A chemical biology approach for targeting of ligand-drug conjugates

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3

Synthesis of pH-activatable red fluorescent BODIPY dyes with distinct functionalities[1]

This chapter describes the synthesis of a series of tunable pH-dependent BODIPY dyes that were functionalized using a Knoevenagel-type condensation reaction. In this fashion, monofunctional dyes containing an alkyne, azide or carboxylic acid (masked as its methyl ester) as ligation sites as well as asymmetrical bifunctional dyes were obtained, without compromising their pH-dependency. In addition, fluorescence excitation and emission maxima for these dyes were shown to be significantly red-shifted in comparison to their tetramethyl precursors.

3.1 Introduction

In recent years fluorescent dyes have found widespread use in chemical biology applications. An important class of dyes are those based on the 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) scaffold. BODIPY dyes usually show excellent photochemical properties, such as high molar absorption coefficients, high quantum yields and narrow emission bandwidths. Moreover, they are relatively stable under physiological conditions.[2–4]

Synthetic efforts have been directed towards the formation of analyte-sensitive dyes and numerous reports can be found in the literature.[4] Particularly useful examples are the pH-sensitive BODIPY dyes that are responsive to protonation.[5–9] Intracellular pH is an important factor in many physiological processes and by using a pH-activatable dye selective visualization of processes in acidic cellular compartments becomes possible. pH-sensitivity is usually accomplished by incorporation of a *p*-(*N,N*-dialkyl)aniline moiety at the *meso*-position of the BODIPY core.[7,10–13] Due to photo-induced electron transfer (PeT) fluorescence is quenched at neutral or basic pH.[13–15] Upon protonation of the aniline nitrogen fluorescence intensity increases drastically.

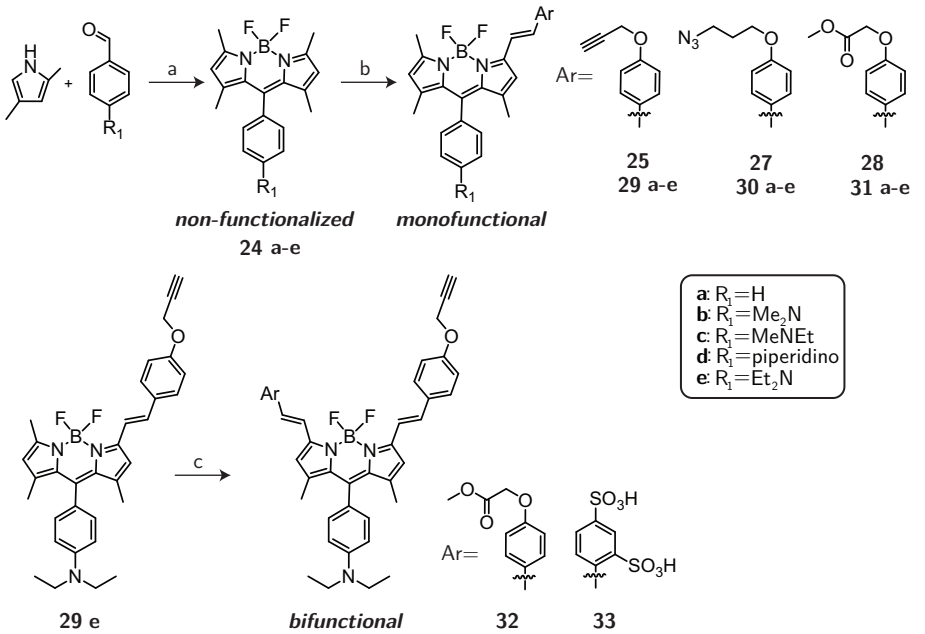
In a recent paper by Urano *et al*., the development of a series of pH-activatable dyes with varying pKa-values is described.[11] These can be easily transformed into bifunctional dyes, but for mono-conjugation they are less convenient due to their symmetry, as described in Chapter 2.[12] Other groups have investigated the tetramethyl-BODIPY scaffold with a *meso*-aniline substituent.[10,13,16] The methyl groups adjacent to the nitrogens are susceptible to a Knoevenagel-type condensation with benzaldehydes, yielding dyes with red-shifted absorption and emission maxima.[5–7,9,17–21] The potential of the Knoevenagel reaction to extend the conjugated system is widely acknowledged and the reaction has been used to construct large libraries of (pH-independent) BODIPY dyes.[22–25] The use of the Knoevenagel reaction to incorporate ligation handles in the BODIPY dye has not been employed, although that would allow covalent attachment to other (bio-active) molecules, which is vital for applications in chemical biology. Often used ligation reactions comprise the Staudinger-Bertozzi ligation (involving azides),[26] the copper(I) catalyzed Huisgen 1,3-cycloaddition (involving azides or alkynes)[27,28] and, in a more traditional fashion, amide/ester formation (involving carboxylic acids). This chapter concerns the synthesis of a series of pH-dependent BODIPY dyes that are functionalized by means of the Knoevenagel reaction. The pH-dependency and spectroscopic properties of these novel dyes are investigated. Finally, one of the dyes is subjected to a ligation reaction to study the synthetic stability of these dyes.

3.2 Results and Discussion

Starting from commercially available *N,N*-dialkylamino benzaldehydes (or benzaldehyde in case of **24a**, the "always on" dye) and 2,4-dimethylpyrrole synthesis of non-functionalized BODIPY dyes **24a-e** proved straightforward, with yields ranging between 32 and 50% (Scheme 7.1).[13,29] To obtain mono-functional BODIPY dyes **29-31a-e**, three benzaldehydes

were synthesized, containing either an alkyne (**25**),[30] azide (**27**)[31] or a methyl ester (**28**)[32] as the functional group. The use of a tert-butyl ester has been reported in a related study,[18] however, literature precedence shows that methyl esters can be saponified using (mild) basic conditions while leaving the BODIPY core intact.[1,11,33] Subsequently, these benzaldehydes were used in a Knoevenagel condensation reaction with BODIPYs **24a-e**.

Scheme 3.1: Synthesis of non-functionalized, monofunctional and bifunctional BODIPY dyes



Reagents and conditions: [**a**] i) cat. TFA, DCM; ii) DDQ; iii) DiPEA, $BF_3 \cdot OEt_2$, 32-50%; [**b**] ArCHO **25**-**28**, pyrrolidine, acetic acid, EtOH or MeOH, microwave, 13-35%; [**c**] ArCHO, pyrrolidine, acetic acid, EtOH or MeOH, microwave, **32**: 42%, **33**: 12%

Reaction times using classical Knoevenagel conditions (refluxing in toluene in the presence of piperidine/acetic acid) can be extensive (12-24 h).[3,4,18] Recent reports show that the use of microwave irradation considerably shortens reaction times (5-20 min).[25,34] Therefore, microwave conditions were used in this study, in order to synthesize various BODIPYs in a relatively short period of time. In case of benzaldehyde **28**, the reaction solvent was changed from ethanol to methanol to prevent transesterification of the methyl ester. Typically, reaction yields of the Knoevenagel reaction are low,[5,6,17,18,34] and although this case forms no exception, the envisaged 15 mono-functional BODIPYs **29-31a-e** were all readily obtained. Importantly, a significant amount (30-50%) of unreacted starting material could easily be recovered by silica column chromatography.

BODIPY dyes **24a-e** contain two reactive methyl groups (at the 3 and 5 positions) and the main byproduct observed in the reaction was indeed the symmetrical distyryl substituted BODIPY. Under these conditions, no formation of tetrastyryl substituted BODIPY

dyes was observed.[19,21] Taking advantage of the acidity of the second methyl group, mono-functional dye **29e** was subjected to another cycle of Knoevenagel condensation with benzaldehyde **28** to obtain bifunctional BODIPY dye **32**, containing two orthogonal ligation handles. As an alternative strategy, the second methyl group can be used to alter the solubility properties of the dye. BODIPY dyes tend to be fairly lipophilic and poorly water-soluble, which can be improved by introduction of polar moieties such as sulfonic acids[37–39] or polyethylene glycol.[19,20] By introduction of a disulfonic acid-containing styryl moiety via the Knoevenagel reaction water-soluble BODIPY dye **33** was synthesized in 12% yield. The low yield of this reaction was mostly due to extensive purification procedures to separate the product from pyrrolidinium acetate.

