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Syntheses and spectroscopic properties of energy transfer systems based on squaraines

Guan-Sheng Jiao,[a] Aurore Loudet,[a] Hong Boon Lee,[a] Stanislav Kalinin,[b] Lennart B.-Å. Johansson[b] and Kevin Burgess[a,*]

[a]*Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, TX 77841-3012, USA*
[b]*Department of Chemistry: Biophysical Chemistry, Umeå University, S-90 187 Umeå, Sweden*

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Abstract—The purpose of this project was to prepare fluorescent dyes that could absorb energy at relatively short wavelengths, and fluoresce in the near-IR region. To achieve this, copper- and palladium-mediated C–N couplings were used to prepare the 'cassettes', i.e the carbazole derivative **3b** and the carbazole-, phenothiazine-, and phenoazine-squaraines **4b**–**d**. These compounds have carbazole, phenothiazine, and phenoazine donor-components that absorb around about 300–320 nm, and squaraine acceptor-parts that fluoresce in the range 650–700 nm. The efficiencies of energy transfer from the donor to the acceptor, and the overall quantum yields of the cassettes were determined.

1. Introduction

'Donor acceptor cassettes' (compounds with a moiety to absorb at low wavelength and another to emit at a higher wavelength) based on through-space fluorescence energy transfer are common in biotechnology.[1–8] In these, a molecular fragment, the 'donor', harvests energy from an excitation source and transmits it through space, via dipole–dipole coupling, to an 'acceptor' that then fluoresces. The donor and acceptor fragments in these cassettes are connected via a non-conjugated linker (Fig. 1(a)). We are investigating another type of cassette in which the donor and acceptor fragments are conjugated.[9,10] This change is apparently subtle, but it can have profound effects on the rates of energy transfer (ET) from the donor to the acceptor, and the 'apparent Stokes' shifts' observed (Fig. 1(b)).[11] Conjugated ET systems of this kind have been investigated extensively in material science.[12,13] Cassettes of this kind could absorb at short wavelength and emit efficiently at much longer wavelengths, and might therefore be useful in applications that involve several multiplexed dyes, excited at one wavelength and observed at well dispersed longer wavelengths. The work described here was undertaken as part of our project to put these ideas to practice. Specifically, this manuscript describes syntheses and spectral investigations of novel through-bond ET cassettes in which donors are directly conjugated to a squaraine acceptor (Fig. 1(c)). Squaraine acceptors are particularly attractive because they emit in the near IR, and this region is far removed from the fluorescence of any biomolecules.[14] We were particularly interested in carbazole and similar heterocycles as donors since these absorb in the UV region at around 300 nm.

2. Results and discussion

2.1. Syntheses

Our preliminary studies led to the preparation of compounds like **1** in which the squaraine fragment was formed from resorcinol derivatives; however, the products had low quantum yields (data not shown). In retrospect, this is unsurprising because the 2,6-dihydroxyl substituents facilitate tautomerization processes, leading to radiationless decay.[15] Similar effects do not apply to the second class of cassettes we prepared, i.e. **2** and others containing 3,3-dimethylindolenine fragments. The improvement in quantum yields was not marked, however, because another detrimental characteristic comes into play for unsymmetrical squaraines; specifically, rotation about the bond labeled '**a**' is facile because resonance favors single bond character here relative to bond '**b**'. This effect makes electronic transmission through the squaraine system more difficult than it would otherwise be, depressing the quantum yield. Consequently, this paper focuses on the symmetrical squaraines **3** and **4** incorporating 3,3-dimethylindolenine and 2-methylbenzothiazole fragments, respectively (Fig. 2).

Keywords: squaraines; fluorescence; energy-transfer; C–N couplings.
* Corresponding author. Tel.: +1-979-845-4345; fax: +1-979-845-8839; e-mail: asymm@tamu.edu

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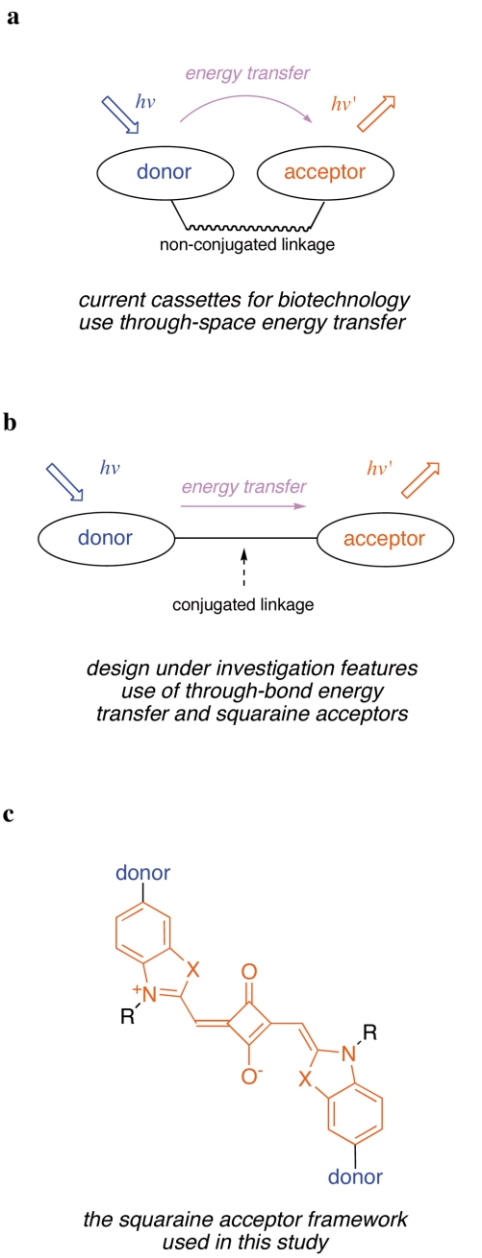


Figure 1. (a) Through-space ET cassettes, (b) through-bond ET cassettes, (c) the acceptor fragments used in this study.

