

Supporting Information

Near-infrared fluorescent aza-BODIPY dyes for sensing and imaging of pH from neutral to highly alkaline range

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Synthesis:

(E)-1-(4-butoxyphenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one (a₁)

1.00 g 4'-butoxyacetophenone (5.20 mmol, 1.00 eq) and 651.0 mg 3-hydroxybenzaldehyde (5.33 mmol, 1.02 eq) were dissolved in 5 mL EtOH in a round bottom flask. 5 mL of an aqueous potassium hydroxide solution (875.5 mg, 15.60 mmol, 3.00 eq) were added dropwise. The solution was stirred for 12 h at room temperature during which it turned orange. The reaction mixture was added dropwise to 40 mL 1 M HCl solution to precipitate a yellow solid. The product was filtered, washed with water and dried. The obtained yellow solid was used for further synthesis without purification. Crude yield: 1.34 g (87 %).

(E)-1,3-bis(4-butoxyphenyl)prop-2-en-1-one (c₁)

c₁ was obtained analogously to **a₁** from 5.40 g 4'-butoxyacetophenone (28.07 mmol, 1.00 eq) and 5.00 g 4-butoxybenzaldehyde (28.08 mmol, 1.00 eq) using 20 mL and 15 mL of an aqueous KOH solution (4.77 g, 85.02 mmol, 3.03 eq). The obtained yellow solid was used for further synthesis without purification. Crude yield: 8.60 g (87 %).

(E)-1-(4-butoxyphenyl)-3-(2-chloro-3-hydroxyphenyl)prop-2-en-1-one (a₂)

a₂ was obtained analogously to **a₁** from 613.9 mg 4'-butoxyacetophenone (3.20 mmol, 1.01 eq) and 498.0 mg 2-chloro-3-hydroxybenzaldehyde (3.18 mmol, 1.00 eq) using 3 mL EtOH and 3 mL of an aqueous KOH solution (539.5 mg, 9.62 mmol, 3.02 eq). The obtained yellow solid was used for further synthesis without purification. Crude yield: 907.9 mg (86 %).

(E)-3-(2,4-dichloro-3-hydroxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (a₃)

a₃ was obtained analogously to **a₁** from 412.7 mg 4'-methoxyacetophenone (2.75 mmol, 1.12 eq) and 469.0 mg 2,4-dichloro-3-hydroxybenzaldehyde (2.46 mmol, 1.00 eq) using 5 mL EtOH and 5 mL of an aqueous KOH solution (649.00 mg, 11.57 mmol, 4.71 eq). The obtained yellow product was used for further synthesis without purification. Crude yield: 691.7 mg (87 %).

(E)-1,3-bis(4-methoxyphenyl)prop-2-en-1-one (c₂)

c₂ was obtained analogously to **a₁** from 998.6 mg 4'-methoxyacetophenone (6.65 mmol, 1.00 eq) and 810 µL 4-methoxybenzaldehyde (6.66 mmol, 1.00 eq) using 5 mL EtOH and 5 mL of an aqueous KOH solution (1.12 g, 19.97 mmol, 3.00 eq). The obtained product (yellow powder) was used for further synthesis without purification. Crude yield: 1.60 g (90 %).

(E)-3-(2,6-difluoro-3-hydroxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (a₄)

a₄ was obtained analogously to **a₁** from 953.7 mg 4'-methoxyacetophenone (6.35 mmol, 1.04 eq) and 964.8 mg 2,6-difluoro-3-hydroxy-benzaldehyde (6.10 mmol, 1.00 eq) using 5 mL EtOH and 5 mL of an aqueous KOH (1.06 g, 18.89 mmol, 3.10 eq). The obtained brown product was used for further synthesis without purification. Crude yield: 1.62 g (91 %), brown powder

(E)-3-(3-hydroxy-4-methoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (a₅)

a₅ was obtained analogously to **a₁** from 998.3 mg 4'-methoxyacetophenone (6.65 mmol, 1.00 eq) and 1.02 g 3-Hydroxy-4-methoxybenzaldehyde (6.69 mmol, 1.01 eq) using 5 mL EtOH and 5 mL of an aqueous KOH solution. The obtained yellow solid was used for further synthesis without purification. Crude yield: 1.49 g (79 %), yellow powder

1-(4-butoxyphenyl)-3-(3-hydroxyphenyl)-4-nitrobutan-1-one (b₁)

In a round bottom flask equipped with a reflux condenser 1.34 g of **a₁** (4.53 mmol, 1.00 eq) and 5.00 mL nitromethane (92.56 mmol, 20.45 eq) were mixed with 10 mL of a potassium hydroxide solution in EtOH (612.0 mg, 10.91 mmol, 2.41 eq) and stirred at 60 °C for 3 h. After cooling to room temperature the solvent was removed under reduced pressure. The brown oily residue was dissolved in 50 mL EtOAc and washed with 1 M HCl solution (3 x 50 mL). The organic phase was dried over

Na₂SO₄ and the solvent removed under reduced pressure. The crude product (brown oil) was used for further synthesis without purification. Crude yield: 2.19 g (135 %, product containing residual solvent).

