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BODIPY Fluorophore Toolkit for Probing Chemical Reactivity and for Tagging Reactive Functional Groups

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The photophysical and synthetic studies of new tether-functionalized boron dipyrromethene (BODIPY) fluorophores as probes for chemical reactions are described. These compounds differ from typically reported probes in that they provide a way to tag and indicate chemical reactions without chemical transformation of the BODIPY core itself; instead the dyes are spectators. The introduction and modification of the tether has expanded the available chemistry and yet these new BODIPY derivatives have similar photophysical properties to their parent substrates. As a result of the chemistry enabled by these probes, a single step of a multistep metal-catalyzed reaction was revealed by a change in the fluorescence resonance energy transfer (FRET) signal. The fundamental knowledge of quantum yield, FRET efficiencies, and reagent compatibility is critical to enabling the broader application of fluorescent probes to study chemical reaction mechanisms by cutting-edge microscopy techniques.

Introduction

An emerging application area for organic fluorophores is as probes for chemical reactions on both the ensemble[1,2] and, in the past 8 years, single-molecule and -particle levels by microscopy.[3–12] Nascent applications include investigations into the mechanisms of organic[13] and transition-metal[1] reactions and homogeneous and heterogeneous catalysis by fluorescence microscopy.[3,4,6,7,12] These studies have been enabled by recent advances in boron dipyrromethene (BODIPY) dye synthesis.[14] These dyes are uniquely suited to probing chemical reactions because unlike other common high-quantum-yield fluorophore dyes, they do not have accessible lone pairs that could bind to transition metals nor protic functional groups that could interfere with a chemical reagent's native reactivity.[15–17]

Consistent with these recent advances, the ability to gain insight into reactivity by fluorescence microscopy is a growing application area with less than 10 reported examples.[14] Essential to the goal of enabling these studies through synthesis is the development of strategies to attach these dyes through tethers, namely unreactive chains such as alkyl groups, to remote reactive groups, for example, alkynes, olefins, alcohols, esters, epoxides, phosphines, and transition-metal complexes. These tethered probes differ from typically reported probes in that they enable tagging and fluorescence indication of chemical reactions without chemical transformation of the BODIPY core itself; instead the probes are spectators.[14] The determination of the photophysical properties of these tethered fluorophore probes is critical for the interpretation of dye behavior in single-molecule or -particle fluorescence microscopy studies.[9,10,14] We herein describe an expansion of the BODIPY toolkit through synthesis and ensemble photophysical studies. In one application that underscores the chemical information available through the exquisite sensitivity of fluorescence resonance energy transfer (FRET), the tethered spectator BODIPY probe signals exactly one fundamental chemical step out of many in a multistep gold-catalyzed reaction.

Results and Discussion

First, a BODIPY FRET pair,[2] **6**, was synthesized (Scheme 1). Molecule **6** exemplifies the strategy of tethering BODIPY fluorophores through inert alkyl tethers to a core reactive functional group. Specifically, the tether removes both BODIPY dyes from the electronic and steric environment of the 1-ester-2-alkynylphenyl group. The key synthetic step in the construction of **6** was a Sonogashira coupling of green BODIPY alkyne **2** (with a tetramethylene tether) and orange BODIPY aryl iodide **5** (with a pentamethylene tether). This cross-coupling reaction showed the compatibility of both tethered BODIPY building blocks towards both copper and palladium catalysts as well as amine bases. Compound **6** displayed a FRET efficiency of 0.94 at an excitation of 488 nm, producing strong emission in the orange region.

We next tested the ability of FRET pair **6** to serve as a color-changing probe for a chemical reaction (Scheme 2). The ability of these dyes to inform on a single step in a multistep gold-catalyzed cyclization to yield isocoumarins