Scheme 1(a) describes the syntheses of the cassettes **3b**. After *N*-alkylation of the iodoindolenine to give **5b**, copper-mediated couplings[16] were used to introduce the carbazole unit giving compounds **6b** in moderate yield (53%). Palladium-mediated couplings to achieve the same result were investigated briefly, but with little success.[17] Compound **6b** was condensed with squaric acid[18] to give the desired product as a blue metallic solid. The control compound **3a** was prepared via an abbreviated version of this sequence. Preparation of the cassettes **4** was achieved via a similar procedure (Scheme 1b) except that a palladium-mediated C–N coupling was used. The most notable difference between the two approaches is that the metal-catalyzed reactions gave higher yields in the second sequence (76–90%).[17]

2.2. Spectroscopic studies

A series of spectroscopic measurements were undertaken to test if compounds **3b** and **4b–d** behave as cassettes (rather than as simple conjugated molecules) and, if so, to quantitate their efficiencies. First, UV spectra of the molecules were recorded. If the UV spectrum of a particular system resembles that which would be obtained by superimposing UV spectra of the corresponding discrete donor and acceptor components, then the molecule is behaving as a cassette. Alternatively, if the UV is different, then the compound is behaving as a conjugated system. Figure 3(a) shows the UV spectra of compound **3b** overlaid with the spectrum of carbazole and **3a**, i.e. the donor and acceptor components, respectively. The UV spectrum of **3b** is very close to that which would be obtained by adding the donor (carbazole) and acceptor (**3a**) components. Consequently, this molecule behaves as a cassette with non-planar donor and acceptor systems in the ground state. Similar data were obtained for **4b–d** indicating they all function as cassettes (Fig. 3).

One of the criteria that define a good cassette is quenching of the donor fluorescence. If this quenching is complete then the ET efficiency is 100%, where:

ET efficiency =

{100 × [1 − (fluorescence intensity of donor in cassette)/

(fluorescence intensity of free donor)]%}

In our systems the fluorescence of the free carbazole donor was so weak that accurate ET efficiency measurements proved to be impossible. In practice, it was also difficult to accurately measure the fluorescence quantum yield when exciting the donor group of the cassettes studied here. Thus, two important characteristics of the cassettes, ET efficiencies and fluorescence quantum yields, could not be measured directly.

ET efficiencies were estimated using another approach to circumvent the experimental difficulties outlined above; this involved the following line of reasoning. The efficiency of ET in a cassette must be close to 100% if the fluorescence quantum yield of the acceptor group is the same when excited at the donor, or at the acceptor absorption. That the ratios of the fluorescence excitation and the UV absorption spectra of the cassettes were constant over the whole spectral region provides evidence for ET efficiencies close to unity. Fluorescence lifetimes of the squaraine acceptor group in all the cassettes were also measured and shown to be very similar irrespective of the excitation wavelength. The results obtained are summarized in Table 1 (details are given in Section 4).

The quantum yields were determined for irradiation of **3b** and **4b–d** in the acceptor region (Table 1). The values (0.23–0.37) show moderate fluorescence efficiencies. Rates of ET between the donor and the acceptor are likely to be fast relative to any thermal energy loss, so the quantum yields of the cassettes when irradiated in the donor region