Table 3.1: Spectroscopic properties measured in TFA (135 mM) /methanol

	λ_{abs} (nm)	λ_{em} (nm)	Φ_f[b,c]	pK_d[e]
24a	497	510	0.63[b]	-
24b	500	514	0.35[b]	4.49[a]/2.14
24c	500	513	0.44[b]	5.10[a]/2.95
24d	500	514	0.40[b]	5.06[a]/3.97
24e	500	513	0.56[b]	5.81[a]/4.05
29a	563	574	1.09[c]	-
29b	567	582	0.66[c]	2.13
29c	569	582	0.84[c]	2.93
29d	568	582	0.85[c]	3.92
29e	568	582	0.84[c]	4.07
30a	564	578	1.01[c]	-
30b	568	586	0.59[c]	2.08
30c	568	586	0.69[c]	2.93
30d	570	586	0.81[c]	3.91
30e	570	587	0.82[c]	4.05
31a	562	574	1.10[c]	-
31b	566	580	0.67[c]	2.08
31c	567	581	0.78[c]	2.96
31d	567	581	0.87[c]	3.94
31e	567	581	0.87[c]	4.10
32	640	655	-[d]	4.10
33	643[a]	660[a]	-[d]	5.81[a]

[a] Measured in citric acid/phosphate buffer.[b] Relative quantum yield (±0.1) with fluorescein ($\Phi_f = 0.925 \pm 0.015$ in 0.1 M NaOH)[35] as standard.[c] Relative quantum yield (±0.1) with rhodamine 101 ($\Phi_f = 1.00 \pm 0.02$ in ethanol)[36] as standard.[d] No standard available.[e] Apparent dissociation constant determined from curves of fluorescence vs p[TFA] (in MeOH) or pH (pK_a = -log K_d).

Evaluation of the spectroscopic properties of the dyes (Table 3.1) revealed small Stokes shifts, characteristic of BODIPY dyes, and moderate to excellent relative quantum yields*. The fluorescence absorption and emission maxima of the dyes were shifted approximately 70 nm towards the red end of the spectrum with each Knoevenagel reaction (Table 3.1, Figure 3.1A). For biological applications such as live-cell fluorescence microscopy this is a great advantage, since levels of autofluorescence are lower at the red end of the spectrum. Besides, as a result of their narrow absorbance and emission peaks, the bifunctional dyes are compatible for use in the same experiment as other pH-dependent dyes, such as the ones described in Chapter 2.

*Some controversy about the actual quantum yield of rhodamine 101 exists in the literature.[40,41] Since quantum yields reported here are relative values, this would explain the values greater than

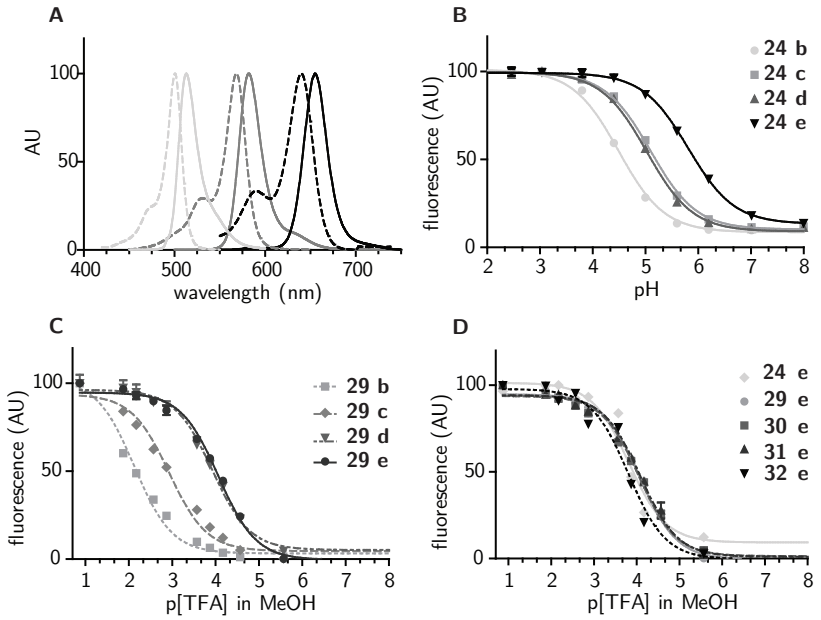


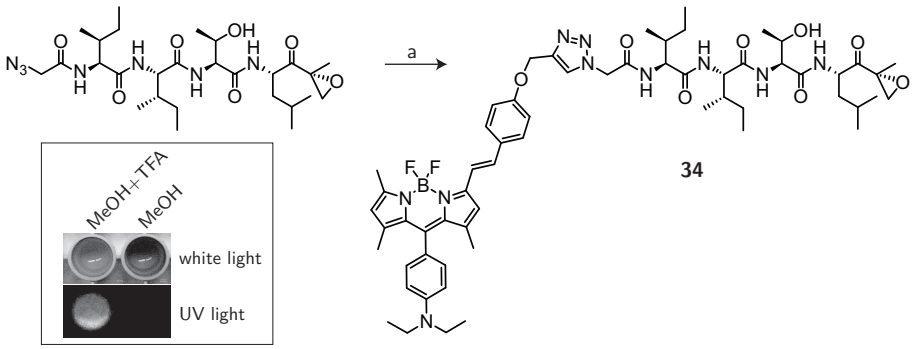
Figure 3.1: A) Absorbance (dashed line) and emission (solid line) spectra of compounds **24a-e** (left), **29-31a-e** (middle) and **32**, **33** (right) in TFA/methanol. B) Fluorescence intensity vs pH curves for compounds **24b-e**. C, D) Fluorescence intensity vs p[TFA] curves for compounds **29b-e** and for non-functionalized (**24e**), monofunctional (**29-31e**) and bifunctional (**32**) diethylaniline derivatives.

To assess whether modifications of the BODIPY core by one or two Knoevenagel reactions would lead to any change in pH-dependency, the fluorescence intensities at different pH-values were measured for all pH-activatable fluorophores (Figure 3.1B-D). Since the water-solubility of dyes **29-31** was low, their fluorescence was determined as a function of the concentration of trifluoroacetic acid (TFA) in methanol. The apparent pK_a (in aq. buffer) or pK_d (in methanol/TFA)[4] of the dyes decreases in the order diethyl > piperidino > ethylmethyl > dimethyl (Figure 3.1B,C, Table 3.1), which corresponds to the electron-donating properties of the substituted amines and the trend found for previously reported pH-dependent dyes (2).[11,12,16] No change in pH-dependency was observed for the various mono- and bifunctional dyes compared to their non-functionalized counterparts (Figure 3.1D, Table 3.1). It is therefore likely that attachment of these dyes via their ligation handle to (bio)active molecules will result in constructs with the same advantageous fluorescence characteristics as the original dyes.

Finally, to examine the stability of the dyes when used in a ligation reaction, dye **29e** was used in a traditional copper(I) catalyzed Huisgen 1,3-cycloaddition[27,28] with the azide-modified proteasome inhibitor epoxomicin.[42] This resulted in the pH-dependent BODIPY-epoxomicin **34** in good yield (Scheme 3.2).

unity (although within the estimated error of measurement) for dyes **29-31a**.

Scheme 3.2: Synthesis of pH-dependent BODIPY-epoxomicin. Inset: pH-dependent fluorescence of compound **34**.



Reagents and conditions: [a] **29e**, sodium ascorbate, $CuSO_4$, H_2O/toluene/*t*-BuOH, 80 °C, 89%

3.3 Conclusion

This chapter describes a series of tunable pH-dependent BODIPY dyes that contain a (bio)-conjugation handle for incorporation in larger constructs. The Knoevenagel-type condensation reaction that was used provides a fast and easy means to a large variety of red-shifted BODIPY dyes. Because of the red-shift in the fluorescence properties of these dyes, they should be more suited for live-cell fluorescence microscopy than their greener counterparts discussed in Chapter 2, as non-invasive tools to selectively image cellular compartments of interest, based on their acidity. Because of the generality of the approach, the methodology can be extended towards the development of many other (analyte-sensitive) BODIPY dyes containing the functionality or property of interest, an example hereof will be discussed in Chapter 8.

