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Complete List of Authors:	Nguyen, Alex; Louisiana State University, Department of Chemistry; Louisiana State University at Alexandria, Mathematics and Physical Sciences Griffin, Kaitlin; Louisiana State University, Department of Chemistry Zhou, Zehua; Louisiana State University, Chemistry Dept Fronczek, Frank; Louisiana State University, Chemistry Dept Smith, Kevin; Louisiana State University, Department of Chemistry Vicente, M Graca H; Louisiana State University, Department of Chemistry

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Syntheses of 1,2,3-Triazole-BODIPYs Bearing up to Three Carbohydrate Units

Alex L. Nguyen,[1,2] Kaitlin E. Griffin,[1] Zehua Zhou,[1] Frank R. Fronczek,[1] Kevin M. Smith[1] and M. Graça H. Vicente[1,*]

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A series of BODIPYs bearing one (**4b,c**), two (**6a**) or three (**3b**) glucose units attached to the meso(8) and the boron atom positions were synthesized using a Cu(I)-catalyzed Huisgen cycloaddition reaction. The resulting 1,2,3-triazole-BODIPYs were characterized by ^{1}H, ^{13}C and ^{11}B NMR, HRMS, and in the case of **4a** and **4b** by X-ray crystallography. Triazole-BODIPYs **3b**, **4b** and **6a** were highly permeable in cells and non-toxic to human HEp2 cells, both in the dark and upon light irradiation (IC_{50} > 100 μM). The time-dependedent cellular uptake increased with the increase of glucose units.

Introduction

4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene dyes, also known as boron dipyrromethenes or BODIPYs, are highly versatile compounds with desirable properties for a range of biological applications, from biological labelling for fluorescence imaging[1] to chemical sensing[2] to cancer therapy.[3] These properties include large molar absorption extinction coefficients, narrow emission bandwidths, high fluorescence quantum yields, long fluorescence lifetimes, relatively high photochemical stability, and high cellular permeability and compatibility.[4] One challenge that remains for their biomedical applications is the development of bioconjugation methods that allow the attachment of BODIPYs to multiple target molecules or ligands, such as amino acids, peptides and carbohydrates. Traditional amide bond formation reactions typically require long reaction times and often produce targeted multimeric conjugates in only low yields.[5] On the other hand, the copper(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition to form triazoles, known as the Huisgen [3+2] cycloaddition or "click" reaction, uses mild conditions and usually produces the targeted conjugates in high yields.[6] We have previously used this methodology to conjugate up to four carbohydrate units (galactose or lactose) to a porphyrin platform.[7] Such carbohydrate-functionalized dyes showed enhanced solubility and cellular uptake, potentially due to enhanced interactions with cell surface glycoprotein receptors, via carbohydrate-mediated cell recognition processes. We have also recently reported the conjugation of BODIPYs to a single glucose or lactose unit via the meso(8) position using a "click" reaction.[8] Other methodologies for conjugating carbohydrate units to BODIPYs include amide bond formation using a succinimidyl ester-functionalized BODIPY[9] and via alkylation of phenol-substituted BODIPYs.[10] Herein we report the conjugation of BODIPY dyes to one, two, or three glucose units by taking advantage of facile functionalization at the boron atom with propargyloxy groups.[11] Although functionalizations at the boron atom have lagged behind those at the carbon atoms of BODIPYs, the nucleophilic substitution of one or both fluorines with alkyl, aryl, alkenyl, alkynyl, alkoxy and aryloxy groups have been reported.[11,12] These reactions require strong nucleophiles, such as Grignard reagents, organolithiums and alkoxides, or alternatively, can be accomplished in the presence of a Lewis acid ($AlCl_3$, $SnCl_4$ or BCl_3) and milder nucleophiles. Therefore, boron functionalization is a convenient methodology for the introduction of additional groups for BODIPY bioconjugation, particularly of propargyloxy groups, which are suitable for "click" reactions with azides under conditions compatible with the BODIPY scaffold. Alternatively, a single glucose unit can also be introduced directly at the boron atom via nucleophilic substitution using two glucose oxygens, albeit in low yield.[13]

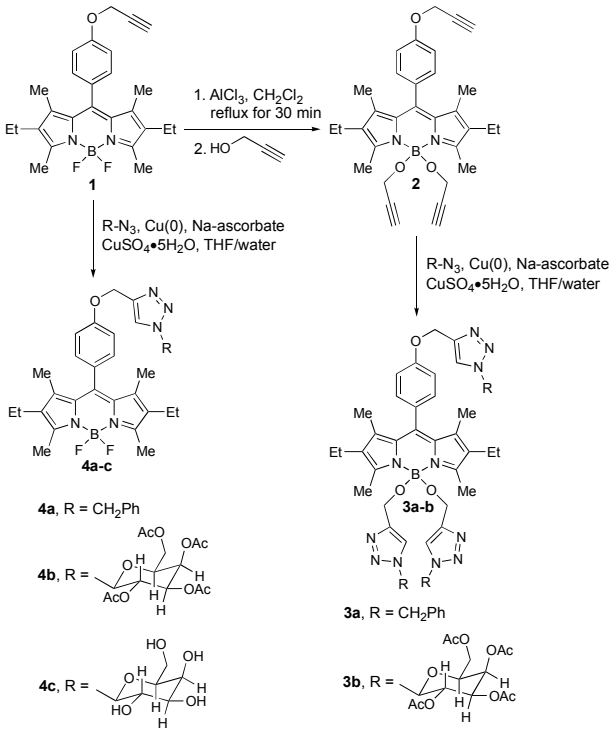
Results and Discussion

Syntheses

BODIPY **2**, bearing three alkyne groups suitable for "click" chemistry, was synthesized from BODIPY **1** as shown in Scheme 1. BODIPY **1** was obtained, in high yield as we have

