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MegaStokes BODIPY-triazoles as environmentally sensitive turn-on fluorescent dyes†

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A novel class of triazole-derivatized BODIPY compounds have been synthesized on solid-phase by employing mild reaction conditions based on the copper-catalyzed azide–alkyne cycloaddition. The resulting BODIPY-triazoles exhibited MegaStokes shifts (up to 160 nm) and remarkable environmentally sensitive quantum yield increments that asserted their potential as turn-on fluorescent sensors. Out of a library of 120 compounds, we identified **BDC-9** as a fluorescent chemosensor with high sensitivity and remarkable species-selectivity towards human serum albumin. These results validate MegaStokes BODIPY dyes as new fluorophores for the development of environmentally sensitive fluorescent probes.

1 Introduction

The development of fluorescent and molecular imaging probes based on the 4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene (BODIPY) scaffold has been widely exploited due to its outstanding fluorescent properties (*e.g.* high photostability, extinction coefficients and quantum yields, tunable excitation and emission spectra)[1] and facile conversion into probes for positron emission tomography (PET) imaging.[2] However, one important shortcoming of BODIPY dyes is their relatively small Stokes shifts, which can lead to experimental limitations, such as self-quenching[3] or direct acceptor excitation in FRET probes.[4] Herein we report the first systematic generation of MegaStokes BODIPY dyes. We prepared a combinatorial library by derivatization of a bifunctional BODIPY scaffold using copper-catalyzed azide–alkyne cycloaddition (CuAAC) and nucleophilic substitution. The incorporation of different triazole moieties paired with a piperidinyl substituent at positions 3 and 5 of the BODIPY core (Scheme 1) afforded 40 BODIPY-triazoles (so-called BoDipy Click, **BDC**) with very large Stokes shifts (*i.e.* from 74 to 160 nm). Further modification of **BDC** compounds led to the corresponding acetyl (**BDCAC**) and chloroacetyl (**BDCCA**) derivatives (Scheme 1). Moreover, the library was synthesized following our previously reported solid-phase strategy, which avoids tedious purification steps and renders BODIPY compounds in high purities.[5]

Weber and co-workers pioneered the preparation of environmentally sensitive dyes by modifying opposite ends of aromatic systems with electron donor and acceptor groups.[6] This approach has been successfully applied to the synthesis of numerous environmentally sensitive fluorophores (*e.g.* dansyl, NBD, ANS, aminophenoxazone).[7] We envisioned that **BDC** compounds, which include an electron-donating piperidinyl group and 40 wide-ranging triazoles at opposed ends of the BODIPY core, may behave as environmentally sensitive compounds and recover the inherent fluorescence of the BODIPY core in specific environments. From the examination of our 120-member library, we identified **BDC-9** as a highly environment-sensitive dye, and demonstrated its potential as a species-selective and FA1 site-specific human serum albumin (HSA) sensor.

2 Results and discussion

Design and synthesis

CuAAC is highly favoured for combinatorial purposes due to its high efficiency, robustness and mild reaction conditions.[8] The resulting triazole ring is chemically stable and, when properly located within a fluorescent core, can modulate the spectral wavelengths of the resulting fluorescent derivatives.[9] A survey of the literature revealed that the incorporation of electron-donating and withdrawing groups at positions 3 and 5 of the BODIPY scaffold can effectively alter its Stokes shifts.[10] Based on these reports, we designed a trifunctional BODIPY aniline (**4**, Scheme 1) for the synthesis of our library. This scaffold

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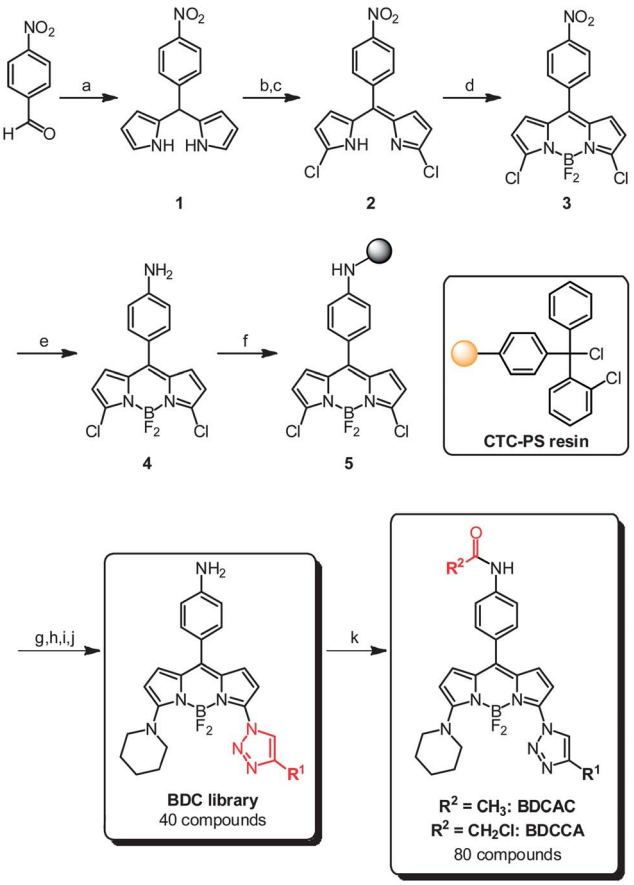
† Electronic supplementary information (ESI) available: General procedures, chemical structures and characterization data of **BDC**, **BDCAC** and **BDCCA**. Full characterization data (NMR, HR-MS) for **BDC-9**. Additional experimental data for **BDC-9**–HSA binding. See DOI: 10.1039/c3sc22166k