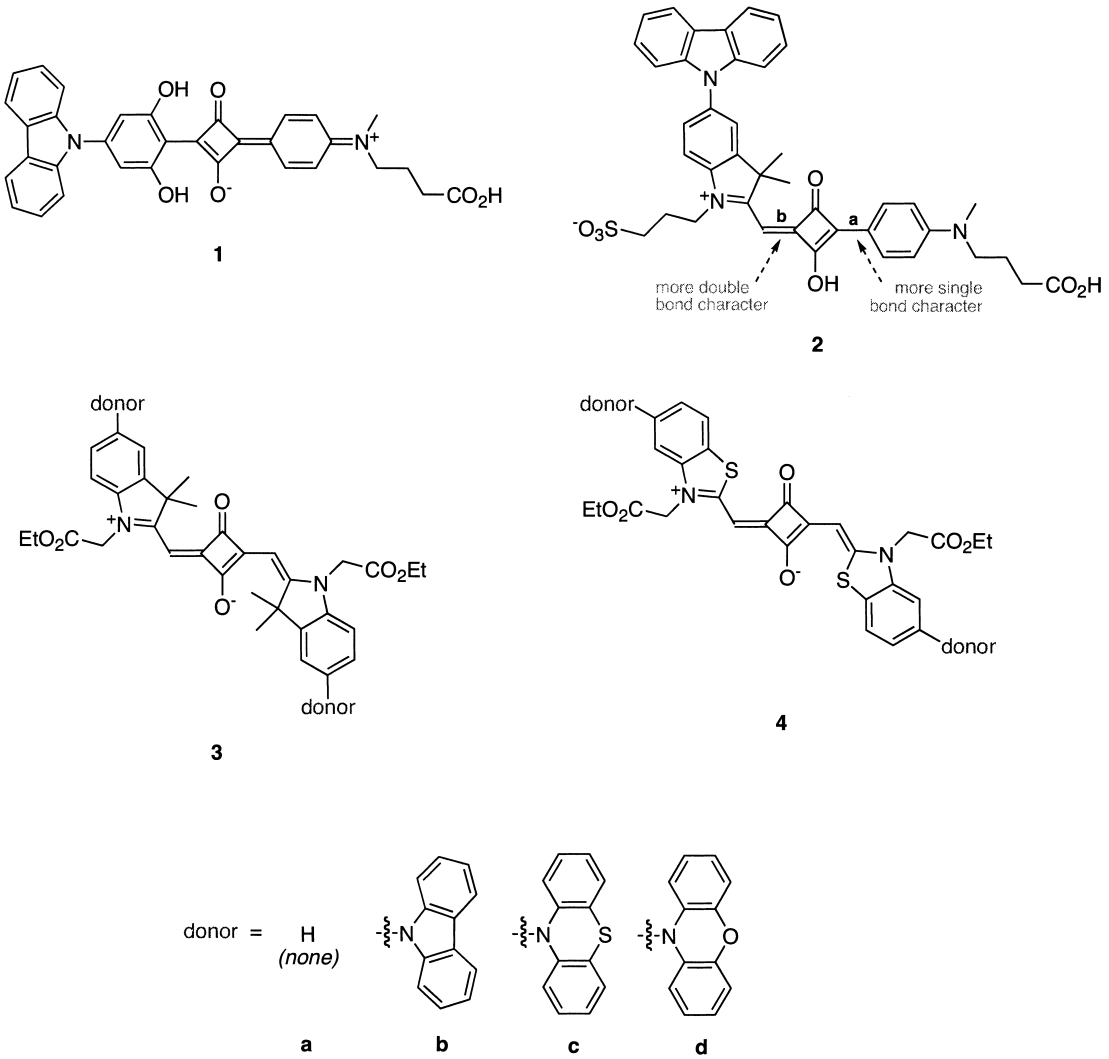


Figure 2. Donor–acceptor cassettes in this research.

are likely to approximate to those of the acceptor components within them.

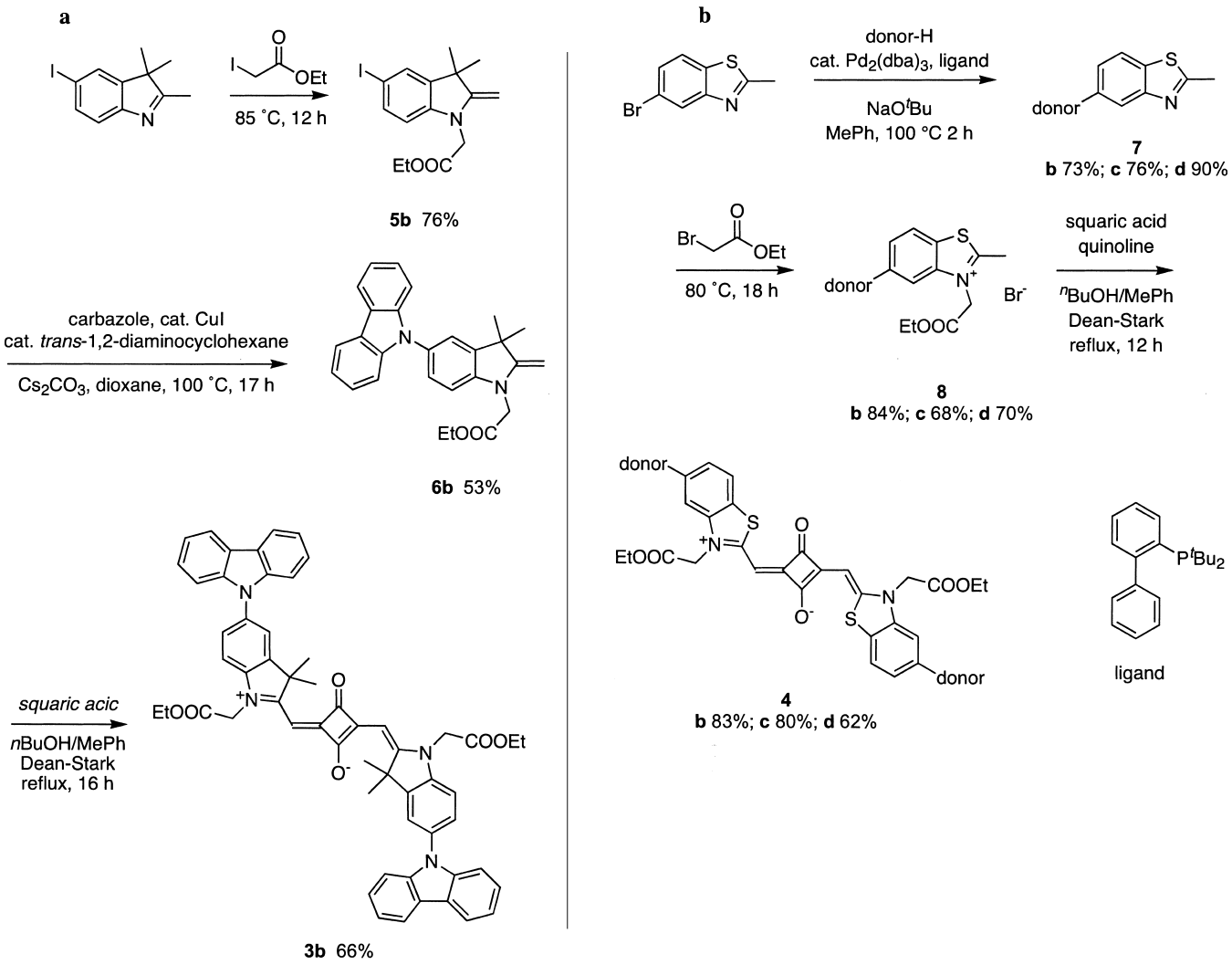
3. Conclusions

In conclusion, several cassettes based on squaraine fragments acting as acceptors (**3b** and **4b**–**d**) were prepared. These cassettes transfer energy very efficiently to their acceptor parts when irradiated in the donor absorption region (ca. 300 nm). With respect to fluorescence emission, the cassettes behave like dyes with a huge Stokes' shift, absorbing around 300 nm and fluorescing in the near IR region (637–693 nm). Emission at such long wavelengths is good for probing biological systems which do not fluoresce in this region, i.e. DNA, RNA, the majority of proteins, and many other biomolecular types. The quantum yields of the acceptors in the cassettes are high, and we infer that they are also high for the cassettes when irradiated in the donor region. Despite these attributes, cassettes **3b** and **4b**–**d** have some disadvantages with respect to tagging biological molecules. First, the donor fragments do not absorb very strongly, hence they cannot supply the acceptor well. Secondly, the molecules have poor water solubilities and this is likely to make their conjugation to biomolecules difficult. Our current research on similar ET cassettes is focused on solving these problems.