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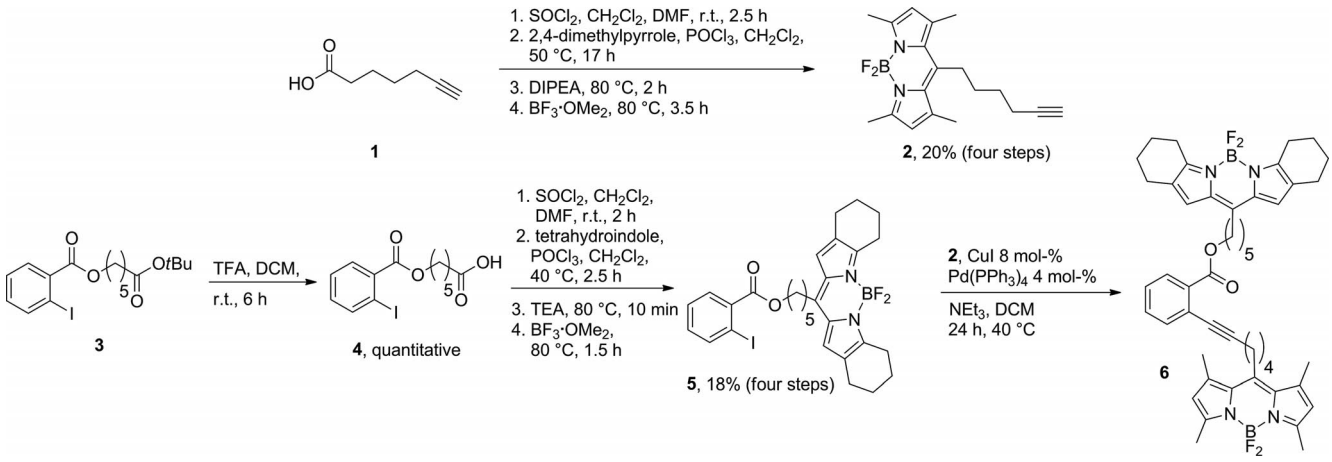
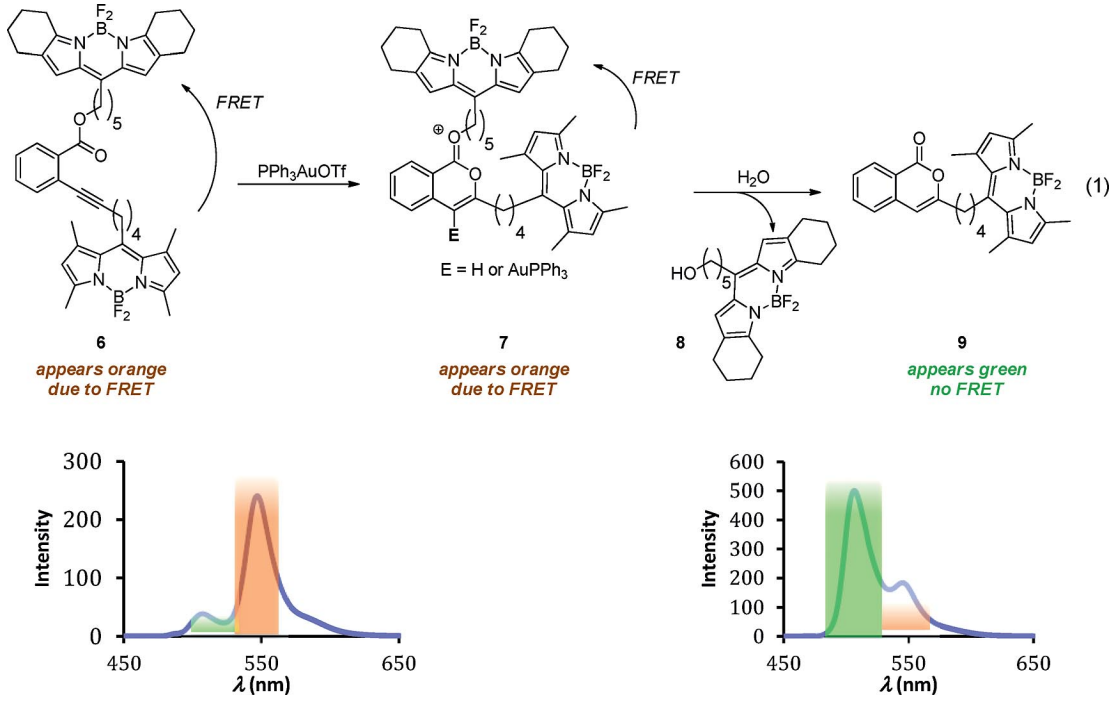


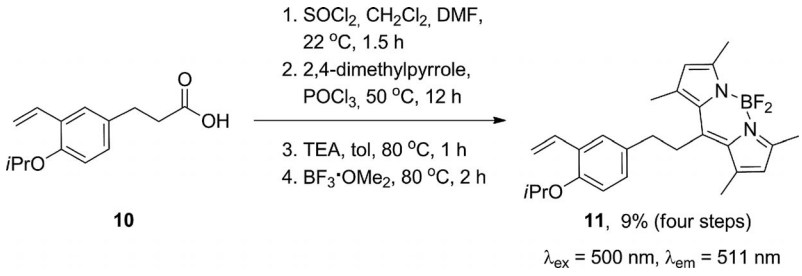
Scheme 1. Synthesis of green BODIPY **2**, orange BODIPY **5**, and BODIPY FRET pair **6** by a key Sonogashira coupling reaction that attaches the fluorophores to the reactive molecular core through spectator tethers.

was investigated. The addition of [PPh_3AuCl]/AgOTF (which generated the corresponding gold triflate in situ) to **6** in wet dichloromethane successfully catalyzed isocoumarin cyclization and hydrolysis to form **9**, as confirmed by ^1H NMR spectroscopy. After 15 min, the ^1H NMR spectrum showed full consumption of **6** and generation of the protodemetalated lactone product **9**. This hydrolysis reaction demonstrated that adventitious water in the solvent was adequate to hydrolyze the oxocarbenium ion intermediate **7** and protodeaurate the gold–carbon bond[18] to generate the demetalated lactone **9**. Overall, the cyclization/hydrolysis of BODIPY-tagged alkynyl esters demonstrated that the tagged reagents participated in analogous gold-catalyzed reactions[19] to their untagged counterparts, a key finding that permitted the interpretation of fluorescence spectra (see below). This result underscores the potential utility of these dyes as probes for individual reaction steps in complicated catalytic reactions, once the appropriate tagging chemistry is developed.

Ensemble fluorescence measurements suggested the viability of the color-change-monitoring strategy to detect one specific step in the multistep catalytic reaction that forms **9**. First, the emission spectrum of probe molecule **6** was acquired before the reaction (Scheme 2, left). Secondly, the emission spectrum of the product mixture containing **9** was acquired after the reaction (Scheme 2, right), which re-



Scheme 2. Application of FRET probe **6** to the ensemble study of gold-catalyzed cyclization/hydrolysis reaction. The fluorescence emission spectra of **6** before the gold-catalyzed cyclization/hydrolysis and after the formation of isocoumarin (**9**) show a fluorescence change that indicates one chemical step (hydrolysis) in the multistep reaction.



Scheme 3. Synthesis of **11** bearing an electron-rich styrenyl moiety.

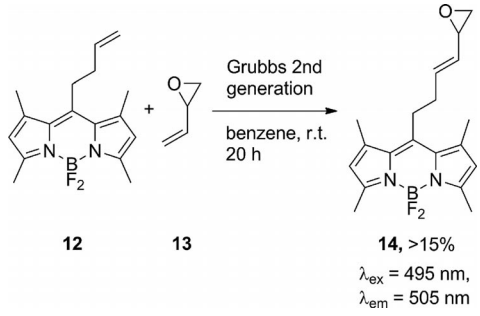
vealed a shift in the fluorescence that confirmed that alcohol **8** was liberated and that the acceptor fluorophore was no longer attached to the substrate, thus permitting emission of the donor molecule [reaction (1) in Scheme 2]. Thus, the change in color from green to orange indicated that a specific single reaction step, alcohol release upon hydrolysis, had occurred.