1,3-bis(4-butoxyphenyl)-4-nitrobutan-1-one (d₁)

d₁ was obtained analogously to **b₁** from 1.01 g **c₁** (2.87 mmol, 1.00 eq) and 3.00 mL nitromethane (55.54 mmol, 19.35 eq) using 5 mL of a KOH solution in EtOH (351.0 mg, 6.26 mmol, 2.18 eq). The crude product (brown oil) was used for further synthesis without purification. Crude yield: 1.29 g (109 %, product containing residual solvent).

1-(4-butoxyphenyl)-3-(2-chloro-3-hydroxyphenyl)-4-nitrobutan-1-one (b₂)

b₂ was obtained analogously to **b₁** from 907.9 mg **a₂** (2.74 mmol, 1.00 eq) and 3.00 mL nitromethane (55.54 mmol, 20.24 eq) using 5 mL of a KOH solution in EtOH (337.0 mg, 6.01 mmol, 2.19 eq). The crude product (brown oil) was used for further synthesis without purification. Crude yield: 1.34 g (125 %, product containing residual solvent).

3-(2,4-dichloro-3-hydroxyphenyl)-1-(4-methoxyphenyl)-4-nitrobutan-1-one (b₃)

b₃ was obtained analogously to **b₁** from 691.7 mg **a₃** (2.14 mmol, 1.00 eq) and 2.5 mL nitromethane (46.28 mmol, 21.62 eq) using 3 mL of a KOH solution in EtOH (340.0 mg, 6.06 mmol, 2.83 eq). The crude product (brown oil) was used for further synthesis without purification. Crude yield: 1.08 g (131 %, product containing residual solvent).

1,3-bis(4-methoxyphenyl)-4-nitrobutan-1-one (d₂)

d₂ was obtained analogously to **b₁** from 1.60 g **c₂** (5.96 mmol, 1.00 eq) and 6.5 mL nitromethane (120.33 mmol, 20.18 eq) using 10 mL of a KOH solution in EtOH (740.0 mg, 13.19 mmol, 2.21 eq) and stirring the reaction at 60 °C for 1 h. The crude product (brown oil) was used for further synthesis without purification. Crude yield: 2.41 g (123 %, product containing residual solvent).

3-(2,6-difluoro-3-hydroxyphenyl)-1-(4-methoxyphenyl)-4-nitrobutan-1-one (b₄)

b₄ was obtained analogously to **b₁** from 1.62 g **a₄** (5.58 mmol, 1.00 eq) and 6.00 mL nitromethane (111.07 mmol, 19.90 eq) using 10 mL of a KOH solution in EtOH (618.00 mg, 11.02 mmol, 1.97 eq) and stirring the reaction at 60 °C for 1 h. The solution turned red-brown and a brown precipitate was formed. After cooling to room temperature the solvent was removed by rotary evaporation. The brown oily residue was dissolved in 50 mL EtOAc and washed with HCl solution (3 x 50 mL). The crude product (brown oil) was used for further synthesis without purification. Crude yield: 1.36 g (120 %, product containing residual solvent).

3-(3-hydroxy-4-methoxyphenyl)-1-(4-methoxyphenyl)-4-nitrobutan-1-one (b₅)

b₅ was obtained analogously to **b₁** 1.49 g **a₅** (5.25 mmol, 1.00 eq) and 5.70 mL nitromethane (105.52 mmol, 20.11 eq) using 10 mL of a potassium hydroxide solution in EtOH (612.0 mg, 10.91 mmol, 2.08 eq) and stirring the reaction at room temperature overnight. The crude product (brown oil) was used for further synthesis without purification. Crude yield: 2.19 g (121 %, product containing residual solvent).

(Z)-3-(2-((3,5-bis(4-butoxyphenyl)-2H-pyrrol-2-ylidene)amino)-5-(4-butoxyphenyl)-1H-pyrrol-3-yl)phenol (e₁)

474.2 mg **b₁** (1.33 mmol, 1.00 eq), 507.2 mg **d₁** (1.23 mmol, 0.92 eq) and 3.58 g ammonium acetate (46.66 mmol, 35.00 eq) were mixed with 25 mL BuOH in a round bottom flask equipped with a reflux condenser and stirred at 95 °C. The reaction conversion was monitored by TLC (DCM) and UV/VIS absorption spectroscopy. After 5 h the reaction mixture was cooled to room temperature and stirred over the weekend.

After removing the solvent under reduced pressure the obtained solid was dissolved in DCM and washed with water (3 x 300 mL). The organic phase was dried over Na₂SO₄ and the solvent was removed. The crude product was purified by column chromatography (silica gel, DCM).

