Electronic Supplementary Material (ESI) for RSC Advances.

A straightforward synthesis of phenyl boronic acid (PBA) containing BODIPY dyes: new functional and modular fluorescent tools for the tethering of the glycan domain of antibodies.

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Supporting Information

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Materials and Methods

Reagents were purchased from commercial suppliers and used without purification. ESI-MS mass spectra were recorded on a LCQ-Fleet Ion Trap equipped with a standard Ionspray interface from Thermo Scientific. NMR spectra were recorded on Varian Gemini 200, Mercury Plus 400, Inova 400, instruments. Elemental analyses were performed with a PerkinElmer series II CHNS/O 2400 Elementary Analyzer. $[\alpha]_D$ values were measured using a JASCO DIP-370 instrument. Melting points were measured with a Melting Point Büchi 510. Fluorescence spectra were recorded using a Perkin-Elmer Spectrophotofluorimeter LS-55, set at 10 nm bandwidth for both excitation and emission monochromator slits. Emission spectra have been corrected for the response of the photomultiplier. Fluorescence spectra (scalar fluorescence spectra) were recorded using JASCO FP_750 spectrofluorometer, absorbance spectra were recordered using Thermo Scientific EVOLUTION 220.

Absorbance and fluorescence spectra of PBA-BODIPY 1-4.

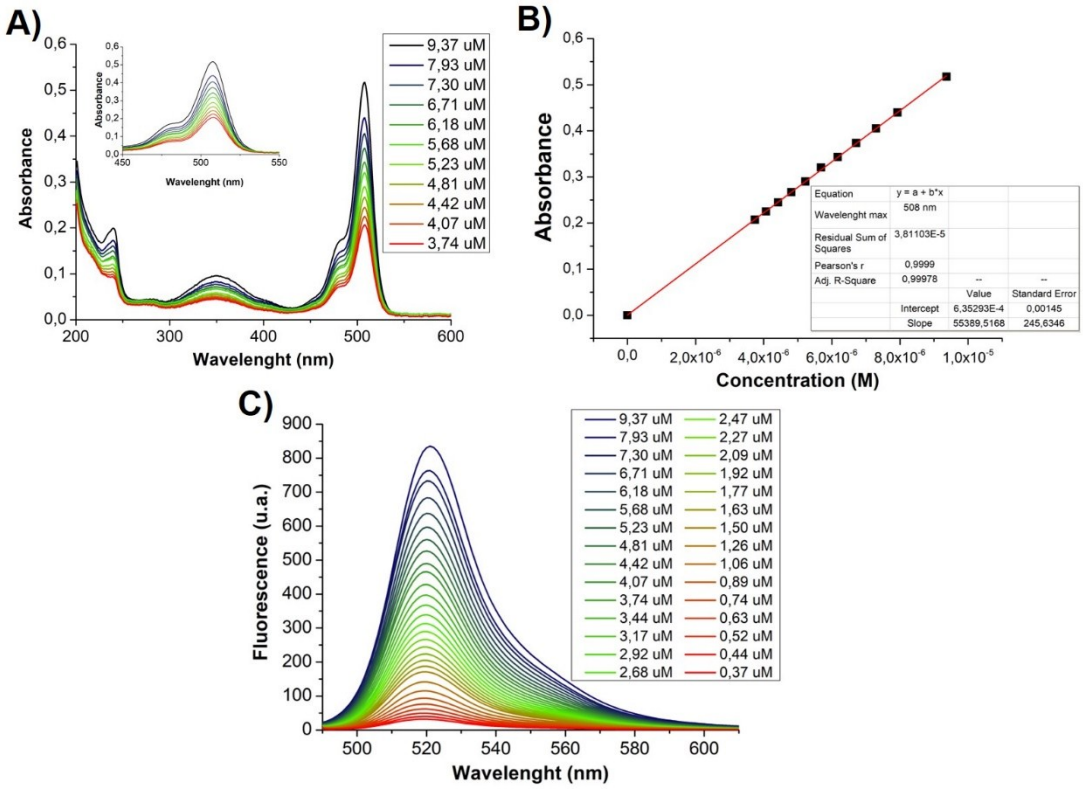

Figure S1. PBA-BODIPY **1** in Methanol: **A)** Scalar absorbance spectra; **B)** Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

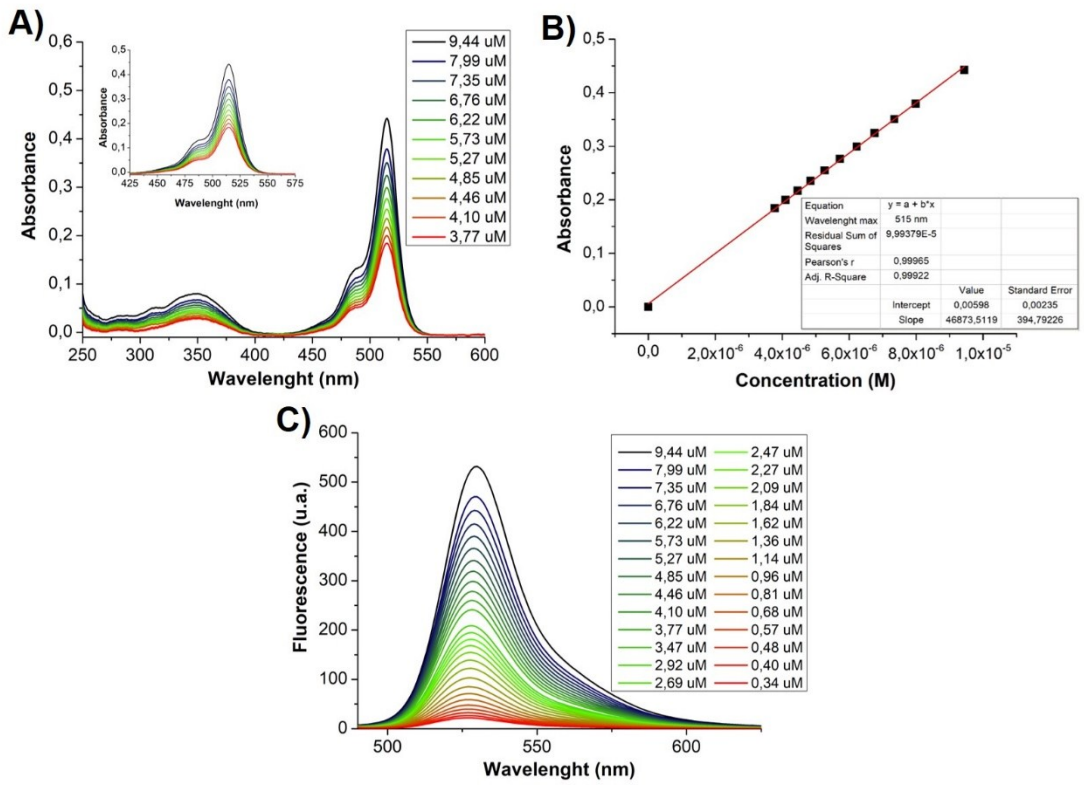

Figure S2. PBA-BODIPY **1** in Dichloromethane**: A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

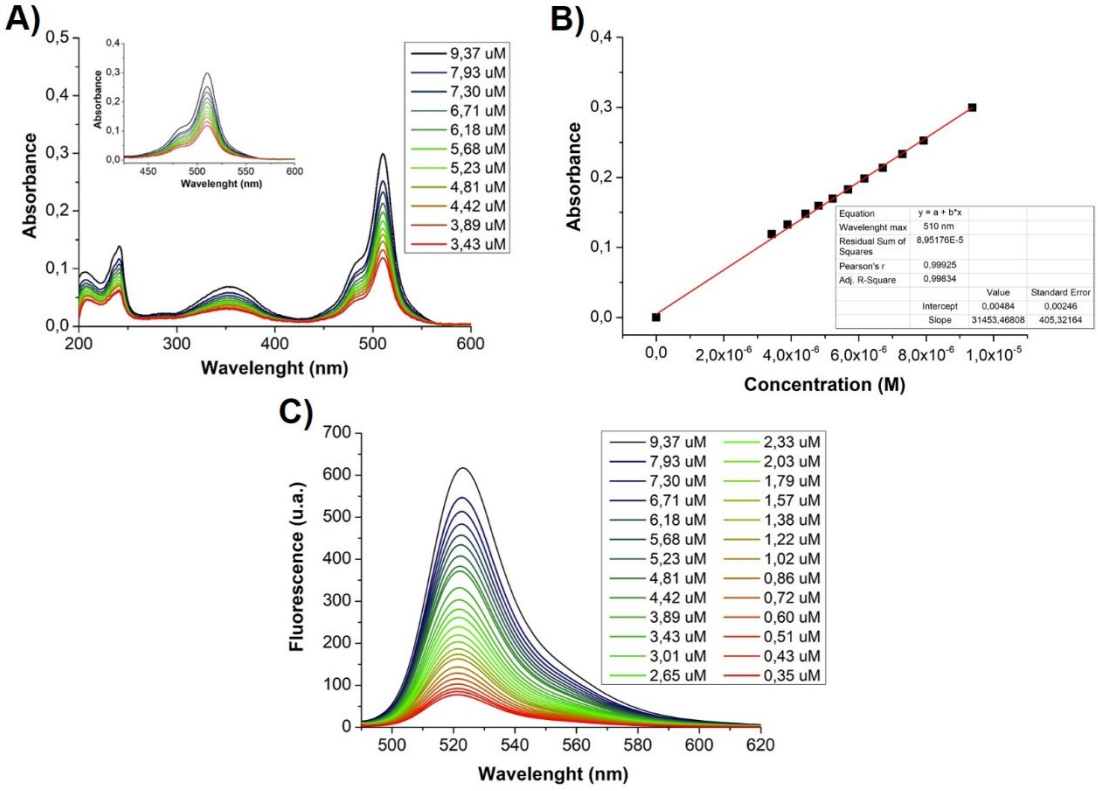

Figure S3. PBA-BODIPY **1** in physiological PBS pH = 7.4**: A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