4. Experimental

4.1. General

4.1.1. Preparation of compound 5b. 5-Iodo-2,3,3-trimethyl-3*H*-indole[19] (1.00 g, 3.50 mmol) and ethyl iodoacetate (0.901 g, 4.21 mmol) were weighed into a sealed tube. The mixture was heated at 85°C for 12 h. After cooling to room temperature, 1N NaOH solution (20 mL) was added and stirred for 1 h. The reaction mixture was extracted with dichloromethane three times. The organic layers were combined and evaporated to a residue which was purified by flash column chromatography in 10% EtOAc/hexane to give the product as a brown oil (0.988 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (dd, J=9.0, 2.0 Hz, 1H), 7.36 (d, J=2.0 Hz, 1H), 6.30 (dd, J=9.0, 2.0 Hz, 1H), 4.19 (q, J=8.0 Hz, 2H), 4.19 (s, 2H), 3.94 (d, J=3.0 Hz, 1H), 3.87



Scheme 1. Syntheses of (a) cassette **3b**; and (b) cassettes **4b–d**.

(d, J=3.0 Hz, 1H),1.35 (s, 6H), 1.24 (t, J=8.0 Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 168.6, 160.8, 145.35, 140.27, 136.30, 131.07, 107.51, 80.71, 75.53, 61.47, 44.41, 44.25, 30.04, 14.34; HR-MS (MALDI) for $C_{15}H_{19}INO_2$ ($M+H^+$) calc'd 372.0461, found 372.0445.

Compound **5a**.[20] 62%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21–7.16 (m, 2H), 6.88 (t, J=7.8 Hz, 1H), 6.57 (d, J=7.8 Hz, 1H), 4.29 (s, 2H), 4.26 (q, J=7.2 Hz, 2H), 3.99 (d, J=2.4 Hz, 1H), 3.91 (d, J=2.4 Hz, 1H), 1.45 (s, 6H), 1.30 (t, J=7.2 Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 169.2, 161.8, 145.7, 137.6, 127.8, 122.3, 119.5, 105.4. 74.7, 61.5, 44.6, 44.5, 30.3, 14.5.

4.1.2. Preparation of compound 6b. Compound **5b** (200 mg, 0.54 mmol), carbazole (90 mg, 0.54 mmol), 1,2-*trans*-diaminocyclohexane (12 mg, 0.11 mmol), CuI (5 mg, 0.03 mmol), and Cs_2CO_3 (369 mg, 1.13 mmol) were weighed into a round-bottom flask, then dioxane (2 mL) was added. The mixture was heated at 100°C for 17 h. After cooling to room temperature, the reaction mixture was diluted with ether and filtered through a pad of celite. The residue after removal of solvent was purified by flash column chromatography using 20% EtOAc/hexane to give the product as a white solid (134 mg, 53%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, J=8.0 Hz, 2H), 7.43–7.26 (m, 8H), 6.68 (d, J=8.0 Hz, 1H), 4.33 (s, 2H), 4.28 (q, J=8.0 Hz, 2H), 4.02 (d, J=3.0 Hz, 1H), 3.96 (d, J=3.0 Hz, 1H), 1.45 (s, 6H), 1.32 (t, J=8.0 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 168.94, 161.48, 144.99, 141.71, 139.22, 136.35, 131.12, 129.38, 126.95, 125.96, 123.21, 121.67, 120.47, 120.41, 119.67, 119.54, 110.76, 110.00, 107.53, 105.79, 75.71, 61.59, 44.63, 44.52, 30.20,14.43.

4.1.3. Preparation of squaraine 3b. Compound **6b** (24 mg, 0.06 mmol) and squaric acid (3 mg, 0.03 mmol) were placed in a round-bottom flask. A mixture of *n*-BuOH/toluene (15 mL, 1:1 v/v) was added. The mixture was refluxed for 16 h. Water was azeotropically removed by using a Dean–Stark trap. After cooling to room temperature, the reaction mixture was filtered to give a dark-blue solid, which was further washed with ether three times (15 mg, 66%). A sample for analysis was obtained by further purification through flash column chromatography. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, J=8.0 Hz, 4H),

7.56–7.27 (m, 16H), 7.10 (d, *J*=8.0 Hz, 2H), 6.02 (s, 2H), 4.33 (q, *J*=7.2 Hz, 4H), 1.83 (br s, 12H), 1.36 (t, *J*=7.2 Hz, 6 H); HR-MS (MALDI) for $C_{58}H_{51}N_4O_6$ ($M+H^+$) calcd 899.3809, found 899.3825.

Compound **3a**. 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (t, *J*=7.2 Hz, 2H), 7.31 (d, *J*=7.2 Hz, 2H), 7.19 (t, *J*=7.2 Hz, 2H), 6.91 (d, *J*=7.2 Hz, 2H), 5.87 (s, 2H), 4.76 (br s, 4H), 4.26 (q, *J*=7.2 Hz, 4H), 1.81 (br s, 12H), 1.29 (t, *J*=7.2 Hz, 6H); HR-MS (MALDI) for $C_{34}H_{37}N_2O_6$ ($M+H^+$) calcd 569.2652, found 569.2627.