Yield: 44.6 mg (5 %), blue solid

(Z)-3-(2-((3,5-bis(4-butoxyphenyl)-2H-pyrrol-2-ylidene)amino)-5-(4-butoxyphenyl)-1H-pyrrol-3-yl)-2-chlorophenol (e₂)

e₂ was prepared analogously to **e₁** from 522.1 mg **b₂** (1.33 mmol, 1.00 eq) and 542.3 mg **d₂** (1.31 mmol, 0.98 eq) using 4.09 g ammonium acetate (53.06 mmol, 39.82 eq) in 25 mL BuOH.

Yield: 62.0 mg (7 %), blue metallic solid.

(Z)-3-(2-((3,5-bis(4-methoxyphenyl)-2H-pyrrol-2-ylidene)amino)-5-(4-methoxyphenyl)-1H-pyrrol-3-yl)-2,6-dichlorophenol (e₃)

e₃ was prepared analogously to **e₁** from 492.3 mg **b₃** (1.28 mmol, 1.00 eq) and 441.9 mg **d₂** (1.34 mmol, 1.05 eq) using 3.69 g ammonium acetate (47.35 mmol, 36.95 eq) in 25 mL BuOH.

Yield: 60.2 mg (8 %), metallic blue solid

(Z)-3-(2-((3,5-bis(4-methoxyphenyl)-2H-pyrrol-2-ylidene)amino)-5-(4-methoxyphenyl)-1H-pyrrol-3-yl)-2,4-difluorophenol (e₄)

e₄ was prepared analogously to **e₁** from 1.13 g **b₄** (3.23 mmol, 1.00 eq) and 1.11 g **d₂** (3.38 mmol, 1.05 eq) using 9.06 g ammonium acetate (117.54 mmol, 36.39 eq) in 50 mL BuOH.

Yield: 74.7 mg (4 %), metallic blue solid

(Z)-5-(2-((3,5-bis(4-methoxyphenyl)-2H-pyrrol-2-ylidene)amino)-5-(4-methoxyphenyl)-1H-pyrrol-3-yl)-2-methoxyphenol (e₅)

e₅ was prepared analogously to **e₁** from 493.4 mg **b₅** (1.43 mmol, 1.00 eq) and 493.8 mg **d₃** (1.45 mmol, 1.01 eq) using 4.11 g ammonium acetate (53.32 mmol, 37.32 eq) in 25 mL BuOH.

Yield: 62.0 mg (7 %), dark green powder

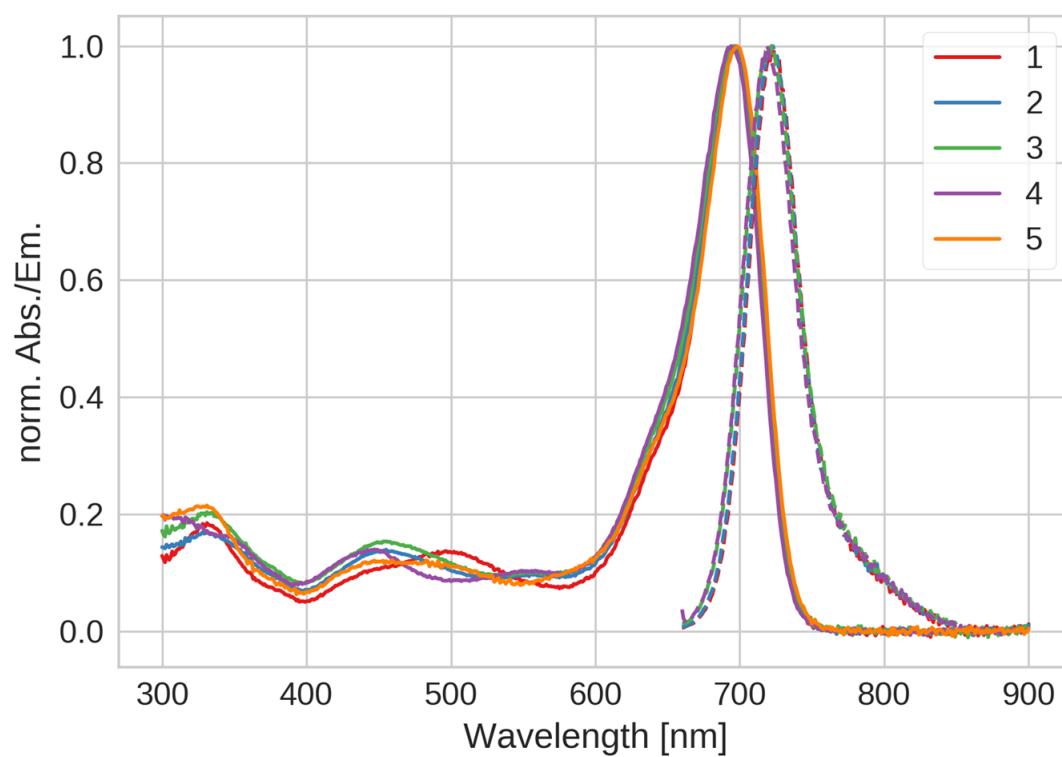


Figure S1: Normalized absorption and emission spectra (protonated form) of the indicators in tetrahydrofuran.