[1]*Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA*
[2]*Present address: Department of Mathematics and Physical Sciences, Louisiana State University, Alexandria, LA 71302, USA*
Electronic Supplementary Information (ESI) available: it contains ^{1}H, ^{11}B and ^{13}C NMR spectra for all BODIPYs. See DOI: 10.1039/x0xx00000x

previously reported,[8] by condensation between commercially available 3-ethyl-2,4-dimethylpyrrole and *p*-propargyloxy-benzaldehyde,[14] followed by DDQ oxidation and boron complexation using BF_3•OEt_2 under basic conditions. Activation of the B-F bonds of BODIPY **1** with aluminium trichloride followed by reaction with excess propargyl alcohol[9] afforded BODIPY **2** in 69% yield. The ^{1}H NMR spectrum of **2** showed the terminal acetylene proton on the meso(8) aryl group at 4.48 ppm, while those on the boron center were found downfield shifted at 3.81 ppm. Furthermore, the characteristic triplet in ^{11}B NMR for the BF_2 group of BODIPY **1** at 0.68 ppm became a singlet at 1.76 ppm for BODIPY **2**. The cycloaddition reaction of BODIPY **2** with commercially available benzyl azide or with 1-azido-1-deoxy-β-D-glucopyranoside tetraacetate, using solution-phase "click" conditions consisting of copper(II) sulfate, copper(0) and sodium ascorbate in THF/water (3:1), gave tri-(1,2,3-triazolo)-BODIPYs **3a** and **3b**, in 76 and 64% yields, respectively. The elemental copper is a convenient reductant for copper(II) to generate copper(I), which is a powerful catalyst for the synthesis of 1,2,3-triazoles from azides and terminal alkynes. Under an inert gas atmosphere, the mixture was refluxed at 70 °C for 6 h and upon reaction completion, the mixture was cooled to room temperature, partitioned between ethyl acetate and water to remove the copper salt by-products, and the crude products were purified by preparative TLC.



Scheme 1. Synthesis of mono- and tri(glucose)-BODIPYs **4b,c** and **3b**.

On the other hand, reaction of BODIPY **1** under Cu(I)-catalyzed azide-alkyne cycloaddition conditions using commercially available benzyl azide or 1-azido-1-deoxy-β-D-glucopyranoside tetraacetate, afforded mono-triazole BODIPYs **4a** and **4b**, in 93 and 82% yields, respectively. Deprotection of the acetate groups of **4b** using sodium methoxide (4.2 equivalents) in methanol gave the corresponding glucose triazole-BODIPY **4c** in 93% yield. Under similar conditions BODIPY **3b** was only partially deprotected, and when a larger amount of sodium methoxide was used (up to 15 equivalents), the corresponding dipyrromethene was the main product obtained due to a deboronation reaction.

The synthesis of di-glucose-BODIPY **6a** from **4a** is shown in Scheme 2. BODIPY **4a** reacted smoothly with excess propargyl alcohol upon activation with $AlCl_3$ to produce **5a** in 73% yield. Under similar "click" reaction conditions using 1-azido-1-deoxy-β-D-glucopyranoside tetraacetate, **5a** afforded **6a** in 72% isolated yield.

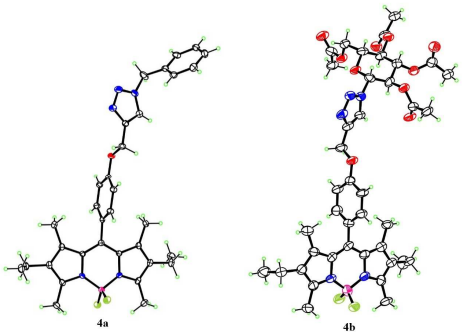
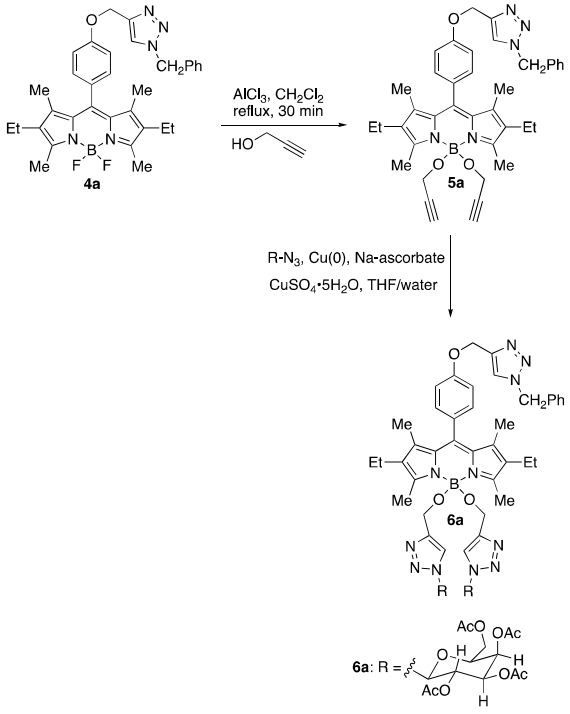


Figure 1. Molecular structures of BODIPYs **4a** and **4b** from X-ray crystal structure analysis, with 50% ellipsoids.