As can be discerned from the fluorescence spectrum of the product mixture of **8** and **9** at an excitation of 488 nm, orange **8** displays reduced but nonzero fluorescence, which indicates overlap between the excitation spectra of the donor and acceptor. Excitation at 488 nm was chosen to mimic the 488 nm argon ion laser line commonly available for microscopy studies. The excitation spectrum overlap is due to the small Stokes shift of BODIPY dyes and reveals an inherent disadvantage of employing these fluorophores as FRET pairs for chemical reaction probes.

BODIPY dyes bearing olefins recently have been employed as spectator probes to investigate ring-opening metathesis polymerization at the single-particle and -molecule level by fluorescence microscopy.[6,12] To broaden the application of olefin-containing BODIPY dyes to studying other types of polymerization processes and mechanisms, dyes bearing pendent styrene moieties (e.g., see Scheme 3) would be desirable given the significant representation of this substitution pattern in polymers.[20]

A potential complication in the synthesis of these compounds would be the incompatibility of the highly electrophilic reagents common to BODIPY synthesis with electron-rich aromatic rings (Scheme 3). The synthesis of **11** proceeded satisfactorily (9% yield over four steps), however, with the employment of thionyl chloride, $POCl_3$, and BF_3 as stoichiometric reagents, and in the presence of reaction byproduct amine-buffered HF. Compound **11** exhibited slightly blueshifted fluorescence excitation and emission spectra relative to the parent tetramethyl-BODIPY, with λ_{ex} = 500 nm and λ_{em} = 511 nm (parent tetramethyl-BODIPY: λ_{ex} = 505 nm and λ_{em} = 515 nm). The small blueshift established that the tethered aromatic ring did not significantly alter the photophysical properties of the core. Thus, the electronic separation provided by the saturated ethylene tether provided sufficient separation of the reactive aromatic ring from the BODIPY core for the BODIPY to serve as a spectator in subsequent reactions.

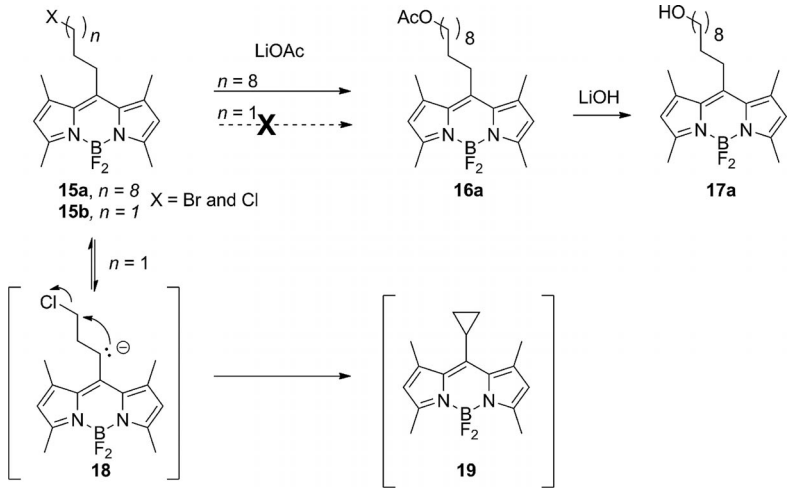
Next, the compatibility of olefin-containing BODIPY compounds with ruthenium carbenes was leveraged (Scheme 4). Specifically, the functionalization of a terminal olefin with butadiene monoepoxide was achieved by the cross-metathesis reaction between BODIPY **12** and epoxide **13** catalyzed by Grubbs' second-generation catalyst, yielding BODIPY-tagged epoxide **14**. The fluorescence excitation and emission spectra of compound **14** were similarly blueshifted relative to the parent tetramethyl-BODIPY, with λ_{ex} = 495 nm and λ_{em} = 505 nm.



Scheme 4. Synthesis and photophysical properties of BODIPY vinylepoxide **14**.

At first, BODIPY dyes appeared compatible with the mildly basic conditions of lithium acetate (Scheme 5). For example, this reagent smoothly transformed bromoalkyl compound **15a** to acetate compound **16a**.[11] Subsequently, the more strongly basic hydrolysis conditions of LiOH transformed acetate compound **16a** into alcohol **17a** in high yield and without apparent disruption of the BODIPY core or tether.

Although the BODIPY core and tether appeared untouched under these basic conditions, an attempt to synthesize the shorter-chain alcohol **17b** from chloropropyl-BODIPY **15b** yielded only cyclopropyl-BODIPY **19**. The generation of compound **19** is consistent with deprotonation of the pseudo-benzylic α position (yielding intermediate **18**), followed by S_N2 displacement of the chloride. Thus, the acidity of this α methylene group can be estimated on the basis of its accessibility to deprotonation by acetate (pK_a of acetic acid in DMSO = 13).[21] The deprotonation of the α methylene is also likely to be occurring reversibly for **15a** under the conditions, however, ring closure is slower



Scheme 5. Synthesis of bromoalkyl-BODIPY compounds and their conversion to the corresponding acetate and cyclopropyl under weakly basic conditions.