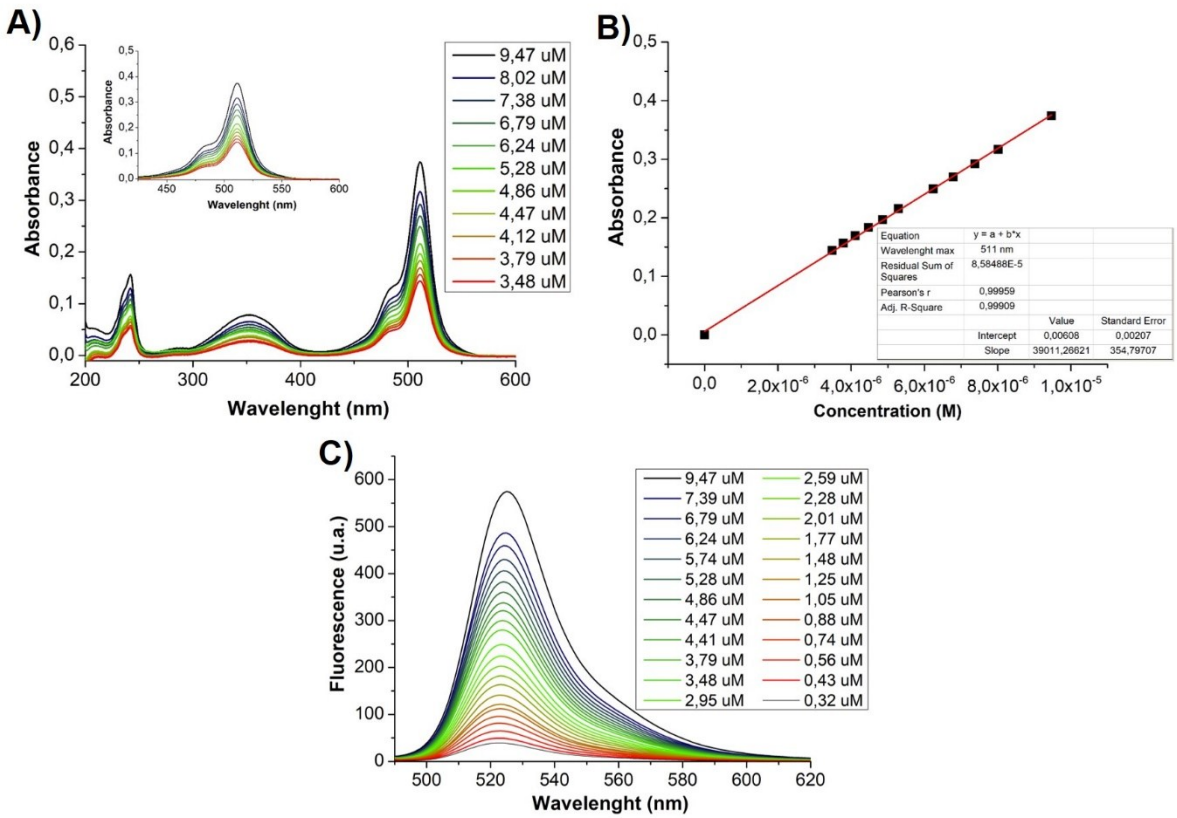

Figure S4. PBA-BODIPY **1** in H_2O**: A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

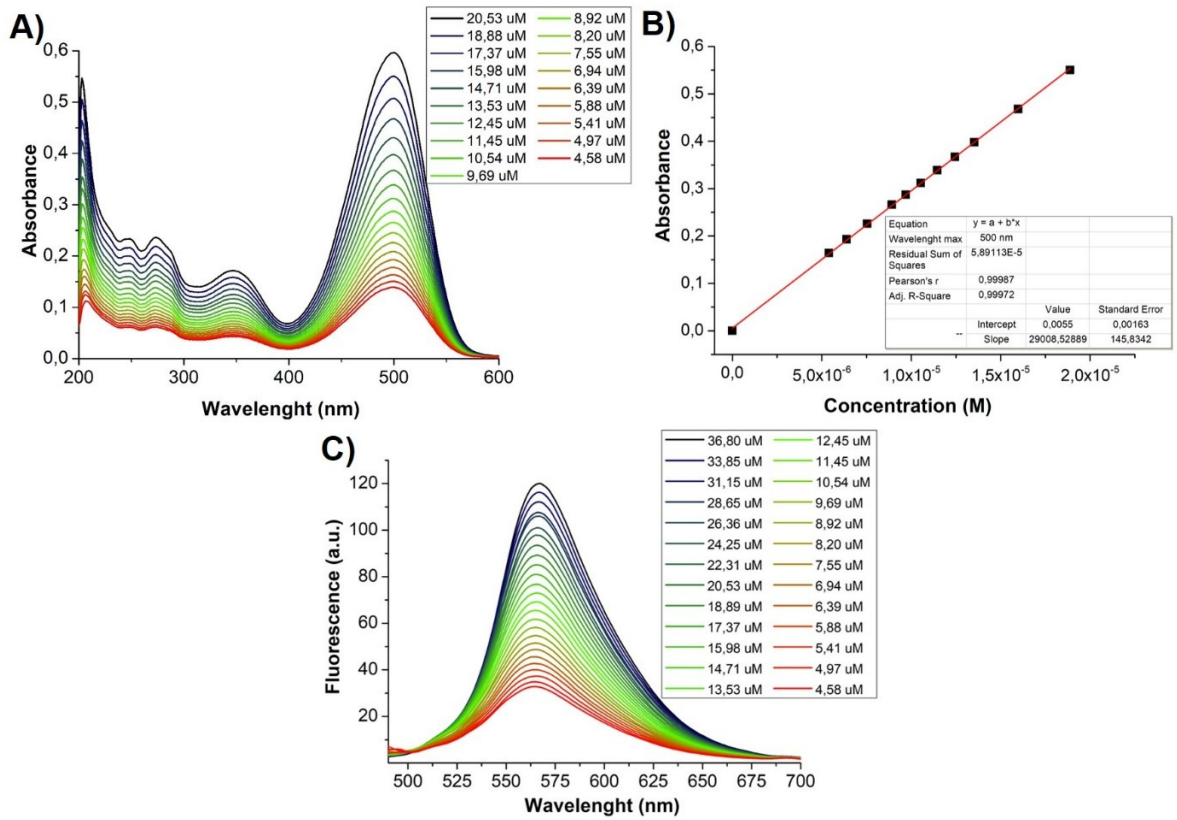

Figure S5. PBA-BODIPY **2** in Methanol: **A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

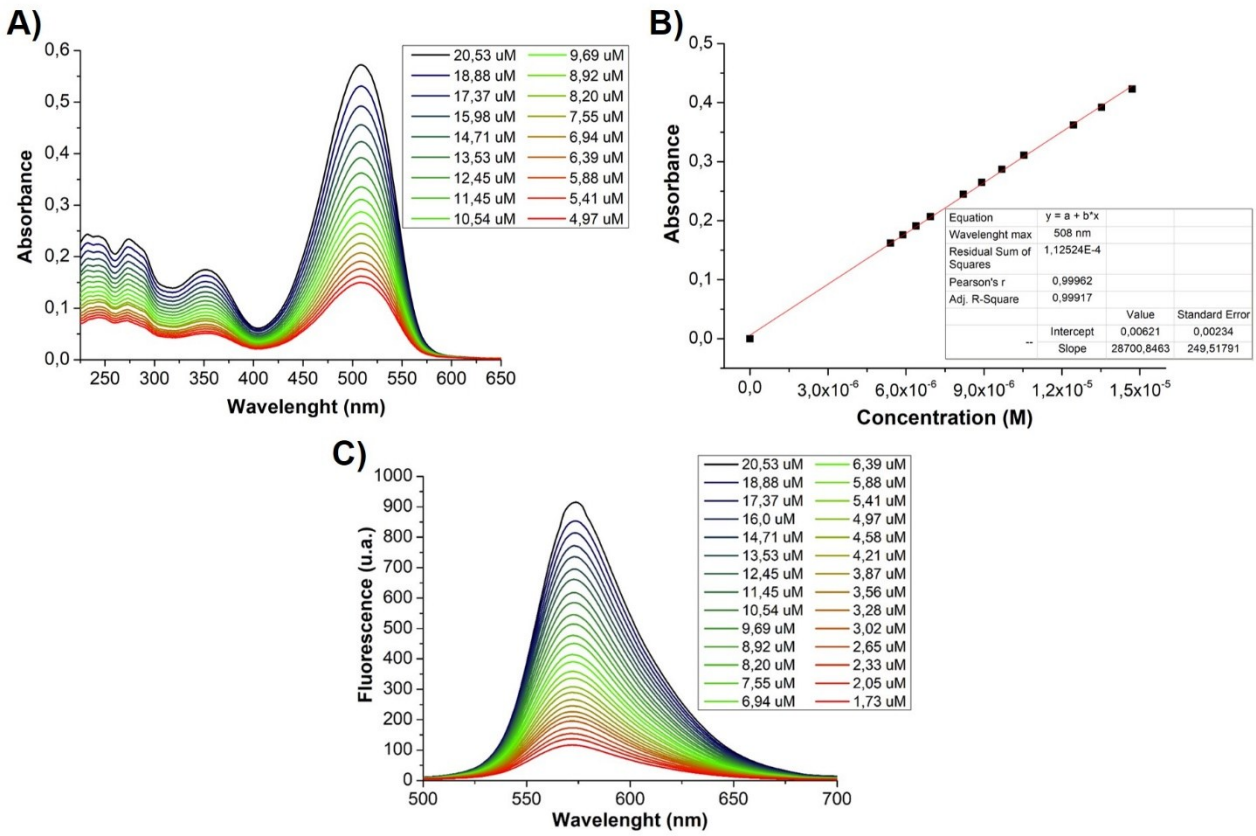

Figure S6. PBA-BODIPY **2** in Dichloromethane**: A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