Scheme 1 Synthesis of the **BDC** library. *Reagents and conditions*: (a) pyrrole, TFA, rt, 3 h; (b) NCS, anhydrous THF, −78 °C to rt, 3 h; (c) DDQ, dry CH_2Cl_2, rt, 1 h; (d) $BF_3{\cdot}OEt_2$, anhydrous CH_2Cl_2, rt, 2 h; (e) Fe activated by 1 M aqueous HCl, CH_3OH–CH_3COOH (10 : 1), reflux, 15 min; (f) CTC-PS resin, DIEA, CH_2Cl_2–DMF, rt, 24 h; (g) NaN_3, DMF, rt, 30 min; (h) DMF–piperidine (4 : 1), rt, 45 min; (i) R–C≡CH, CuI, ascorbic acid, rt, 30 min; (j) 0.5% TFA in CH_2Cl_2, 10 min; (k) R^2COCl, sat. $NaHCO_3$, rt, 10 min.

possesses two electrophilic sites at positions 3 and 5, which can be modified *via* CuAAC and nucleophilic substitution,[11] and one aniline group to construct the library using solid-phase synthesis. Amino-functionalized BODIPYs can be successfully loaded onto chlorotrityl chloride polystyrene resin (CTC-PS), which allows their cleavage under weakly acidic conditions and preserve the integrity of the fluorophore (Scheme 1).[5] The aniline group also provided chemical stability to the BODIPY core[1e] and an additional point for further chemical derivatization.

The synthesis of **4** consisted of four steps with an overall yield of 37%. **1** was readily prepared from *p*-nitrobenzaldehyde and pyrrole following reported methods.[12] Chlorination with *N*-chlorosuccinimide (NCS) followed by oxidation with DDQ afforded **2**, which was subsequently treated with $BF_3{\cdot}OEt_2$ to obtain the highly stable BODIPY analogue **3**.[12b,13] Reduction with activated iron rendered **4** in quantitative yields. The aniline was then loaded onto CTC-PS resin using standard coupling conditions. Treatment with sodium azide followed by piperidine generated a monosubstituted intermediate upon which diversity was introduced *via* CuAAC. 40 chemically diverse alkynes were used as building blocks for the construction of the library. Final cleavage with 0.5% trifluoroacetic acid (TFA) in CH_2Cl_2 rendered 40 **BDC** derivatives with average purities of 95% after minimum purification steps. Subsequent acetylation and chloroacetylation of the aniline group afforded the corresponding **BDCAC** and **BDCCA** compounds (see Fig. S8 and Tables S2–S4 in ESI† for chemical structures and detailed characterization).

General photophysical properties of BODIPY-triazoles

Most BODIPY-triazoles exhibited similar absorbance wavelength maxima (**BDC**: 465–478 nm; **BDCAC** and **BDCCA**: 458–468 nm), whereas their emission maxima spanned across a much broader range (**BDC**: 546–616 nm; **BDCAC** and **BDCCA**: 542–612 nm) (Tables S2–S4 in ESI†). Notably, we observed that the spectral properties of MegaStokes BODIPY dyes might be fine-tuned with different alkyne building blocks. For instance, electron-rich alkynes (*e.g.* 4-ethynyl-*N*,*N*-dimethylaniline) generated compounds with longer absorption wavelengths than alkynes containing electron-withdrawing groups (*e.g.* 4-(trifluoromethoxy)phenylacetylene) (Fig. S8–S10 in ESI†). While **BDC** compounds exhibited low quantum yields in polar solvents due to photoinduced electron transfer (PeT) effect,[14] the acetylation and chloroacetylation of the aniline group partially recovered their fluorescence emission with an average 6-fold fluorescence increase. These properties affirm the potential of **BDC** compounds to behave as fluorescent turn-on sensors.

Solvatochromic effects

BODIPY dyes substituted at positions 3 and 5 have been reported to display absorption and emission shifts correlated to solvent polarity.[16] In order to examine the potential of BODIPY-triazoles as environmentally sensitive fluorophores, we analyzed the absorbance and fluorescence spectra of a representative MegaStokes BODIPY-triazole (**BDC-9**, Fig. 1) in different solvents covering a wide range of polarities and viscosities (Table 1).

The shape of the absorption spectrum of **BDC-9** is similar to most BODIPY dyes. Two absorption bands are observed: a S_0–S_1 transition band with a maxima spanning across 465 to 515 nm and a weaker S_0–S_2 band at approximately 400 nm (Fig. 2a). These bands are less distinct in polar solvents due to band broadening by solvent interactions. The absorption band corresponding to the S_0–S_1 transition showed a pronounced dependence on the solvent polarity, and absorption maxima shifted to shorter wavelengths from hexane (515 nm) to acetonitrile (465 nm) (Fig. 2a, Table 1). This shift suggests that the dipole moment of the ground state may be larger than in the excited state. We detected a similar phenomenon in mixtures of cyclohexane and ethanol (see Fig. S1a in ESI†). In very polar solvents such as DMSO and water, a slight reversal to longer wavelengths was detected. On the other hand, the position of the S_0–S_2 transition band was less affected by the solvent polarity. These environment dependence shifts in the absorption maximum may be attributed to strong dipolar interactions