Scheme 2. Synthesis of di(glucose)-BODIPY **6a**.

All BODIPYs were characterized by ^{1}H, ^{11}B and ^{13}C NMR, HRMS and by UV-Vis spectrophotometry (see ESI for the spectra). In the ^{1}H NMR spectra of BODIPYs containing benzyl groups, triazole peaks were observed between 7.23–7.60 ppm while for the glucose-containing BODIPYs the peaks were within the range 8.12–8.43 ppm. All BODIPYs showed absorption bands centered at ca. 525 nm and fluorescence emission bands at ca. 535 nm in DMSO solutions (see representative spectra in ESI). BODIPYs **4a** and **4b** were further characterized by X-ray crystallography, as shown in Figure 1. The central C_3N_2B ring of the BODIPY in **4a** has a slight envelope distortion from planarity, with the boron atom lying 0.224 Å out of the plane of the other five atoms. The phenyl group at the *meso* position is roughly perpendicular to the central plane, forming a dihedral angle of 84.6° with it. The triazole plane has dihedral angles of 73.1° with the *meso* phenyl and 24.9° with the central ring. In **4b**, the central C_3N_2B ring is much more planar, with mean deviation of 0.023 Å. The *meso* phenyl is somewhat less orthogonal to the central plane than in **4a**, with dihedral angle 75.3°. The triazole plane has dihedral angles of 75.2° with the *meso*-phenyl and 31.6° with the central ring, similar to those in **4a**. The glucose ring has the expected chair conformation, with mean endocyclic torsion angle magnitude 58.7°.

Cellular Properties

The dark and photo cytotoxicity of BODIPYs **1**, **3b**, **4b** and **6a** bearing one, two, three or zero glucose units, were investigated in human carcinoma HEp2 cells using a Cell Titer Blue viability assay (Promega),[15] at concentrations up to 100 μM for each BODIPY. None of the BODIPYs were toxic to cells, as summarized in Table 1. The low dark and phototoxicity observed for these BODIPYs are in agreement with our previous investigations of carbohydrate-functionalized BODIPYs.[8]

Table 1. Cytotoxicity of selected triazole-BODIPYs (CellTiter Blue assay, 1.5 J/cm^2).

BODIPY	Dark toxicity (IC_{50}, μM)	Phototoxicity (IC_{50}, μM)
1	> 200	> 100
3b	> 200	> 100
4b	> 200	> 100
6b	> 200	> 100

The time-dependent uptake of BODIPYs **4b, 6a, 3b** and **1** bearing respectively, one, two, three or zero glucose units, was also evaluated at a concentration of 10 μM in HEp2 cells over a period of 24 h, and the results are shown in Figure 2. BODIPY **1** accumulated the most within cells in the first 8 h. This may be attributed to the lower molecular weight and enhanced hydrophobic character of this compound. However, after 24 h, BODIPY **3b** bearing three glucose units showed the highest cellular uptake, indicating that the glucose units do influence the cellular permeability of the BODIPYs. Different uptake kinetics was also observed. While the uptake for BODIPYs **1** and **4b** reached a plateau at 8 h, the uptake of **3b** and **6a** continued to increase over the 24 h period investigated. Among the triazole-BODIPYs, the cellular uptake at 24 h followed the order of increasing number of glucose units: **3b > 6a > 4b**. This result suggests that the glucose moieties enhance cell internalization, probably due to increased receptor-mediated recognition processes.

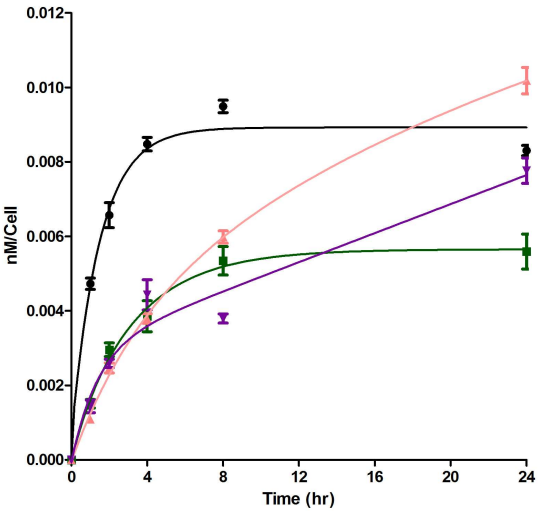


Figure 2. Time-dependent of BODIPYs **1** (black), **3b** (pink), **4b** (green) and **6a** (purple) at 10 μM by HEp2 cells.

