Copper-assisted azide–alkyne cycloaddition chemistry as a tool for the production of emissive boron difluoride 3-cyanoformazanates†

Stephanie M. Barbon,a,b Samantha Novoa,a,b Desiree Bender,a,b Hilary Groom,c Leonard G. Luyt*a,d,c and Joe B. Gilroy*a,b

The synthesis and characterization of emissive boron difluoride (BF₂) complexes of 3-cyanoformazanate ligands produced using copper-assisted azide–alkyne cycloaddition (CuAAC) chemistry is described. Detailed spectroscopic and electrochemical characterization of benzyl-functionalized complexes served as models and demonstrated that triazole formation at the N-aryl substituents of the formazanate ligand scaffold led to red-shifted absorption and emission maxima and more difficult electrochemical reduction compared to alkyne-substituted precursors. CuAAC chemistry was also used to append ferrocene and tetraethylene glycol substituents to the formazanate backbone. In the case of the ferrocene-substituted complexes, fluorescence was quenched and a reversible oxidation feature (in addition to the reduction features associated with formazanate complexes) was observed using cyclic voltammetry. Treatment with NOBF₄ oxidized ferrocene to ferrocenium and resulted in the reestablishment of fluorescence. Tetraethylene glycol substitution produced the first water soluble BF₂ formazanate, which was shown to distribute throughout the cytoplasm and nucleus of mouse fibroblast cells when studied as a fluorescence imaging agent.

Introduction

Fluorophores are a very useful class of molecules as they provide a handle that allows for the visualization of chemical phenomena at the molecular, macromolecular and bulk size regimes.1–6 For this and other reasons, the development of new classes of fluorophores has been the focus of a significant amount of research.7,8 One of the major hurdles to overcome in the field was the facile synthetic modification of fluorophores in order to utilize them for an expanded range of applications.9–11 Significant impact was made in this area with the reports on copper-assisted azide–alkyne cycloaddition (CuAAC) chemistry.12,13 Since the original reports appeared, the number of alkyne- and azide-modified fluorophores such as those based on anthracene,14,15 pyrene,16,17 boron dipyrromethenes (BODIPYs),18–20 rhodamine,21,22 and coumarin23,24 has expanded dramatically (e.g., 1–3). The onward CuAAC chemistry of these and other fluorophores has led to their use, for example, as protein labels,25 in photosensitizers,26 as molecular switches,27 as probes of DNA hybridization,28 and in radiolabeling.29 CuAAC chemistry has also been used to create a variety of fluorescence sensors for anions,30 acids and bases,31,32 nitroxide radicals,33 hydroxyl radicals,34 and various metal ions including mercury35,36 and zinc.37,38

In addition, CuAAC chemistry has been utilized to modify fluorophores for biological imaging applications. This strategy has been used both to impart water solubility (e.g., 4),39,40 and functionalities that interact with specific biological targets (e.g., 5).41–44 More recently, this strategy has been extended to include copper-free, strain-promoted azide–alkyne cyclo-
addition chemistry with specific utility in biological systems.\textsuperscript{45,46} Transition metal\textsuperscript{47–53} and boron\textsuperscript{54–56} complexes of formazanate ligands have received significant attention due to their rich optical and electrochemical properties. More specifically, boron difluoride (BF\textsubscript{2}) complexes of 3-cyanoformazanates have recently been shown to have many of the desirable properties associated with common fluorophores, including high fluorescence quantum yields, large Stokes shifts, facile high-yielding syntheses, and perhaps most importantly, emission in the red to near-IR region of the electromagnetic spectrum.\textsuperscript{57–59} These complexes have shown promise as fluorescence cell-imaging agents,\textsuperscript{58} as precursors to B(1)-carbenoid intermediates\textsuperscript{60} and stable radicals,\textsuperscript{61} as efficient electrochemiluminescence emitters,\textsuperscript{62} aggregation-induced emission luminogens (AIEgens),\textsuperscript{63} and building blocks for functional polymeric materials.\textsuperscript{64,65} Herein, we expand the scope of BF\textsubscript{2} formazanate chemistry further by producing a variety of emissive materials through the use of CuAAC chemistry that cannot be readily synthesized by other methods.