4.1.4. Preparation of compound 7b. Carbazole (184 mg, 1.1 mmol), 5-bromo-2-methylbenzothiazole (228 mg, 1.00 mmol), and sodium *tert*-butoxide (134 mg, 1.40 mmol) were placed in a Schlenk tube. Tris(dibenzylideneacetone)-dipalladium (23 mg, 0.02 mmol) and 2-(di-*t*-butylphosphino)-biphenyl (12 mg, 0.04 mmol) were weighed in glove box, the tube was removed from the box, then toluene (2 mL) was added. The mixture was heated at 100°C for 2 h. After cooling to room temperature, the reaction mixture was diluted with ether and filtered through a pad of celite. The residue after removal of solvent was purified by flash column chromatography using 20% EtOAc/hexane to give the product as a white solid (256 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23–8.19 (m, 3H), 8.02 (d, *J*=8.7 Hz, 1H), 7.58 (dd, *J*=8.1, 1.8 Hz, 1H), 7.51–7.42 (m, 4H), 7.35 (td, *J*=8.1, 1.8 Hz, 2H), 2.93 (s, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 169.23, 154.83, 141.29, 136.23, 126.36, 126.08, 124.18, 123.74, 122.80, 121.19, 120.67, 120.41, 110.02, 20.62; HR-MS (ESI) for $C_{20}H_{15}N_2S$ ($M+H^+$) calcd 315.0956, found 315.0926.

Compound **7c**. 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.02 (d, *J*=5.7 Hz, 1H), 7.37 (dd, *J*=8.4, 1.8 Hz, 1H), 7.07–7.04 (m, 2H), 6.86–6.82 (m, 4H), 6.29–6.25 (m, 2H), 2.90 (s, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 169.09, 155.52, 144.51, 139.44, 135.71, 127.42, 127.14, 127.08, 124.97, 123.80, 122.94, 120.76, 116.60, 20.59; HR-MS (ESI) for $C_{20}H_{15}N_2S_2$ ($M+H^+$) calcd 347.0671, found 347.0653.

Compound **7d**. 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, *J*=8.4 Hz, 1H), 7.98 (d, *J*=1.8 Hz, 1H), 7.34 (dd, *J*=8.4, 1.8 Hz, 1H), 6.75–6.56 (m, 6H), 5.96 (dd, *J*=8.4, 1.5 Hz, 1H), 2.90 (s, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 168.95, 155.77, 144.25, 137.40, 136.09, 134.68, 127.26, 125.30, 124.11, 123.47, 121.70, 115.73, 113.63, 20.45; HR-MS (ESI) for $C_{20}H_{15}N_2OS$ ($M+H^+$) calcd 331.0899, found 331.0867.

4.1.5. Preparation of compound 8b. Compound **7b** (250 mg, 0.800 mmol) and ethyl bromoacetate (160 mg, 0.960 mmol) were weighed into a sealed tube. The mixture was heated at 80°C for 18 h. After cooling to room temperature, the reaction mixture was poured into ether and filtered to give a pale green solid which was washed with ether 2×(322 mg, 84%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.74 (d, *J*=6.9 Hz, 1H), 8.31 (d, *J*=7.5 Hz, 2H), 8.13 (d, *J*=7.2 Hz, 1H), 7.48 (d, *J*=3.9 Hz, 4H), 7.39–7.34 (m, 2H), 5.92 (s, 2H), 4.25 (q, *J*=7.2 Hz, 2H), 3.29 (s, 3H), 1.25 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 181.90, 166.07, 143.18, 140.49, 138.86, 127.88, 127.58, 127.31, 127.22, 123.80, 121.58, 121.42, 115.68, 110.30, 63.27, 50.62, 17.91, 14.59; HR-MS (MALDI) for $C_{24}H_{21}N_2O_2S$ ($M+H^+$) calcd 401.1324, found 401.1264.

Compound **8a**.[21] 86%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.52 (d, *J*=7.8 Hz, 1H), 8.30 (d, *J*=7.8 Hz, 1H), 7.90 (t, *J*=7.8 Hz, 1H), 7.82 (t, *J*=7.8 Hz, 1H), 5.91 (s, 2H), 4.24 (q, *J*=7.2 Hz, 2H), 3.23 (s, 3H), 1.25 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 180.31, 166.00, 141.78, 130.38, 129.29, 129.04, 125.62, 117.34, 63.30, 50.45, 17.84, 14.60.

Compound **8c**. 68%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.66 (dd, *J*=8.7, 1.8 Hz, 1H), 8.36 (d, *J*=1.8 Hz, 1H), 7.82 (dd, *J*=8.7, 1.8 Hz, 1H), 7.24 (dd, *J*=7.2, 1.8 Hz, 2H), 7.07–6.97 (m, 4H), 6.39 (dd, *J*=7.2, 1.8 Hz, 2H), 5.84 (s, 2H), 4.17 (q, *J*=7.2 Hz, 2H), 3.25 (s, 3H), 1.18 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 180.89, 165.19, 143.14, 142.76, 142.59, 128.63, 127.53, 127.35, 127.12, 124.01, 123.80, 122.27, 118.30, 115.98, 62.53, 49.87, 17.16, 13.84; HR-MS (MALDI) for $C_{24}H_{21}N_2O_2S_2$ ($M+H^+$) calcd 433.1045, found 433.1066.