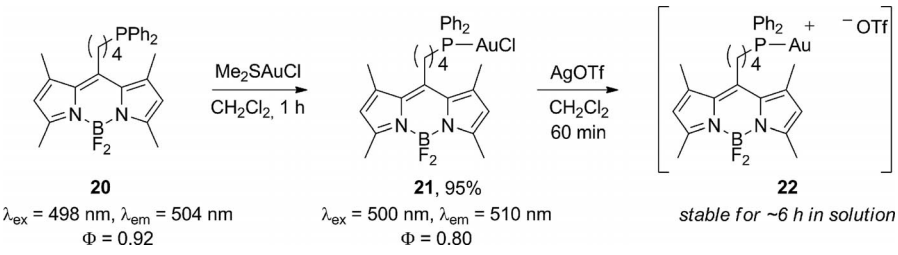
for the 10-membered ring involved in the formation of **16a** than for the three-membered ring.[22] The slower cyclization reaction would then make this α-methylene deprotonation reversible and nonproductive.

Gold complexes, especially the formally cationic gold(I) complexes, are an expanding class of catalysts in organic synthesis. Therefore the ability to synthesize and gain fundamental knowledge of the photophysical properties of fluorophore-tagged gold complexes would facilitate application to the study of catalysis by fluorescence microscopy. Ligand exchange between BODIPY-phosphine **20** and dimethyl sulfide in the complex [Me_2SAuCl] cleanly produced BODIPY-tagged gold complex **21** (95%; Scheme 6). Coordination of **20** to gold resulted in minor shifts of the absorbance and fluorescence maxima ($\Delta\lambda$ = 2 and 6 nm, respectively). The high quantum yield of the core was also mostly retained upon coordination (Φ = 0.92 and 0.80 for **20** and **21**, respectively). The reduction in quantum yield suggests the possibility that partial quenching of the BODIPY core by gold(I) occurs despite the tetramethylene tether and the full solubility of the complex in dichloromethane; this photophysical effect contrasts the absence of quenching in compound **20**. To the best of our knowledge, this is the first BODIPY-tagged gold complex.

Treatment of gold chloride **21** with AgOTf produced the formally cationic gold(I) complex **22**, as observed by ^{1}H NMR spectroscopy (>95% conversion); gold complexes with weakly coordinating triflate anions are often produced in situ from the corresponding chloride for employment as "cationic" gold catalysts.[19,23–26]

Monitoring solutions of triflate **22** over the course of 6 h by ^{1}H and ^{31}P NMR spectroscopy probed the chemical stability of the BODIPY core towards the electrophilic gold(I) center. Although mostly inert, the compound began to decompose in solution after 2 h, as characterized by a small decomposition peak in the ^{31}P NMR spectrum that increased with time (<4% after 2 h and 8% after 6 h). Thus, the BODIPY core was substantially inert to irreversible single-electron transfer to the electrophilic gold center[27] and gold Lewis acid promoted attack of nucleophiles on the core during the timescale of many gold-catalyzed reactions (including the reaction shown in Scheme 2, which was complete in less than 15 min). Long-scale chemostability, however, was lacking. Due to the instability of **22**, this complex was not isolated for further photophysical study.

For comparison, the excitation and emission spectra of BODIPY-styrene **11**, BODIPY-vinyl epoxide **14**, and BODIPY-gold(I) complex **21** are shown in Figure 1. The modification of the *meso* position of the BODIPY core led only to a marginal change in the photophysical properties compared with the parent substrates and other similarly tethered functional groups. López Arbeloa and co-workers reported that an ester tethered through a single methylene unit at the *meso* position changed the spectral properties



Scheme 6. Synthesis of **21** and **22**, showing retention of the high quantum yield of the BODIPY core upon coordination to gold(I) chloride and the stability towards salt metathesis conditions and a π Lewis acidic gold(I) cation.

and quantum yield significantly.[28] In contrast, the longer methylene linker examined in this study left the photophysical properties largely intact.

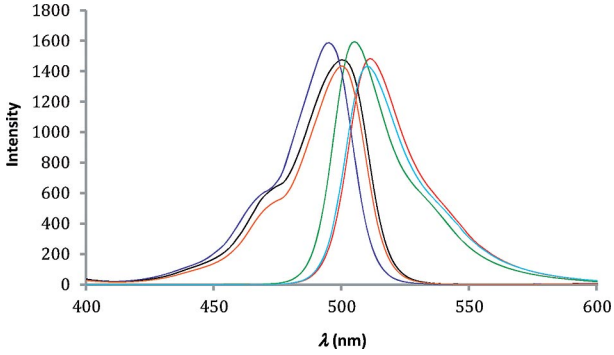


Figure 1. Fluorescence emission and excitation spectra of BODIPY styrene **11** (λ_{ex} = 500 nm) in CH_2Cl_2, BODIPY-vinyl epoxide **14** (λ_{ex} = 495 nm) in CH_3CN, and BODIPY-gold complex **21** (λ_{ex} = 500 nm) in CH_2Cl_2 (red: emission of **11**, black: excitation of **11**, green: emission of **14**, dark blue: excitation of **14**, light blue: emission of **21**, orange: excitation of **21**).

Conclusions

We have expanded the toolkit of spectator BODIPY fluorophores tethered through unreactive methylene chains to reactive functional groups. The introduction of methylene chains prevented changes in the photophysical properties of the modified fluorophores. It was possible to reveal a single step of a multistep metal-catalyzed reaction with these probes. The reported findings provide fundamental compatibility, reactivity, and photophysical information that enable the broader application of fluorescent probes to the study of chemical reactions by state-of-the-art fluorescence microscopy techniques.[3,4,6,9,11,12]

Experimental Section

General: Unless otherwise noted, all reagents and solvents were used as received from their respective suppliers. Reactions were monitored by TLC on precoated glass-backed plates (Merck F250) and components were visualized by using UV light. Flash chromatography was performed on Dynamic Absorbents 43–60 micron silica gel. *tert*-Butyl 6-hydroxyhexanoate was purchased from Santa Cruz Biotechnology, 2-iodobenzioc acid and chloro(dimethyl sulfide)gold were purchased from Sigma Aldrich, and [2-(diphenylphosphanyl)ethyl]triethoxysilane was purchased from Gelest.