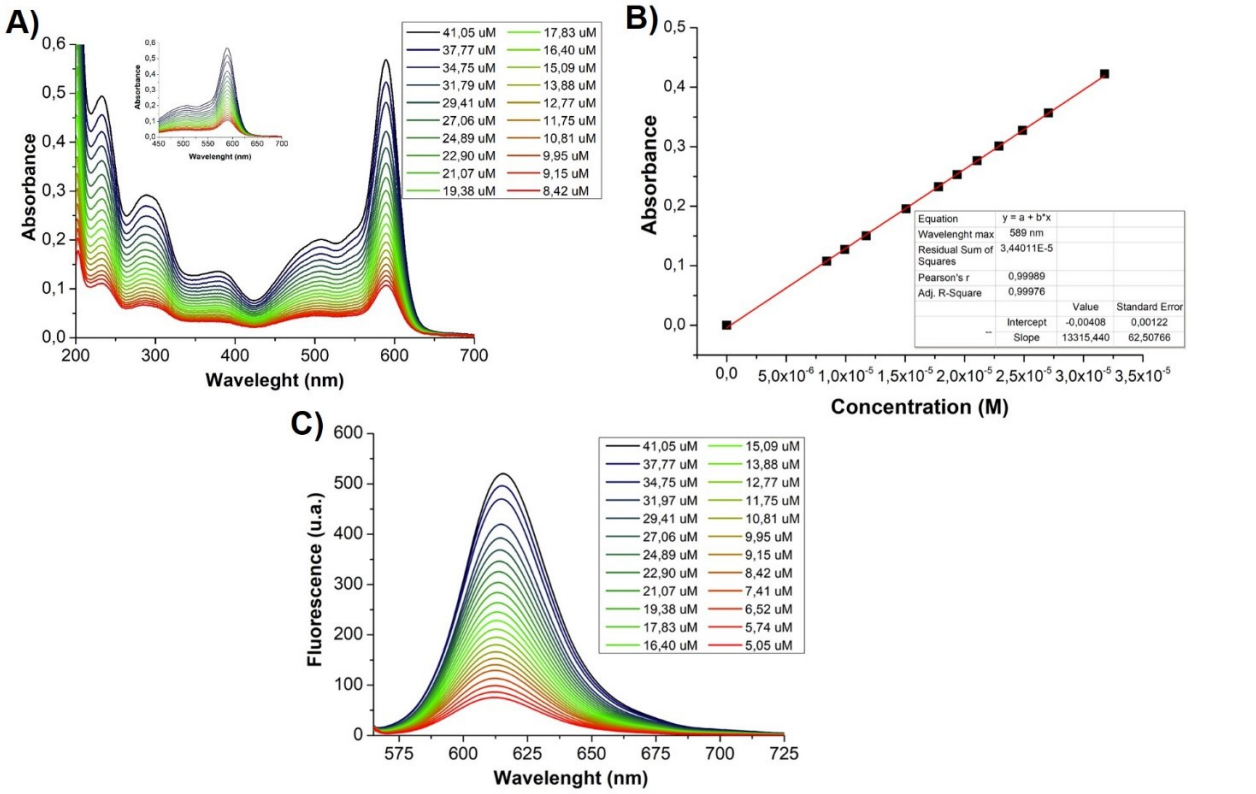

Figure S7. PBA-BODIPY **3** in Methanol: **A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

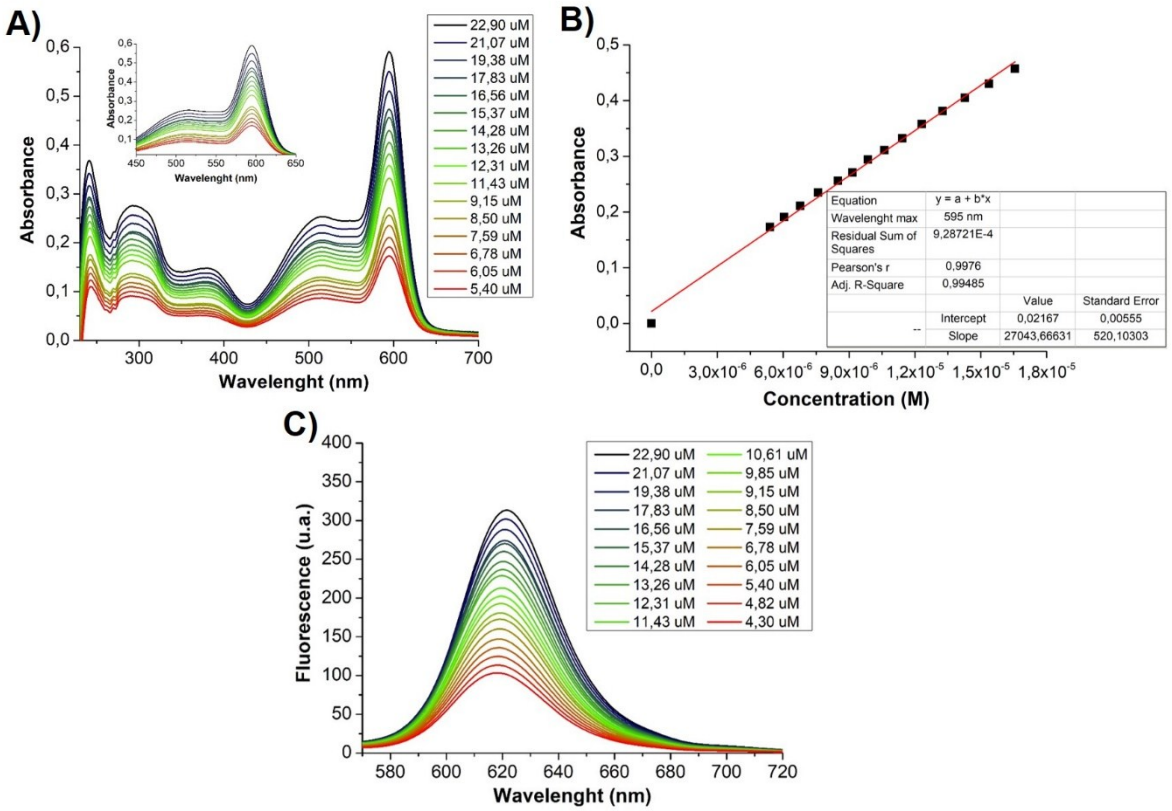

Figure S8. PBA-BODIPY **3** in Dichloromethane**: A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

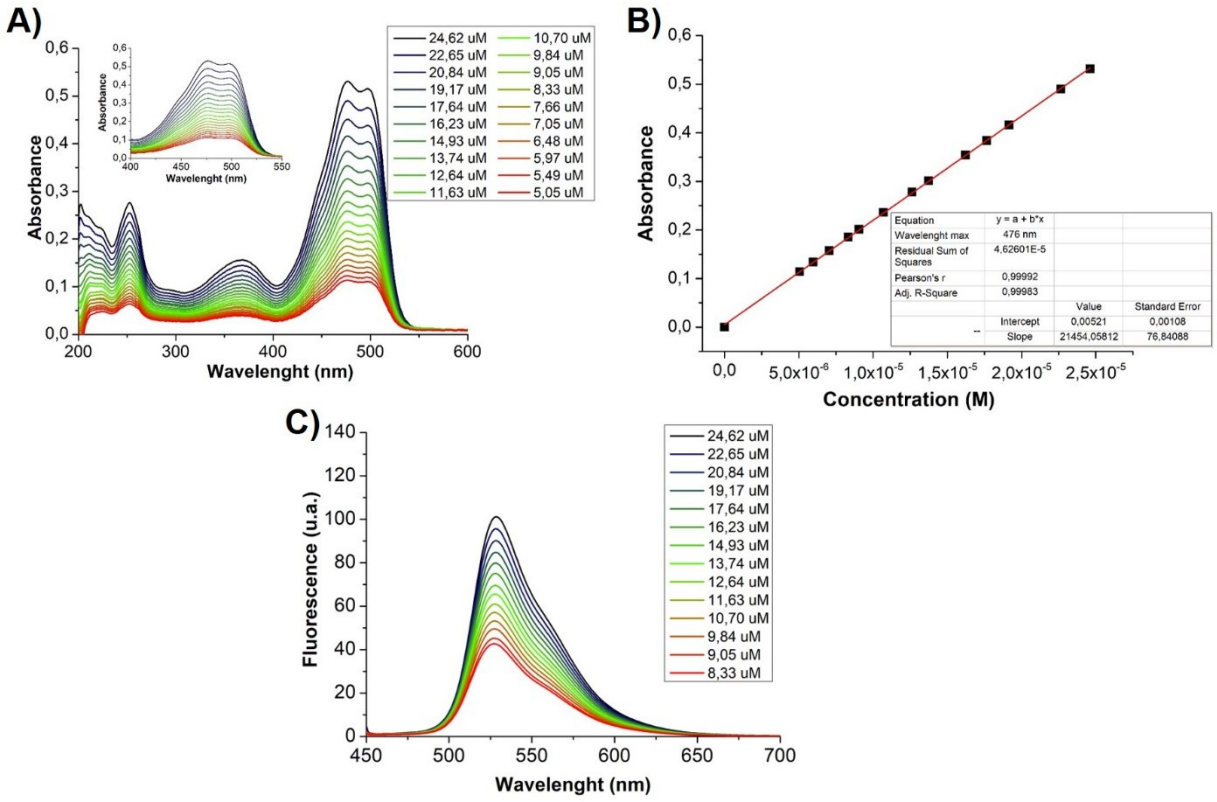

Figure S9. PBA-BODIPY **4** in Methanol: **A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