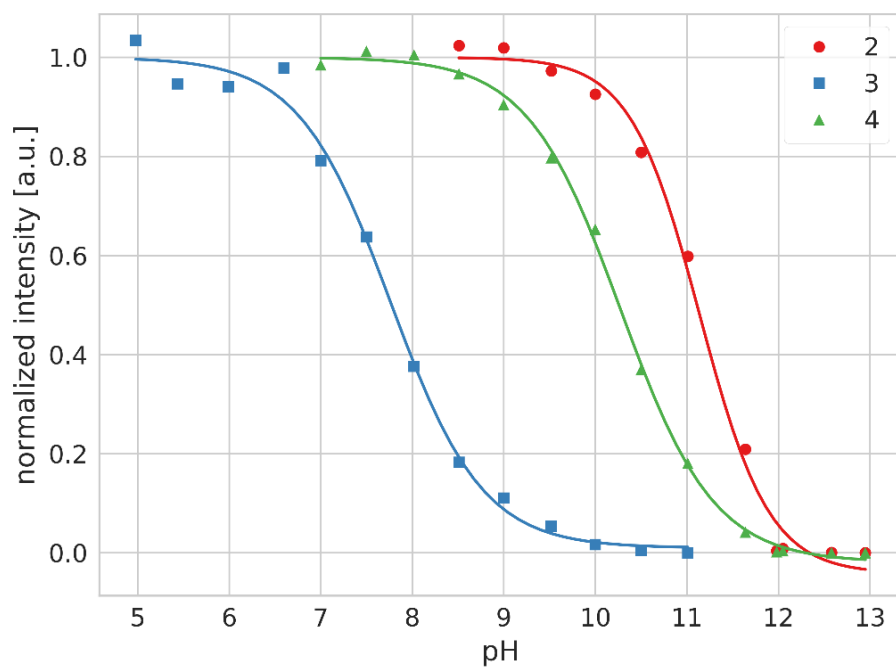


Figure S2: Calibration curves of the indicators in EtOH/buffer 1:1.

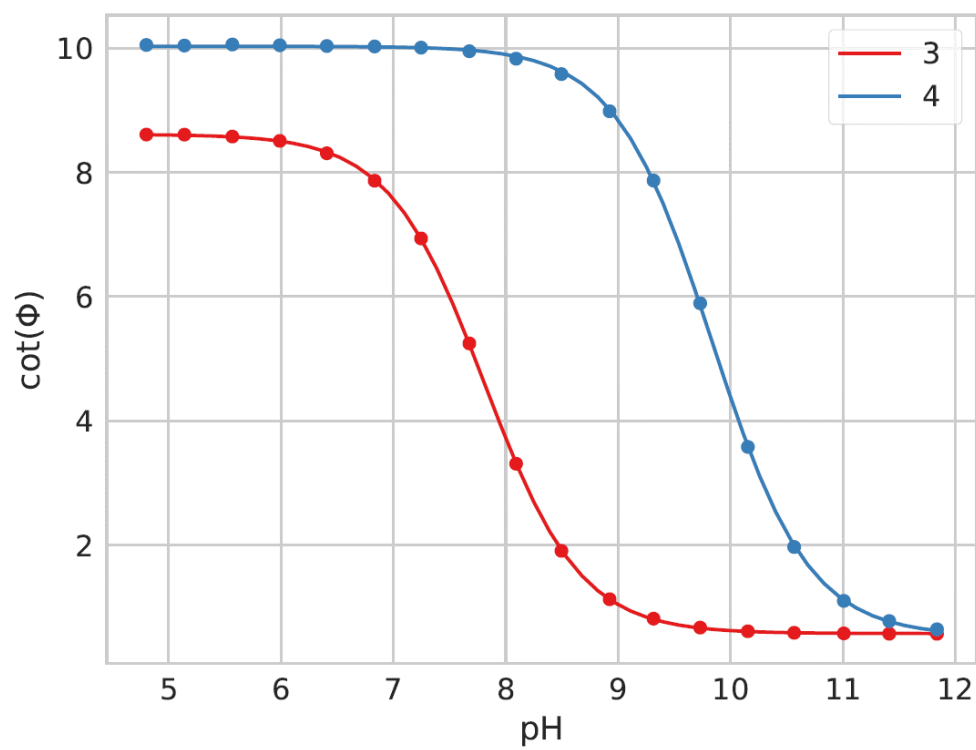


Figure S3: Calibration curves for the sensors based on indicators **3** and **4** which additionally contained Egyptian blue micropowder as a DLR reference.