**Results and discussion**

**Benzyl-functionalized BF\textsubscript{2} complexes of 3-cyanoformazanates**

Formazans 6 and 10 were prepared via aryl diazonium coupling reactions in basic solutions of cyanoacetic acid by adapting a reported method.\textsuperscript{66} Formazan 6 was prepared from a mixture of alkyne- and methoxy-substituted aryl diazonium salts and separated from the symmetrically-substituted byproducts using column chromatography. In order to study the effect of triazole formation on the photophysical properties of BF\textsubscript{2} complexes of 3-cyanoformazanate ligands, formazans 6 and 10 were converted to mono-alkyne-substituted complex 7 and bis-alkyne-substituted complex 11 by adaptation of established methods (Scheme 1).\textsuperscript{57} Complex 7 was first characterized by single crystal X-ray diffraction (Fig. 1), and crystallized on a two-fold rotation axis. As a result, the alkyne and methoxy substituents were disordered. However, the structure does confirm the expected connectivity and planarity throughout the molecule (dihedral angle between N\textsubscript{4} plane and aryl substituents: 7.2°). All other structural metrics were consistent with the solid-state structures of previously reported BF\textsubscript{2} adducts of 3-cyanoformazanate ligands.\textsuperscript{57}

The alkyne-substituted complexes 7 and 11 underwent CuAAC chemistry with benzyl azide when combined in the presence of copper(i) iodide and N,N,N′,N″,N″-pentamethylenetriamine (PMDETA) in THF to form mono- and bis-benzyl-functionalized compounds 8 and 12 (Scheme 1). The successful conversion of alkyne-substituted complexes 7 and

**Scheme 1** Synthesis of (a) mono- and (b) bis-functionalized BF\textsubscript{2} complexes of 3-cyanoformazanates.
to benzyl-functionalized complexes 8 and 12 was confirmed by ¹H, ¹³B, ¹³C{¹H} and ¹⁹F NMR spectroscopy (Fig. 2 and S1–S17†), UV-vis absorption/emission and IR spectroscopy, and high resolution mass spectrometry. CuAAC chemistry was also used to synthesize BF₂ formazanate complexes with appended ferrocene substituents (9 and 13) and water-solubilizing tetraethylene glycol substituents (14). Initial attempts to synthesize the water-soluble complex 14 were unsuccessful, likely due to the TEG chains outcompeting PMDETA for the ligation of copper(i) when all of the reagents were combined simultaneously. We circumvented this issue by stirring CuI and PMDETA in THF for 30 min before the TEG azide was added.

The electrochemical properties of complexes 7, 8, 11 and 12 were studied using cyclic voltammetry in THF (Fig. 3 and Table 1). Each voltammogram showed two reversible one-electron reduction waves within the electrochemical window of the solvent. Considering the first reduction wave, which corresponds to the formation of a ligand-centered radical anion, bis-alkyne-substituted complex 11 is significantly easier to reduce than mono-alkyne-substituted complex 7 (−0.50 V and −0.67 V relative to the ferrocene/ferrocenium redox couple, respectively) due to the presence of the electron donating methoxy substituent in 7. The benzyl-functionalized complexes were more difficult to reduce than their alkyne-substituted precursors (8: −0.68 V; 12: −0.62 V). We have previously demonstrated that extending electronic conjugation in BF₂ complexes of 3-cyanoformazanate ligands results in complexes that are easier to reduce due to a narrowing of the HOMO–LUMO gap.⁵⁹ However, in the case of complexes 8 and 12, the more negative reduction potentials observed are likely due to the electron donating nature of the benzyl-substituted triazoles, which appear to be better electron donors than alkyne substituents.

![Solid-state structure of 7. Anisotropic displacement ellipsoids are shown at 50% probability and hydrogen atoms have been removed for clarity. Selected bond lengths (Å): B1–N1 1.5759(15), N1–N2 1.5023(15), N2–C1 1.3351(13), O1–C11 1.418(5), C9–C10 1.191(7). Selected bond angles (°): B1–N1–N2 124.57(10), N1–N2–C1 117.24(11).](image)

![¹H NMR spectra of (a) benzyl azide (blue), mono-alkyne-substituted complex 7 (red) and mono-benzyl-functionalized complex 8 (black) and (b) benzyl azide (blue), bis-alkyne-substituted complex 11 (red) and bis-benzyl-functionalized complex 12 (black) in CDCl₃. The asterisks denote residual solvent signals. The blue squares denote the benzyl azide CH₂ signals, the red triangles denote the alkyne CH signals, the black circles denote the triazole CH signals, and the black squares denote the CH₂ signal from the benzyl azide moieties in complexes 8 and 12.](image)

![Cyclic voltammograms of (a) mono-alkyne-substituted complex 7 (black) and mono-benzyl-functionalized complex 8 (blue) and (b) bis-alkyne-substituted complex 11 (red) and bis-benzyl-functionalized complex 12 (purple) recorded at 100 mV s⁻¹ in 1 mM THF solutions containing 0.1 M [nBu₄N][PF₆] as supporting electrolyte.](image)
The second reduction waves, which correspond to the formation of ligand-centred dianions, followed the same trend.

In both the UV-vis absorption and emission spectra, the wavelength of maximum absorption (\(\lambda_{\text{max}}\)) and emission (\(\lambda_{\text{em}}\)) red-shift by approximately 10 nm when comparing mono-benzyl-functionalized complex 8 and mono-alkyne-substituted complex 7. As may be expected, a more drastic red-shift was observed when the spectra of bis-benzyl-functionalized complex 11 and bis-alkyne-substituted complex 12 were compared. In this case, red-shifts of approximately 30 nm (absorption) and 40 nm (emission) were observed (Fig. 4 and Table 1).