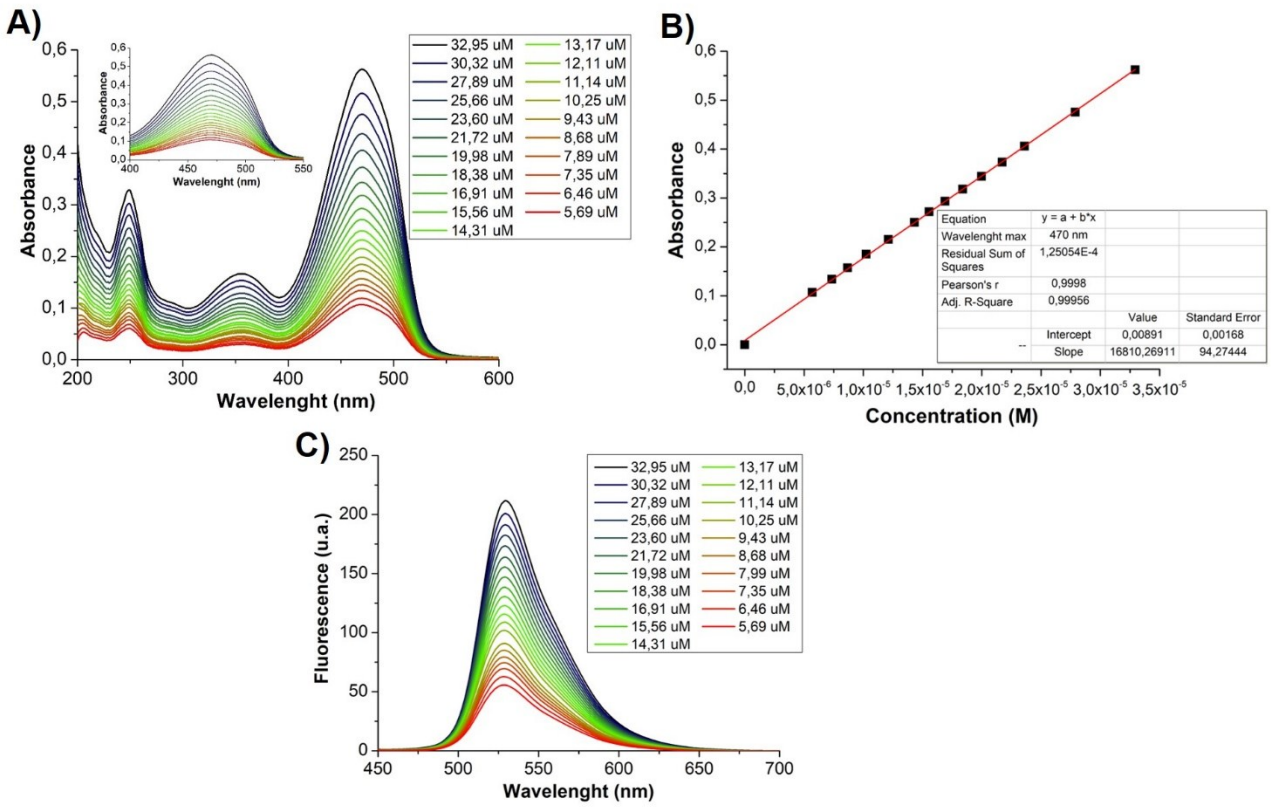

Figure S10. PBA-BODIPY **4** in physiological PBS pH = 7.4**: A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

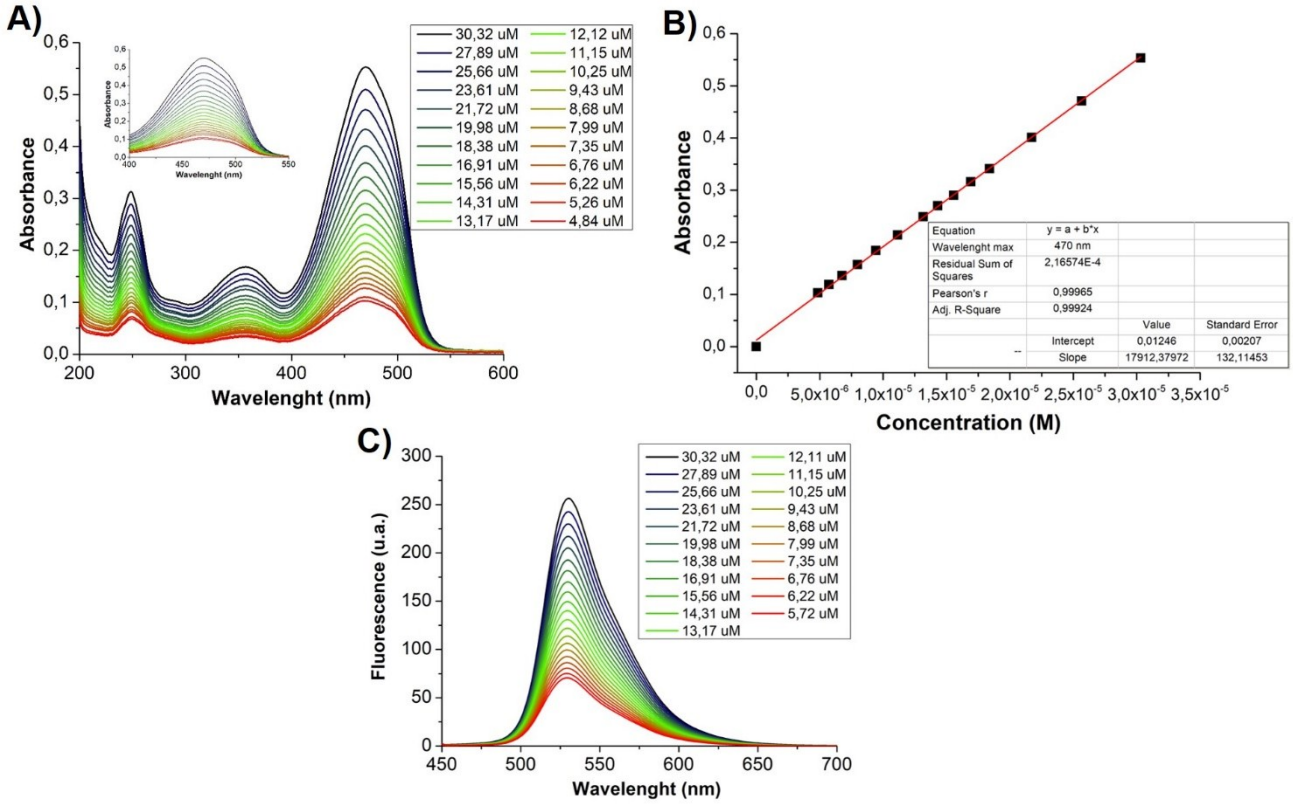

Figure S11. PBA-BODIPY **4** in H_2O**: A**) Scalar absorbance spectra; **B**) Molar extinction coefficient (ε); **C**) Scalar fluorescence spectra.

Experimental procedures

Synthesis of 4-(di(1H-pyrrol-2-yl)methyl)phenyl)boronic acid (5). To an ice-cooled suspension of 4-formylphenylboronic acid **6** (650 mg, 4.34 mmol) in neat pyrrole (8.72 g, 9.0 mL, 130.1 mmol), TFA (49 mg, 0.033 mL, 0.43 mmol) was added under Ar atmosphere. The reaction mixture was warmed at room temperature, then after 25' NEt_3 (88 mg, 0.120 mL, 0.87 mmol) was added. The excess of pyrrole was removed under vacuum to give a crude mixture, which was dissolved in ethyl acetate and filtered over a pad of Celite. The filtrate was washed with H_2O (3 x 30 mL) and Brine (3 x 30 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness to give 1.50 g of a yellow oil. Then, the crude was dissolved in ethyl acetate and filtered over a pad of Celite to give 1.250 g of a crude brown solid which was finally triturated with a mixture of ethyl acetate/cyclohexane mixture (1:1.2) to afford **5** (840 mg, 3.23 mmol) as a light tan solid. (Yield 73%). Mp: 129-132°C. ^{1}H-NMR (400 MHz, CD_3OD): δ 7.52 (d, J_{a-b} = 8.1 Hz, 2H), 7.19 (d, J_{b-a} = 7.9 Hz, 2H), 6.63-6.62 (m, 2H), 5.98-5.96 (m, 2H), 5.70-5.69 (m, 2H), 5.39 (s, 1H); ^{13}C-NMR (100 MHz, CD_3OD) δ: 145.11, 133.04, 127.56, 116.47, 106.69, 106.10, 44.04. ESI-MS m/z: calcd for $C_{16}H_{16}BN_2O_4$ [M+HCOOH-H] 311,12 found 310.97. Elementary analysis calcd for $C_{15}H_{15}BN_2O_2$ C67.7 N10.53 H 5.68 found C 68.89 N 9.39 H 5.20.