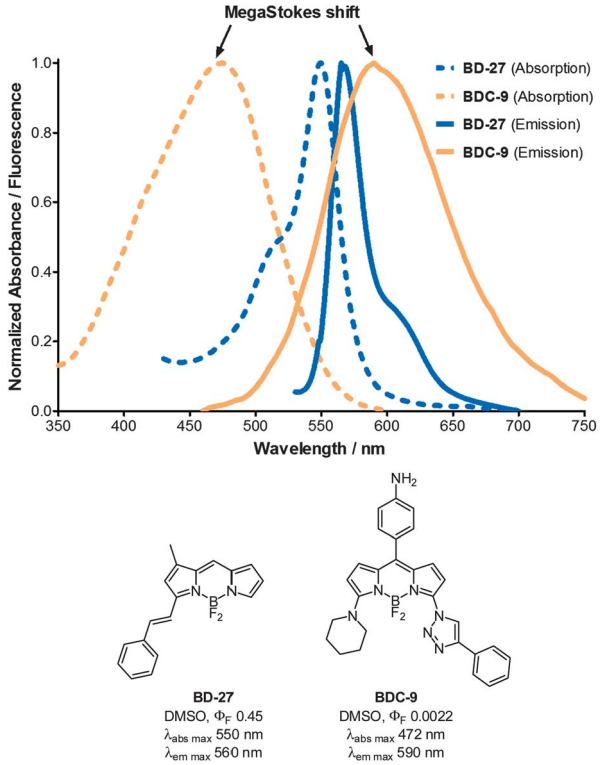


Fig. 1 Normalized absorbance and emission spectra of a representative BODIPY-triazole (**BDC-9**) in DMSO at rt. The corresponding spectra of a reported BODIPY (in DMSO) (**BD-27**) are also included for comparison.[15] *Blue*: **BD-27**; *Orange*: **BDC-9**.

from the asymmetric and charged-separated resonance forms of the **BDC** structure (see Fig. S3 in ESI†).

The fluorescence maximum wavelength of **BDC-9** displayed minor changes in different organic solvents (Table 1), whereas its quantum yield (Φ_F) showed a remarkable dependence on the nature of the solvent (Fig. 2b, Table 1). In order to examine the contribution of solvent polarity and viscosity in this dependency, we first analysed the fluorescence intensity of **BDC-9** in cyclohexane–ethanol mixtures, and observed that the emission intensity decreased as solvent polarity increased from cyclohexane to ethanol (see Fig. S1b in ESI†). Secondly, we measured the emission spectra of **BDC-9** in saturated alcohols with different viscosity, and noticed a strong correlation between fluorescence intensity and viscosity (see Fig. S2 in ESI†). Increased solvent viscosity reduces bond rotation at positions 3 and 5 of the BODIPY core, which might minimize non-radiative energy loss and lead to higher quantum yields.[11e] The strong environment dependence of **BDC** compounds confirms their potential as environmentally sensitive turn-on sensors.

Sensor development

Next we examined the fluorescence properties of **BDC**, **BDCAC** and **BDCCA** to identify environmentally sensitive compounds that could restore the strong fluorescence of BODIPY in specific environments. We performed a primary screen of our library (Table S1 in ESI†), and observed that a number of BODIPY-triazoles showed a significant turn-on effect upon interaction with the hydrophobic regions of different proteins. From this screening we selected the compound **BDC-9**, which displayed a remarkable selectivity towards HSA when compared to other proteins and analytes (Fig. 3).

In addition to its high selectivity, **BDC-9** showed a significant 220-fold increase in fluorescence upon binding to HSA (Fig. 4), and a hypsochromic shift of its emission maximum wavelength (from 585 to 575 nm) (see Fig. S4 in ESI†). The response of **BDC-9** proved to be linear within a dynamic range between 0.37 and 31 μg mL^{-1}, and we determined the limit of detection of HSA ($S/N = 3$) as 0.3 μg mL^{-1} (see Fig. S5 in ESI†).

We further investigated the selectivity of **BDC-9** towards serum albumins from different species. Serum albumins represent a family of proteins with a high degree of similarity in their primary sequence across various species (~75% homology).[18] However, some albumin ligands have been found to show species-dependent binding differences, and the development of species-selective probes may be useful for structural

Table 1 Photophysical properties of **BDC-9** in various solvents[a]

Solvent	Dielectric constant, ε	Viscosity, η/mPa s	$\lambda_{abs,max}$/nm	ε_{max}/ 10^4 M^{-1} cm^{-1}	$\lambda_{em,max}$/nm	Φ_F (%)	$\Delta\tilde{\nu}$/cm^{-1}
Hex	1.88	0.29	515	1.7	575	5.4	2027
CyH	2.02	0.90	515	1.5	580	1.4	2177
Tol	2.38	0.55	495	2.5	584	1.2	3079
$CHCl_3$	4.81	0.54	485	2.5	585	1.3	3525
EA	6.02	0.43	483	2.5	585	0.77	3610
ACN	37.5	0.34	465	2.6	585	0.22	4412
DMSO	46.7	2.00	472	2.5	590	0.22	4148
n-OctOH	3.4	7.66[17]	490	2.5	585	2.7	3315
n-HexOH	13.3	4.59[17]	485	2.5	585	1.9	3525
n-BuOH	18.0	2.59	480	2.6	585	1.2	3740
EtOH	24.6	1.08	475	2.6	585	0.55	3959
MeOH	33.0	0.54	465	2.4	585	0.31	4412
H_2O–MeOH (99 : 1)	80.1	0.89	480	1.5	615	0.40	4574

[a] Hexane: Hex; cyclohexane: CyH; toluene: Tol; ethyl acetate: EA; acetonitrile: ACN.