Complexes 7, 8, 11 and 12 exhibit moderate fluorescence quantum yields in THF, CH\(\text{2Cl}_2\), and toluene (Table 1). Monoalkyne-substituted complex 7 has a quantum yield of 30% in CH\(\text{2Cl}_2\), which is almost double that of bis-alkyne-substituted complex 11 (\(\Phi_F = 18\%\) in CH\(\text{2Cl}_2\)), likely due to its asymmetric structure.\(^{65}\) Mono-benzyl-functionalized complex 8 has the same quantum yield as complex 7, while bis-benzyl-functionalized complex 12 showed the most intense emission at a quantum yield of 46%. The Stokes shifts observed (79–133 nm; 2143–3394 cm\(^{-1}\)) for these complexes were typical of other BF\(_2\) complexes of 3-cyanoformazanates.\(^{57}\)

### Table 1  Optical and electrochemical properties of BF\(_2\) formazanate complexes 7–9 and 11–14

| Solvent     | \(\lambda_{\text{max}}\) (nm) | \(\epsilon\) (M\(^{-1}\) cm\(^{-1}\)) | \(\lambda_{\text{em}}\) (nm) | \(\Phi_F\) (%) | \(\nu_{\text{ST}}\) (nm) | \(\nu_{\text{ST}}\) (cm\(^{-1}\)) | \(E_{\text{red1}}\) (V) | \(E_{\text{red2}}\) (V) |
|-------------|-------------------------------|-----------------------------------|---------------------------|---------------|-------------------|-------------------------------|----------------|----------------|
| 7 CH\(\text{2Cl}_2\) | 552                           | 30 400                            | 647                       | 30            | 95                | 2660                          | —              | —              |
| THF         | 550                           | 32 500                            | 650                       | 27            | 100               | 2797                          | —              | -0.67          |
| Toluene     | 569                           | 26 800                            | 648                       | 36            | 79                | 2143                          | —              | -1.78          |
| 8 CH\(\text{2Cl}_2\) | 560                           | 31 000                            | 660                       | 30            | 100               | 2706                          | —              | -0.68          |
| THF         | 560                           | 33 700                            | 672                       | 25            | 112               | 2976                          | —              | -1.81          |
| Toluene     | 579                           | 37 400                            | 661                       | 37            | 82                | 2143                          | —              | -1.80          |
| 9 \(^c\) CH\(\text{2Cl}_2\) | 562                           | 28 600                            | —                         | —             | —                 | —                             | 0.20           | -0.67          |
| THF         | 562                           | 31 700                            | —                         | —             | —                 | —                             | —              | -1.80          |
| Toluene     | 578                           | 23 900                            | —                         | —             | —                 | —                             | —              | —              |
| 11 CH\(\text{2Cl}_2\) | 530                           | 32 700                            | 635                       | 18            | 105               | 3120                          | —              | -0.50          |
| THF         | 529                           | 29 600                            | 638                       | 19            | 109               | 3230                          | —              | -1.58          |
| Toluene     | 547                           | 41 700                            | 637                       | 36            | 90                | 2583                          | —              | —              |
| 12 CH\(\text{2Cl}_2\) | 558                           | 39 600                            | 679                       | 46            | 121               | 3194                          | —              | -0.62          |
| THF         | 563                           | 34 100                            | 696                       | 34            | 133               | 3394                          | —              | -1.72          |
| Toluene     | 577                           | 33 700                            | 680                       | 72            | 103               | 2625                          | —              | —              |
| 13 \(^c\) CH\(\text{2Cl}_2\) | 569                           | 30 900                            | —                         | —             | —                 | —                             | 0.21           | -0.56          |
| THF         | 569                           | 26 100                            | —                         | —             | —                 | —                             | —              | -1.67          |
| Toluene     | 582                           | 30 700                            | —                         | —             | —                 | —                             | —              | —              |
| 14 CH\(\text{2Cl}_2\) | 542                           | 16 500                            | 680                       | 53            | 138               | 3474                          | —              | -0.58          |
| THF         | 563                           | 11 900                            | 697                       | 20            | 134               | 3415                          | —              | -1.68          |
| Toluene     | —                              | —                                 | —                         | —             | —                 | —                             | —              | —              |

\(\Phi_F\): Quantum yield; \(\nu_{\text{ST}}\): Stokes shift.