^1H and ^{13}C NMR spectra were acquired with either a Bruker DRX-500 or DRX-500 spectrometer equipped with a cryogenic probe, and ^{31}P NMR spectra were acquired with a Bruker DRX-400 spectrometer. Chemical shifts (δ) are reported in parts per million [ppm] and referenced to residual protiated solvent peaks as reported in the literature. The spectral data are reported with the following abbreviations: app, apparent; br., broad; m, multiplet; q, quartet; s, singlet; t, triplet; td, triplet of doublets, sept, septet. HRMS was performed at the facility operated by the Department of Chemistry at the University of California, Irvine. Ultrapure water with >18 MW resistance and a total organic content of <5 ppb was obtained from a Milli-Q Gradient A-10 water purifier (Millipore, Billerica, MA) using a Q-Gard 2 purification pack and a Quantum EX Ultrapure Organex cartridge. Fluorescence spectra were recorded with an F-4500 fluorescence spectrofluorimeter maintained at the facility operated by the Department of Chemistry at the University of California, Irvine. All solvents used for single-molecule studies were of spectrophotometric grade.

BODIPY Donor 2: An oven-dried 100-mL round-bottomed flask was charged with 6-heptynoic acid (**1**; 1.00 g, 7.92 mmol) and a stirring bar, and then placed under nitrogen. Dry CH_2Cl_2 (20 mL) and 2 drops of dry DMF were added to the flask. Thionyl chloride (0.860 mL, 11.8 mmol) was added through a syringe causing a slow evolution of gas. After the evolution of gas had subsided, the solution was stirred at room temperature for 2.5 h. It was then concentrated in vacuo to yield a yellow oil and then placed on a high-vacuum line for 2 h to remove excess thionyl chloride. Without further purification, the acid chloride was redissolved in dry CH_2Cl_2 (25 mL), and 2,4-dimethylpyrrole (2.00 mL/g, 19.8 mmol) and phosphorus oxychloride (0.810 mL, 8.71 mmol) were added under N_2. The resulting solution was heated at reflux with stirring overnight. The solution was then cooled to room temperature and concentrated in vacuo. To remove nonpolar impurities, the residue was completely submerged in reagent-grade hexanes (90 mL) and stored at –35 °C overnight. The hexanes then were decanted and the residue was placed under high vacuum for 1 h and used in the next step without further purification.

The residue was dissolved in dry toluene (55 mL) under N_2 and treated with diisopropylethylamine (4.14 mL, 23.7 mmol). The solution was stirred for 2 h at 80 °C. Boron trifluoride–dimethyl etherate (2.55 mL, 27.7 mmol) was added and the mixture was stirred at 80 °C for an additional 3.5 h. The red solution was cooled to room temperature and transferred to a separatory funnel. Reagent-grade toluene was swirled over the residue remaining in the flask to extract residual product and added to the separatory funnel. The solution was washed with brine, dried with sodium sulfate, and concentrated in vacuo. The resulting residue was purified by flash chromatography [CH_2Cl_2/hexanes (60:40), R_f = 0.3] to afford **2**. The product was then recrystallized from reagent-grade toluene/pentanes to yield **2** as dark-green/orange crystals (500 mg, 20%). For recrystallization, the product was dissolved in reagent-grade toluene (1 mL), layered with reagent-grade pentanes (4 mL), and stored at –35 °C overnight. The product was spectroscopically identical to the compound reported in the literature:[29] λ_{em} = 498 nm. (excitation at λ = 488 nm).

6-(*tert*-Butoxy)-6-oxohexyl 2-Iodobenzoate (3): In an oven-dried 100-mL round-bottomed flask, 2-iodobenzoic acid (1.2 g, 4.8 mmol), *tert*-butyl 6-hydroxyhexanoate (1.0 g, 5.3 mmol), and 4-(dimethylamino)pyridine (0.060 g, 0.48 mmol) were dissolved in dry dichloromethane (45 mL). The mixture was cooled in an ice bath, dicyclohexylcarbodiimide (1.1 g, 5.5 mmol) was added, and the mixture was stirred for 48 h with warming to room temperature as the ice-bath melted. Dicyclohexenylurea (DCU) precipitated and was removed by filtration. The filtered solution was concentrated in vacuo to yield a yellow oil with some unremoved DCU as a white powder. The residue was then dissolved in diethyl ether (100 mL) and residual DCU was filtered off. Traces of DCU were then removed on a short silica gel column (diethyl ether) to yield 1.98 g (99%) of **3** as a pale-yellow oil. The product was then used in the next step without further purification. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.96 (d, J = 7.9 Hz, 1 H), 7.78 (dd, J = 1.5, 7.7 Hz, 1 H), 7.38 (t, J = 7.4 Hz, 1 H), 7.13 (dt, J = 1.5, 7.7 Hz, 1 H), 4.32 (t, J = 6.6 Hz, 2 H), 2.23 (t, J = 7.4 Hz, 2 H), 1.81–1.75 (m, 2 H), 1.67–1.61 (m, 2 H), 1.50–1.45 (m, 2 H), 1.42 (s, 9 H) ppm.

6-(2-iodobenzoyl)oxyhexanoic acid (4): In a glovebox, an oven-dried 25-mL round-bottomed flask was charged with 6-(*tert*-butoxy)-6-oxohexyl 2-iodobenzoate (**3**) (0.50 g, 1.20 mmol) dissolved in dry dichloromethane (15 mL). Trifluoroacetic acid (5.5 mL, 72 mmol) was added and the resulting solution was stirred for 6 h. The reaction mixture was removed from the glovebox, concentrated in vacuo, and the residue was stripped with toluene (3 × 10 mL) to afford a clear oil. The oil was placed under high vacuum overnight and a white solid was formed. The product **4** (0.43 g, quantitative) was then used in the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 11.5 (br. s, 1 H), 8.01 (d, J = 7.9 Hz, 1 H), 7.80 (dd, J = 1.5, 7.7 Hz, 1 H), 7.43 (t, J = 7.4 Hz, 1 H), 7.19 (dt, J = 1.5, 7.8 Hz, 1 H), 4.37 (t, J = 6.6 Hz, 2 H), 2.42 (t, J = 7.4 Hz, 2 H), 1.87–1.81 (m, 2 H), 1.79–1.70 (m, 2 H), 1.59–1.48 (m, 2 H) ppm.