Synthesis of (Z)-(4-((5-chloro-1H-pyrrol-2-yl)(5-chloro-2H-pyrrol-2-ylidene)methyl)phenyl)boronic acid (7). A stirred solution of **5** (800 mg, 3.00 mmol) in dry THF (65 mL) was cooled at -78°C, then a solution of *N*-chlorosuccinimide (802 mg, 6.00 mmol) in dry THF (60 mL) wad slowly added under N_2 atmosphere. The reaction mixture was stirred for 1h at -78 °C, then it was slowly warmed at -20°C and placed in the freezer overnight at -20 °C. The reaction mixture was warmed at r.t. and then, the solvent was removed under vacuum. The crude product was used in the subsequent reaction without further purification. To a solution of crude **8** (1.0 g) in dry dichloromethane (38 mL) the 2,3-dichloro-5,6-dicyano-p-benzoquinone (682 mg, 3.00 mmol) was added at r.t.. The reaction mixture was stirred at r.t. for 24 h and then diluted with dichloromethane (600 mL) and filtered over a pad of Celite. The filtrate was washed with water (4 x 40 mL) and Brine (4 x 40 mL). The organic phase was dried over Na_2SO_4, and the solvent removed under vacuum to afford **7** (900 mg) as a brown solid. The solid was suspended in isopropanol (30 mL) at 0°C and then filtered through a pad of Celite to afford pure **7** (738 mg, 2.21 mmol, 73%) as brown solid. Mp: 150-153 °C. ^{1}H-NMR (400 MHz, CD_3OD): δ 7.73 (d, J_{a-b} = 7.7 Hz, 2H), 7.45 (d, J_{b-a} = 7.9 Hz, 2H), 6.61 (d, J_{6-7} = 2.2, 2H), 6.37 (d, J_{7-6} = 4.3 Hz, 2H). ^{13}C-NMR (100 MHz, CD_3OD): δ 144.13, 139.99, 132.80, 130.30, 120.05, 116.74. ESI-MS m/z: calcd for $C_{15}H_{10}BCl_2N_2O_2$ [M+H] 332,02 found: 333,31. Elementary analysis calcd for $C_{15}H_{11}BCl_2N_2O_2$ C 54.11 N 8.41 H 3.33 found C 53.90 N 8.37 H 3.31.

Synthesis of 3,5-dichloro-4,4-difluoro-8-(4-boronophenyl)-4-bora-3a,4a-diaza-*s*-indacene (1). A solution of **7** (358 mg, 1.07 mmol) in dry dichloromethane (6.0 mL) was cooled at -10°C. Then, BF_3OEt_2 (638 mg, 4.5 mmol) and N,N'-Diisopropylethylendiamine (580 mg, 4.5 mmol) were added under N_2 atmosphere. The reaction mixture was stirred at -10°C for 1h 30' and then diluted with water (10 mL) and $CHCl_3$ (10 mL). The reaction mixture was warmed to r.t. and filtered on a pad of Celite. The filtrate was washed with water (3 x 20 mL), with a solution of HCl (0.5 M in H_2O, 1 x 20 mL) and with Brine (1 x 20 mL). The organic phase was dried over Na_2SO_4, and concentrated to dryness to give 0.400 g of a crude which was dissolved in $CHCl_3$ and filtered on ion exchange resin (Dowex® Marathon™ C) to afford **1** as a red solid (307 mg, 0.81 mmol, 75%). Mp: 235-237. ^{1}H-NMR (400 MHz, CD_3OD): δ 7.82 (d, J_{a-b} = 7.0 Hz, 2H), 7.58 (d, J_{b-a} = 7.7 Hz, 2H), 6.98 (d, J_{6-7} = 4.0 Hz, 2H), 6.58 (d, J_{7-6} = 4.4 Hz, 2H); ^{13}C-NMR (100 MHz, CD_3OD): δ 144.31, 133.58, 133.28, 131.86, 129.65, 118.79. HRMS m/z: calcd. for $C_{15}H_{11}B_2Cl_2F_2N_2O_2$ 381.0463 Found 381.03378.

Synthesis of 5-chloro-3-phenylamino-4,4-difluoro-8-(4-boronophenyl)-4-bora-3a,4a-diaza-*s*-indacene (2). To a solution of **1** (50 mg, 0.13 mmol) in dry dichloromethane (3.0 mL) aniline (100 uL, 1.04 mmol) was added. The reaction mixture was stirred at 60°C for 48 h and then diluted with chloroform (200 mL) and washed with water (4 x 15 mL), with a solution of HCl (0.5 M in H_2O, 2 x

10 mL) and Brine (1 x 10 mL). The organic phase was dried over Na_2SO_4, and the solvent removed under vacuum to afford 60 mg of a crude orange solid. The crude product was filtered on silica gel (petroleum ether: ethyl acetate 3:2) to obtain **2** as an orange solid (33 mg, 0.076 mmol, 58%). Mp: 118-122 °C. ^{1}H-NMR (400 MHz, CD_3OD): δ 7.76 (d, 2H), 7.50-7.30 (m, 7H), 6.97 (d, $J_{2\text{-}1}$ = 4.2 Hz, 1H), 6.44 (d, $J_{1\text{-}2}$ = 4.8 Hz, 1H), 6.36-6.35 (bs, 1H), 6.20 (d, $J_{6\text{-}7}$ = 3.5 Hz, 1H).; ^{13}C-NMR (100 MHz, CD_3OD): δ 137.73, 137.29, 133.43, 133.18, 131.54, 129.26, 126.32, 123.89, 119.60, 113.07, 112.14. ESI-MS: m/z per $C_{21}H_{15}B_2ClF_2N_3O_2^-$ [M-H]$^-$ calcd 436.10, found 436.39. Elementary analysis calcd for $C_{21}H_{16}B_2ClF_2N_3O_2$ C 57.66 N 9.61 H 3.69 found C 57.44 N 9.57 H 3.67.

Synthesis of 4,4-difluoro-3,5-bis(phenylamino)-8-(4-boronophenyl)-4-bora-3a,4a-diaza-*s*-indacene (3). A solution of **1** (66 mg, 0.17 mmol) in 600 μL of aniline (612 mg, 6.58 mmol) was warmed 140°C for 1 h. The reaction mixture switches from red to intense blue colour, then it is cooled at r.t. diluted with DCM (200 mL) and washed with H_2O (5 × 15 mL). The organic phase was dried over Na_2SO_4 filtered and concentrated to dryness to give 76 mg of crude blue solid. The crude was purified by flash cromatography on silica gel (petroleum ether:ethyl acetate 5:1) to give 30 mg (0.061 mmol, 34%) of intense blues solid. Mp: 109-110°C. ^{1}H-NMR (400 MHz, CD_3OD): δ 7.50-7.48 (m, 6H), 7.37 (at, 5H), 7.31-7.29 (m, 5H), 7.11 (at, 2H), 6.62 (d, *J* = 4.0 Hz, 2H), 6.22 (d, *J* = 4.0 Hz, 2H). ^{13}C-NMR (100 MHz, CD_3OD): δ.143.5, 139.9, 130.3. 129.5, 129.0, 128.7, 128.1, 123.5, 122.4, 120.1, 118.2. ESI-MS m/z calcd for $C_{27}H_{22}B_2F_2N_4NaO_2$ [M+Na] 517.18 found 517.84. Elementary analysis calcd for $C_{27}H_{22}B_2F_2N_4O_2$: N 11.34, C 65.63, H 4.49; found N 10.48, C 71.47, H 5.85.

Synthesis of 3-(2-Aminoethyl)amino-5-chloro-4,4-difluoro-8-(4-boronophenyl)-4-bora-3a,4a-diaza-*s*-indacene (4). A solution of **1** (50 mg, 0.13 mmol) in 500 μL of ethylendiamine (449 mg, 7.48 mmol) was stirred at r.t. for 45 min, then, the reaction mixture was concentrated to dryness to give 99 mg of a red solid. The crude product was diluted in ethyl acetate and washed with water (4 × 10 mL) and brine (1 × 10 mL). The organic phase was dried over Na_2SO_4, and the solvent removed under vacuum to afford 49 mg of an orange solid. The product was then filtered on silica gel (DCM:MeOH 4:1 + 1% NEt_3) to obtain **4** (23 mg, 0.057 mmol, 44%) as an orange solid Mp: 115-117 °C. ^{1}H-NMR (400 MHz, CD_3OD): δ 7.32-7.29 (m, 2H), 7.04 (d, J = 5.0 Hz, 1H), 6.91-6.89 (m, 2H), 6.52 (d, *J* = 4.7 Hz, 1H), 6.36 (d, *J* = 3.6 Hz, 1H), 6.16 (d, *J* = 3.6 Hz, 1H), 3.69 (t, *J* = 6.1 Hz, 2H), 3.13 (t, *J* = 6.2 Hz, 2H). ^{13}C-NMR (100 MHz, CD_3OD): δ 158.77, 135.92, 131.49, 124,57, 119.45, 114.84, 111.94, 110.88, 42.20, 39.40. Elementary analysis calcd for $C_{17}H_{17}B_2ClF_2N_4O_2$ C 50.49, N 13.85, H 4.24 found C 50.01, N 13.72, H 4.19.