The emission properties in CH\(\text{2Cl}_2\) (Fig. 5). NOBF\(_4\) was chosen as an oxidant as the gaseous byproducts were assumed not to contribute to the spectra collected upon oxidation and the oxidation potential (ca. 1.0 V relative to the ferrocene/ferrocinium redox couple)\(^{74}\) was not in a range that would oxidize the 3-cyanoformazanate ligand backbone. In CH\(\text{2Cl}_2\), the absorption maxima associated with the \(\pi \rightarrow \pi^*\) transition of the BF\(_2\) formazanate backbone of complexes 9 and 13 were blue-shifted, by up to 20 nm in complex 13, upon sequential addition of NOBF\(_4\). The blue-shifts appear to arise due to the removal of electron density from the ferrocene moieties upon oxidation, rendering the complexes less electron rich overall. This effect has been observed previously for similar complexes, whereby the introduction of electron withdrawing groups blue-shifted absorption maxima.\(^{57}\) Perhaps more significant, was the observation that, upon conversion of ferrocene to ferrocinium, the solutions of 9 and 13 that had been treated with NOBF\(_4\) became emissive. The emission intensity gradually increased with the subsequent addition of oxidizing agent until a maximum intensity (\(\Phi_F\) = 7% for 9 and 14% for 13) was reached when a full equivalent of NOBF\(_4\) per ferrocene unit
had been added, while $\lambda_{em}$ was unchanged. These observations corroborate our hypothesis that the emission of these complexes was quenched as a result of excited-state electron transfer from the formazanate backbone to ferrocene.

BF$_2$ complexes of formazanate ligands generally exhibit very well-behaved reduction chemistry. However, their oxidation is rarely observed within the electrochemical window of most organic solvents. Ferrocene can be reversibly oxidized, and so the redox properties of compounds that contain both ferrocene and formazanate moieties may have unusual properties, including charge-transfer characteristics. Similar compounds based on different fluorophores have been previously used as viscosity probes, redox-active fluorescent switches, and ion pair recognition receptors.

Cyclic voltammetry studies of complexes 9 and 13 revealed two characteristic BF$_2$ formazanate reduction waves, and indicated that each complex was more difficult to reduce than the alkyne-substituted precursors (7 and 11), due to the electron-rich nature of ferrocene (Table 1). Oxidation waves corresponding to one electron for 9 and two electrons for 13 were observed at potentials of 0.20 V and 0.21 V, which corresponds to the oxidation of the ferrocene groups (Fig. 6). The coincident appearance of the ferrocene waves in the cyclic voltammogram of 13 confirmed that there was little to no electronic communication between the ferrocene units via the BF$_2$ formazanate spacer. This observation was consistent with those noted for polymers and model compounds derived from BF$_2$ complexes of triarylformazanate ligands, whereby triazole groups were shown to limit the overall degree of electronic conjugation along the polymer backbone.

Tetraethylene glycol-functionalized BF$_2$ complexes of 3-cyanoformazanates

Complex 14, which was targeted in an effort to maximize hydrophilicity, is soluble in both polar organic solvents and water. Interestingly, the optical properties were highly dependent on the polarity of the solvent used. In relatively non-polar solvents such as CH$_2$Cl$_2$ and THF, 14 was moderately fluorescent ($\Phi_F$: 53% and 20%, respectively). However, the fluorescence intensity dropped off significantly in more polar solvents ($\Phi_F$: 5% in H$_2$O), and there was also a blue-shift in the wavelength of maximum emission (Table 2, Fig. 7), as is often observed for fluorescent dyes in highly polar solvents.

While the emission intensity of 14 was relatively low in water, many other fluorophores with relatively low quantum yields have shown promise as cell-imaging agents, especially dyes which emit in the red to near-IR region. With these factors in mind, complex 14 was introduced into mouse fibroblast cells and its utility as an imaging agent studied using fluorescence confocal microscopy.
Complex 14 was successfully incorporated into the cells, as evidenced by the fluorescence micrographs shown in Fig. 8. Specifically, complex 14 was distributed throughout the cell structure and clearly penetrated the cell nucleus. The dark spots visualized in Fig. 8c are believed to be DNA-free nucleoli, and appear to be the only features of the cells that were not stained by 14. Despite the widespread incorporation of complex 14 throughout the cell, Fig. 8b and d demonstrate our ability to differentiate between cytoplasm and nucleus when 4′,6-diamidino-2-phenylindole (DAPI), a selective nuclear stain, was employed concurrently. These results differed significantly when compared to previous studies involving BF₂ complexes of 3-cyanoformazanates bearing p-anisole substituents. The hydrophobic nature of the anisole-based dye resulted in selective staining of the cell cytoplasm and required the use of DMSO to achieve cellular uptake. While we have not produced a site-specific imaging agent as part of this study, we have demonstrated our ability to create hydrophilic analogs of the parent complexes and shown that, in doing so, we can drastically alter their cellular uptake. This work sets the stage for future iterations whereby site-specific staining will be possible.