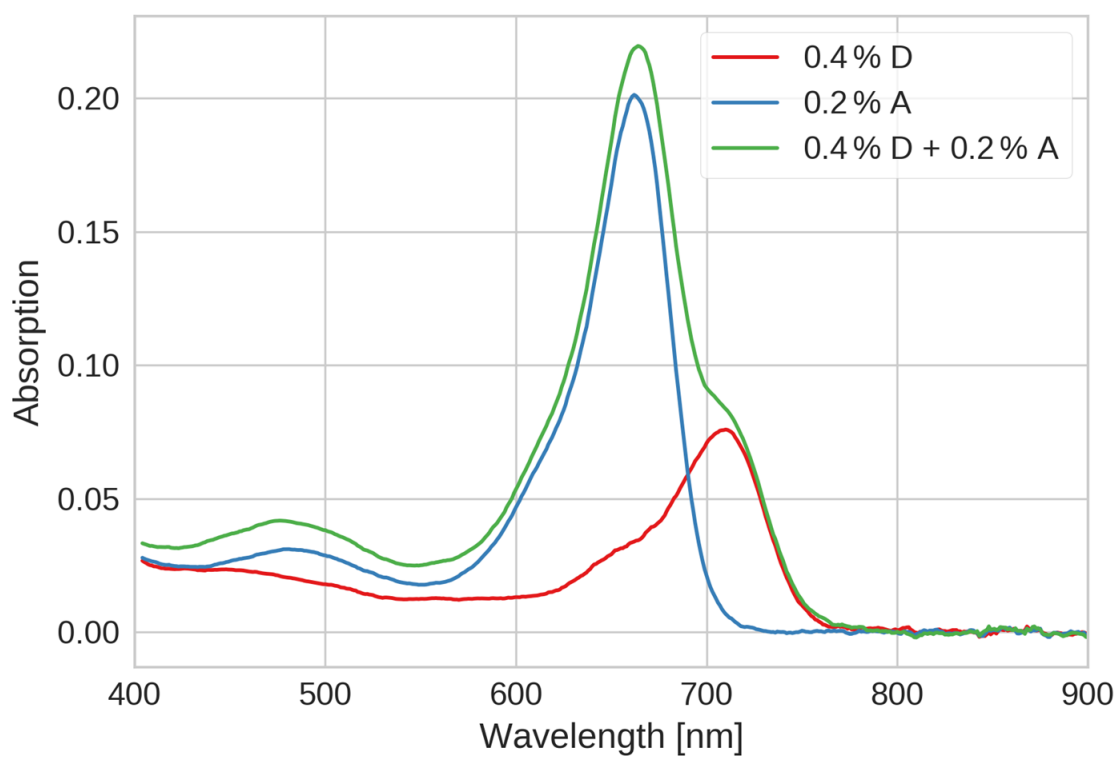


Figure S4: Absorption spectra of the light harvesting foils in hydrogel D4. D = Energy donor (tetraphenyl aza-BODIPY), A = Energy Acceptor (Indicator **3**).

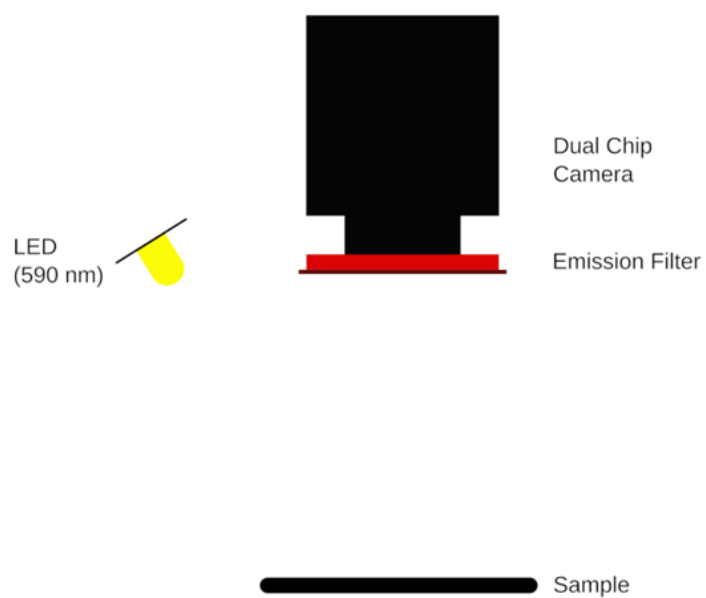


Figure S5: Scheme of the set-up used for ratiometric imaging of pH distribution.