BODIPY Acceptor 5: Dry DMF (2 drops) and thionyl chloride (0.120 mL, 1.65 mmol) were added through a syringe to a solution of **4** (0.400 g, 1.10 mmol) in dry CH_2Cl_2 (15 mL) causing a slow evolution of gas. After the evolution of gas had subsided, the solution was stirred at room temperature for 2 h. The solution was then concentrated in vacuo to yield a pale-yellow oil and then put on a high-vacuum line for 2 h to remove excess thionyl chloride. Without further purification, the acid chloride was redissolved in dry CH_2Cl_2 (6 mL) and tetrahydroindole (0.340 g, 2.8 mmol) in dry CH_2Cl_2 (6 mL) was added under N_2. Phosphorus oxychloride (0.120 mL, 1.28 mmol) was added and the solution was heated to 40 °C and stirred for 2.5 h. The mixture was then concentrated in vacuo to yield a red/green gum. To remove nonpolar impurities, the residue was then covered with reagent-grade hexanes (90 mL) and stored at –35 °C overnight. The mixture was decanted and the residue placed on a high-vacuum line for 1 h to remove residual hexanes. The remaining residue was dissolved in dry toluene (10 mL) and dry triethylamine (0.500 mL, 3.58 mmol) was added. The mixture was then heated to 80 °C and stirred for 10 min. Boron trifluoride–dimethyl etherate (0.320 mL, 3.50 mmol) was added and the solution stirred for an additional 1.5 h. The resulting dark-red/pink solution was transferred to a separatory funnel and reagent-grade toluene was swirled over the residue remaining in the flask to extract residual product and added to the separatory funnel. The solution in the separatory funnel was washed with brine (3 × 50 mL) and the organic layer was dried with sodium sulfate, filtered, and concentrated in vacuo to yield a dark-red residue. The crude product was purified by flash chromatography [ethyl acetate/hexanes (1:9), R_f = 0.4] and the product recrystallized from reagent-grade DCM/hexanes to yield **5** as dark-green/orange crystals (122 mg, 18%). For recrystallization, the product was dissolved in reagent-grade DCM (1 mL), layered with reagent-grade hexanes (4 mL), and stored at –35 °C overnight. The product was then used in the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.05 (d, J = 7.9 Hz, 1 H), 7.78 (dd, J = 1.5, 7.3 Hz, 1 H), 7.46 (t, J = 7.4 Hz, 1 H), 7.72 (dt, J = 1.5, 7.7 Hz, 4 H), 6.78 (s, 2 H), 4.40–4.37 (m, 2 H), 3.0 (t, J = 6.1 Hz, 4 H), 2.82 (t, J = 7.7 Hz, 2 H), 2.60 (t, J = 6.2 Hz, 4 H), 1.84–1.73 (m, 9 H), 1.59–1.54 (m, 2 H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 186.8, 156.5, 143.1, 141.2, 1345.6, 133.8, 132.6, 130.8, 128.6, 127.9, 122.9, 93.9, 65.4, 33.0, 30.1, 28.4, 26.5, 24.6, 23.2, 22.9, 22.4 ppm. HRMS (ESI): calcd. for $[M + Na]^+$ 639.1473; found 639.1458. λ_{em} = 540 nm (excitation at λ = 488 nm).

FRET Pair 6: A 50-mL round-bottomed flask was charged with of $[Pd(PPh_3)_4]$ (0.009 g, 0.008 mmol), CuI (0.003 g, 0.02 mmol), and dry triethylamine (8 mL). A solution of BODIPY acceptor **5** (0.120 g, 0.194 mmol) and BODIPY donor **2** (0.076 g, 0.23 mmol) dissolved in dry DCM (4 mL) was added to the resulting suspension. The reaction could not be run in neat triethylamine due to the insolubility of BODIPY donor **2** in triethylamine. The solution was stirred at reflux under N_2 overnight. The resulting mixture was filtered through a glass frit and then added to a separatory funnel. It was then washed with saturated NH_4Cl (2 × 15 mL) and brine (2 × 15 mL). The organic layer was dried with Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography with 10% ethyl acetate in hexanes (R_f = 0.3). The resulting red oil was then further purified by recrystallization from reagent-grade DCM/hexanes to afford 30 mg (19% containing less than 1 ppm hydrocarbon grease) of FRET pair **6**. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.85 (d, J = 7.8 Hz, 1 H), 7.49–7.47 (m, 1 H), 7.45–7.41 (m, 1 H), 7.36–7.32 (m, 1 H), 6.77 (s, 2 H), 6.04 (s, 2 H), 4.24 (t, J = 6.6 Hz, 2 H), 3.04–3.01 (m, 6 H), 2.75 (t, J = 7.5 Hz, 2 H), 2.57–12.51 (m, 12 H), 2.42 (s, 6 H), 1.83–1.85 (m, 6 H), 1.78–1.73 (m, 6 H), 1.55–1.52 (m, 12 H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 186.2, 156.5, 153.9, 146.0, 140.4, 134.3, 133.8, 131.9, 131.5, 131.4, 130.1, 128.6, 127.4, 124.2, 122.8, 121.6, 94.7, 64.7, 80.0, 64.8, 33.2, 31.6, 30.9, 30.1, 29.2, 28.4, 28.0, 26.4, 24.6, 23.2, 22.9, 22.4, 19.7, 16.4, 14.5 ppm. HRMS (ESI): calcd. for $[M + Na]^+$ 839.4282; found 839.4286. λ_{em} = 547 nm, FF = 0.94 (excitation at λ = 488 nm).