Table 2: Optical properties of complex 14 in different solvents

| Solvent | λₘₐₓ (nm) | ε (M⁻¹ cm⁻¹) | λₑₘ (nm) | Φₓ (%) | ν_ST (nm) | ν_ST (cm⁻¹) |
|---------|-----------|--------------|-----------|--------|-----------|-------------|
| CH₂Cl₂  | 542       | 16 500       | 680       | 53     | 138       | 3744        |
| THF     | 563       | 11 900       | 697       | 20     | 134       | 3415        |
| MeOH    | 521       | 19 900       | 681       | 6      | 160       | 4510        |
| MeCN    | 524       | 18 200       | 697       | 10     | 173       | 4737        |
| DMSO    | 526       | 17 600       | 716       | <1     | 190       | 5045        |
| H₂O     | 527       | 15 100       | 698       | 5      | 171       | 4649        |

Quantum yields were measured according to published protocols using [Ru(bpy)₃]²⁺[PF₆]³⁻ as a relative standard and corrected for wavelength-dependent detector sensitivity (Fig. S18).
achieved through the introduction of hydrophilic, tailor-made peptide chains.

Conclusions

As a result of this work, we have demonstrated the effect of triazole formation on the spectroscopic and electrochemical properties of BF₂ complexes of 3-cyanoformazanates. Complexes functionalized with benzyl groups showed that the formation of triazole rings resulted in red-shifted wavelengths of maximum absorption and emission, as well as increased fluorescence quantum yields. Triazole formation also resulted in BF₂ complexes that were more difficult to reduce electrochemically. We further demonstrated how CuAAC chemistry could be used to expand the scope of BF₂ formazanate chemistry. The introduction of ferrocene into the BF₂ formazanate scaffold via CuAAC resulted in the formation of a non-emissive complex with rich electrochemistry. Specifically, oxidation waves corresponding to one electron per ferrocene and two reduction waves (one electron each) originating from the formazanate backbones were observed by cyclic voltammetry. We also demonstrated that the stepwise conversion of ferrocene to ferrocenium was accompanied by an increase in emission intensity, confirming our hypothesis that quenching arose due to excited-state electron transfer from the formazanate backbone to ferrocene. Finally, CuAAC chemistry was used to append water-solubilizing TEG chains to a BF₂ formazanate complex. The resulting complex was used to image mouse fibroblast cells, and demonstrated our ability to control cellular uptake via the modification of hydrophobicity/hydrophilicity.

Experimental section

General considerations

Reactions and manipulations were carried out under a nitrogen atmosphere using standard Schlenk techniques unless otherwise stated. Solvents were obtained from Caledon Laboratories, dried using an Innovative Technologies Inc. solvent purification system, collected under vacuum and stored under a nitrogen atmosphere over 4 Å molecular sieves. Reagents were purchased from Sigma-Aldrich or Alfa Aesar and used as received. TEG-N₃ and Fc-N₃ were prepared according to literature procedures. NMR spectra were recorded on 400 MHz (¹H: 399.8 MHz, ¹³B: 128.3 MHz, ¹⁹F: 376.1 MHz) or 600 MHz (¹H: 599.5 MHz, ¹³C{¹H}: 150.8 MHz) Varian INOVA instruments. ¹H NMR spectra were referenced to residual CHCl₃ (7.26 ppm) or DMSO-d₆ (2.50 ppm) and ¹³C{¹H} NMR spectra were referenced to CDCl₃ (77.2 ppm) or DMSO-d₆ (39.5 ppm). ¹¹B spectra were referenced to BF₃·OEt₂ at 0 ppm and ¹⁹F spectra were referenced to CFCl₃ at 0 ppm. Mass spectrometry data were recorded in positive-ion mode using a high-resolution Finnigan MAT 8200 spectrometer using electron impact ionization or a Micromass LCT electrospray time-of-flight mass spectrometer. UV-vis absorption spectra were recorded using a Cary 5000 instrument. Four separate concentrations were run for each sample and molar extinction coefficients were determined from the slope of a plot of absorbance against concentration. FT-IR spectra were recorded on a KBr disk or using an attenuated total reflectance (ATR) attachment using a Bruker Vector 33 FT-IR spectrometer. Emission spectra were obtained using a Photon Technology International QM-4 SE spectrofluorometer. Excitation wavelengths were chosen based on
$\lambda_{\text{max}}$ from the respective UV-vis absorption spectrum in the same solvent. Emission quantum yields were estimated relative to [Ru(bpy)$_3$]$_2^+$[PF$_6$]$_2$ and corrected for wavelength dependent detector sensitivity (Fig. S18†).  

Electrochemical methods

Cyclic voltammetry experiments were performed with a Bioanalytical Systems Inc. (BASi) Epsilon potentiostat and analyzed using BASi Epsilon software. Electrochemical cells consisted of a three-electrode setup including a glassy carbon working electrode, platinum wire counter electrode and silver wire pseudo reference electrode. Experiments were run at scan rates of 100 mV s$^{-1}$ in degassed THF solutions of the analyte (~1 mM) and supporting electrolyte (0.1 M nBu$_4$PF$_6$). Cyclic voltammograms were referenced against an internal standard (~1 mM ferrocene) and corrected for internal cell resistance using the BASi Epsilon software.