Fluorescence quantum yield

Fluorescence quantum yields have been determined by measuring fluorescence spectra for a series of methanol solutions with absorbance ranging from 0 to 0.1 in 1 cm optical path length quartz cuvette. For each solution, the integrated fluorescence intensities were plotted as a function of the solution absorption ($1\text{-}10^{-A}$). The resulting data point were fitted with a straight line, whose slope was proportional to the sample fluorescence quantum yield (J.R. Lakowicz, "Principles of fluorescence spectroscopy", Springer, New York, 2006, Third Edition). Absolute values are calculated using standard samples which have known quantum yield Φ_{ST}, being η the refractive index of the solvent:

$$\Phi_x = \Phi_{ST}\left(\frac{\text{Slope}_x}{\text{Slope}_{ST}}\right)\left(\frac{\eta_x^2}{\eta_{ST}^2}\right)$$

The reference standard should match the absorption and the emission wavelength region of the sample. For this reason, Rhodamine 6G and DODCI were chosen as standard for BODIPY dyes **1**, **2**, **4** and **3**, respectively.

Binding monitoring by Quartz Crystal Microbalance

The ability of the boronic acid derivative to bind glycosylated chains present on antibodies was evaluated by gravimetric based sensing, i.e. Quartz Crystal Microbalance (QCM). In particular, the binding was assessed by monitoring the resonance frequency decrease on 9.5 MHz crystals, carrying the boronic acid derivative **4** modified surface, induced by rabbit anti-streptavidin antibody. The binding of the antibody causes a mass increase on the quartz crystal, inducing the recorded frequency shift. The QCM presents the advantage to be a real time and label free approach for monitoring bio-specific interactions. Quartz crystals (9.5 MHz AT-cut, 14 mm, sandwiched between two gold electrodes) were purchased from Elbatech (Marciana (LI), Italy). The experiments were run in real time on quartz crystal analyzer model QC Magic 4 Channels (Elbatech, Marciana (LI)) in parallel onto 2 channels. The first one dedicated to the crystal modified with **4** (crystal 1), while the second one was exploited for the negative control (without **4**, crystal 2), as showed in Figure 1. The quartz crystals were firstly cleaned for 10 min with piranha solution (3:1 mixture of H_2SO_4 and H_2O_2), in order to remove any organic residue from the gold surface. After washing with deionized water and drying under nitrogen flush, crystal 1 was immersed for 16 h in a mixture containing 10 mM CS_2 and 10 mM **4** in methanol, to allow the condensation of the primary amines with CS_2 on the gold surface. Crystal 2 was instead immersed in only 10 mM CS_2. After rinsing with methanol and drying with nitrogen, the modified crystals were fixed into methacrylate cells, by exposing only one side of the crystal to the following treatments. The surface was kept in contact with a 10 mM solution of ethanolamine (EA) in methanol three times for 10 min, in order to deactivate non-reacted CS_2, and avoiding non-specific adsorption. After gently washing with methanol, the crystals were conditioned with 20 mM Hepes pH 8.5 until the baseline resulted stabilized. Both crystals were exposed to a 1 mg L^{-1} solution of rabbit anti-streptavidin IgG in 20 mM Hepes pH 8.5 until stabilization of the signal (ca. 1 h 40 min), to allow the binding between the glycosylated portion of the antibody and the boronic acid functions of **4** present on crystal 1. The surfaces were then rinsed with buffer to remove non-specifically bound antibody. After baseline equilibration, the recorded frequency shifts were 60 Hz and 1 Hz for crystal 1 and 2, respectively, confirming that the antibody specifically binds the crystal functionalized with the boronic acid, likely through the glycosylated portions (Figure 2).

Figure S12. ^{1}H-NMR (400MHz compound **5**)

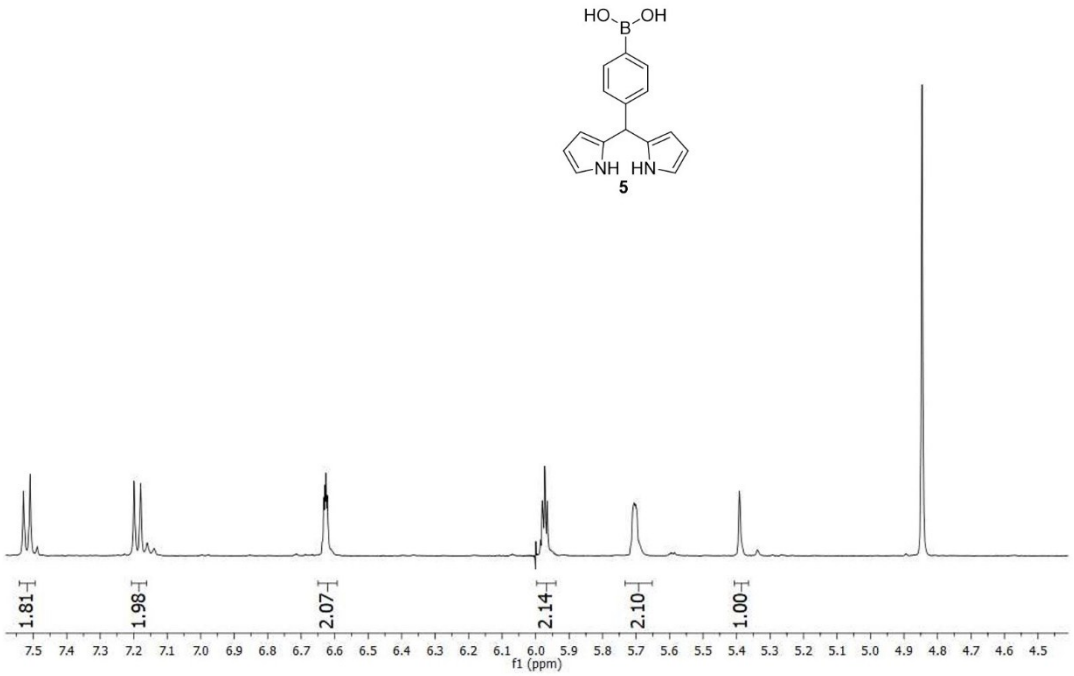

Figure S13. ^{13}C-NMR (100MHz compound **5**)

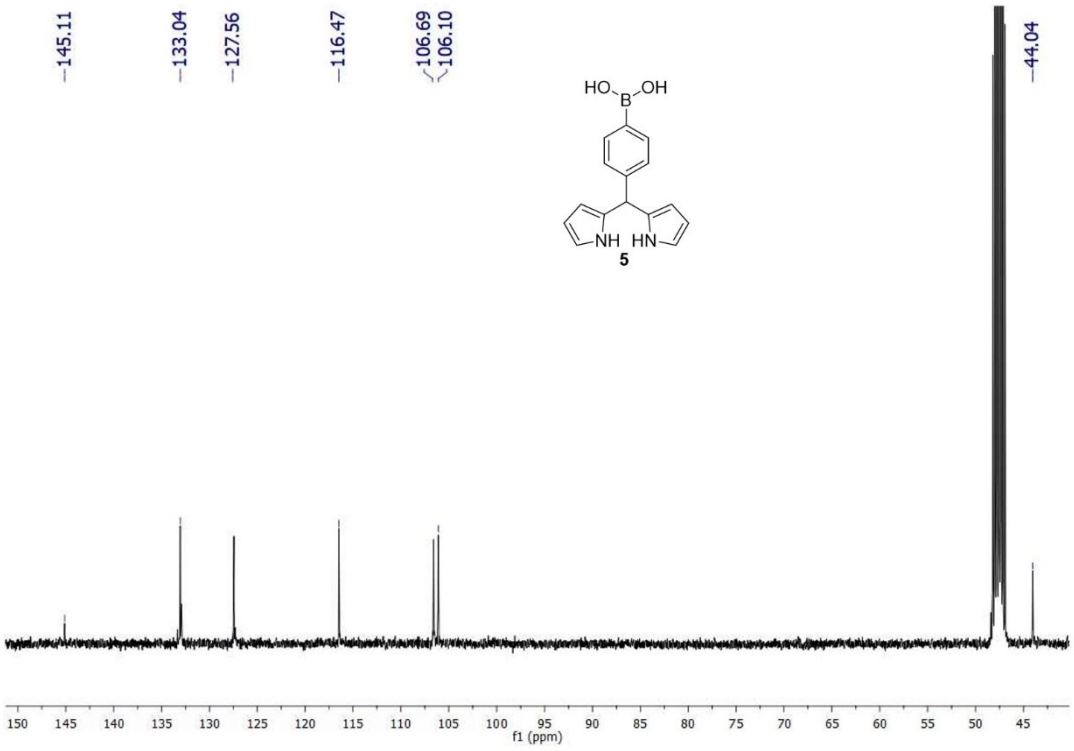

Figure S14. ^{1}H-NMR (400MHz compound **1**)