BODIPY-Styrene 11: Dry DMF (2 drops) and thionyl chloride (0.019 mL, 0.26 mmol) were added through a syringe to a solution of **10** (0.050 g, 0.21 mmol) in dry CH_2Cl_2 (5 mL) causing a slow evolution of gas. After the evolution of gas had subsided, the solution was stirred at room temperature for 1.5 h. The solution was concentrated in vacuo to yield a pale-yellow oil and then put on a high-vacuum line to remove excess thionyl chloride. Without further purification, the acid chloride was redissolved in dry CH_2Cl_2 (5 mL) and 2,4-dimethylpyrrole (0.057 mL, 0.53 mmol) was added under N_2 to give a yellow solution. Phosphorus oxychloride (0.022 mL, 0.23 mmol) was added and the solution was heated to 50 °C and stirred for 12 h. After cooling to room temperature, the mixture was concentrated in vacuo. To remove nonpolar impurities, the residue was then covered with reagent-grade hexanes. The mixture was decanted and the thick red oil was placed on a high-vacuum line, redissolved in dry toluene (5 mL) and heated to 80 °C. Dry triethylamine (0.095 mL, 0.682 mmol) was added and the mixture was stirred at 80 °C for 1 h. Boron trifluoride–dimethyl etherate (0.059 mL, 0.639 mmol) was added and the solution stirred for an additional 2 h. The resulting red solution was cooled to room temperature and washed with brine. The organic layer was dried with sodium sulfate, filtered, and concentrated in vacuo to yield a dark-red/black oil. The crude product was purified by flash chromatography [ethyl acetate/hexanes (1:10)]. The product was recrystallized from reagent-grade DCM/hexanes to yield **11** as dark-orange crystals (8 mg, 9%). For recrystallization, the product was dissolved in reagent-grade DCM, layered with reagent-grade hexanes, and stored at –35 °C overnight. ^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.36 (d, J = 2.1 Hz, 1 H), 7.12 (dd, J = 2.25, 8.4 Hz, 1 H), 7.03 (dd, J = 11.2, 17.8 Hz, 1 H), 6.86 (d, J = 8.4 Hz, 1 H), 6.08 (s, 2 H), 5.71 (dd, J = 1.4, 17.8 Hz, 1 H), 5.23 (dd, J = 1.4, 11.2 Hz, 1 H), 4.53 (sept, J = 6.1 Hz, 1 H), 3.32–3.29 (m, 2 H), 2.93–2.89 (m, 2 H), 2.50 (s, 12 H), 1.34 (d, J = 6.1 Hz, 2 H) ppm. Observable ^{13}C NMR (126 MHz, CD_2Cl_2): δ = 154.3, 132.5, 132.3, 128.5, 128.2, 126.3, 122.0, 114.9, 114.3, 71.4, 37.0, 30.1, 22.3, 16.9, 14.6 ppm. HRMS (ESI): calcd. for $[M + Na]^+$ 495.2400; found 495.2385. λ_{ex} = 500 nm, λ_{em} = 511 nm.

BODIPY-Vinyl Epoxide 14: Butadiene oxide (**13**; 0.230 g, 3.28 mmol) and Grubbs II catalyst (0.054 g, 0.066 mmol) were added to a solution of **12** (0.200 g, 0.661 mmol) in C_6H_6 (25 mL). The resulting solution was stirred at room temperature for 20 h.

The reaction was then quenched with vinyl ethyl ether (1.5 mL). The crude product was purified by flash chromatography [CH_2Cl_2/hexanes (60:40), R_f = 0.4] to yield the title product **14** (15%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.10–6.04 (m, 3 H), 5.31 (dd, J = 7.7, 15.5 Hz, 1 H), 3.38–3.35 (m, 1 H), 3.09–3.06 (m, 2 H), 3.00–2.98 (m, 1 H), 2.69–2.67 (m, 1 H), 2.52 (s, 6 H), 2.45–2.39 (m, 8 H) ppm. Observable ^{13}C NMR (500 MHz, $CDCl_3$): δ = 134.1, 129.1, 121.8, 52.1, 48.9, 34.0, 27.2, 16.5, 14.5 ppm. HRMS (ESI): calcd. for $[M + Na]^+$ 367.1773; found 367.1768. λ_{ex} = 495 nm, λ_{em} = 505 nm.

Chloropropyl-BODIPY 15b: Dry DMF (50 μL) and thionyl chloride (0.66 mL, 9.1 mmol) were added to a solution of 4-bromobutyric acid (1.23 g, 7.40 mmol) in dry CH_2Cl_2 (40 mL) and the solution was stirred at room temperature for 1 h. It was then concentrated in vacuo to yield a pale-yellow oil, which was redissolved in toluene (few drops) and then subsequently put on a high-vacuum line to remove the solvent and excess thionyl chloride. Without further purification, the acid chloride was redissolved in dry CH_2Cl_2 (50 mL) and 2,4-dimethylpyrrole (1.94 mL, 18.4 mmol) was added under N_2 to give a yellow solution. Phosphorus oxychloride (0.78 mL, 8.3 mmol) was added and the solution heated to 50 °C and stirred for 8 h. After cooling to room temperature, the mixture was concentrated in vacuo. To remove nonpolar impurities, the residue was then covered with reagent-grade hexanes. The mixture was decanted and the thick red oil was placed on a high-vacuum line, redissolved in dry toluene (50 mL), and heated to 80 °C. Dry triethylamine (1.6 mL, 11 mmol) was added and the mixture was stirred at 80 °C for 1 h. Boron trifluoride–dimethyl etherate (1.0 mL, 11 mmol) was added and the solution was stirred for an additional 1 h. The resulting red solution was cooled to room temperature and washed with brine. The organic layer was dried with sodium sulfate, filtered, and concentrated in vacuo to yield a dark-red/black oil. The crude product was purified by flash chromatography [ethyl acetate/hexanes (1:4)] to yield **15b** (0.55 g, 23%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.07 (s, 2 H), 3.70 (t, J = 6.1 Hz, 2 H), 3.13–3.10 (m, 2 H), 2.52 (s, 6 H), 2.43 (s, 6 H), 2.10–2.04 (m, 2 H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 154.4, 144.5, 140.4, 131.5, 121.9, 44.8, 34.0, 25.9, 16.62, 14.51 ppm. HRMS (ESI): calcd. for $[M + Na]^+$ 347.1277; found 347.1279. $C_{16}H_{20}BClF_2N_2$ (324.14): C 59.20, H 6.21, N 8.63; found C 59.12, H 6.36, N 8.58. λ_{ex} = 495 nm, λ_{em} = 502 nm, Φ = 0.84.