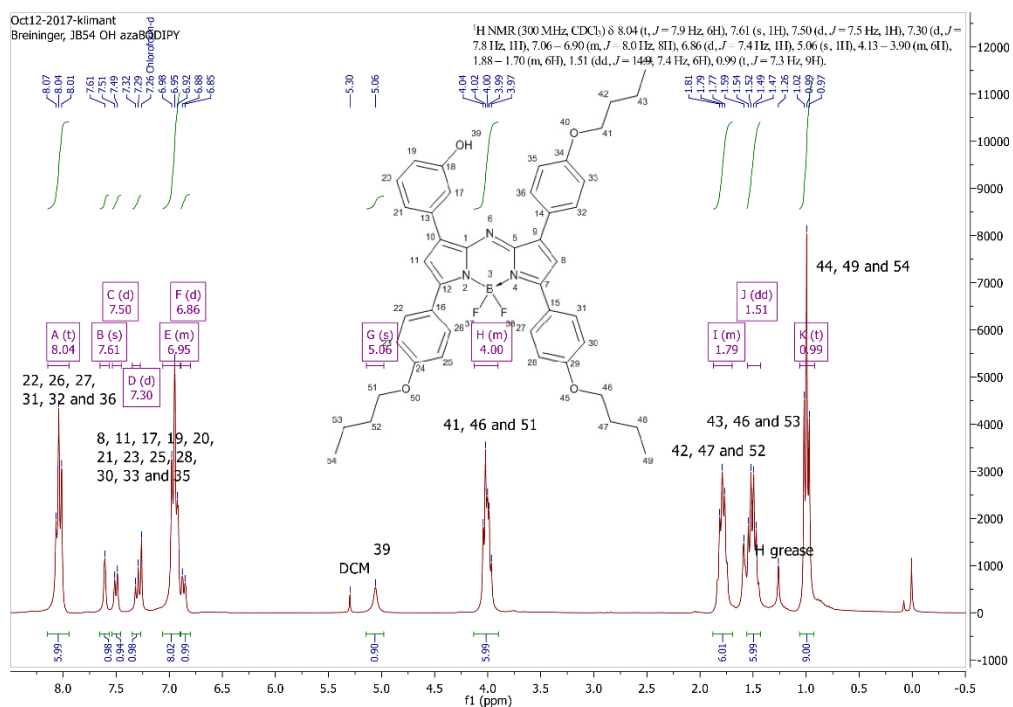


Figure S6: ¹H NMR spectrum of indicator **1**.

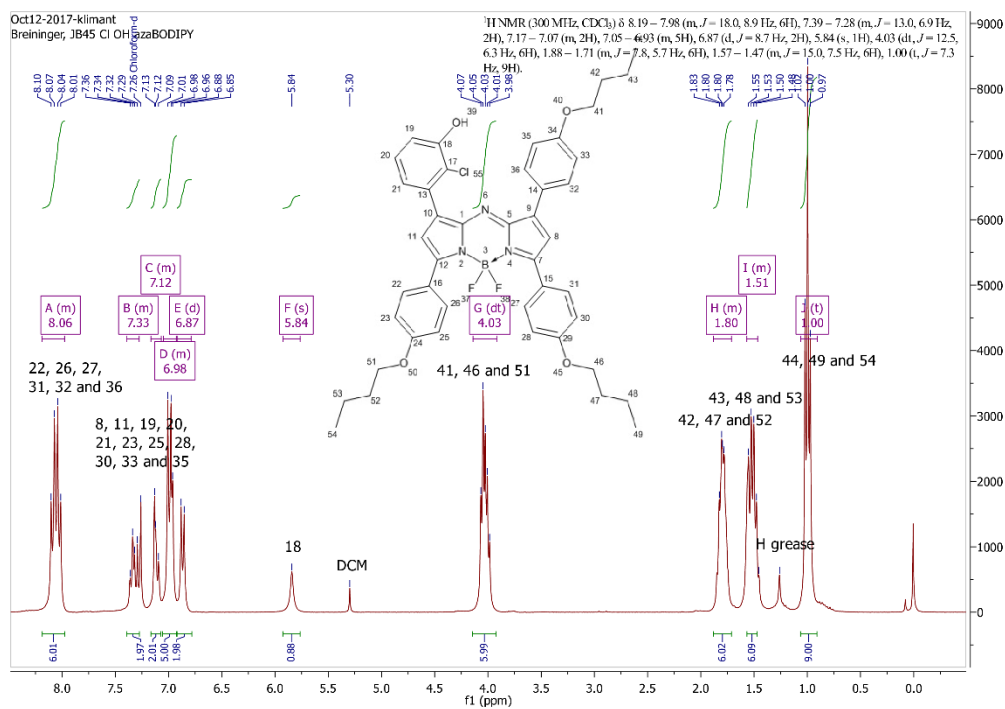


Figure S7: ¹H NMR spectrum of indicator **2**.