3.4 Experimental Section

Absorption and Fluorescence Spectroscopy. All spectroscopic experiments were performed in 135 mM TFA in methanol (spectroscopic grade) or a citric acid/phosphate buffer pH 1.8, at a concentration of 1 μM (absorption) or 100 nM (fluorescence). For fluorescence experiments the excitation wavelength was set equal to the maximum of the corresponding absorption spectrum and the emission spectrum recorded. Measurements were conducted on a Shimadzu UV1700 pharmaspec UV-VIS spectrophotometer (absorbance) and a Shimadzu RF-5301PC spectrofluorometer (fluorescence). For the determination of relative quantum yields the slit width was set to 3 nm for both excitation and emission. For the determination of relative quantum yields the slit width was set to 3 nm for both excitation and emission. Quantum yields were calculated using equation 3.1*:

*Quantum yields were determined according to the method described in: http://www.jobinyvon.com/usadivisions/Fluorescence/applications/quantumyieldstrad.pdf

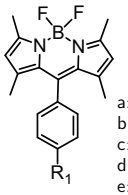
$$\Phi_x = \Phi_{st} \frac{Grad_x}{Grad_{st}} \frac{\eta_x^2}{\eta_{st}^2} \tag{3.1}$$

where x denotes the unknown and st the standard. Φ is the quantum yield, $Grad$ is the gradient from the plot of integrated fluorescence vs absorbance (absorbances between 0.01 and 0.1) and η is the refractive index of the solvent used (1.3288 for methanol, 1.36 for ethanol and 1.333 for water). Quantum yields were determined with rhodamine 101 ($\Phi_f = 1.00 \pm 0.02$ in ethanol)[36] and fluorescein ($\Phi_f = 0.925 \pm 0.015$ in 0.1 M NaOH)[35] as standards. The error in the measurements is estimated to be ± 0.1.

Determination of pH-dependency. Fluorescence intensity was measured on a FLUOstar Optima platereader (BMG Labtech) using the following filter settings:
Dyes **24b-e**: λ_{ex} 480-12; λ_{em} 505-10; Dyes **29**-**31b-e**: λ_{ex} 520-10; λ_{em} 570-10; Dyes **32**, **33**: λ_{ex} 620-10; λ_{em} 660-10. All compounds were prepared as a stock solution in DMSO (100 μM). Intensity was measured of 1 μM solutions of compounds **24a-e**, **29a-e**, **30a-e**, **31a-e** and **32** in freshly prepared solutions of trifluoroacetic acid in methanol in a 96-wells plate. Alternatively, for compounds **24a-e** and **33** measurements were conducted in a citric acid/phosphate buffer of different pH, containing 0.2% SDS. All experiments were conducted at least in triplicate. Data was analyzed using GraphPad Prism 5.0 and curves were corrected for background fluorescence of the solvent.

Synthesis

General. All reagents were of commercial grade and used as received unless stated otherwise. Reaction solvents were of analytical grade and when used under anhydrous conditions stored over flame-dried 3 Å molecular sieves. Dichloromethane was distilled over CaH_2 prior to use. Solvents used for column chromatography were of technical grade and distilled before use. All moisture and oxygen sensitive reactions were performed under an argon atmosphere. Flash chromatography was performed on silica gel (Screening Devices BV, 0.04-0.063 mm, 60 Å). Reactions were routinely monitored by TLC analysis on DC-alufolien (Merck, Kieselgel60, F254) with detection by UV-absorption (254/366 nm) where applicable and spraying with a solution of $(NH_4)_6Mo_7O_{24} \cdot 4\,H_2O$ (25 g/l) and $(NH_4)_4Ce(SO_4)_4 \cdot 2\,H_2O$ (10 g/l) in 10% sulfuric acid in water followed by charring at ∼150 °C. ^1H and ^{13}C NMR spectra were recorded on a Bruker AV-400 (400 MHz) or Bruker DMX-600 (600 MHz). Chemical shifts are given in ppm (δ) relative to the residual solvent peak or TMS (0 ppm) as internal standard. Coupling constants are given in Hz. Peak assignments are based on 2D ^1H-COSY and ^{13}C-HSQC NMR experiments. IR measurements (thin film) were conducted on an IRaffinity-1 apparatus and evaluated using IRSolutions software (Shimadzu, Kyoto, Japan). LC-MS measurements were conducted on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a standard C18 (Gemini, 4.6 mmD x 50 mmL, 5μ particle size, Phenomenex) analytical column and buffers A: H_2O, B: ACN, C: 0.1% aq.TFA. High resolution mass spectra were recorded on a LTQ Orbitrap (Thermo Finnigan) mass spectrometer equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL min^{-1}, capillary temperature 250 °C) with resolution R=60000 at m/z 400 (mass range m/z=150-2000) and dioctylphtalate (m/z = 391.28428) as a "lock mass". The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). For reversed-phase HPLC purification of the final compounds an automated HPLC system equipped with a C18 semiprep column (Gemini C18, 250x10 mm, 5μ particle size, Phenomenex) was used. Microwave syntheses were conducted in a Personal Chemistry Emrys Optimizer automated microwave synthesizer.



a: R_1=H
b: R_1=Me_2N
c: R_1=MeNEt
d: R_1=piperidino
e: R_1=Et_2N

General procedure for the synthesis of non-functionalized BODIPYs (24a-e).

To a solution of 2,4-dimethylpyrrole (0.5 mL, 0.46 g, 4.4 mmol, 2 eq) in dry DCM (100 mL) the appropriate benzaldehyde (2.2 mmol, 1 eq) was added followed by a catalytic amount of trifluoroacetic acid (TFA). After overnight stirring of the resulting reddish solution, 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 1.2 eq) was added and the mixture stirred for 1 h. If TLC analysis (4:1 PE:EtOAc + 1% triethylamine) revealed incomplete oxidation, another 0.5 eq of DDQ was added. All reactions were complete after 2 h, upon which *N,N*-diisopropylethylamine (DIPEA, 3 mL) and $BF_3.OEt_2$ (3.5 mL) were added. After 1 h the mixture was concentrated *in vacuo*, redissolved in EtOAc and washed with water. The water layer was extracted with EtOAc and the combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Silica column chromatography (toluene) yielded **24a-e** as orange powders. R_f = 0.5 (4:1 PE:EtOAc)

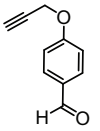
1, 3, 5, 7-Tetramethyl-8-phenyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (24a). Yield: 287 mg, 0.89 mmol, 40%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 - 7.36 (m, 3H, 3 x CH_{ar}), 7.38 - 7.10 (m, 2H, 2 x CH_{ar}), 5.98 (s, 2H, 2 x CH), 2.56 (s, 6H, 2 x CH_3), 1.37 (s, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.55, 143.29, 141.85, 135.11, 131.56, 129.26, 129.06, 128.05, 121.33, 14.73, 14.48. FT-IR (thin film) υ 2922, 1533, 1504, 1301, 1177, 1150, 1051, 966, 718 cm^{-1}. ESI-HRMS (*m/z*): calcd. for $[C_{19}H_{19}BF_2N_2 + H]^+$ 325.16821; obsd. 325.16822.

1, 3, 5, 7-Tetramethyl-8-[4-(*N,N*-dimethylamino)phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (24b). Yield: 350 mg, 0.95 mmol, 32%; based on 3 mmol benzaldehyde. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15 - 7.00 (m, 2H, 2 x CH_{ar}), 6.77 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 5.97 (s, 2H, 2 x CH), 3.02 (s, 6H, 2 x NCH_3), 2.55 (s, 6H, 2 x CH_3), 1.49 (s, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.86, 150.83, 143.37, 132.34, 128.88, 122.33, 120.96, 112.47, 40.46, 14.82, 14.69. FT-IR (thin film) υ 2916, 2854, 1610, 1504, 1302, 1182, 1153, 1074, 1047, 974,806 cm^{-1}. ESI-HRMS (*m/z*): calcd. for $[C_{21}H_{24}BF_2N_3 + H]^+$ 368.21041; obsd. 368.21077.