X-ray crystallography details

Single crystals of mono-alkyne-substituted complex 7 suitable for X-ray diffraction studies were grown by slow evaporation of a concentrated CH$_2$Cl$_2$ solution. The sample was mounted on a MiTeGen polyimide micromount with a small amount of Paratone N oil. X-ray measurements were made on a Bruker Kappa Axis Apex2 diffractometer at a temperature of 110 K. The data collection strategy included a number of $\omega$ and $\phi$ scans which collected data over a range of angles, 20. The frame integration was performed using SAINT.  

The resulting raw data was scaled and absorption corrected using a multi-scan averaging of symmetry equivalent data using SADABS. The structure was solved by using a dual space methodology using the SHELXT program. All non-hydrogen atoms were obtained from the initial solution. The hydrogen atoms were introduced at idealized positions and were allowed to refine isotropically. The structural model was fit to the data using full matrix least-squares based on $F^2$. The calculated structure factors included corrections for anomalous dispersion from the usual tabulation. The structure was refined using the SHELXL-2014 program from the SHELXT suite of crystallographic software. See Table 3 and CCDC 1500180 for refinement details for mono-alkyne-substituted complex 7.

Cell imaging studies

Mouse fibroblast C3H/10T1/2 cells (ATCC) were released from the tissue culture flask and seeded onto cover slips in a 12-well tissue culture plate at approximately 50,000 cells per well. The cells were incubated overnight in Dulbecco’s modified Eagle’s medium (DMEM, Sigma-Aldrich) containing 10% fetal bovine serum (FBS, Sigma-Aldrich) with penicillin streptomycin at 37 °C in a 5% CO$_2$ atmosphere. Cell media was aspirated and the cells were washed once with serum free media and twice with phosphate buffer saline (PBS, Sigma-Aldrich). Water-soluble complex 14 (8 mg) was dissolved into 5 mL DMEM (serum free), filter sterilized with a 0.2 μm filter and diluted to 100 μM with serum free DMEM. The dye stock was incubated at 37 °C for 1 h with the cells. The cells were washed twice with PBS, fixed with 4% paraformaldehyde and mounted onto slides containing Pro-Long Antifade mounting medium with DAPI (ThermoFisher Scientific). Fluorescence microscopy images were obtained using an Olympus Fluoview FV 1000 confocal microscope. Images based on the fluorescence of complex 14 were obtained using 10% laser strength, with excitation at 405 nm and emission collected between 425 and 475 nm. Images based on the fluorescence of complex 14 were obtained using 10% laser strength, with excitation at 579 nm and emission collected between 425 and 475 nm.

Mono-alkyne-substituted formazan 6

In air, cyanoacetic acid (1.00 g, 11.7 mmol) and NaOH (4.70 g, 117 mmol) were mixed with deionized H$_2$O (60 mL) and the solution was stirred in an ice bath for 20 min. Meanwhile, in a separate flask, 4-ethynylaniline (1.10 g, 9.36 mmol) was mixed with 12 M HCl (2.3 mL, 28 mmol) in deionized H$_2$O (2.3 mL). The solution was cooled in an ice bath for 15 min before a cooled solution of sodium nitrite (0.75 g, 10 mmol) in deionized H$_2$O (7 mL) was added dropwise. The resulting reaction mixture, which contained the corresponding diazonium salt, was stirred in an ice bath for an additional 20 min. In a separate flask, $p$-anisidine (1.4 g, 12 mmol) was mixed with 12 M HCl (2.3 mL, 35 mmol) in deionized H$_2$O (2.3 mL). The solution was cooled in an ice bath for 15 min before a cooled solution of sodium nitrite (0.93 g, 13 mmol) in deionized H$_2$O (8 mL) was added dropwise. The resulting reaction mixture, which contained diazonium salt, was stirred in an ice bath for an additional 20 min. The diazonium salt solutions were then mixed together and stirred in an ice bath for 10 min. This solution was then added dropwise to the cyanoacetic acid solution described above. The solution turned dark red after approxi-
mately 2 min. After complete addition, the mixture was stirred in an ice bath for an additional 60 min before it was neutralized with 1 M HCl. The resulting red-brown solid was isolated by vacuum filtration and purified by flash chromatography using a gradient strategy (starting at 1:1 n-hexanes:CH2Cl2 and ending with 2:8 n-hexanes:CH2Cl2) where the second coloured fraction contained the desired product. Removal of the solvent in vacuo afforded mono-alkyne-substituted forma-
monobenzyl-functionalized complex 8