Conclusions

1,2,3-Triazole-BODIPYs bearing one, two or three glucose groups were synthesized in good yields using "click" cycloaddition reactions between alkyne-functionalized BODIPYs and 1-azido-1-deoxy-β-D-glucopyranoside tetraacetate. Deacetylation of glucose tetracetate-BODIPY **4b** using sodium methoxide in methanol gave the corresponding **4c** in high yield, while **3b** decomposed under similar conditions. *In vitro* cellular investigations of BODIPYs **1**, **3b**, **4b** and **6a** using human carcinoma HEp2 cells showed that all BODIPYs are non-cytotoxic, both in the absence and presence of light (IC_{50} > 100 μM, 1 J/cm^2). The cellular uptake of these BODIPYs increased with the number of glucose moieties, and was highest at 24 h for **3b**, bearing three glucose groups.

Experimental Section

General

All commercially available reagents were purchased from Sigma-Aldrich and were used without further purification. All anhydrous reactions were carried out with oven-dried glassware under a dry argon atmosphere. All the reactions were monitored by using Sorbent Technologies pre-coated polyester backed TLC (200 μm silica gel with indicator UV-254 nm). Flash column chromatography was performed using Sorbent Technologies 60 Å neutral alumina (230-400 mesh). All

preparative-TLC was performed with Silica Gel 60 Å silica gel 20 x 20 cm (210-270 µm). All ^{1}H-, ^{13}C-, ^{11}B-NMR spectral were collected using an AV-400 spectrometer (operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) and deuterated acetone or chloroform as solvents. The $CDCl_3$ chemical shifts (δ) are reported in ppm with 7.27 for proton and 77.16 for carbon NMR as reference. The $(CD_3)_2CO$ chemical shifts (δ) are reported in ppm with 2.05 for proton and 29.84 and 206.26 for carbon NMR as references. The $BF_3{\bullet}OEt_2$ chemical shift (δ) is used as 0.00 for boron NMR as reference. Coupling constants are reported in Hertz (Hz). All the mass spectra were collected using an Agilent 6210 ESI-TOF mass spectrometer. BODIPY **1** was synthesized according to the literature procedure.[11]

General procedure for boron functionalization

In an oven-dried flask, BODIPY (1.0 equiv) and $AlCl_3$ (2.0 equiv) were dissolved in anhydrous CH_2Cl_2 (10 mL). The mixture was refluxed for 10 min before propargyl alcohol (278.0 equiv) was added. The final reaction mixture was refluxed for 15 min. The mixture was cooled to room temperature and then concentrated under reduced pressure. The resulting residue was purified by TLC, using the eluents indicated below.

BODIPY 2: Eluent 80:20 hexane/EtOAc; yield: 80.6 mg, 69%; recrystallized from 1:1 hexane/dichloromethane. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.21 (d, *J* = 8 Hz, 2H), 7.09 (d, *J* = 8 Hz, 2H), 4.78 (s, 2H), 3.81 (s, 4H), 2.57 (s, 6H), 2.31 (q, *J* = 7.3 Hz, 4H), 2.04 (s, 2H), 1.33 (s, 6H), 0.98 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.86, 154.72, 139.39, 137.15, 132.71, 132.30, 129.69, 129.38, 115.44, 83.27, 78.15, 75.80, 70.44, 56.06, 49.80, 17.11, 14.71, 12.75, 11.89; ^{11}B NMR ($CDCl_3$, 128 MHz) δ 1.76 (s); HRMS (ESI-TOF) *m/z* 528.2669 $[M + Na]^+$ calculated for $C_{32}H_{35}BN_2O_3$: 528.2681.

BODIPY 5a: Eluent 50:50 hexane/EtOAc; yield: 37.3 mg, 73%; recrystallized from 1:1 hexane/EtOAc. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.59 (s, 1H), 7.39–7.32 (m, 5H), 7.19 (d, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 5.58 (s, 2H), 5.25 (s, 2H), 3.80 (s, 4H), 2.57 (s, 6H), 2.30 (q, *J* = 7.3 Hz, 4H), 2.04 (s, 2H), 1.30 (s, 6H), 0.98 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.64, 154.75, 144.26, 139.56, 137.21, 134.52, 132.77, 132.40, 129.82, 129.28, 129.11, 128.97, 128.25, 122.80, 115.28, 83.35, 70.55, 62.18, 54.41, 49.88, 17.20, 14.81, 12.83, 11.96; ^{11}B NMR ($CDCl_3$, 128 MHz) δ 1.77 (s); HRMS (ESI-TOF) *m/z* 661.3309 $[M + Na]^+$ calculated for $C_{39}H_{42}BN_5O_3$: 661.3318.

General procedure for "click" reactions

Alkynyl-BODIPY (1.0 equiv), azido-compound (15 equiv) and Cu(0) (1.0 equiv) were added to a mixture of THF/water (8 mL, 3:1), under an inert gas atmosphere. A solution of $CuSO_4{\cdot}5H_2O$ (0.2 equiv.) and sodium-ascorbate (0.5 equiv.) in THF/water (8 mL, 3:1) was added (after sonication for 30 min) and the reaction mixture was heated from 6 to 22 h at 70 °C. Once TLC indicated complete consumption of starting material, the mixture was cooled to room temperature and partitioned between ethyl acetate (30 mL) and water (30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude product was then purified by preparative thin-layer chromatography to obtain the desired product.