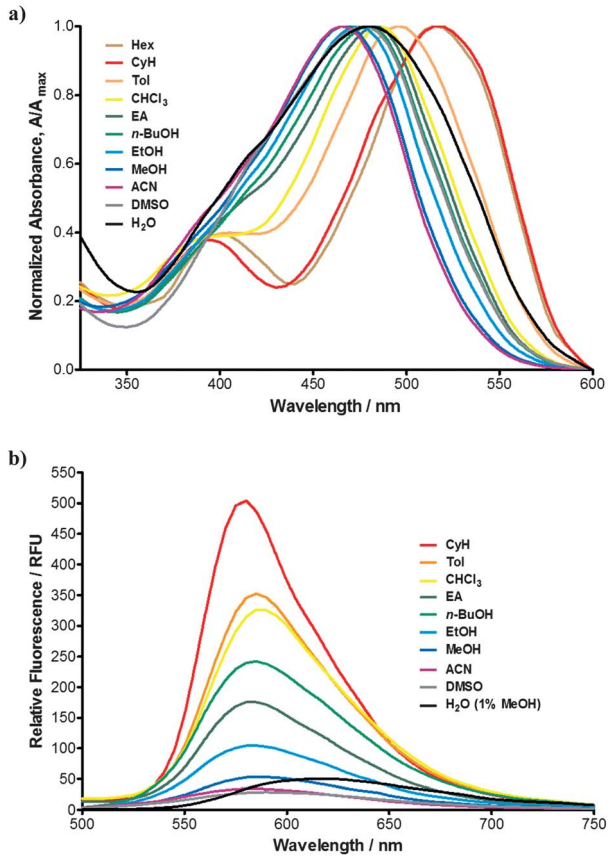


Fig. 2 Spectra of a representative BODIPY-triazole (**BDC-9**, 100 μM) in various organic solvents ordered according to polarity. Measurements were taken at rt. (a) Normalized absorption spectra in various organic solvents; (b) emission spectra.

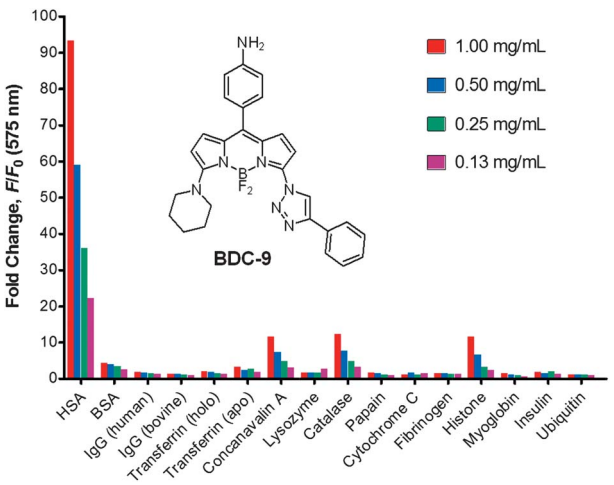


Fig. 3 Fluorescence response (*F*) of **BDC-9** (10 μM) toward 16 different proteins (0.13, 0.25, 0.50 and 1.00 mg mL^{-1}); 10 mM HEPES buffer (pH = 7.4) (λ_{exc}: 460 nm, λ_{em}: 575 nm). F_0 is the fluorescence intensity of **BDC-9** in HEPES buffer. Values are represented as means ($n = 4$).

studies of albumin binding sites.[19] We evaluated the fluorescence response of **BDC-9** against serial concentrations of serum albumins from rat (RSA), porcine (PSA), rabbit (RbSA), sheep (SSA) and bovine (BSA). As shown in Fig. 5, **BDC-9** displayed a marked selectivity only towards HSA with marginal response on the other species.[20]

Analysis of the binding of BDC-9 at HSA

Following experiments were aimed at studying the binding properties of **BDC-9** on HSA. HSA contains two main drug binding sites, namely Sudlow sites I and II.[21] Recently, X-ray crystallographic structures of HSA revealed at least three additional sites with affinity for drug compounds.[22] These drug binding sites correspond to the fatty acid (FA) sites 1, 3/4 (Sudlow site II), 5, 6 and 7 (Sudlow site I).[23] In order to corroborate the specific binding of **BDC-9** and determine its binding site, we performed competition assays using drugs binding to the different sites (*e.g.* hemin (FA1 site),[22b,k] dansyl-L-norvaline (FA3/4 sites),[22i] propofol (FA3/4 and FA5 sites),[22g] ibuprofen (FA3/4 and FA6 sites)[22d] and warfarin (FA7 site)[22d]) were performed.[22b,24]

As illustrated in Fig. 6a, only the competition with hemin induced a significant decrease in the fluorescent response of **BDC-9** while the competition with all other drugs did not affect its fluorescence. In addition, the competition with hemin reversed the hypsochromic shift observed upon HSA binding, which confirms that hemin is able to displace **BDC-9** from the FA1 site (Fig. 6b). These observations clearly indicate that the environmental turn-on response of **BDC-9** is due to its binding to the FA1 site (heme site) of HSA. There have been several reports of fluorescent dyes binding to FA3/4,[25] FA6[26] and FA7[27] sites. However, to the best of our knowledge, this is the first experimentally proven fluorescent dye binding to the FA1 site,[28] and in which binding is not dependent on contacts with bound lipid.[25a] **BDC-9** is therefore a valuable probe for examining the heme region on HSA.

In addition, we performed a Job plot analysis to determine the stoichiometry of the complex formed by **BDC-9** and HSA, and confirmed the results from site-selectivity studies. The fluorescence response of **BDC-9** peaked at a 1 : 1 proportion of **BDC-9** : HSA, which indicates that **BDC-9** binds mainly at one site of the protein (see Fig. S6 in ESI†).[29] A one-site binding model was fitted to the titration of HSA (0.67 mg mL^{-1}) with serial concentrations of **BDC-9**, and we determined the dissociation constant (K_D) of **BDC-9** as 12.7 ± 0.4 μM (see Fig. S7 in ESI†).