Compound **8d**. 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (d, *J*=8.7 Hz, 1H), 8.62 (d, *J*=1.8 Hz, 1H), 7.90 (dd, *J*=8.7, 1.8 Hz, 1H), 6.84–6.64 (m, 6H), 5.87 (dd, *J*=7.5, 1.5 Hz, 2H), 5.86 (s, 2H), 4.20 (q, *J*=7.2 Hz, 2H), 3.26 (s, 3H), 1.19 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 181.09, 164.87, 143.22, 143.12, 139.69, 133.25, 130.60, 128.64, 127.79, 123.55, 122.07, 119.62, 115.47, 113.33, 62.36, 49.82, 17.11, 13.67; HR-MS (MALDI) for $C_{24}H_{21}N_2O_3S$ ($M+H^+$) calcd 417.1273, found 417.1276.

4.1.6. Preparation of squaraine 4b. Compound **8b** (100 mg, 0.21 mmol), quinoline (27 mg, 0.21 mmol), and squaric acid (12 mg, 0.10 mmol) were placed in a round-bottom flask. A mixture of *n*-BuOH/toluene (15 mL, 1:1 v/v) was added. The mixture was refluxed for 12 h while the water formed was azeotropically removed by using a Dean–Stark trap. After cooling to room temperature, the reaction mixture was filtered to give a dark-green solid, which was further washed with ether 3×(81 mg, 83%). A sample for analysis was obtained by purification through flash column chromatography using 40% EtOAc/CH_2Cl_2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J*=7.8 Hz, 4H), 7.75 (d, *J*=8.1 Hz, 2H), 7.48–7.21 (m, 16H), 5.88 (s, 2H), 4.78 (s, 4H), 4.29 (q, *J*=7.2 Hz, 4H), 1.32 (t, *J*=7.2 Hz, 6H); HR-MS (MALDI) for $C_{52}H_{39}N_4O_6S_2$ ($M+H^+$) calcd 879.2311, found 879.2334.

Compound **4a**. 96%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, *J*=7.5 Hz, 2H), 7.36 (t, *J*=7.5 Hz, 2H), 7.23 (t, *J*=7.5 Hz, 2H), 7.02 (d, *J*=7.5 Hz, 2H), 5.81 (s, 2H), 4.80 (s, 4H), 4.29 (q, *J*=7.2 Hz, 4H), 1.32 (t, *J*=7.2 Hz, 6H); HR-MS (ESI) for $C_{28}H_{25}N_2O_6S_2$ ($M+H^+$) calcd 549.1154, found 549.1132.

Compound **4c**. 80%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, *J*=8.4 Hz, 2H), 7.26 (dd, *J*=8.4, 1.8 Hz, 2H), 7.11–7.08 (m, 4H), 7.02 (d, *J*=1.8 Hz, 2H), 6.96–6.86 (m, 8H), 6.32 (dd, *J*=8.1, 1.8 Hz, 4H), 5.85 (s, 2H), 4.75 (s, 4H), 4.27 (q, *J*=7.2 Hz, 4H), 1.29 (t, *J*=7.2 Hz, 6H); HR-MS (MALDI) for $C_{52}H_{39}N_4O_6S_4$ ($M+H^+$) calcd 943.1753, found 943.1709.