BODIPY 3a: Eluent: 20:80 hexane/EtOAc; yield: 23.8 mg, 76%; recrystallized from 1:3 acetone/hexanes. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.59 (s, 1H), 7.38–7.23 (m, 15H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.07 (d, *J* = 8 Hz, 2H), 5.57 (s, 2H), 5.42 (s, 4H), 5.25 (s, 2H), 4.22 (s, 4H), 2.39 (s, 6H), 2.22 (q, *J* = 7.12 Hz, 4H), 1.28 (s, 6H), 0.93 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.67, 153.79, 149.71, 144.31, 140.18, 137.32, 134.93, 134.51, 132.72, 132.53, 129.85, 129.31, 129.24, 129.11, 129.00, 128.90, 128.69, 128.27, 128.24, 122.78, 121.65, 115.35, 62.21, 56.84, 54.43, 54.10, 21.15, 17.20, 14.81, 14.30, 12.62, 11.99; ^{11}B NMR ($CDCl_3$, 128 MHz) δ 2.10 (s); HRMS (ESI-TOF) *m/z* 927.4589 $[M + Na]^+$ calculated for $C_{53}H_{56}BN_{11}O_3$: 927.4593.

BODIPY 3b: Eluent: 100% EtOAc to 95:5 EtOAc/MeOH; yield: 56.3 mg, 64%; recrystallized from EtOAc. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz) δ 8.43 (s, 1H), 8.12 (s, 1H), 7.98 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 8.0 Hz, 2H), 6.30 (d, *J* = 8.0 Hz, 1H), 6.22 (d, *J* = 8.1 Hz, 1H), 6.17 (d, *J* = 8.1 Hz, 1H), 5.64 (d, *J* = 8.0 Hz, 2H), 5.62 (s, 6H), 5.54 (d, 2H), 5.29 (d, 2H), 4.36–4.32 (m, 4H), 4.26–4.23 (m, 4H), 4.22–4.19 (m, 4H), 2.54 (s, 6H), 2.35 (q, *J* = 7.52 Hz, 4H), 2.04 (s, 9H), 2.01 (s, 9H), 1.97 (s, 9H), 1.79 (s, 9H), 1.41 (s, 6H), 0.98 (t, *J* = 7.44 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.79, 170.34, 170.14, 169.30, 154.93, 141.63, 137.82, 133.43, 130.86, 123.73, 121.79, 121.42, 116.44, 86.06, 85.88, 75.42, 75.32, 73.65, 71.53, 71.46, 68.93, 68.86, 62.91, 62.8, 57.10, 55.03, 20.71, 20.68, 20.59, 20.28, 20.23, 17.73, 15.14, 12.98, 12.33; ^{11}B NMR ($CDCl_3$, 128 MHz) δ 2.05 (s); HRMS (ESI-TOF) *m/z* 1647.6033 $[M + Na]^+$ calculated for $C_{74}H_{92}BN_{11}O_{30}$: 1647.6019.

BODIPY 4a: Eluent: 50:50 hexane/EtOAc; yield: 36.4 mg, 93%; recrystallized from 1:3 dichloromethane/hexane. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (s, 1H), 7.39–7.28 (m, 5H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 5.58 (s, 2H), 5.25 (s, 2H), 2.54 (s, 6H), 2.30 (q, *J* = 7.4 Hz, 4H), 1.31 (s, 6H), 1.00 (t, *J* = 7.16 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.75, 153.69, 144.24, 140.11, 138.49, 134.52, 132.81, 131.23, 129.66, 129.30, 129.00, 128.63, 128.26, 122.83, 115.41, 62.18, 54.42, 17.18, 14.74, 12.59, 11.91; ^{11}B NMR ($CDCl_3$, 128 MHz) δ 0.68 (t, *J* = 33.7 Hz); HRMS (ESI-TOF) m/z 589.2910 $[M + Na]^+$ calculated for $C_{33}H_{36}BF_2N_5O$: 589.2895.

BODIPY 4b: Eluent 50:50 hexane/EtOAc; yield: 76.5 mg, 82%; recrystallized from 1:3 dichloromethane/hexane. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz) δ 8.43 (s, 1H), 7.30–7.25 (m, 4H), 6.30 (d, *J* = 8.1 Hz, 1H), 5.67 (t, *J* = 9.4 Hz, 1H), 5.57 (t, *J* = 9.4 Hz, 1H), 5.31 (s, 2H), 4.40–4.36 (m, 1H), 4.30–4.25 (m, 1H), 4.21–4.18 (m, 1H), 2.49 (s, 6H), 2.35 (q, *J* = 7.5 Hz, 4H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.83 (s, 3H), 1.39 (s, 6H), 0.99 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR ($(CD_3)_2CO$, 100 MHz) δ 170.76, 170.35, 170.17, 169.40, 160.18, 154.24, 144.92, 141.80, 139.29, 133.58, 131.97, 130.56, 129.01, 123.76, 116.55, 86.07, 75.43, 73.52, 71.53, 68.84, 62.78, 62.63, 20.67, 20.58, 20.27, 17.56, 15.04, 12.66, 12.19; ^{11}B NMR ($CDCl_3$, 128 MHz) δ 0.67 (t, *J* = 33.9 Hz); HRMS (ESI-TOF) *m/z* 829.3391 $[M + Na]^+$ calculated for $C_{40}H_{48}BF_2N_5O_{10}$: 829.3384.