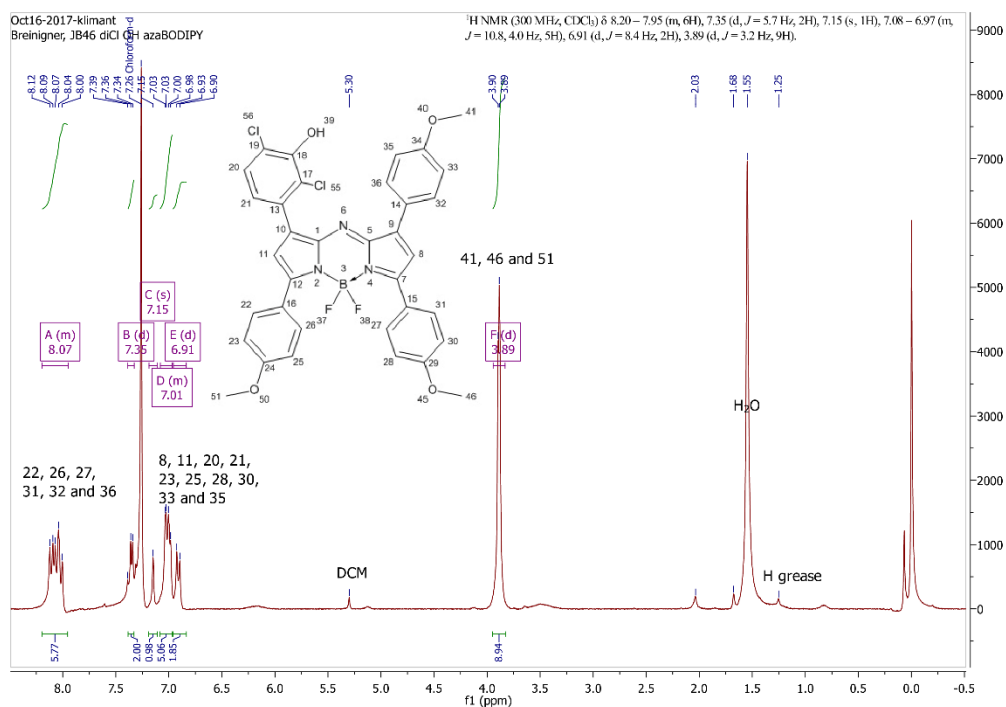


Figure S8: $^1\text{H NMR}$ spectrum of indicator **3**.

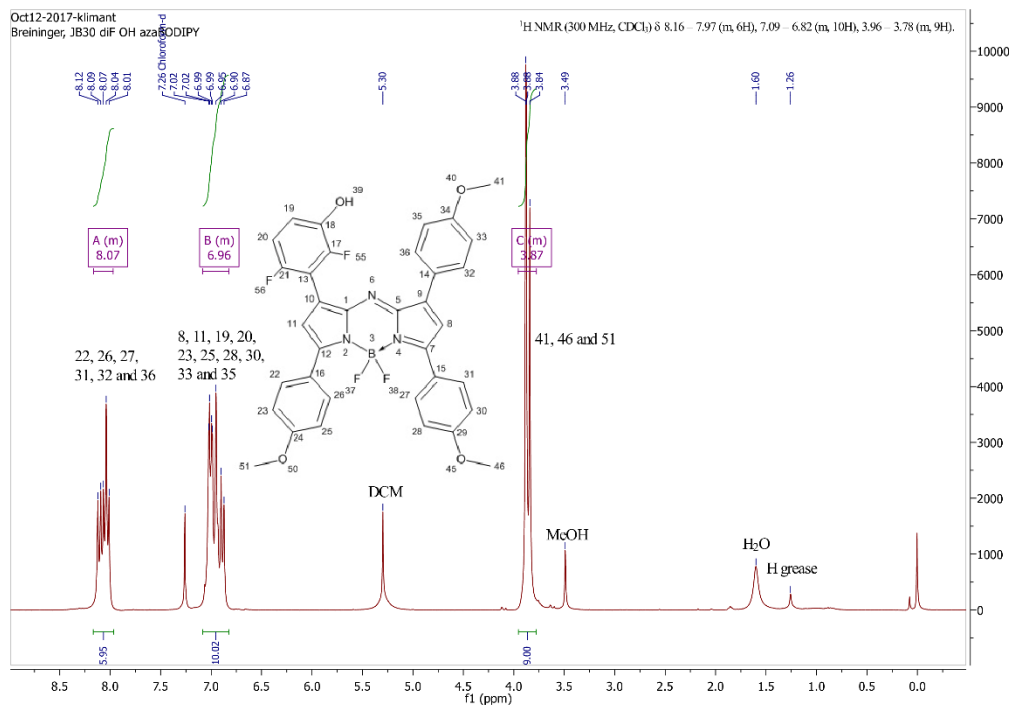


Figure S9: $^1\text{H NMR}$ spectrum of indicator **4**.