d was stirred at 22 ºC for 16 h. The THF solution was then purified by flash chromatography (THF, neutral alumina) and recrystallized from MeOH to afford mono-benzyl-functionalized complex 8 as a dark-purple microcrystalline solid. Yield = 0.14 g, 51%. M.p. 188–189 ºC. 1H NMR (400.1 MHz, CDCl3): δ 7.95–7.93 (m, 6H, aryl CH), 7.75 (s, 1H, CH=CH), 7.42–7.40 (m, 3H, aryl CH), 7.34–7.32 (m, 2H, aryl CH), 7.00 (d, 3JHH = 9 Hz, 2H, aryl CH) 5.60 (s, 2H, CH2), 3.90 (s, 3H, CH3). 13C([H] NMR (150.7 MHz, CDCl3): δ 162.7, 146.6, 142.6, 136.8, 134.4, 132.9, 129.2, 128.9, 128.1, 126.4, 125.3, 123.3, 120.5, 114.9, 114.3. 55.8, 54.3. 11B NMR (128.3 MHz, CDCl3): δ −0.7 (t, 2JBF = 29 Hz). 19F NMR (376.1 MHz, CDCl3): δ −134.2 (q, JFF = 29 Hz). FT-IR/ATR): 3123 (m), 2849 (m), 2250 (m), 1603 (m), 1579 (m), 1479 (m), 1367 (m). UV-Vis (CH2Cl2): λmax 560 nm (ε = 31 000 M⁻¹ cm⁻¹). Mass Spec. (EI, +ve mode): exact mass calculated for [C24H19BF2N4O]⁺: 484.1743; exact mass found: 484.1759; difference: +3 ppm.

Mono-ferrocene-functionalized BF2 complex 9

A solution of PMDETA (0.002 g, 0.002 mL, 0.01 mmol) in THF (3 mL) was degassed via 3 freeze–pump–thaw cycles before CuI (0.002 g, 0.01 mmol) was added and the resulting mixture was stirred for 30 min. Ferrocenyl azide (0.024 g, 0.11 mmol) and mono-alkyne-substituted complex 7 (0.037 g, 0.11 mmol) were then added and the reaction mixture was stirred at 50 ºC for 18 h. Upon cooling to 22 ºC, the resulting dark purple solution was filtered through a plug of celite and purified by flash chromatography (CH2Cl2, silica gel) to afford ferrocene-functionalized complex 9 as a dark purple powder. Yield = 0.04 g, 72%. Melting point not observed (m.p. >250 ºC). 1H NMR (400.1 MHz, CDCl3): δ 8.06 (s, 1H, triazole CH), 8.02–7.95 (m, 6H, aryl CH), 7.01–7.00 (m, 2H, aryl CH), 4.90 (s, 2H, ferrocene CH), 4.32 (s, 2H, ferrocene CH), 4.26 (s, 6H, ferrocene CH), 3.91 (s, 3H, OCH3). 13C([H] NMR (150.7 MHz, CDCl3): δ 162.9, 146.3, 142.9, 137.0, 132.9, 126.7, 125.4, 123.6, 120.0, 115.1, 114.4, 93.6, 70.4, 67.1, 62.4, 62.3, 56.0. 11B NMR (128.3 MHz, CDCl3): δ −0.7 (t, 2JBF = 31 Hz). 19F NMR (376.1 MHz, CDCl3): δ −134.1 (q, JFF = 30 Hz). FT-IR/ATR): 3282 (m), 2928 (s), 2840 (s), 2240 (m), 1593 (s), 1505 (m), 1407 (s), 1343 (s), 1328 (s), 1307 (s), 1262 (s), 1166 (s), 1138 (s) cm⁻¹. UV-Vis (CH2Cl2): λmax 562 nm (ε = 28 600 M⁻¹ cm⁻¹). Mass Spec. (EI, +ve mode): exact mass calculated for [C27H21BF2FeN8O]⁺: 758.1249; exact mass found: 758.1261; difference: +2.1 ppm.

Bis-alkyne-substituted formazan 10

In air, cyanooacetic acid (0.47 g, 5.5 mmol) was dissolved in de-
ionized H2O (75 mL) containing NaOH (2.20 g, 55 mmol). This colourless solution was stirred for 45 min in an ice bath. Meanwhile, 4-ethynylaniline (1.30 g, 12 mmol) was mixed with concentrated HCl (2.85 mL) in deionized H2O (30 mL). This solution was cooled in an ice bath for 10 min before a solution of sodium nitrite (1.01 g, 15 mmol) in deionized H2O (15 mL) was added slowly to the 4-ethy
nylaniline solution over a 10 min period. This mixture was stirred in an ice bath for 30 min, and then added slowly to the basic cyanooacetic acid solution. A dark red/orange colour per-
sisted almost immediately, and a dark red/orange precipitate formed after a few min. The mixture was stirred in an ice bath for an additional 16 h after ethyl acetate (250 mL) was added and the organic layer was isolated, washed with deionized H2O (3 × 100 mL), dried over MgSO4, gravity filtered and concentrated in vacuo. The resulting residue was purified by flash chromatography (CH2Cl2, neutral alumina) to a yield = 1.55 g, 94%. Melting point not observed (>250 °C).