BODIPY 6a: Eluent 100% EtOAc to 50:50 EtOAc/acetone; yield: 12.1, 72%; recrystallized from acetone. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz) δ 8.12 (s, 2H), 7.97 (s, 2H), 7.39–7.36 (m, 5H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 8.2 Hz, 2H), 6.17 (d, *J* = 8.0 Hz, 2H), 5.68 (s, 2H), 5.64 (t, *J* = 9.48 Hz, 2H), 5.53 (t, *J* = 9.48 Hz, 2H), 5.29 (s, 2H), 4.16 (s, 4H), 2.54 (s, 6H), 2.34 (q, *J* = 7.32 Hz, 4H), 2.04 (s, 6H), 2.01 (s, 6H), 1.97 (s, 6H), 1.79 (s, 6H), 1.41 (s, 6H), 0.98 (t, *J* = 7.48 Hz, 6H); ^{13}C NMR ($(CD_3)_2CO$, 100 MHz) δ 200.13, 199.00, 197.50, 196.91, 189.04, 181.45, 170.60, 169.75, 167.30, 159.01, 155.38, 142.14, 133.87, 130.27, 129.70, 129.47, 121.88, 116.89, 86.36, 75.80, 74.12, 71.94,

69.41, 57.60, 54.81, 50.28, 21.20, 21.07, 20.76, 15.63, 13.45, 12.77; ^{11}B NMR ($(CD_3)_2CO$, 128 MHz) δ 2.16 (s); HRMS (ESI-TOF) *m/z* 1407.5552 $[M + Na]^+$ calculated for $C_{67}H_{80}BN_{11}O_{21}$: 1407.5546.

Deprotection of acetate groups

BODIPY 4c: BODIPY **4b** (22.5 mg, 0.028 mmol, 1.0 equiv.) and NaOMe (6.3 mg, 0.117 mmol, 4.2 equiv.) were dissolved in MeOH (5 mL) at 0 °C. The mixture was stirred for 30 min and then warmed to room temperature. Once TLC indicated complete consumption of starting material, Dowex 50WX8 H^+ resin was added and the mixture was stirred until the solution was neutral. The resin was filtered off and crude product was concentrated by reduced pressure. The crude product was purified using alumina column chromatography (eluent 50:50 dichloromethane/acetone) to afford BODIPY **4c** (16.5 mg, 93% yield) and was recrystallized from 1:6 water/dichloromethane. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz) δ 8.31 (s, 1H), 7.29 (s, 4H), 5.70 (d, 1H), 5.30 (s, 2H), 4.71 (d, *J* = 4.0 Hz, 2H), 4.64 (d, *J* = 4.0 Hz, 2H), 4.46 (d, *J* = 4.0 Hz, 2H), 4.04–3.98 (m, 1H), 3.89–3.82 (m, 2H), 3.74–3.63 (m, 3H), 3.60–3.54 (m, 1H), 2.49 (s, 6H), 2.35 (q, *J* = 7.56 Hz, 4H), 1.39 (s, 6H), 0.99 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR ($(CD_3)_2CO$, 100 MHz) δ 160.63, 154.58, 139.65, 133.91, 132.32, 130.92, 129.27, 124.43, 116.81, 89.39, 81.17, 78.92, 74.19, 71.41, 62.98, 62.90, 17.91, 15.39, 13.02, 12.55; ^{11}B NMR ($CDCl_3$, 128 MHz) δ 0.60 (t, *J* = 34.3 Hz); HRMS (ESI-TOF) *m/z* 639.3149 $[M + H]^+$ calculated for $C_{32}H_{40}BF_2N_5O_6$: 639.3109.

X-ray structural determination

Crystal structures were determined using low-temperature data from a Bruker Kappa APEX-II DUO diffractometer with either Mo Kα or Cu Kα radiation. H atoms were located from difference maps but constrained in calculated positions during refinement. Crystal data **4a**: $C_{33}H_{36}BF_2N_5O$, *M* = 567.48, triclinic, *a* = 11.1039(3), *b* = 11.3734(3), *c* = 12.9720(4) Å, α = 67.466(2), β = 72.869(2), γ = 84.364(2)˚, *U* = 1445.82(7) Å^3, *T* = 90 K, space group P-1, *Z* = 2, Dc = 1.304 g cm^{-3}, μ(Mo Kα) = 0.09 mm^{-1}, 26387 reflections measured, θ_{max} = 30.5˚, 8843 unique (R_{int} = 0.040). The final *R* = 0.053 (5772 *I>2σ(I)* data), *wR(F^2)* = 0.129 (all data), CCDC 1580552. Crystal data **4b**: $C_{40}H_{48}BF_2N_5O_{10}$, *M* = 807.64, monoclinic, *a* = 13.3965(15), *b* = 9.2534(10), *c* = 17.1816(19) Å, β = 104.822(8)˚, *U* = 2059.0(4) Å^3, *T* = 90 K, space group $P2_1$, *Z* = 2, Dc = 1.303 g cm^{-3}, μ (Cu Kα) = 0.83 mm^{-1}, 9382 reflections measured, θ_{max} = 59.0˚, 4680 unique (R_{int} = 0.060). The final *R* = 0.062 (2472 *I>2σ(I)* data), *wR(F^2)* = 0.131 (all data), Flack parameter -0.3(3) for 1559 Friedel pairs, in agreement with starting materials, CCDC 1580553.