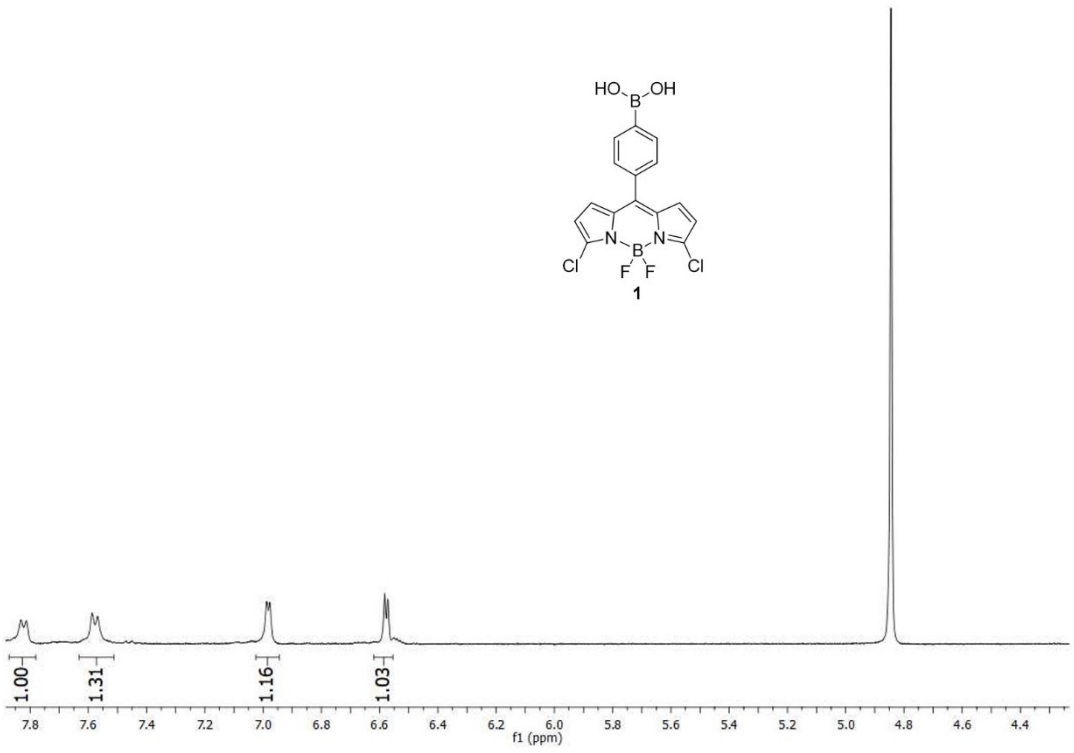

Figure S15. ^{13}C-NMR (100MHz compound **1**)

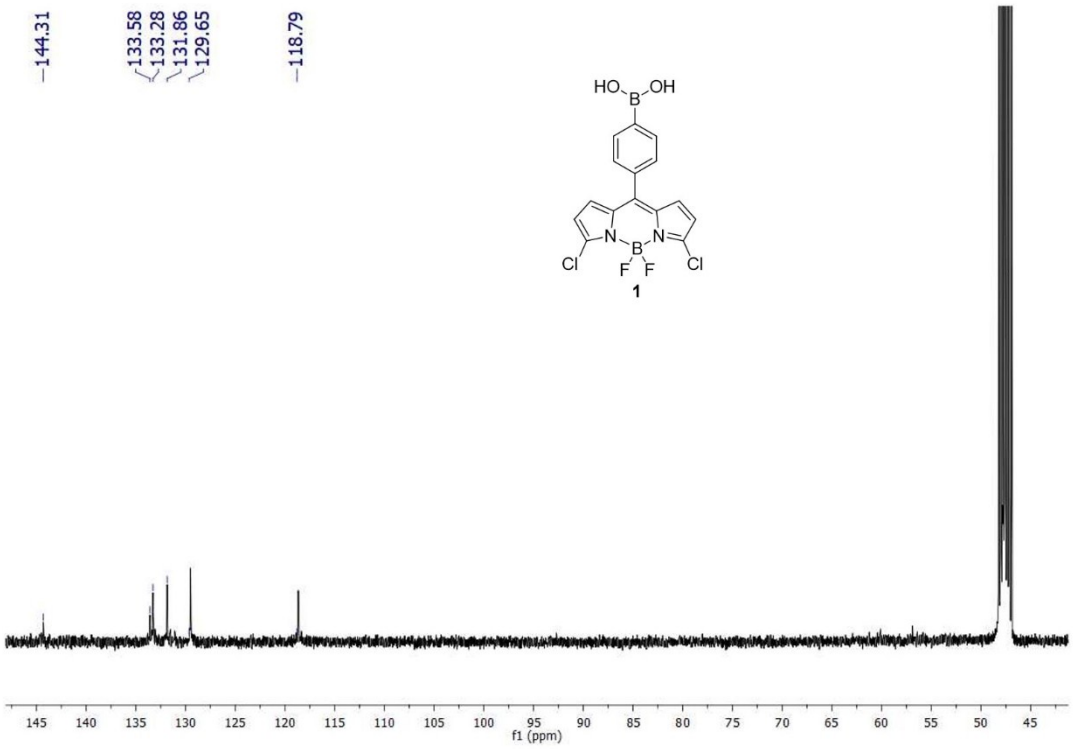

Figure S16. ^{1}H-NMR (400MHz compound **2**)

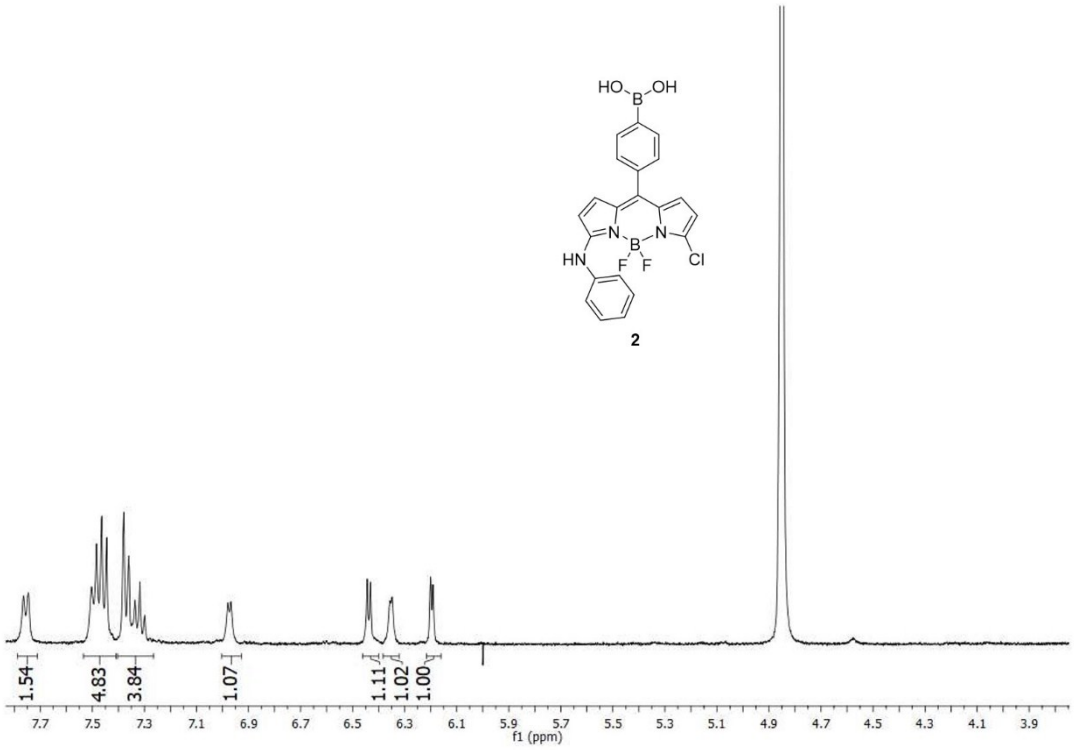

Figure S17. ^{13}C-NMR (100MHz compound **2**)