Application of BDC-9 to quantify HSA in urine samples

In order to examine the behaviour of **BDC-9** as an HSA sensor in complex matrices, we evaluated the application of **BDC-9** to quantify the amount of HSA in urine samples. The detection and quantification of HSA in biofluids is of great clinical importance.[30] Microalbuminuria, which involves an albumin excretion rate of 15–40 μg mL^{-1},[31] is a well-established cardiovascular risk marker and an indication of liver and renal disease.[32] We analysed the fluorescence response of **BDC-9** in urine that was spiked with different amounts of HSA, up to 1 mg mL^{-1}. We observed an excellent linear correlation between the fluorescence response of **BDC-9** and the amount of HSA in a

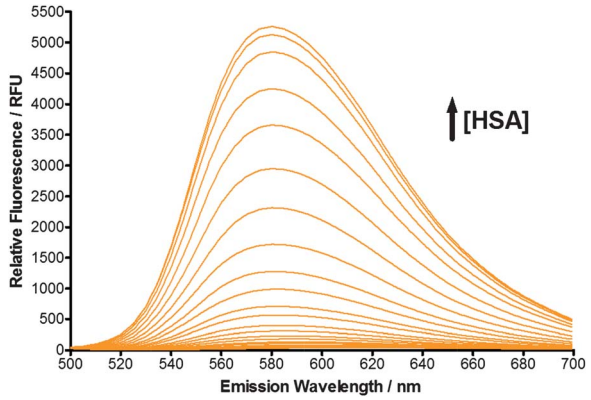


Fig. 4 Fluorescence response of **BDC-9** (10 μM) upon incubation with serial dilutions of HSA (from 1.2×10^{-4} to 4 mg mL^{-1}) in 10 mM phosphate buffer (pH = 7.3); λ_{exc}: 460 nm. Φ_F (in 4 mg mL^{-1} HSA) = 0.41. Values are represented as means ($n = 3$). Measurements were taken at rt.

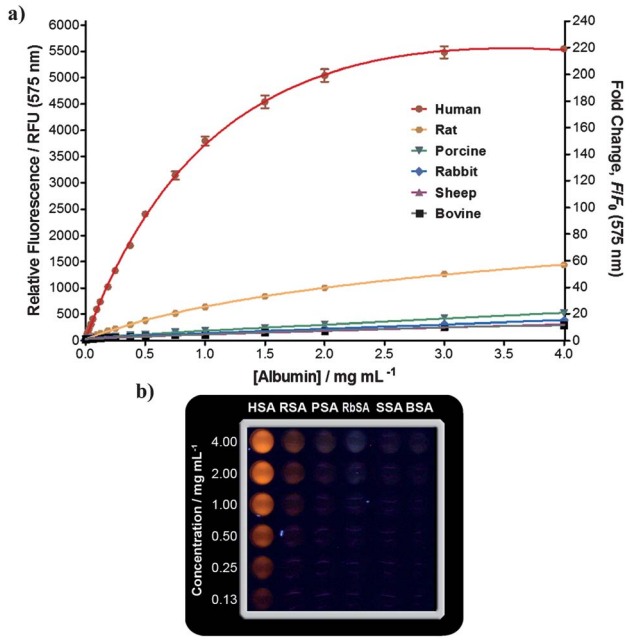


Fig. 5 Species selectivity of **BDC-9**. (a) Fluorescence response of **BDC-9** (10 μM) upon interaction with serial dilutions of the respective albumins (from 1.2×10^{-4} to 4 mg mL^{-1}) in 10 mM phosphate buffer (pH = 7.3); λ_{exc}: 460 nm, λ_{em}: 575 nm. Values are represented as means and error bars as standard deviations ($n = 3$). Measurements were taken at rt. (b) Photographic image of **BDC-9** (10 μM) mixed with respective albumins. Irradiation with a handheld UV lamp at 365 nm.

clinically significant range (Fig. 7), which proves the potential of **BDC-9** to quantify HSA levels in urine samples.

3 Conclusions

In conclusion, we prepared a solid-phase 120-member library of BODIPY-triazoles (**BDC**, **BDCAC** and **BDCCA**) *via* CuAAC with minimum purification steps and high purities. The incorporation of triazole and piperidinyl moieties at positions 3 and 5 of a bifunctional BODIPY core resulted in dyes with very large Stokes shifts, and represents the first systematic generation of Mega-Stokes dyes based on the BODIPY scaffold. The fluorescence emission of **BDC** compounds shows a strong dependence on environmental properties, such as solvent polarity and viscosity. We screened our library and identified **BDC-9** as a highly sensitive and species-selective HSA chemosensor. **BDC-9** showed a remarkable 220-fold fluorescence increase, species-selectivity over other serum albumins and binding site specificity at the FA1 site (heme site) of HSA. Furthermore, we proved that **BDC-9** can be used for clinical applications to quantify HSA in urine samples within the concentration limits of micro-albuminuria. Altogether, these results validate MegaStokes BODIPY dyes as new fluorophores for the development of environmentally sensitive fluorescent probes.