Bis-alkyne-substituted BF2 complex 11 was stirred at 50 °C for 18 h. Upon cooling to 22 °C, the resulting dark purple solution was subjected to 10 000 scans (8 h) on a 600 MHz spectrometer, a publication quality Mass Spec. (EI, +ve mode) exact mass calculated for [C18H10N5BF2]+: 345.0997; exact mass found: 345.1012; dispersion: +1.3 ppm. 

Bis-TEG-functionalized BF2 complex 14 as a dark purple powder. Yield = 0.080 g, 90%. Melting point not observed (>250 °C). 1H NMR (599.5 MHz, CDCl3) δ 7.99–7.94 (m, 8H, aryl CH), 7.76 (s, 2H, triazole CH), 7.43–7.38 (m, 6H, aryl CH), 7.34–7.33 (m, 4H, aryl CH), 5.60 (s, 4H, benzyl CH2). 13C{1H} NMR (150.7 MHz, CDCl3): δ 146.7, 142.8, 134.4, 133.8, 129.4, 129.2, 128.3, 126.7, 123.8, 120.7, 114.2, 54.6. 11B NMR (128.3 MHz, CDCl3): δ 0–7 (t, JBF = 29 Hz). 19F NMR (376.1 Hz, CDCl3): δ −133.1 (q, JFBF = 29 Hz). FT-IR (KBr): 3109 (m), 3100 (s), 2915 (m), 2915 (m), 2850 (m), 2246 (s), 1608 (s), 1497 (m), 1456 (s), 1326 (s), 1226 (m), 1074 (s), 1027 (s), 976 (m), 826 (m) cm−1. UV-vis (CHCl3): λmax = 558 nm (ε = 39 600 M−1 cm−1). Mass Spec. (EI, +ve mode): exact mass calculated for [C13H29N3BF2]2+: 612.2356; exact mass found: 612.2362; difference: +0.1 ppm.

Bis-ferrocene-functionalized BF2 complex 13 
A solution of PMDETA (0.0019 g, 0.0023 mL, 0.011 mmol) in THF (4 mL) was degassed via freeze–pump–thaw cycles before CuI (0.021 g, 0.011 mmol) was added and the resulting mixture was stirred for 30 min. Ferrocenyl azide (0.050 g, 0.22 mmol) and bis-alkyne-substituted complex 11 (0.038 g, 0.11 mmol) were then added and the reaction mixture was stirred at 50 °C for 18 h. The resulting dark purple solution was filtered through a plug of celite and purified by flash chromatography (CH2Cl2, silica gel) to afford ferrocene-functionalized complex 13 as a dark purple powder. Yield = 0.080 g, 90%. Melting point not observed (>250 °C). 1H NMR (599.5 MHz, CDCl3) δ 8.05 (s, 2H, triazole CH), 8.05 (s, 8H, aryl CH), 4.91 (m, 4H, ferrocene CH), 4.34–4.33 (m, 4H, ferrocene CH), 4.28–4.26 (m, 10H, ferrocene CH). Due to the poor solubility of 13, and despite a saturated solution in THF-d8 being subjected to 10 000 scans (8 h) on a 600 MHz spectrometer, a publication quality Mass Spec. (EI, +ve mode) exact mass calculated for [C18H10N5BF2]2+: 800.1367; exact mass found: 800.1380; difference: +1.6 ppm.

Bis-TEG-functionalized BF2 complex 14 
A solution of PMDETA (0.008 g, 0.009 mL, 0.04 mmol) in THF (3 mL) was degassed via freeze–pump–thaw cycles before CuI (0.008 g, 0.04 mmol) was added and the resulting solution was stirred for 30 min. TEG-azide (0.038 g, 1.3 mmol) and bis-alkyne-substituted complex 11 (0.15 g, 0.44 mmol) were then added and the reaction mixture was stirred at 50 °C for 18 h. The resulting dark purple solution was filtered through a plug of celite and purified by precipitation of a concentrated THF solution into n-hexanes thrice to yield a dark purple solid. Yield = 0.24 g, 71%. 1H NMR (599.5 MHz, CDCl3) δ 8.22 (s, 2H, triazole CH), 8.00 (s, 8H, aryl CH), 4.63–4.61 (m, 4H, ethylene glycol CH2), 3.93–3.90 (m, 4H, ethylene glycol CH2), 3.71–3.57 (m, 24H, ethylene glycol CH2). 13C{1H} NMR (150.7 MHz, CDCl3): 145.9, 142.4, 134.0, 126.5, 123.6, 122.6, 114.1, 72.4, 70.5, 70.4, 70.3, 70.1, 69.4, 61.5, 50.5 ppm. 11B NMR
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