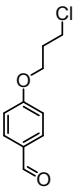
1, 3, 5, 7-Tetramethyl-8-[4-(*N,N*-ethylmethylamino)phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (24c). Yield: 420 mg, 1.1 mmol, 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.76 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 5.97 (s, 2H, 2 x CH), 3.45 (q, J = 7.1 Hz, 2H, NCH_2), 2.96 (s, 3H, NCH_3), 2.55 (s, 6H, 2 x CH_3), 1.50 (s, 6H, 2 x CH_3), 1.15 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.75, 149.45, 143.36, 132.31, 128.93, 121.86, 120.93, 112.43, 46.90, 37.51, 14.82, 14.69, 11.17. FT-IR (thin film) υ 2918, 2850, 1732, 1543, 1263, 1195, 1083, 734, 704 cm^{-1}. ESI-HRMS (*m/z*): calcd. for $[C_{22}H_{26}BF_2N_3 + H]^+$ 382.22606; obsd. 382.22656.

1, 3, 5, 7-Tetramethyl-8-[4-(piperidino)phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (24d). Yield: 382 mg, 0.94 mmol, 43%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.07 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 7.00 (d, J = 8.8 Hz, 2H, 2 x CH_{ar}), 5.96 (s, 2H, 2 x CH), 3.28 - 3.15 (m, 4H, 2 x NCH_2), 2.54 (s, 6H, 2 x CH_3), 1.80 - 1.69 (m, 4H, 2 x CH_2), 1.66 - 1.55 (m, 2H, CH_2), 1.46 (s, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.98, 152.56, 143.35, 142.88, 132.12, 128.76, 124.87, 121.02, 116.32, 50.19, 25.75, 24.36, 14.75, 14.67. FT-IR (thin film) υ 2916, 2850, 1730, 1543, 1263, 1157, 1060, 732, 704 cm^{-1}. ESI-HRMS (*m/z*): calcd. for $[C_{24}H_{28}BF_2N_3 + H]^+$ 408.24171; obsd. 408.24179.

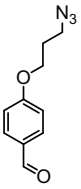
1, 3, 5, 7-Tetramethyl-8-[4-(*N,N*-diethylamino)phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (24e). Yield: 332 mg, 0.84 mmol, 38%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.73 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 5.96 (s, 2H, 2 x CH), 3.40 (q, J = 7.0 Hz, 4H, 2 x NCH_2), 2.54 (s, 6H, 2 x CH_3), 1.51 (s, 6H, 2 x CH_3), 1.19 (t, J = 7.0 Hz, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.68, 148.28, 143.38, 132.38, 129.02, 121.27, 120.90, 112.04, 44.47, 14.86, 14.69, 12.51. FT-IR (thin film) υ 2924, 2878, 1726, 1603, 1501, 1466, 1265, 1192, 1044, 970, 814 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{23}H_{28}BF_2N_3 + H]^+$ 396.24171; obsd. 396.24170.



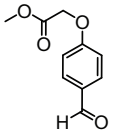
4-(propargyloxy)-benzaldehyde (25).[30] 4-hydroxybenzaldehyde (6.1 g, 50 mmol) was dissolved in acetone (250 mL). Potassium carbonate (9.7 g, 70 mmol, 1.4 eq) was added and the mixture was refluxed for 30 minutes before propargylbromide (11.1 mL, 100 mmol, 2 eq) was added. After 2 h of refluxing, the solvent was evaporated *in vacuo*. The residue was dissolved in water and extracted with EtOAc (4 x 75 mL). The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was purified by silica column chromatography (0 $\rightarrow$ 10% EtOAc in toluene) resulting in compound **25** in 98% yield (7.8 g, 48.9 mmol). R_f = 0.8 (5:1 toluene:EtOAc) ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.91 (s, 1H, HCO), 7.93 - 7.80 (m, 2H, 2 x CH_{ar}), 7.10 (d, J = 7.2 Hz, 2H, 2 x CH_{ar}), 4.79 (s, 2H, CH_2), 2.58 (s, 1H, CH). ^{13}C NMR (101 MHz, $CDCl_3$): δ 190.91, 162.47, 132.02, 130.71, 115.29, 109.74, 77.66, 76.50, 56.07. FT-IR (thin film) υ 3205, 2121, 1678, 1600, 1573, 1238, 1168, 1006, 825, 651 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{10}H_8O_2 + H]^+$ 161.05971; obsd. 161.05952.



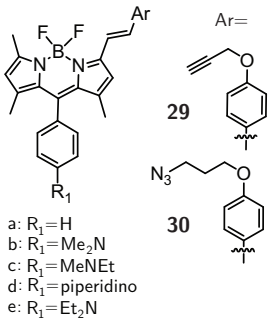
4-(3-chloropropoxy)benzaldehyde (26). To a solution of 4-hydroxybenzaldehyde (6.1 g, 50 mmol) in acetone (50 mL) K_2CO_3 (8.1 g, 55 mmol, 1.1 eq) was added and the solution was heated to reflux. After 30 minutes 1-bromo-3-chloropropane (5.45 mL, 55 mmol, 1.1 eq) was added and the mixture was refluxed overnight. The solvent was evaporated *in vacuo*, the residue dissolved in EtOAc, washed with water, dried ($MgSO_4$), filtered and concentrated. Silica column chromatography (0 $\rightarrow$ 10% EtOAc in PE) afforded pure compound **26** (9.76 g, 49 mmol) as an oil in 98% yield. R_f = 0.7 (3:1 PE:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.89 (s, 1H, COH), 7.99 - 7.70 (m, 2H, 2 x CH_{ar}), 7.10 - 6.87 (m, 2H, 2 x CH_{ar}), 4.21 (t, J = 5.9 Hz, 2H, CH_2), 3.76 (t, J = 6.2 Hz, 2H, CH_2), 2.28 (p, J = 6.0 Hz, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ 190.87, 163.80, 132.10, 130.19, 114.85, 64.70, 41.32, 32.08. FT-IR (thin film) υ 1686, 1600, 1578, 1508, 1253, 1160 cm^{-1}.



4-(3-azidopropoxy)benzaldehyde (27).[31] Compound **26** (9.65 g, 48.7 mmol) was dissolved in DMF (50 mL). Sodium azide (3.9 g, 60 mmol, 1.2 eq) was added and the mixture heated to 95 °C for 16 h. The solvent was evaporated under reduced pressure, the residue was taken up in DCM (50 mL), washed with water and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica column chromatography (0 $\rightarrow$ 13% EtOAc in PE) resulting in compound **27** as a yellowish oil in 88% yield (8.87 g, 43 mmol). R_f = 0.55 (4:1 PE:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.86 (s, 1H, COH), 7.82 (d, textitJ = 7.9 Hz, 2H, 2 x CH_{ar}), 6.99 (d, J = 7.8 Hz, 2H, 2 x CH_{ar}), 4.12 (d, J = 5.4 Hz, 2H, CH_2), 3.53 (d, J = 6.0 Hz, 2H, CH_2), 2.08 (d, J = 5.8 Hz, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ 190.62, 163.56, 131.84, 129.95, 114.63, 64.81, 47.91, 28.45. FT-IR (thin film) υ 2094 (N_3), 1684, 1598, 1576, 1508, 1250, 1157 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{10}H_{11}N_3O_2 + H]^+$ 206.09240; obsd. 206.09220.