Cell culture experiments

The HEP2 cell line used in this study was purchased from ATCC. The culture medium and other reagents were purchased from Life Technologies. HEP2 cells were cultured in the medium (DMEM:Advanced = 1:1) containing 10% FBS and 1% antibiotic (Penicillin-streptomycin). A 32 mM compound stock solution was prepared by dissolving the compound in 95% DMSO and 5% Cremophor. The working concentrations were made by diluting the stock solution with growing medium.

Dark Toxicity: The HEp2 cells were placed in a 96-well plate as above, with the compound concentration of 200, 100, 50, 25, 12.5, 6.25, and 0 μM, five repetitions for each concentration, and then incubated at 37 °C. After 24h incubation, the compound was removed by washing cells with 1X PBS and replaced with the media containing 20 % CellTiter-Blue® (Promega). The cells were incubated for an additional 4 h at 37 °C. The viable cells were measured by fluorescence at 570/615 nm using a FluoStar Optima micro-plate reader. The dark toxicity was expressed in terms of the percentage of viable cells.

Phototoxicity: The concentration range of 100, 50, 25, 12.5, 6.25, 3.125, and 0 μM was used for the phototoxicity experiments. HEp2 cells were placed in 96 well plates as described before, and treated with compound for 24 h at 37 °C. After 24 h treatment, the loading media was removed. The cells were washed with 1X PBS, and then refilled with fresh media. The cells were exposed to light for 20 min to achieve an approximate 1.5 J/cm^2 light dose. After exposure to light, the cells were returned to the incubator for 24 h. After this 24 h incubation, the medium was removed and replaced with media containing 20 % cell titer blue. The cells were incubated for an additional 4 h. The viable cells were measured by fluorescence at 570/615 nm using a FluoStar Optima micro-plate reader. The phototoxicity was expressed in terms of the percentage of viable cells

Time-Dependent Cellular Uptake: HEP2 cells were plated at 15000 cells per well in a Costar 96-well plate (BD biosciences) and grown overnight. The cells were treated by adding 100 μL /well of 10μM working solution at different time periods of 0, 1, 2, 4, 8, and 24h. The loading medium was removed at the end of the treatments. The cells were washed with 1X PBS, and solubilized by adding 0.25% Triton X-100 in 1 PBS. A compound standard curve, 10 μM, 5 μM, 2.5 μM, 1.25 μM, 0.625 μM, 0.3125 μM, was made by diluting stock solution with 0.25% Triton X-100 (Sigma-Aldrich) in 1X PBS. A cell standard curve was prepared using 10000, 20000, 40000, 60000, 80000, and 100000 cells per well. The cells were quantified by using the CyQuant Cell Proliferation Assay (Life Technologies). The compound and cell number were determined using a FluoStar Optima micro-plate reader (BMG LRBTEH), with wavelengths 485/520nm. Cellular uptake was expressed in terms of nM compound per cell.

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Notes and references

1 (a) R. Ziessel, G. Ulrich and A. Harriman, *New J. Chem.,* 2007, **31**, 496; (b) L.-Y. Niu, Y.-S. Guan, Y.-Z. Chen, L.-Z. Wu, C.-H.

Tung and Q.-Z. Yang, *J. Am. Chem. Soc.*, 2012, **134**, 18928; (c) N. Boens, V. Leen and W. Dehaen, *Chem. Soc. Rev.* 2012, **41**, 1130; (d) Y. Ni and J. Wu, *Org. Biomol. Chem.,* 2014, **12**, 3774; (e) T. Kowada, H. Maeda and K. Kikuchi, *Chem. Soc. Rev.,* 2015, **44**, 4953-4972.

2 (a) H. N. Kim, W. X. Ren, J. S. Kim and J. Yoon, *Chem. Soc. Rev.,* 2012, **41**, 3210; (b) M. Zhang, Y. Wu, S. Zhang, H. Zhu, Q. Wu, L. Jiao and E. Hao, *Chem. Commun.,* 2012, **48**, 8925; (c) X. Kong, F. Su, L. Zhang, J. Yaron, F. Lee, Z. Shi, Y. Tian and D. R. Meldrum, *Angew. Chem. Int. Ed.,* 2015, **54**, 12053; (d) F. Wang, L. Zhou, C. Zhao, R. Wang, Q. Fei, S. Luo, Z. Guo, H. Tian and W.-H. Zhu, *Chem. Sci.,* 2015, **6**, 2584; (e) S. T. Manjare, J. Kim, Y. Lee and D. G. Churchill, *Org. Lett.,* 2013, **16**, 520; (f) T. Wang, E. F. Douglass, K. J. Fitzgerald and D. A. Spiegel, *J. Am. Chem. Soc.,* 2013, **135**, 12429; (g) L.-Y. Niu, Y.-S. Guan, Y.-Z. Chen, L.-Z. Wu, C.-H. Tung and Q.-Z. Yang, *J. Am. Chem. Soc.,* 2012, **134**, 18928.

3 (a) A. Kamkaew, S. H. Lim, H. B. Lee, L. V. Kiew, L. Y. Chung and K. Burgess, *Chem. Soc. Rev.,* 2013, **42**, 77; (b) J. H. Gibbs, Z. Zhou, D. Kessel, F. R. Fronczek, S. Pakhomova and M. G. H. Vicente, *J. Photochem. Photobiol. B: Biol.,* 2015, **145**, 35; (c) A. Kamkaew and K. Burgess, *J. Med. Chem.,* 2013, **56**, 7608; (c) S. Xuan, N. Zhao, Z. Zhou, F. R. Fronczek and M. G. H. Vicente, *J. Med. Chem.,* 2016, **59**, 2109.