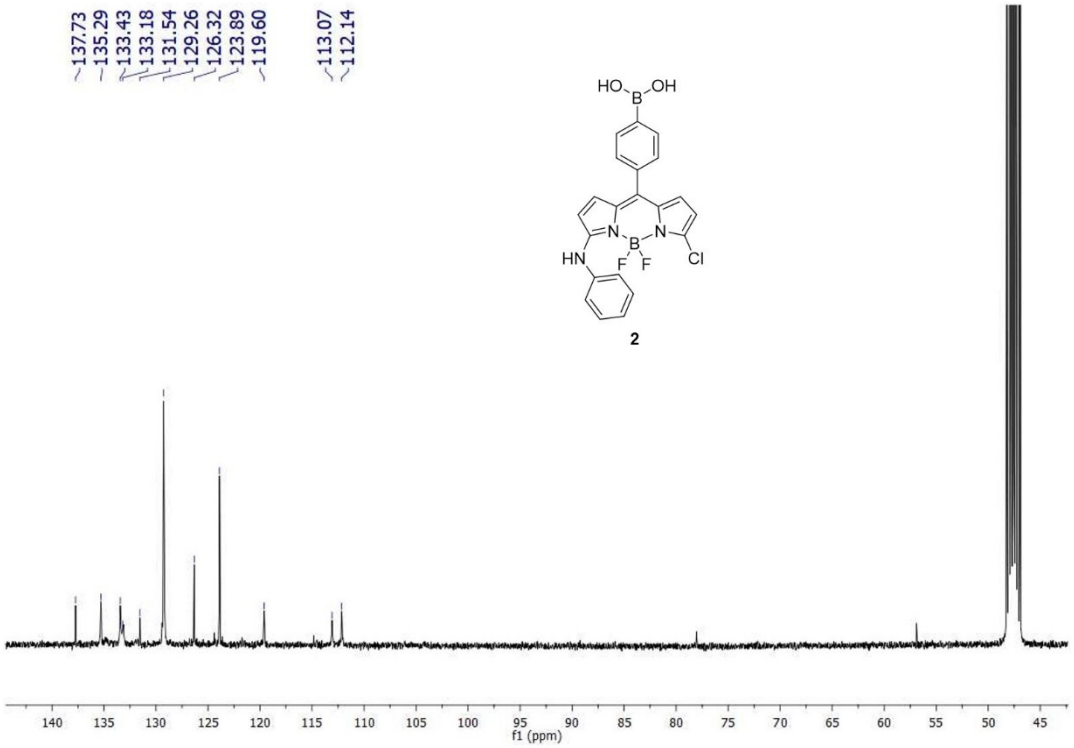

Figure S18. gDQCOSY (compound **2**)

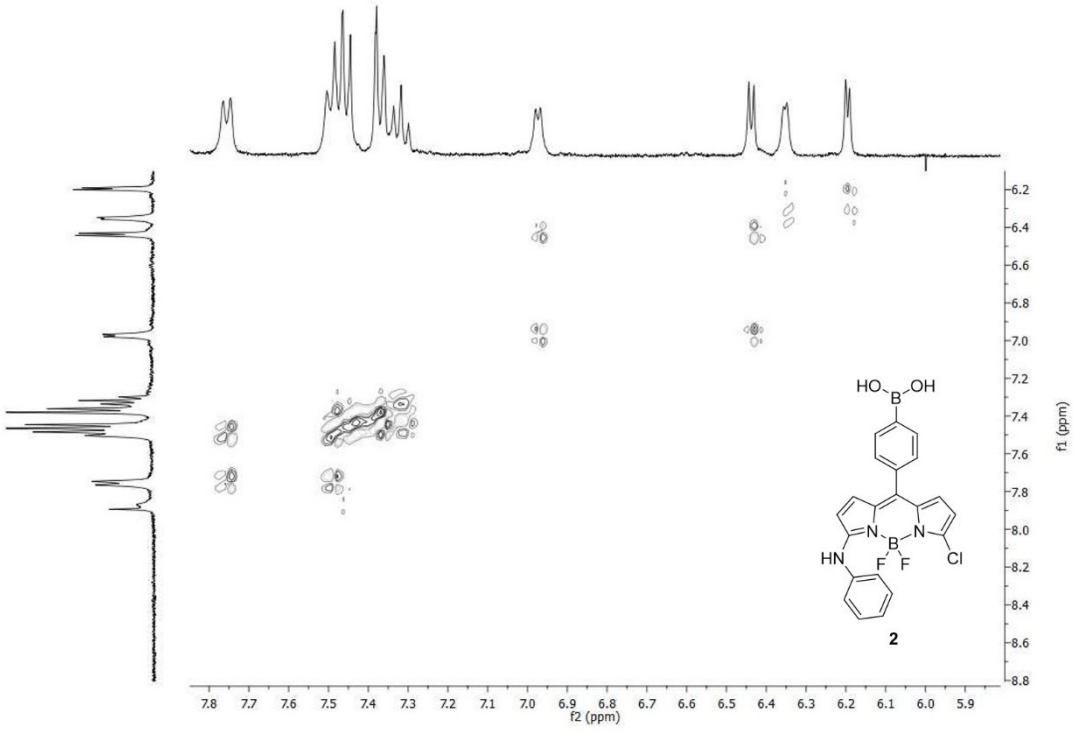

Figure S19. ^{1}H-NMR (400MHz compound **4**)

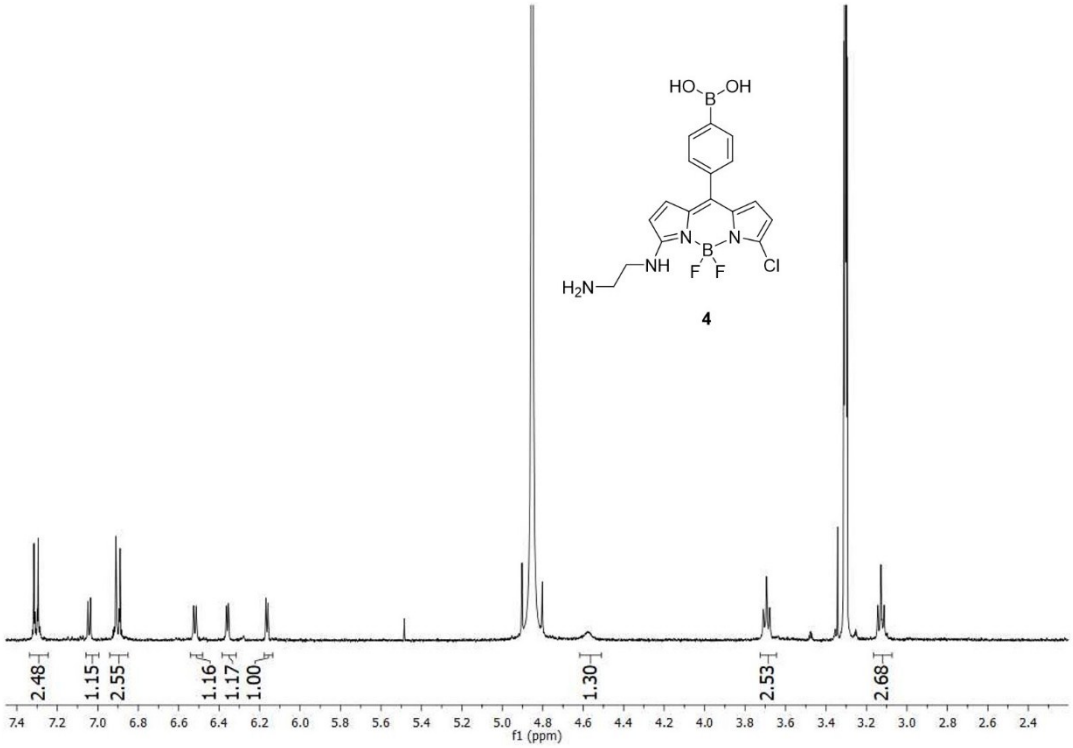

Figure S20. ^{13}C-NMR (100MHz compound **4**)

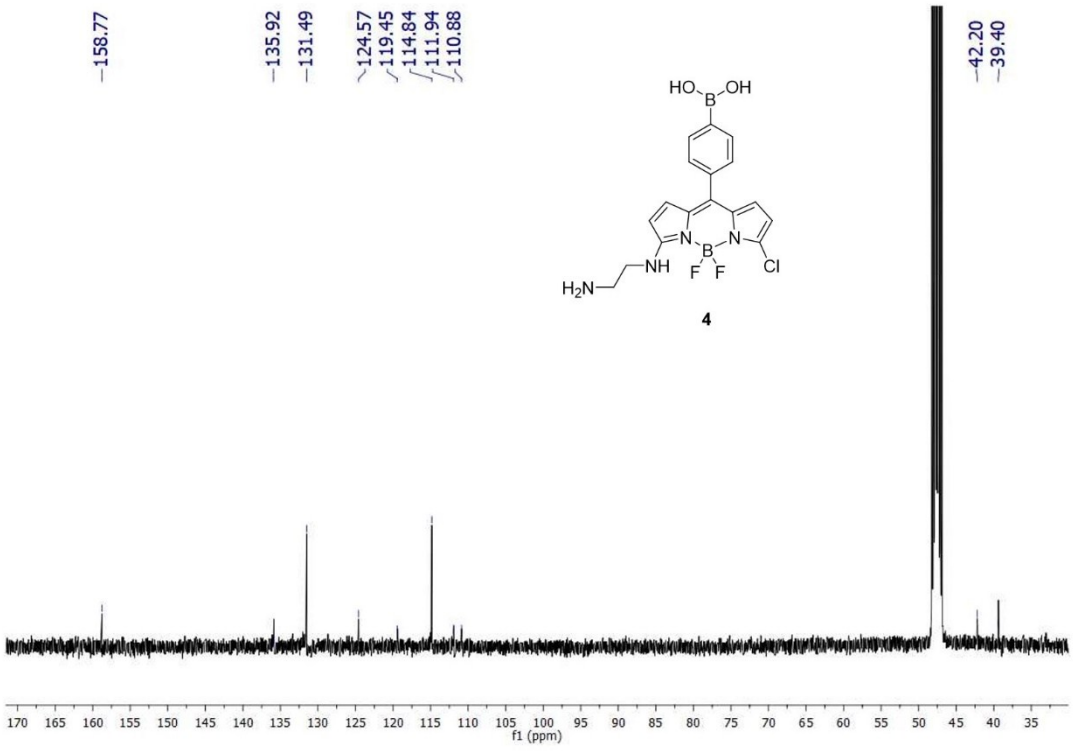

Figure S21. gDQCOSY (compound **4**)