4-((1-methylacetate)oxy)-benzaldehyde (28).[32] To a solution of 4-hydroxybenzaldehyde (6.1 g, 50 mmol) in acetone (125 mL) potassium carbonate (11 g, 80 mmol, 1.6 eq) was added and the mixture was stirred vigorously. Methylbromoacetate (5.7 mL, 60 mmol, 1.2 eq) was added and the mixture was stirred for 3.5 h at room temperature. The mixture was concentrated *in vacuo*, dissolved in EtOAc, washed with H_2O, dried (Na_2SO_4), filtered and concentrated under reduced pressure yielding the product as a colorless oil that solidified over time (9.7 g, 50 mmol, quant). R_f = 0.4 (6:1 toluene:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.90 (s, 1H, HCO), 7.85 (d, J = 8.8 Hz, 2H, 2 x CH_{ar}), 7.01 (d, J = 8.8 Hz, 2H, 2 x CH_{ar}), 4.73 (s, 2H, CH_2), 3.82 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 190.68, 168.50, 162.53, 131.97, 130.78, 114.87, 65.08, 52.45. FT-IR (thin film) υ 2959, 1749, 1693, 1682, 1600, 1580, 1508, 1207, 1163, 1080, 820, 613 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{10}H_{10}O_4 + H]^+$ 195.06519; obsd. 195.06509.



a: R_1=H
b: R_1=Me_2N
c: R_1=MeNEt
d: R_1=piperidino
e: R_1=Et_2N

General procedure for the synthesis of monofunctional BODIPYs (29 and 30a-e).
BODIPY **24a-e** (∼0.15 mmol) was dissolved in dry ethanol (0.7 mL). Benzaldehyde **25** or **27** (1 eq) was added, followed by acetic acid (10 eq) and pyrrolidine (10 eq). The mixture was put under an argon atmosphere before it was subjected to microwave irradiation (10 min, 130 °C, 1 min pre-stirring). After removal of the solvent under reduced pressure the mixture was purified by silica column chromatography (2 (starting material) → 4 (product) → 10% (double substituted) acetone in PE) In most cases 40-50% unreacted starting material could be recovered. Mono-substituted products compound-pHBDPalk**a-e** and **30a-e** were crystallized from DCM/hexanes to give purple crystals. R_f = 0.35 (4:1 PE: EtOAc)

1, 5, 7-Trimethyl-3-[4-(propargyloxy)styryl] -8-phenyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (29a). Yield: 16 mg, 0.035 mmol, 23%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (s, 3H, 2 x CH_{ar}, CH=), 7.48 (s, 3H, 3 x CH_{ar}), 7.30 (s, 2H, 2 x CH_{ar}), 7.19 (d, J = 15.9 Hz, 1H, CH=), 6.98 (s, 2H, 2 x CH_{ar}), 6.58 (s, 1H, CH), 6.00 (s, 1H, CH), 4.72 (s, 2H, CH_2), 2.59 (s, 3H, CH_3), 2.54 (s, 1H, CH), 1.42 (s, 3H, CH_3), 1.38 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.24, 154.95, 153.11, 142.52, 140.17, 135.66, 135.13, 132.80, 131.74, 130.26, 129.08, 128.92, 128.21, 121.18, 117.56, 117.46, 115.19, 78.26, 75.80, 55.86, 14.72, 14.60, 14.36. FT-IR (thin film) υ 2965, 1597, 1535, 1493, 1296, 1196, 1173, 938, 725 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{29}H_{25}BF_2N_2O + H]^+$ 467.21008; obsd. 467.20989.

1, 5, 7-Trimethyl-3-[4-(propargyloxy)styryl] -8-[4-(*N,N*-dimethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (29b). Yield: 16 mg, 0.031 mmol, 21%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 - 7.51 (m, 3H, 2 x CH_{ar}, CH=), 7.17 (d, J = 16.3 Hz, 1H, CH=), 7.08 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.97 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.78 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.58 (s, 1H, CH), 5.99 (s, 1H, CH), 4.72 (d, J = 2.3 Hz, 2H, CH_2), 3.02 (s, 6H, 2 x NCH_3), 2.58 (s, 3H, CH_3), 2.54 (s, 1H, CH), 1.53 (s, 3H, CH_3), 1.50 (s, 3H, CH_3).^{13}C NMR (101 MHz, $CDCl_3$): δ 158.23, 154.54, 152.62, 150.81, 142.89, 142.79, 141.86, 135.18, 133.70, 132.68, 130.55, 129.09, 128.96, 122.44, 121.00, 117.92, 117.22, 115.30, 112.44, 78.43, 75.90, 56.01, 40.49, 15.09, 14.86. FT-IR (thin film) υ 2887, 1614, 1523, 1462, 1232, 1198, 1157, 1045, 818 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{31}H_{30}BF_2N_3O + H]^+$ 510.25228; obsd. 510.25240.

1, 5, 7-Trimethyl-3-[4-(propargyloxy)styryl] -8-[4-(*N,N*-ethylmethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (29c). Yield: 15 mg, 0.029 mmol, 19%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 - 7.50 (m, 3H, 2 x CH_{ar}, CH=), 7.17 (d, J = 16.3 Hz, 1H, CH=), 7.06 (d, J = 8.5 Hz, 2H, 2 x CH_{ar}), 6.97 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.77 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.58 (s, 1H, CH), 5.99 (s, 1H, CH), 4.72 (d, J = 2.0 Hz, 2H, CH_2), 3.46 (q, J = 6.9 Hz, 2H, NCH_2), 2.96 (s, 3H, NCH_3), 2.58 (s, 3H, CH_3), 2.54 (s, 1H, CH), 1.55 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.16 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.21, 154.48, 152.56, 149.50, 142.88, 142.80, 141.97, 135.14, 133.70, 132.70, 130.55, 129.18, 128.94, 122.05, 120.98, 117.91, 117.21, 115.28, 112.46, 78.43, 75.90, 55.99, 46.93, 37.53, 15.07, 14.84, 11.19. FT-IR (thin film) υ 2924, 1734, 1463, 1373, 1265, 1242, 1045, 734, 704 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{32}H_{32}BF_2N_3O + H]^+$ 524.26793; obsd. 524.26814.

1, 5, 7-Trimethyl-3-[4-(propargyloxy)styryl] -8-[4-(piperidino)phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (29d). Yield: 11 mg, 0.02 mmol, 16%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 - 7.54 (m, 3H, 2 x CH_{ar}, CH=), 7.21 (d, J = 16.3 Hz, 1H, CH=), 7.13 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 7.06 - 6.97 (m, 4H, 4 x CH_{ar}), 6.60 (s, 1H, CH), 6.02 (s, 1H, CH), 4.75 (d, J = 2.3 Hz, 2H, CH_2), 3.31 - 3.21 (m, 4H, 2 x NCH_2), 2.61 (s, 3H, CH_3), 2.57 (t, J = 2.3 Hz, 1H, CH), 1.82 - 1.72 (m, 4H, 2 x CH_2), 1.69 - 1.60 (m, 2H, CH_2), 1.55 (s, 3H, CH_3), 1.51 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.12, 154.53, 152.63, 152.45, 142.71, 141.24, 135.20, 133.31, 132.34, 130.36, 128.88, 128.84, 124.91, 120.94, 117.70, 117.17, 116.19, 115.15, 78.28, 75.79, 55.86, 50.11, 25.65, 24.26, 14.91, 14.67. FT-IR (thin film) υ 2926, 1735, 1458, 1373, 1236, 1157, 1045, 985, 736 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{34}H_{34}BF_2N_3O + H]^+$ 550.28358; obsd. 550.28326.

1, 5, 7-Trimethyl-3-[4-(propargyloxy)styryl] -8-[4-(*N,N*-diethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (29e). Yield: 22 mg, 0.041 mmol, 27%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 - 7.53 (m, 3H, 2 x CH_{ar}, CH=), 7.17 (d, J = 16.3 Hz, 1H, CH=), 7.03 (d, J = 8.4 Hz, 2H, 2 x CH_{ar}), 6.97 (d, J = 8.5 Hz, 2H, 2 x CH_{ar}), 6.74 (d, J = 8.5 Hz, 2H, 2 x CH_{ar}), 6.58 (s, 1H, CH), 5.99 (s, 1H, CH), 4.72 (d, J = 1.8 Hz, 2H, CH_2), 3.40 (q, J = 7.0 Hz, 4H, 2 x NCH_2), 2.58 (s, 3H, CH_3), 2.54 (s, 1H, CH), 1.56 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.20 (t, J = 7.0 Hz, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.22, 154.44, 152.51, 148.34, 142.91, 142.81, 142.17, 135.07, 133.74, 132.75, 130.60, 129.28, 128.94, 121.48, 120.97, 117.97, 117.19, 115.31, 112.10, 111.87, 78.45, 75.89, 56.01, 44.50, 15.10, 14.88, 12.54. FT-IR (thin film) υ 2974, 1604, 1487, 1196, 1159, 986, 808 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{33}H_{34}BF_2N_3O + H]^+$ 538.28358; obsd. 538.28383.