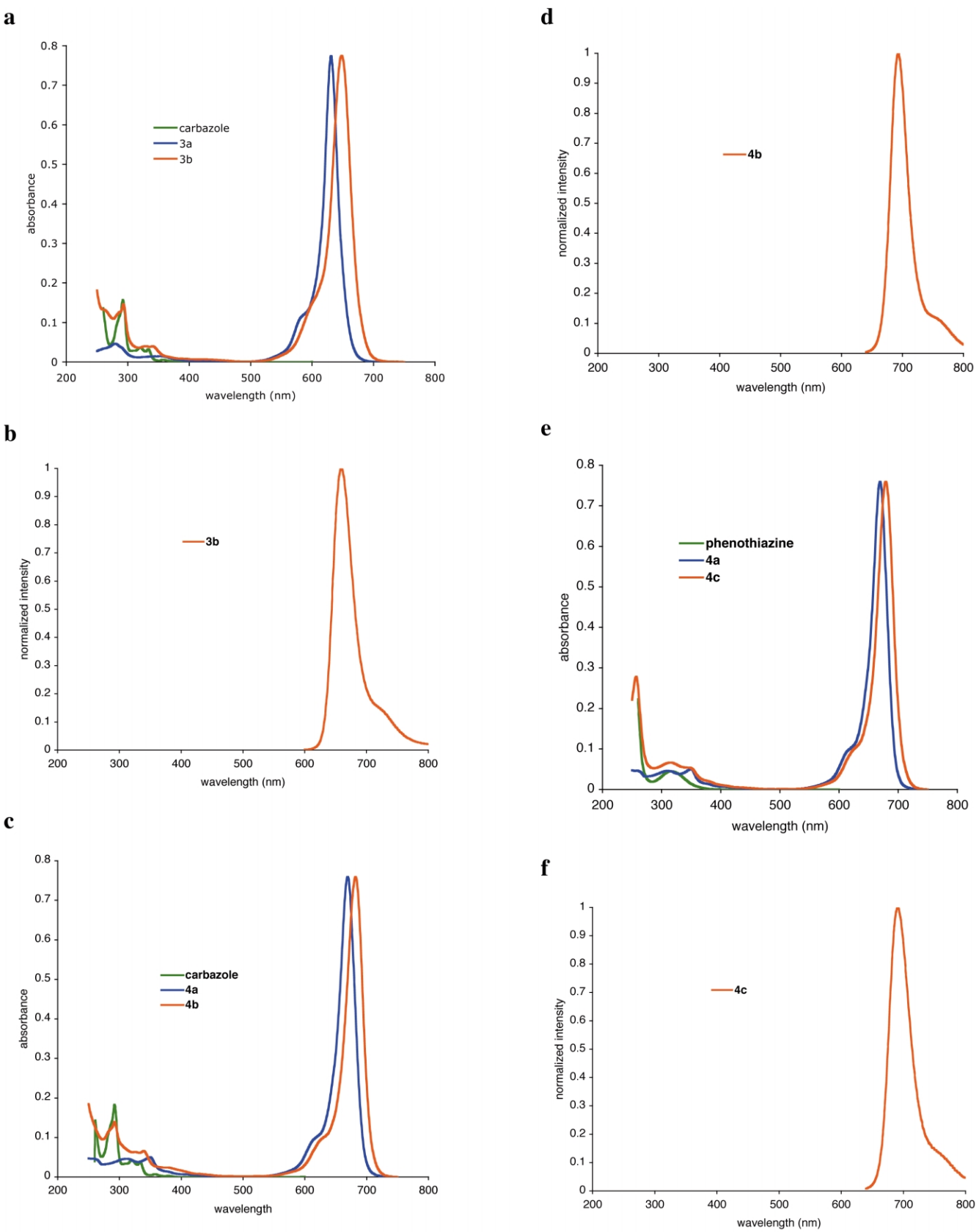


Figure 3 (*continued*)

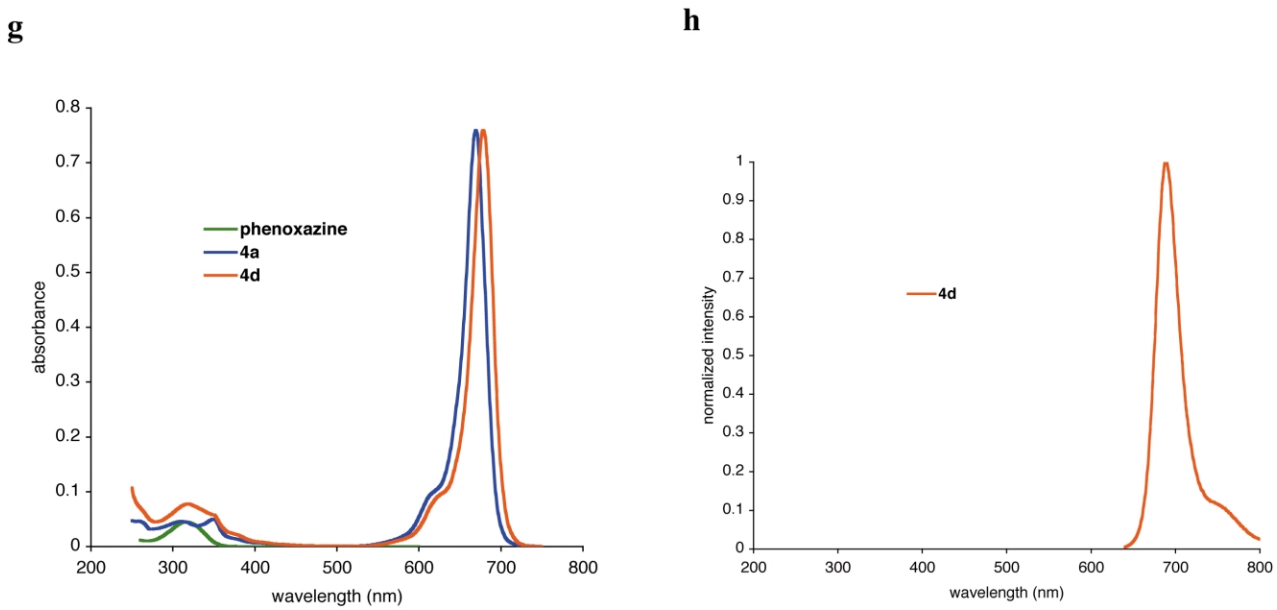


Figure 3. (a), (c), (e) and (g) Overlaid absorption spectra of the donor fragments, the acceptor fragments, and the complete cassettes; all in $CHCl_3$ solution. (b), (d), (f) and (h) Fluorescence spectra of the cassettes; all excited at ~300 nm or 590 (630) nm in $CHCl_3$ solution.

Table 1. Key spectroscopic data for the compounds **3a**,**b** and **4b**–**d**

Compound	Absorption λ_{max} below 350 nm (nm)	Fluorescence emission λ_{max} (nm)	Apparent Stokes' shift (nm)	Energy transfer efficiency, ET (%)[a]	Quantum yield Φ^b
3a	279, 344	637	6[c]	–	0.23
3b	293, 340	659	366, 319	~100	0.31
4a	310, 350	679	10[c]	–	0.33
4b	292, 339	693	401, 354	~100	0.37
4c	257, 315	691	434, 376	~100	0.33
4d	319	688	369	~100	0.36

[a] Determined indirectly, see Section 4.
[b] Excited at 590 nm for **3a**,**b** and 630 nm for **4a**–**d**.
[c] Actual Stokes' shift for **3a** and **4a**.

Compound **4d**. 62%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J=8.4 Hz, 2H), 7.23 (dd, J=8.4, 1.8 Hz, 2H), 7.02 (d, J=1.8 Hz, 2H), 6.75–6.60 (m, 14H), 5.94 (dd, J=8.1, 1.5 Hz, 4H), 5.86 (s, 2H), 4.77 (s, 4H), 4.27 (q, J=7.2 Hz, 4H), 1.30 (t, J=7.2 Hz, 6H); HR-MS (MALDI) for $C_{52}H_{39}N_4O_8S_2$ ($M+H^+$) calcd 911.2209, found 911.2254.

4.2. Indirect determination of ET efficiencies

In practice it is extremely difficult to directly measure the ET efficiency of the cassettes **3b** and **4b**–**d** because of the very low absorbance in the donor region as compared to the acceptor region. However, the donor absorption can be increased by increasing the concentration of the cassettes, but then the fluorescence spectra become distorted due to the re-absorption in the acceptor region. Therefore, we used the following procedures to indirectly estimate the ET efficiency of the cassettes **3b** and **4b**–**d**.