4 Experimental

Materials

All commercially available reagents, solvents and proteins were purchased from Sigma Aldrich, Alfa Aesar, Fluka, Merck or Acros, and used as received unless otherwise stated. CH_2Cl_2 (Fisher Scientific, analytical grade) was freshly distilled from P_2O_5 under nitrogen. Anhydrous THF was purchased from Alfa Aesar and used without further purification. Phosphate buffer (10 mM) was prepared following a modified recipe of Dulbecco's PBS buffer (1×): 0.2 g KH_2PO_4, 2.17 g $Na_2HPO_4 \cdot 7H_2O$, 1000 mL MilliQ™ H_2O, pH 7.3.

Measurements and analysis

Spectroscopic and quantum yield data were measured on a SpectraMax M2 spectrophotometer (Molecular Devices). Data analysis was performed using GraphPrism 5.0. Mixtures of **BDC-9** with HSA were prepared in phosphate buffer (pH 7.3, 1% DMSO) at room temperature and fluorescence measurements were taken after incubation for 2 h unless otherwise stated. Analytical characterization was performed on a HPLC-MS (Agilent-1200 series) with a DAD detector and a single quadrupole mass spectrometer (6130 series) with an ESI probe. Analytical HPLC method: eluents, A: H_2O (0.1% HCOOH), B: CH_3CN (0.1% HCOOH), gradient 5% B to 95% B (10 min). Reverse-phase Phenomenex C_{18} Luna column (4.6 × 50 mm^2, 3.5 μm particle size), flow rate: 1 mL min^{-1}. ^{1}H-NMR, ^{19}F-NMR and ^{13}C-NMR spectra were recorded on Bruker ACF300 (300 MHz) and AMX500 (500 MHz) spectrometers and reported in δ (ppm). ^{1}H NMR spectra were referenced to residual proton signals in $CDCl_3$ ($\delta = 7.26$); ^{13}C NMR spectra were referenced to solvent resonances of $CDCl_3$ ($\delta = 77.0$ ppm); ^{19}F NMR spectra were referenced to external TFA ($\delta = -76.55$ ppm *vs.* $CFCl_3$ at 0.00 ppm).

Quantum yield measurements. Quantum yields were calculated by measuring the integrated emission area of the fluorescent spectra in its respective solvents and comparing to the area measured for Acridine Yellow ($\Phi_F = 0.47$) in EtOH ($\eta = 1.361$) when excited at 430 nm. Quantum yields for the **BDC**, **BDCAC** and **BDCCA** libraries were calculated using the equation:

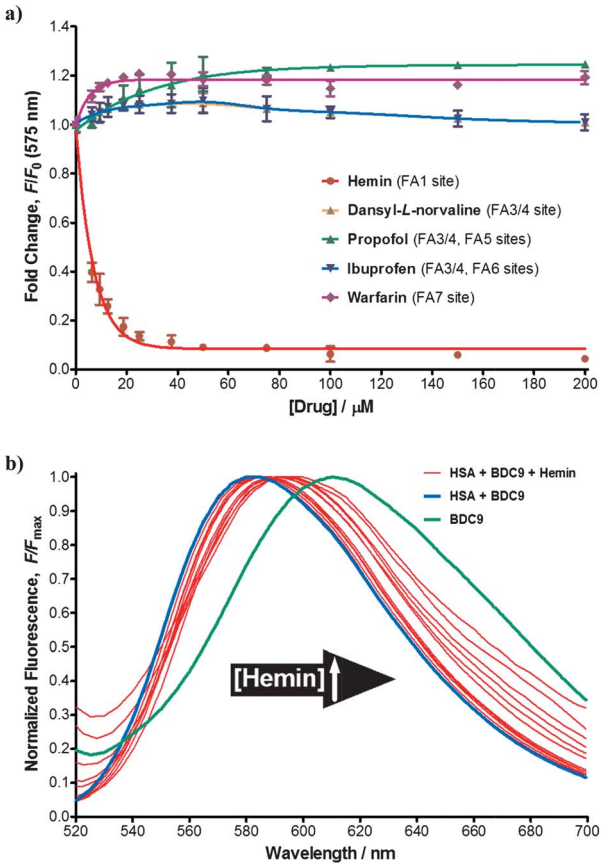


Fig. 6 Competition with site-selective HSA-binding drugs. HSA (fatty-acid free) (0.34 mg mL^{-1}, 5 μM) was preincubated at rt for 2 h in 10 mM phosphate buffer (pH = 7.3) with different concentrations of the drugs (up to 200 μM) before **BDC-9** (10 μM) was added. (a) Fluorescence fold-change at 575 nm. *F* is the fluorescence intensity at the indicated drug concentration and F_0 is the fluorescence intensity with no drug added. (b) Red-shift in emission spectra of **BDC-9** upon competition with hemin (FA1 site); λ_{exc}: 460 nm. Values are represented as means and error bars as standard deviations ($n = 3$). Measurements were taken at rt.

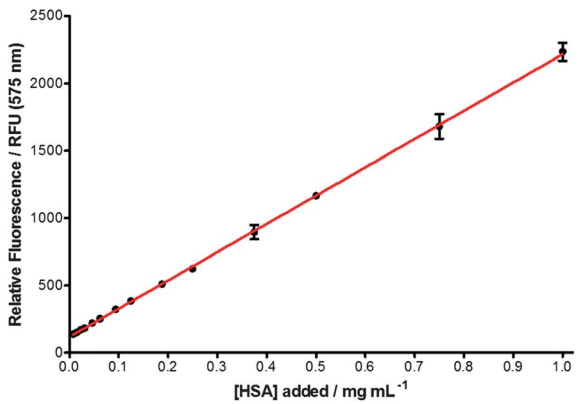


Fig. 7 Fluorescence response of **BDC-9** in urine samples. HSA (0–1 mg mL^{-1}) was added to 10% urine from healthy individuals in 10 mM phosphate buffer (pH = 7.3). **BDC-9** was added at 10 μM concentration; λ_{exc}: 460 nm, λ_{em}: 575 nm. Measurements were taken at rt. Values represented as means ($n = 3$) and error bars as standard deviations; linear regression: $R^2 = 0.998$.

$$\Phi_F^{sample} = \Phi_F^{reference}\left(\frac{F^{sample}}{F^{reference}}\right)\left(\frac{\eta^{sample}}{\eta^{reference}}\right)\left(\frac{Abs^{reference}}{Abs^{sample}}\right)$$

where *F* represents the area of fluorescent emission, η is the refractive index of the solvent, and Abs is absorbance at the excitation wavelength selected for standards and samples. Emission was integrated between 460 and 750 nm.