1, 5, 7-Trimethyl-3-[4-((3-azidopropyl)oxy)styryl] -8-phenyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (30a). Yield: 26 mg, 0.051 mmol, 35%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 - 7.42 (m, 6H, 5 x CH_{ar}, CH=), 7.33 - 7.27 (m, 2H, 2 x CH_{ar}), 7.19 (d, J = 16.3 Hz, 1H, CH=), 6.89 (d, J = 8.5 Hz, 2H, 2 x CH_{ar}), 6.58 (s, 1H, CH), 5.99 (s, 1H, CH), 4.08 (t, J = 5.9 Hz, 2H, CH_2), 3.53 (t, J = 6.6 Hz, 2H, CH_2), 2.59 (s, 3H, CH_3), 2.11 - 2.01 (m, 2H, CH_2), 1.42 (s, 3H, CH_3), 1.38 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.63, 154.89, 153.46, 142.74, 142.51, 140.21, 136.07, 135.29, 132.97, 131.83, 129.78, 129.21, 129.18, 129.05, 128.37, 121.26, 117.61, 117.32, 114.90, 64.76, 48.36, 28.91, 14.84, 14.73, 14.47. FT-IR (thin film) υ 2927, 2097 (N_3), 1599, 1537, 1497, 1196, 1173, 1157, 986 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{29}H_{28}BF_2N_5O + H]^+$ 512.24277; obsd. 512.24259.

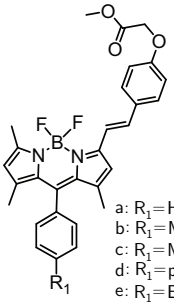
1, 5, 7-Trimethyl-3-[4-((3-azidopropyl)oxy)styryl] -8-[4-(*N,N*-dimethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (30b). Yield: 16 mg, 0.029 mmol, 20%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 - 7.48 (m, 3H, 2 x CH_{ar} , CH=), 7.17 (d, J = 16.3 Hz, 1H, CH=), 7.08 (d, J = 8.6 Hz, 2H,

2 x CH_{ar}), 6.88 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.78 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.57 (s, 1H, CH), 5.99 (s, 1H, CH), 4.08 (t, J = 5.9 Hz, 2H, CH_2), 3.53 (t, J = 6.6 Hz, 2H, CH_2), 3.02 (s, 6H, 2 x NCH_3), 2.59 (s, 3H, CH_3), 2.11 - 1.99 (m, 2H, CH_2), 1.53 (s, 3H, CH_3), 1.50 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.47, 154.33, 152.81, 150.80, 142.85, 142.73, 141.76, 135.45, 133.69, 132.61, 129.90, 129.09, 129.07, 122.45, 120.93, 117.51, 117.23, 114.85, 112.43, 64.73, 48.36, 40.46, 28.90, 15.08, 14.82. FT-IR (thin film) υ 2926, 2096 (N_3), 1600, 1537, 1524, 1494, 1196, 1173, 1159, 986 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{31}H_{33}BF_2N_6O + H]^+$ 555.28497; obsd. 555.28508.

1, 5, 7-Trimethyl-3-[4-((3-azidopropyl)oxy)styryl] -8-[4-(*N,N*-ethylmethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (30c). Yield: 19 mg, 0.033 mmol, 22%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 - 7.49 (m, 3H, 2 x CH_{ar} , CH=), 7.17 (d, J = 16.3 Hz, 1H, CH=), 7.06 (d, J = 8.5 Hz, 2H, 2 x CH_{ar}), 6.88 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.77 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.58 (s, 1H, CH), 5.99 (s, 1H, CH), 4.08 (t, J = 5.9 Hz, 2H, CH_2), 3.53 (t, J = 6.6 Hz, 2H, CH_2), 3.46 (q, J = 7.0 Hz, 2H, NCH_2), 2.96 (s, 3H, NCH_3), 2.59 (s, 3H, CH_3), 2.06 (m, 2H, CH_2), 1.55 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.16 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.48, 154.30, 152.78, 149.53, 142.86, 142.75, 141.89, 135.42, 133.74, 132.66, 129.93, 129.21, 129.07, 122.10, 120.93, 117.55, 117.23, 114.87, 112.47, 64.75, 48.37, 46.94, 37.53, 28.92, 15.08, 14.83, 11.19. FT-IR (thin film) υ 2926, 2096 (N_3), 1601, 1537, 1524, 1510, 1495, 1198, 1173, 1159, 986 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{32}H_{35}BF_2N_6O + H]^+$ 569.30062; obsd. 569.30086.

1, 5, 7-Trimethyl-3-[4-((3-azidopropyl)oxy)styryl] -8-[4-(piperidino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (30d). Yield: 12 mg, 0.020 mmol, 17%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 - 7.49 (m, 3H, 2 x CH_{ar}, CH=), 7.17 (d, J = 16.3 Hz, 1H, CH=), 7.10 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 7.01 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.89 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.57 (s, 1H, CH), 5.99 (s, 1H, CH), 4.08 (t, J = 5.9 Hz, 2H, CH_2), 3.53 (t, J = 6.6 Hz, 2H, CH_2), 3.27 - 3.19 (m, 4H, 2 x NCH_2), 2.58 (s, 3H, CH_3), 2.11 - 2.00 (m, 2H, CH_2), 1.79 - 1.70 (m, 4H, 2 x CH_2), 1.64 - 1.59 (m, 2H, CH_2), 1.52 (s, 3H, CH_3), 1.48 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.50, 154.45, 152.95, 152.57, 142.83, 142.69, 141.27, 135.60, 133.50, 132.41, 129.86, 129.09, 129.03, 125.05, 121.00, 117.45, 117.31, 116.32, 114.86, 64.73, 50.25, 48.36, 28.91, 25.78, 24.39, 15.04, 14.83, 14.78. FT-IR (thin film) υ 2927, 2096 (N_3), 1600, 1539, 1496, 1197, 1172, 985 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{34}H_{37}BF_2N_6O + H]^+$ 595.31627; obsd. 595.31622.

1, 5, 7-Trimethyl-3-[4-((3-azidopropyl)oxy)styryl] -8-[4-(*N,N*-diethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (30e). Yield: 18 mg, 0.031 mmol, 21%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 - 7.50 (m, 3H, 2 x CH_{ar} , CH=), 7.17 (d, J = 16.3 Hz, 1H, CH=), 7.03 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.88 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.74 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.58 (s, 1H, CH), 5.99 (s, 1H, CH), 4.07 (t, J = 5.9 Hz, 2H, CH_2), 3.53 (t, J = 6.6 Hz, 2H, CH_2), 3.40 (q, J = 7.0 Hz, 4H, 2 x NCH_2), 2.59 (s, 3H, CH_3), 2.11 - 2.02 (m, 2H, CH_2), 1.56 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.20 (t, J = 7.0 Hz, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.43, 154.19, 152.67, 148.29, 142.85, 142.74, 142.05, 135.34, 133.75, 132.67, 129.90, 129.25, 129.05, 121.44, 120.88, 117.52, 117.18, 114.83, 112.04, 64.71, 48.35, 44.48, 28.90, 15.12, 14.87, 12.52. FT-IR (thin film) υ 2930, 2097 (N_3), 1601, 1540, 1522, 1510, 1497, 1198, 1173, 1159, 988 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{33}H_{37}BF_2N_6O + H]^+$ 583.31627; obsd. 583.31633.