4 (a) A. Loudet and K. Burgess, *Chem. Rev.,* 2007, **107**, 4891; (b) G. Ulrich, R. Ziessel and A. Harriman, *Angew. Chem. Int. Ed.*, 2008, **47**, 1184; (c) N. Boens, V. Leen and W. Dehaen, *Chem. Soc. Rev.,* 2012, **41**, 1130; (d) H. Lu, J. Mack, Y. Yang and Z. Shen, *Chem. Soc. Rev*., 2014, **43**, 4778 (e), T. Kowada, H. Maeda and K. Kikuchi, *Chem. Soc. Rev.,* 2015, ***44***, 4953.

5 (a) M. D. Witte, C. V. Descals, S. V. P. de Lavoir, B. I. Florea, G. A. van der Marel and H. S. Overkleeft, *Org. Biomol. Chem.,* 2007*,* **5**, 3690; (b) B. G. de la Torre, V. Hornillos, J. R. Luque-Ortega, M. A. Abengózar, F. Amat-Guerri, A. Ulises Acuña, L. Rivas and D. Andreu, *Amino Acids,* 2014, **46**, 1047; (c) N. Kondo, T. Temma, J. Deguchi, K. Sano, M. Ono and H. Saji, *J. Controlled Release,* 2015, **220**, *Part A*, 476; (d) D. Wang, J. Fan, X. Gao, B. Wang, S. Sun and X. Peng, *J. Org. Chem.,* 2009, **74**, 7675.

6 (a) A. Kamkaew and K. Burgess, *J. Med. Chem.,* 2013, **56**, 7608; (b) A. M. Hansen, A. L. Sewell, R. H. Pedersen, D.-L. Long, N. Gadegaard and R. Marquez, *Tetrahedron,* 2013, **69**, 8527; (c) P. Verwilst, C. C. David, V. Leen, J. Hofkens, P. A. M. de Witte and W. M. de Borggraeve, *Bioorg. Med. Chem. Lett.,* 2013, **23**, 3204; (d) N. Maindron, M. Ipuy, C. Bernhard, D. Lhenry, M. Moreau, S. Carme, A. Oudot, B. Collin, J.-M. Vrigneaud, P. Provent, F. Brunotte, F. Denat and C. Goze, *Chem. Eur. J.,* 2016, **22**, 12670.

7 E. Hao, T. J. Jensen and M. G. H. Vicente, *J. Porphyrins Phthalocyanines,* 2009, **13**, 51.

8 T. Uppal, N. V. S. D. K. Bhupathiraju and M. G. H. Vicente, *Tetrahedron,* 2013, **69**, 4687.

9 T. V. Ovchinnikova, A. V. Pshezhetsky, A. B. Tuzikov and N. V. Bovin, *Mendeleev Commun.*, 2015, **25**, 422.

10N. Shivran, M. Tyagi, S. Mula, P. Gupta, B. Saha, B. S. Patro and S. Chattopadhyay, Eur. J. Med. Chem., 2016, **122**, 352.

11A. L. Nguyen, P. Bobadova-Parvanova, M. Hopfinger, F. R. Fronczek, K. M. Smith and M. G. H. Vicente, *Inorg. Chem.,* 2015, **54**, 3228.

12(a) G. Ulrich, C. Goze, M. Guardigli, A. Roda and R. Ziessel, *Angew. Chem. Int. Ed.,* 2005, **44**, 3694; (b) C. Goze, G. Ulrich, L. J. Mallon, B. D. Allen, A. Harriman and R. Ziessel, *J. Am. Chem. Soc.,* 2006, **128**, 10231; (c) L. Li, B. Nguyen and K. Burgess, *Bioorg. Med. Chem. Lett.,* 2008, **18**, 3112; (d) D. A. Smithen, A. E. G. Baker, M. Offman, S. M. Crawford, T. S. Cameron and A. Thompson, *J. Org. Chem.,* 2012, **77**, 3439; (e) B. Brizet, C. Bernhard, Y. Volkova, Y. Rousselin, P. D. Harvey, C. Goze and F. Denat, *Org. Biomol. Chem.,* 2013, **11**, 7729.

13B. Liu, N. Novikova, M. C. Simpson, M. S. M. Timmer, B. L. Stocker, T. Söhnel, D. C. Ware and P. J. Brothers, *Org. Biomol. Chem*., 2016, **14**, 5205.

14J.-B. Giguère, D. Thibeault, F. Cronier, J.-S. Marois, M. Auger and J.-F. Morin, *Tetrahedron Lett.,* 2009, **50**, 5497.

15J. H. Zhang, T. D. Chung and K. R. Oldenburg, *J. Biomol. Screen.,* 1999*,* **4**, 67.

TOC

Syntheses of 1,2,3-Triazole-BODIPYs Bearing up to Three Carbohydrate Units

Alex L. Nguyen, Kaitlin E. Griffin, Zehua Zhou, Frank R. Fronczek, Kevin M. Smith and M. Graça H. Vicente*

BODIPYs bearing up to three glucose units were synthesized and their cytotoxicity and uptake investigated in human HEp2 cells.