***In vitro* fluorescence screening.** BODIPY-triazoles (10 μM) were screened in 10 mM HEPES buffer, pH 7.4, containing 1% DMSO. Fluorescence intensities were measured using a SpectraMax M2 plate reader in 384-well plates. Excitation was provided at each compound's excitation range, and emission spectrum was obtained starting from at least 30 nm beyond the excitation wavelength. All the analytes were tested at four serial concentrations (see Table S1 in ESI†).

Determination of dissociation constant for BDC-9 with HSA. HSA (10 μM) was titrated against serial concentrations of **BDC-9** (0.47 to 60 μM) and the fluorescence intensities were measured at 575 nm ($\lambda_{exc} = 460$ nm). The fluorescence intensity of bound **BDC-9** at each concentration was determined using the equation:

$$F_{bound} = \frac{F_{sat}(F - F_0)}{(F_{sat} - F_0)}$$

where *F* and F_0 are the fluorescence intensities of a given concentration of **BDC-9** with and without HSA respectively. F_{sat} is the fluorescence intensity at the same concentration of **BDC-9** when fully bound.

Synthesis

General procedure for the preparation of the BDC library. 2-Chlorotrityl chloride resin (220 mg, 0.275 mmol) was swollen in CH_2Cl_2 (3.0 mL) for 15 min. A solution of **4** (30 mg, 0.085 mmol) and DIEA (200 μL, 1.15 mmol) in DMF–CH_2Cl_2 (1 : 1, 2 mL) was added to the resin suspension and shaken at rt for 24 h, after which the resin was capped with MeOH (0.4 mL, 1.8 mL g^{-1} resin) and DIEA (100 μL) for 12 h. The resin was filtered, washed with DMF (4 × 10 mL) and CH_2Cl_2 (4 × 10 mL) and dried. The loaded resin was resuspended in CH_2Cl_2 and shaken at rt for 15 min and washed with DMF. A suspension of NaN_3 (50 mg, 0.77 mmol) in DMF (3.0 mL) was added and the reaction mixture further shaken at rt for 30 min. After washing with DMF (4 × 10 mL), a solution of DMF–piperidine (4 : 1) (4.0 mL) was added and the reaction shaken for 45 min. The alkyne (0.854 mmol), CuI (65 mg, 0.34 mmol) and ascorbic acid (60 mg, 34 mmol) were subsequently added and the reaction mixture shaken for another 30 min. The resin was filtered, washed with DMF (4 × 10 mL) and CH_2Cl_2 (4 × 10 mL) following which cleavage was performed with 0.5% TFA in CH_2Cl_2 (3 × 15 mL, 10 min each). The organic extracts were recovered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (eluent: CH_2Cl_2 to CH_2Cl_2–MeOH (98 : 2)) to afford the corresponding **BDC** library compound as an orange solid (*ca.* 16 mg, 0.03 mmol, 70% yield) (see Table S2 in ESI† for chemical structures and detailed characterization).

General procedure for the preparation of BDCAC and BDCCA libraries. To a solution of the respective **BDC**

compounds (6 mg, *ca.* 0.011 mmol) in CH_2Cl_2 (1.5 mL), 6 drops of saturated aqueous $NaHCO_3$ were added and cooled to 0 °C. Acetyl chloride (10 μL, 0.11 mmol) was added in portions over 1 min and the resulting mixture was stirred at rt and monitored by analytical TLC. Upon reaction completion, the reaction was diluted with CH_2Cl_2 (15 mL), washed with water (2 × 15 mL), saturated $NaHCO_3$ (1 × 15 mL), saturated brine (1 × 15 mL) and dried over anhydrous Na_2SO_4. The organic extract was evaporated to afford the corresponding **BDCAC** or **BDCCA** compounds as an orange solid (*ca.* 6 mg, >90% yield) (see Tables S3 and S4 in ESI† for chemical structures and detailed characterization).

2,2′-((4-Nitrophenyl)methylene)bis(1*H*-pyrrole) (1). To a solution of 4-nitrobenzaldehyde (1.0 g, 6.6 mmol) in pyrrole (10 mL, 144 mmol) was added TFA (0.51 mL, 0.66 mmol) and stirred at rt for 3 h. The mixture was concentrated *in vacuo*, and purified by flash column chromatography on silica gel (hexane–EtOAc, 4 : 1) to afford **1** as a bright yellow solid (1.63 g, 6.10 mmol, 92% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (d, J = 8.7 Hz, 2H), 8.00 (br s, 2H), 7.38 (d, J = 8.7 Hz, 2H), 6.75 (d, J = 5.8 Hz, 2H), 6.18 (dd, J = 5.8, 2.9 Hz, 2H), 5.87 (br s, 2H), 5.58 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.4, 147.7, 131.5, 129.9, 124.5, 118.7, 109.5, 108.5, 44.5; MS (ESI): m/z $[M + H]^+$ = 268.1.