(1) The absorption spectrum $\{A(\lambda_{ex})\}$ and the corrected excitation spectrum $\{F(\lambda_{ex})\}$ were compared. If the ET efficiency is 1, then one expects to find a ratio between these spectra which is constant at all wavelengths. The ratios obtained are listed in Table 2. These data suggest that the ET efficiency is close to 1 for cassettes **3b**, **4b**, and **4d**. The deviation for **4c** can

Table 2. The ratios of absorptions and fluorescence excitation spectra $\{A(\lambda_{ex})/F(\lambda_{ex})\}$ and the average lifetimes of the acceptor group upon excitation of the donor and the acceptors of cassettes **3b** and **4b**–**d**

Compound	Average $A(\lambda_{ex})/F(\lambda_{ex})$, λ_{ex}=300–400 nm[a]	Average lifetime (ns), λ_{ex}=300–350 nm	Average lifetime (ns), λ_{ex}=630 nm
4b	1.03	2.78	2.60
4c	1.33	3.04	2.83
4d	0.88	2.95	2.79
3b	0.99	1.56	1.46

[a] The spectra are scaled so that the average ratio $A(\lambda_{ex})/F(\lambda_{ex})$ at λ_{ex}=600–690 nm is 1.

be explained by a lower ET efficiency, or by presence of pure donor.

(2) We further measured the fluorescence lifetimes of the cassettes **3b** and **4b–d** upon excitation in the donor and acceptor regions. If the timescale of ET is ~100 ps or slower, we expect to observe negative components in the fluorescence decay. No such component was found. It is also true that the average lifetimes must be longer when the donor is excited directly. We obtained the average lifetimes shown in Table 2. The experimental accuracy suggests that the average lifetimes are very similar.

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References

1. Hung, S.-C.; Ju, J.; Mathies, R. A.; Glazer, A. N. *Anal. Biochem.* **1996**, *243*, 15–27.
2. Lee, L. G.; Spurgeoun, S. L.; Rosenblum, B. *Energy Transfer Dyes with Enhanced Fluorescence*; Perkin–Elmer: US, 1997; p 3.
3. Lee, L. G.; Rosenblum, B.; Spurgeoun, S. L. *Fluorescent Energy Transfer Dyes—Useful for Labelling Dideoxynucleotides, Oligonucleotides; etc*. Perkin–Elmer: US, 1998; p 2.
4. Berti, L.; Xie, J.; Medintz, I. L.; Glazer, A. N.; Mathies, R. A. *Anal. Biochem.* **2001**, *292*, 188–197.
5. Berti, L.; Medintz, I. L.; Tom, J.; Mathies, R. A. *Bioconjugate Chem.* **2001**, *12*, 493–500.
6. Nampalli, S.; Zhang, W.; Rao, T. S.; Xiao, H.; Kotra, L. P.; Kumar, S. *Tetrahedron Lett.* **2002**, *43*, 1999–2003.
7. Lee, L. G.; Spurgeon, S. L.; Heiner, C. R.; Benson, S. C.; Rosenblum, B. B.; Menchen, S. M.; Graham, R. J.; Constantinescu, A.; Upadhya, K. G.; Cassel, J. M. *Nucleic Acids Res.* **1997**, *25*, 2816–2822.
8. Rosenblum, B. B.; Lee, L. G.; Spurgeon, S. L.; Khan, S. H.; Menchen, S. M.; Heiner, C. R.; Chen, S. M. *Nucleic Acids Res.* **1997**, *25*, 4500–4504.
9. Burgess, K.; Burghart, A.; Chen, J.; Wan, C.-W. *New Chemistry of BODIPY Dyes, and BODIPY Dye Cassettes Featuring Through-Bond Energy Transfer; SPIE BiOS 2000*. The International Symposium on Biomedical Optics, San Jose, CA, 2000.
10. Burghart, A.; Thoresen, L. H.; Chen, J.; Burgess, K.; Bergström, F.; Johansson, L. B.-A. *Chem. Commun.* **2000**, 2203–2204.
11. Speiser, S. *Chem. Rev.* **1996**, *96*, 1953–1976.
12. Wagner, R. W.; Lindsey, J. S. *J. Am. Chem. Soc.* **1994**, *116*, 9759–9760.
13. McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537–2574.
14. Slavik, J. In *Near-Infrared Dyes for High Technology Applications*; Daehne, S., Resch-Genger, U., Wolfbeis, O. S., Eds.; Application of Red Fluorescent Probes for the Measurement of Individual Cell Cytoplasmic Ph Values. Kluwer Academic: Dordrecht, 1998; Vol. 52, pp 115–124.
15. Law, K.-Y. In *Organic Photochemistry*, Ramamurthy, V., Ed.; Absorption, Fluorescence Emission, and Photophysics of Squaraines. Marcel Dekker: New York, 1997; Vol. 1, pp 519–584.
16. Klapars, A.; Antilla, J. C.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 7727–7729.
17. Old, D. W.; Harris, M. C.; Buchwald, S. L. *Org. Lett.* **2000**, *2*, 1403–1406.
18. Sprenger, H.-E.; Ziegenbein, W. *Angew. Chem. Int. Ed.* **1967**, *6*, 553–554.
19. Moreau, M. F.; Lapalus, F.; Meyniel, G. *Eur. J. Med. Chem.-Chim. Ther.* **1974**, *9*, 274–280.
20. Tilford, C. H. *J. Med. Chem.* **1971**, *14*, 1020–1021.
21. Galera, C.; Vaquero, J. J.; Navio, J. L. G.; Alvarez-Builla, J. *J. Heterocycl. Chem.* **1986**, *23*, 1889–1892.