General procedure for the synthesis of monofunctional BODIPYs (31a-e). BODIPY **24a-e** (∼0.15 mmol) was dissolved in dry methanol (0.7 mL). Benzaldehyde **28** (1 eq) was added, followed by acetic acid (10 eq) and pyrrolidine (10 eq). The mixture was put under an argon atmosphere before it was subjected to microwave irradiation (10 min, 110 °C, 1 min pre-stirring). After removal of the solvent under reduced pressure the mixture was purified by silica column chromatography (2 (starting material) → 4 (product) → 10% (double substituted) acetone in PE) In most cases 40-50% unreacted starting material could be recovered. Mono-substituted products **31a-e** were crystallized from DCM/hexanes to give purple crystals. R_f = 0.30 (4:1 PE: EtOAc)

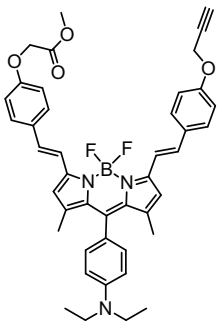
1, 5, 7-Trimethyl-3-[4-((1-methylacetate)oxy)styryl] -8-phenyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (31a). Yield: 16 mg, 0,032 mmol, 21%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 - 7.52 (m, 3H, 2 x CH_{ar} , CH=), 7.51 - 7.45 (m, 3H, 3 x CH_{ar}), 7.32 - 7.28 (m, 2H, 2 x CH_{ar}), 7.18 (d, J = 16.3 Hz, 1H, CH=), 6.91 (d, J = 8.8 Hz, 2H, 2 x CH_{ar}), 6.58 (s, 1H, CH), 6.00 (s, 1H, CH), 4.66 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 2.59 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.38 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.24, 158.52, 155.17, 153.12, 142.73, 142.65, 140.35, 135.60, 135.22, 132.91, 131.88, 130.62, 129.22, 129.13, 129.06, 128.32, 121.35, 117.82, 117.58, 115.02, 65.42, 52.48, 14.85, 14.72, 14.49. FT-IR (thin film) v 2924, 1767 (C=O), 1597, 1535, 1492, 1196, 1173, 1155, 1061, 976 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{29}H_{27}BF_2N_2O_3 + H]^+$ 501.21556; obsd. 501.21548.

1, 5, 7-Trimethyl-3-[4-((1-methylacetate)oxy)styryl] -8-[4-(*N,N*-dimethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (31b). Yield: 12 mg, 0.022 mmol, 17%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 - 7.50 (m, 3H, 2 x CH_{ar}, CH=), 7.16 (d, J = 16.3 Hz, 1H, CH=), 7.08 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.90 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.78 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.57 (s, 1H, CH), 5.99 (s, 1H, CH), 4.67 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 3.02 (s, 6H, 2 x NCH_3), 2.58 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.50 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.18, 158.25, 154.50, 152.28, 150.67, 142.82, 142.60, 141.78, 134.85, 130.66, 128.94, 128.91, 122.28, 120.90, 117.93, 117.06, 114.87, 112.30, 65.32, 52.35, 40.34, 14.95, 14.73. FT-IR (thin film) v 2916, 1767 (C=O), 1597, 1508, 1485, 1194, 1171, 1080, 986 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{31}H_{32}BF_2N_3O_3 + H]^+$ 544.25776; obsd. 544.25758.

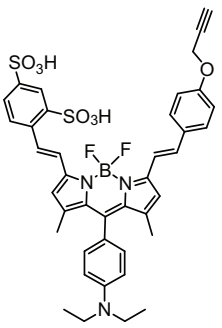
1, 5, 7-Trimethyl-3-[4-((1-methylacetate)oxy)styryl] -8-[4-(*N,N*-ethylmethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (31c). Yield: 9 mg, 0.017 mmol, 13%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 - 7.51 (m, 3H, 2 x CH_{ar}, CH=), 7.16 (d, J = 16.3 Hz, 1H, CH=), 7.06 (d, J = 8.5 Hz, 2H, 2 x CH_{ar}), 6.90 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.77 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.57 (s, 1H, CH), 5.99 (s, 1H, CH), 4.67 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 3.46 (q, J = 7.0 Hz, 2H, NCH_2), 2.97 (s, 3H, NCH_3), 2.58 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 1.16 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.30, 158.38, 154.60, 152.45, 149.52, 142.99, 142.77, 142.03, 134.95, 130.80, 129.18, 129.04, 122.04, 121.03, 118.08, 117.20, 115.00, 112.46, 65.46, 52.49, 46.94, 37.54, 15.07, 14.85, 11.20. FT-IR (thin film) v 2924, 1761 (C=O), 1521, 1508, 1197, 1174, 1080, 987 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{32}H_{34}BF_2N_3O_3 + H]^+$ 558.27341; obsd. 558.27325.

1, 5, 7-Trimethyl-3-[4-((1-methylacetate)oxy)styryl] -8-[4-(piperidino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (31d). Yield: 17 mg, 0.029 mmol, 23%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 - 7.51 (m, 3H, 2 x CH_{ar} , CH=), 7.17 (d, J = 16.3 Hz, 1H, CH=), 7.10 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 7.01 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.90 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.57 (s, 1H, CH), 5.99 (s, 1H, CH), 4.67 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 3.27 - 3.19 (m, 4H, 2 x NCH_2), 2.58 (s, 3H, CH_3), 1.75 (m, 4H, 2 x CH_2), 1.66 - 1.59 (m, 2H, CH_2), 1.52 (s, 3H, CH_3), 1.48 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.28, 158.43, 154.79, 152.60, 142.94, 142.76, 141.45, 135.14, 132.52, 130.77, 129.07, 129.02, 125.03, 121.12, 118.03, 117.29, 116.32, 115.02, 65.47, 52.49, 50.25, 25.79, 24.41, 15.03, 14.81. FT-IR (thin film) υ 2924, 1764 (C=O), 1595, 1489, 1298, 1157, 1080, 989 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{34}H_{36}BF_2N_3O_3 + H]^+$ 584.28906; obsd. 584.28900.

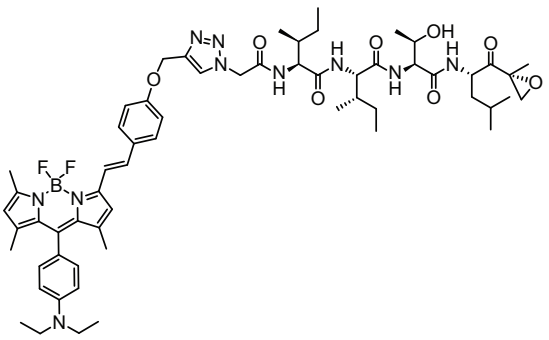
1, 5, 7-Trimethyl-3-[4-((1-methylacetate)oxy)styryl] -8-[4-(*N,N*-diethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (31e). Yield: 11 mg, 0.019 mmol, 15%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 - 7.49 (m, 3H, 2 x CH_{ar} , CH=), 7.16 (d, J = 16.3 Hz, 1H, CH=), 7.03 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 6.90 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.74 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.57 (s, 1H, CH), 5.99 (s, 1H, CH), 4.67 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 3.40 (q, J = 7.0 Hz, 4H, 2 x NCH_2), 2.58 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.20 (t, J = 7.0 Hz, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.29, 158.37, 154.52, 152.37, 148.33, 142.98, 142.22, 134.88, 130.82, 129.26, 129.03, 121.43, 121.00, 118.11, 117.17, 115.00, 112.08, 65.46, 52.48, 44.50, 15.10, 14.89, 12.53. FT-IR (thin film) υ 2924, 1765 (C=O), 1597, 1524, 1510, 1492, 1194, 1159, 1078, 988 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{33}H_{36}BF_2N_3O_3 + H]^+$ 572.28906; obsd. 572.28959.