Compounds **16a** and **17a** were synthesized as previously reported.[11]

Cyclopropyl-BODIPY 19: Chloropropyl-BODIPY **15b** (0.20 g, 0.54 mmol) and potassium acetate (0.21 g, 2.2 mmol) were dissolved in dry DMF (10 mL) and the solution was stirred at 50 °C for 3 d. After cooling to room temperature, the reaction mixture was diluted in EtOAc (40 mL) and washed with H_2O (30 mL). The organic phase was washed with 80% brine (3× 50 mL) and also satd. brine. The organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.07 (s, 2 H), 2.52 (s, 6 H), 2.48 (s, 2 H), 1.94–1.91 (m, 1 H), 1.29–1.25 (m, 2 H), 0.84–0.81 (m, 2 H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 154.0, 147.0, 141.4, 134.8, 121.2, 29.8, 16.6, 14.6, 13.1, 12.1 ppm.

BODIPY–Au–Cl Complex 21: BODIPY-phosphine **20** was synthesized according to a procedure[2] previously published by our group and therefore not characterized by ^{1}H and ^{13}C NMR spectroscopy. A solution of [Me_2SAuCl] (0.055 g, 0.185 mmol) in dry CH_2Cl_2 (2 mL) was added to BODIPY-phosphine **20** (0.09 g, 0.19 mmol) and dry CH_2Cl_2 (1 mL) was swirled over the residue remaining in the vial and added to the BODIPY-phosphine solution. The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the orange solid obtained was dried under high vacuum to yield 0.125 g (95%) of **21**. ^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.70–7.64 (m, 4 H), 7.57–7.49 (m, 6 H), 7.35–7.33 (m, 3 H), 6.08–6.06 (m, 2 H), 2.97–2.94 (m, 2 H), 2.52–2.50 (m, 2 H), 2.48–2.44 (m, 6 H), 2.35–2.34 (m, 6 H), 1.79–1.78 (m, 4 H) ppm. ^{13}C NMR (500 MHz, CD_2Cl_2): δ = 154.3, 145.8, 141.0, 133.6, 133.5, 132.5, 132.4, 131.6, 129.8, 129.7, 129.2, 122.0, 33.1, 32.9, 28.3, 28.0, 26.7, 26.6, 16.8, 14.7 ppm. ^{31}P NMR (400 MHz, CD_2Cl_2): δ = 29.5 ppm. HRMS (ESI): calcd. for $[M + Na]^+$ 743.1621; found 743.1592. λ_{ex} = 500 nm, λ_{em} = 510 nm, Φ = 0.80.

BODIPY–Au–OTf Complex 22: AgOTf (0.013 g, 0.050 mmol, 1.6 equiv.) was added to a solution of **21** (0.022 g, 0.031 mmol) in CD_2Cl_2 (0.6 mL) and the reaction mixture was stirred at room temperature. After 1 h the precipitated Ag salts were filtered through a Whatman® filter and the formation and stability of the AgOTf complex **22** were monitored in situ by ^{1}H and ^{31}P NMR spectroscopy. ^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.67–7.54 (m, 10 H), 6.09 (s, 2 H), 2.95 (s, 2 H), 2.76–2.28 (m, 14 H), 1.80 (s, 2 H) ppm. ^{31}P NMR (400 MHz, CD_2Cl_2): δ = 25.5 ppm. HRMS (ESI): calcd. for $[M - SO_3CF_3]^+$ 685.2035; found 685.2013.

FRET Experiment – Gold-Catalyzed Isocoumarin Rearrangement: A 20-mL scintillation vial was charged with [PPh_3AuOTf] (2.2 mg, 0.0036 mmol), a solution of **6** (3.0 mg, 0.0036 mmol) dissolved in CD_2Cl_2 (2 mL) was added, and the mixture stirred at room temperature. After 3 h, MeOD (0.7 mL) and H_2O (0.1 mL) were added and the mixture was stirred at room temperature for an additional 15 min. The reaction progress was monitored by ^{1}H NMR spectroscopy, which confirmed the formation of protodemetalated lactone product **9** after 15 min. The NMR spectra from this experiment are included in the Supporting Information. The fluorescence of **6** and the product **9** was evaluated following the procedure described below.

Fluorescence Spectrophotometer: Fluorescence spectra were recorded with a Hitachi F-4500 FL spectrophotometer. Solutions of BODIPY donor **2**, BODIPY acceptor **5**, and BODIPY FRET pair **6** were prepared in spectrophotometric-grade DCM at concentrations of 2×10^{-6} M (intramolecular FRET is not significant at this concentration).[2] The solutions were excited at 488 nm and the emission spectra recorded. The samples were scanned from 300 to 700 nm at a scan speed of 240 nm/min.

Supporting Information (see footnote on the first page of this article): ^{1}H, ^{13}C, and ^{31}P NMR spectra.

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