] From $\phi^{DA} = \phi^{A}E_{expt}(\tau^{A,DA})(\tau^{A})^{-1}$ where $\tau^{A,DA}$ and τ^{A} denote the fluorescence lifetime of the acceptor in the presence and absence of the donor, respectively. [g] Determined using fluorescence and absorption spectra.[9] As references of fluorescence quantum yields were used; *N*,*N*′-bis(1-hexylheptyl)-3,4:9,10-perylene-bis(dicarboximide) in CH_2Cl_2 (Φ = 0.99[12]), 3,3′,4,4′-difluoro-1,3,5,7-tetramethyl-4-borata-3a-azonia-4a-aza-*s*-indacene in MeOH (Φ = 0.94[13]) and Cresyl violet in MeOH (Φ = 0.54[14]).

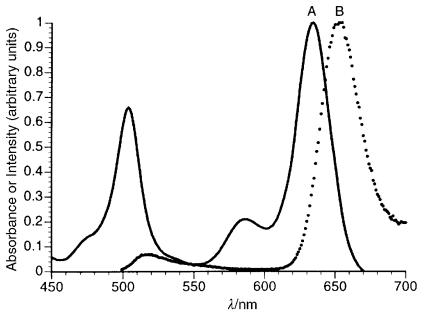


Fig. 1 Absorption ——— (A) and emission •••••• (B) spectra for cassette **4**.

lower than the calculated values, perhaps because the calculations assume energy transfer *via* the dipole–dipole coupling mechanism, which is not a good approximation when distances of interaction are compatible with the molecular size.[7] For all systems studied, the fluorescence decays were not perfectly well described by a single exponential function. Instead bi-exponential fits were found to be acceptable (data not shown). Weighted residuals, reduced χ^2 parameter and the Durbin-Wattson parameter judged upon the goodness of fit. Values considered acceptable were, of the reduced χ^2 parameter < 1.2 and of the Durbin-Wattson parameter > 1.7. The origin to the non-exponential relaxation is not known, but a possible explanation could be connected to a non-homogeneous distribution of the mutual donor and acceptor orientations. Average fluorescence lifetimes varied from 0.9 to 4.2 ns. The fluorescence quantum yields upon exciting the donor (Φ^{DA}) were calculated in two ways for the cassettes, namely; from fluorescence lifetime data (see table text) and by using fluorescence and absorption spectra.[9] The corresponding fluorescence quantum yields (Table 1) are lower than those of the acceptor fragments (data not shown). The fact that there is also a discrepancy in fluorescence lifetimes may partly be assigned to non-radiative processes, other than the Förster mechanism. Despite this, the energy transfer efficiencies are high.

Data from cyclic voltammetry studies demonstrated that the donor and acceptor parts of the cassettes do not communicate electronically in the ground state. For example, cassette **4** displays four distinct redox waves (in CH_3CN at 100 mVs^{-1}, glassy carbon electrode). These are two oxidative ($E_{1/2}$ = +0.62 and +0.78 V *vs.* ferrocene), and two reductive ($E_{1/2}$ = −1.19 and −1.52 V) waves; all four of which can be assigned to redox processes of the single dye portions. This confirms the conclusion from the spectroscopic studies that the donor and acceptors behave as distinct entities, rather than a unified, conjugated system.

In conclusion, this work shows that the BODIPY® fluorophores conjugated in cassettes **1**–**4** exhibit extremely high energy transfer from the donor to the acceptor giving enhanced resolution and intensities for the system when irradiated at the donor absorption maxima. The *pseudo*-Stokes' shifts for the dyes vary between 40 and 148 nm, and their quantum yields are from 0.12 to 0.60. It appears that in these systems *para* or *meta*-orientations (*cf.* **1** and **3**) make no significant difference. Even though these dyes have donor and acceptor fragments that are connected *via* a conjugated linker, steady-state and time-resolved fluorescence measurements are consistent with highly efficient energy transfer according to the Förster mechanism alone.

We thank Ben Lane for running the ^{11}B NMR spectra for this study. Financial support for this work was provided by the NIH (HG01745), The Swedish Natural Science Research Council (NFR K6501981724/2000), The Kempe Foundation, and The Robert A. Welch Foundation.

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