1,7-dimethyl-3-[4-(propargyloxy) styryl] -5-[4-((1-methylacetate)oxy) styryl] -8-[4-(*N,N*-diethylamino) phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (32). BODIPY **29e** (16 mg, 0.03 mmol) and benzaldehyde **28** (29 mg, 0.15 mmol, 5 eq) were dissolved in methanol (0.5 mL) and acetic acid (17 µL, 0.3 mmol, 10 eq) and pyrrolidine (25 µL, 0.3 mmol, 10 eq) were added. The mixture was put under an argon atmosphere before it was subjected to microwave irradiation (10 min, 110 °C, 1 min pre-stirring). After removal of the solvent under reduced pressure the mixture was purified by repeated silica column chromatography (3 (starting material) → 10% (product) acetone in PE and 0 → 0.25% methanol in DCM). After crystallization (DCM/hexanes) product **32** was obtained as blue crystals in 42% yield (9 mg, 0.013 mmol). R_f = 0.7 (1:1 PE:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 - 7.54 (m, 6H, 4 x CH_{ar} , 2 x CH=), 7.19 (d, J = 16.3, 1H, CH=), 7.18 (d, J = 16.3, 1H, CH=), 7.05 (d, J = 8.6 Hz, 2H, 2 x CH_{ar}), 7.01 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.93 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.75 (d, J = 8.7 Hz, 2H, 2 x CH_{ar}), 6.60 (s, 2H, 2 x CH), 4.74 (d, J = 2.3 Hz, 2H, CH_2), 4.68 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.41 (q, J = 7.0 Hz, 4H, 2 x NCH_2), 2.55 (t, J = 2.3 Hz, 1H, CH), 1.58 (s, 6H, 2 x CH_3), 1.21 (t, J = 7.0 Hz, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.32, 158.37, 158.22, 152.25, 152.05, 148.31, 142.37, 142.26, 140.48, 135.06, 134.80, 134.19, 130.94, 130.68, 129.49, 129.08, 129.00, 121.55, 118.27, 118.09, 117.31, 115.32, 115.03, 112.05, 78.46, 75.92, 65.47, 56.01, 52.50, 44.51, 15.12, 12.54. FT-IR (thin film) υ 2926, 1755 (C=O), 1595, 1510, 1450, 1196, 1157, 986 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{43}H_{42}BF_2N_3O_4 + H]^+$ 714.33092; obsd. 714.33139.



1,7-dimethyl-3-[4-(propargyloxy) styryl] -5-[styryl-2,4-disulfonic acid]-8-[4-(*N,N*-diethylamino)phenyl] -4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (33). BODIPY **29e** (27 mg, 0.05 mmol) was dissolved in ethanol (0.7 mL). 4-Formyl-1,3-benzenedisulfonic acid, disodium salt hydrate (78 mg, 0.25 mmol, 5 eq) was added followed by acetic acid (41 µL, 0.5 mmol, 10 eq) and pyrrolidine (27 µL, 0.5 mmol, 10 eq). The mixture was put under an argon atmosphere before it was subjected to microwave irradiation (10 min, 130 °C, 1 min pre-stirring). After removal of the solvent under reduced pressure and co-evaporation with toluene, the mixture was purified by size exclusion chromatography (Sephadex, methanol) and crystallization from DCM/hexanes. This yielded blue compound **33** in 12% yield (5 mg, 6 µmol). R_f = 0.35 (1:1:1:1 n-BuOH:H_2O:EtOAc:AcOH). ^{1}H NMR (400 MHz, MeOD): δ 10.95 (s, 1H, SO_3H), 8.58 - 8.50 (m, 2H, CH_{ar}, SO_3H), 8.40 (d, J = 16.2 Hz, 1H, CH=), 8.05 - 7.97 (m, 2H, 2 x CH_{ar}), 7.76 (d, J = 16.5 Hz, 1H, CH=), 7.67 - 7.55 (m, 3H, 2 x CH_{ar} , CH=), 7.41 (d, J = 16.3 Hz, 1H, CH=), 7.14 - 7.04 (m, 4H, 4 x CH_{ar}), 6.91 - 6.86 (m, 3H, CH_{ar}, CH), 6.82 (s, 1H, CH), 4.81 (d, J = 1.9 Hz, 2H, CH_2), 3.48 (q, J = 7.0 Hz, 4H, 2 x NCH_2), 3.01 (t, J = 2.2 Hz, 1H, CH), 1.65 (s, 3H, CH_3), 1.64 (s, 3H, CH_3), 1.22 (t, J = 7.0 Hz, 6H, 2 x CH_3).^{13}C NMR (101 MHz, MeOD): δ 160.15, 154.93, 151.99, 149.83, 144.73, 142.89, 142.49, 137.77, 137.62, 132.98, 131.50, 130.53, 129.92, 128.88, 127.40, 126.79, 126.37, 122.26, 118.93, 116.47, 113.21, 77.02, 56.72, 45.36, 15.25, 15.05, 12.68. FT-IR (thin film) v 3600-3200 (br, OH), 2922, 1599, 1487, 1198, 1163, 1022, 991, 690 cm^{-1}. ESI-HRMS (m/z): calcd. for $[C_{40}H_{38}BF_2N_3O_7S_2 + H]^+$ 786.22851; obsd. 786.22818.



pH-dependent BODIPY-epoxomicin (34). BODIPY **29e** (7 mg, 0.013 mmol) and azido-epoxomicin[43] (8 mg, 0.014 mmol, 1.1 eq) were dissolved in toluene/*t*-BuOH/H_2O (1:1:1 v/v) and the solution was degassed (sonication) under argon. Sodium ascorbate (aq solution, 0.013 mmol, 1 eq) and copper sulfate (aq solution, 0.0013 mmol, 10 mol%) were added and the mixture was heated to 80 °C for 1 hour. TLC analysis showed complete conversion of the BODIPY dye, so the mixture was concentrated and purified by silica column chromatography (0 → 2.5% methanol in DCM). Additional Sephadex size exclusion (methanol) and recrystallization (DCM/hexanes) purification steps resulted in purple compound **34** in 89% yield (13 mg, 0.011 mmol). R_f = 0.2 (10:1 DCM:methanol). ^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ 7.95 (s, 1H, CH_{trz}), 7.60 - 7.50 (m, 3H, 2 x CH_{ar} , CH=), 7.21 (d, J = 16.4 Hz, 1H, CH=), 7.05 (d, J = 8.2 Hz, 2H, 2 x CH_{ar}), 7.00 (d, J = 8.3 Hz, 2H, 2 x CH_{ar}), 6.78 (d, J = 8.2 Hz, 2H, 2 x CH_{ar}), 6.62 (s, 1H, CH), 6.02 (s, 1H, CH), 5.23 (s, 2H, OCH_2), 5.15 (q, J = 12.6 Hz, 2H, CH_2), 4.54 (d, J = 10.6 Hz, 1H, CH), 4.26 - 4.18 (m, 2H, 2 x CH), 4.14 - 4.04 (m, 1H, CH), 3.90 (s, 1H, CH), 3.49 - 3.40 (m, 4H, 2 x NCH_2), 3.31 (d, J = 4.6 Hz, 1H, CH_2-H^a), 2.92 (d, J = 4.6 Hz, 1H, CH_2-H^b), 2.57 (s, 3H, CH_3), 1.84 (s, 2H, 2 x CH), 1.68 (s, 1H, CH), 1.64 - 1.44 (m, 11H, 3 x CH_3, 2 x CH_2-H^a), 1.39 - 1.26 (m, 2H, CH_2-H^b, OH), 1.21 (t, J = 6.7 Hz, 6H, 2 x CH_3), 1.14 (d, J = 6.1 Hz, 4H, CH_3 , CH_2-H^b), 0.99 - 0.79 (m, 18H, 6 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$/MeOD): δ 208.46, 171.81, 171.77, 170.32, 165.69, 158.68, 153.99, 152.27, 148.18, 143.73, 142.80, 142.01, 135.06, 133.44, 132.41,

130.02, 128.93, 128.70, 124.93, 121.05, 120.72, 117.21, 116.97, 114.88, 111.96, 66.84, 61.45, 59.04, 58.09, 57.60, 52.23, 51.92, 50.63, 44.21, 39.15, 36.68, 36.38, 24.97, 24.68, 24.63, 22.98, 20.75, 18.23, 16.45, 15.01, 14.95, 14.66, 14.43, 14.25, 12.03, 10.64, 10.59. ESI-HRMS (m/z): calcd. for $[C_{60}H_{81}BF_2N_{10}O_8 + H]^+$ 1119.63727; obsd. 1119.63914.

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