1,1′-Dichloro-5-(4-nitrophenyl)dipyrromethene (2).[13] A solution of **1** (1.1 g, 4.1 mmol) in anhydrous THF (35 mL) was stirred under N_2 atmosphere at −78 °C for 15 min. *N*-Chlorosuccinimide (1.4 g, 10.3 mmol) in anhydrous THF (35 mL) was added dropwise using a pressure equalizing funnel and the resulting mixture was stirred at rt for 3 h. Upon completion, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (1.12 g, 4.92 mmol) was added and the reaction mixture stirred at rt for an additional 2 h. THF was removed *in vacuo* and the resulting mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 70 mL). The combined organic extracts were washed with saturated brine, dried over anhydrous Na_2SO_4 and the solvents removed *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (hexane–EtOAc, 10 : 1) to afford **2** as a dark orange solid (0.78 g, 2.3 mmol, 57% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.33 (d, J = 4.3 Hz, 2H), 7.63 (d, J = 4.3 Hz, 2H), 6.42 (d, J = 2.1 Hz, 2H), 6.29 (d, J = 2.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.1, 143.7, 142.7, 138.7, 137.1, 132.3, 130.1, 123.8, 118.5; MS (ESI): m/z $[M + H]^+$ = 334.0.

3,5-Dichloro-8-(4′-nitrophenyl)-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacence (3).[13] To a solution of **2** (1.0 g, 2.97 mmol) in anhydrous CH_2Cl_2 (70 mL) was added *N*,*N*-diisopropylethylamine (3.1 mL, 17.8 mmol) stirred at 0 °C for 10 min. Boron trifluoride diethyl etherate (2.2 mL, 17.8 mmol) was added dropwise and the resulting mixture was stirred at rt for 2 h. Upon completion, solvents were removed *in vacuo* and the residue purified by flash column chromatography on silica gel (hexane–EtOAc–MeOH, 10 : 1 : 0.1) to afford **3** as a deep reddish-purple solid (0.81 g, 2.14 mmol, 72% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.39 (d, J = 4.4 Hz, 2H), 7.69 (d, J = 4.4 Hz, 2H), 6.75 (d, J = 2.2 Hz, 2H), 6.48 (d, J = 2.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.2, 146.6, 140.2, 138.5, 133.4, 131.3, 131.1, 123.8, 119.8, 120.8, 114.9, 111.3; ^{19}F NMR (282 MHz, $CDCl_3$): δ −148.07 (q, J = 28 Hz); MS (ESI): m/z $[M + H]^+$ = 382.9.

3,5-Dichloro-8-(4′-aminophenyl)-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacence (4). A suspension of iron powder (1.46 g, 26.2 mmol) was activated in 1 M aqueous HCl for 1 min, rinsed with absolute EtOH and used as such. A solution of **3** (500 mg, 1.3 mmol) in EtOH (80 mL) and acetic acid (8 mL) was added the activated iron and refluxed. The reaction mixture was monitored by TLC. Upon completion, iron was removed and the solvent was evaporated *in vacuo*. The residue was diluted with water and extracted with CH_2Cl_2 (3 × 70 mL). The combined organic extracts were washed with saturated Na_2CO_3, saturated brine, dried over anhydrous Na_2SO_4 and the solvents removed *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc–MeOH–NH_3: 6 : 3 : 1 : 0.05) to afford **4** as a dark red solid (197 mg, 0.56 mmol, 98% yield); mp 134–135 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 (dt, J = 8.5, 2.6 Hz, 2H), 6.93 (d, J = 3.8 Hz, 2H), 6.76 (dt, J = 8.5, 2.6 Hz, 2H), 6.43 (d, J = 3.8 Hz, 2H), 4.08 (br s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.7, 144.9, 143.2, 133.4, 132.7, 131.1, 122.3, 118.1, 114.4; ^{19}F NMR (282 MHz, $CDCl_3$): δ −148.65 (q, J = 28 Hz); HRMS ($C_{15}H_{11}BCl_2F_2N_3$): calc. $[M + H]^+$: 352.0386, found $[M + H]^+$: 352.0215.

10-(4-Aminophenyl)-5,5-difluoro-7-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-3-(piperidin-1-yl)-5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*][1,3,2]diazaborinin-4-ium-5-uide (BDC-9). Orange solid (17 mg, 0.032 mmol, 72% yield); mp 129–131 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.56 (s, 1H), 7.95 (d, J = 6.9 Hz, 2H), 7.45 (t, J = 7.6, Hz, 2H), 7.34 (t, J = 7.6 Hz, 1H), 7.27 (d, 8.2 Hz, 2H), 6.94 (d, J = 5.7 Hz, 1H), 6.80 (d, J = 8.2 Hz, 2H), 6.62 (d, J = 3.8 Hz, 1H), 6.42 (d, J = 3.8 Hz, 1H), 6.32 (d, J = 5.1 Hz, 1H), 5.30 (s, 1H), 3.78–3.88 (m, 4H), 1.64–1.80 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.2, 146.7, 136.0, 135.7, 134.9, 131.8, 131.3, 131.0, 130.8, 129.6, 128.7, 127.9, 126.0, 125.0, 122.4, 122.3, 116.9, 115.4, 115.0, 114.9, 109.7, 53.4, 52.0, 26.3, 24.0; ^{19}F NMR (282 MHz, $CDCl_3$): δ −132.37 (q, J = 35 Hz); HRMS ($C_{28}H_{27}BF_2N_7$): calc. $[M + H]^+$: 510.2384, found $[M + H]^+$: 510.2399.

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