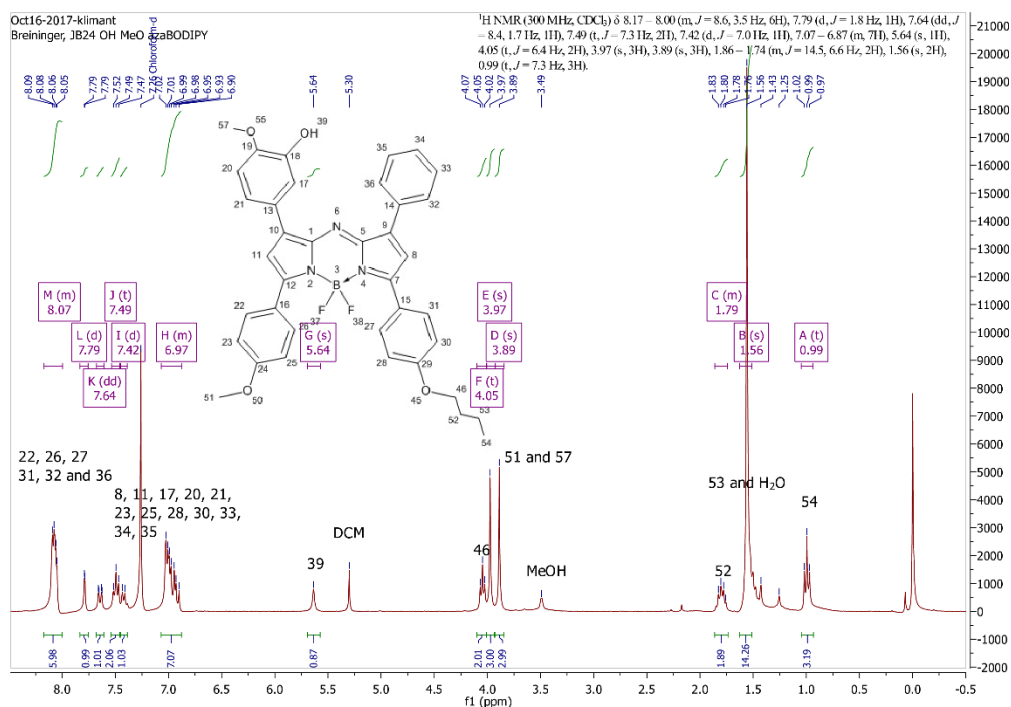


Figure S10: ¹H NMR spectrum of indicator **5**.

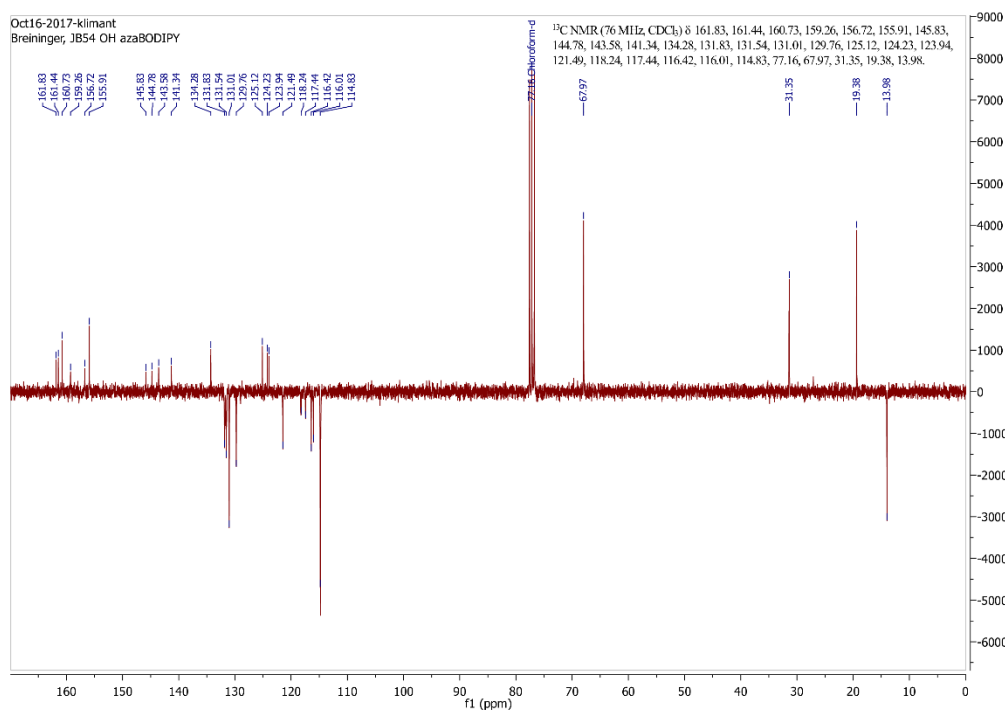


Figure S11: ¹³C APT NMR spectrum of indicator **1**.

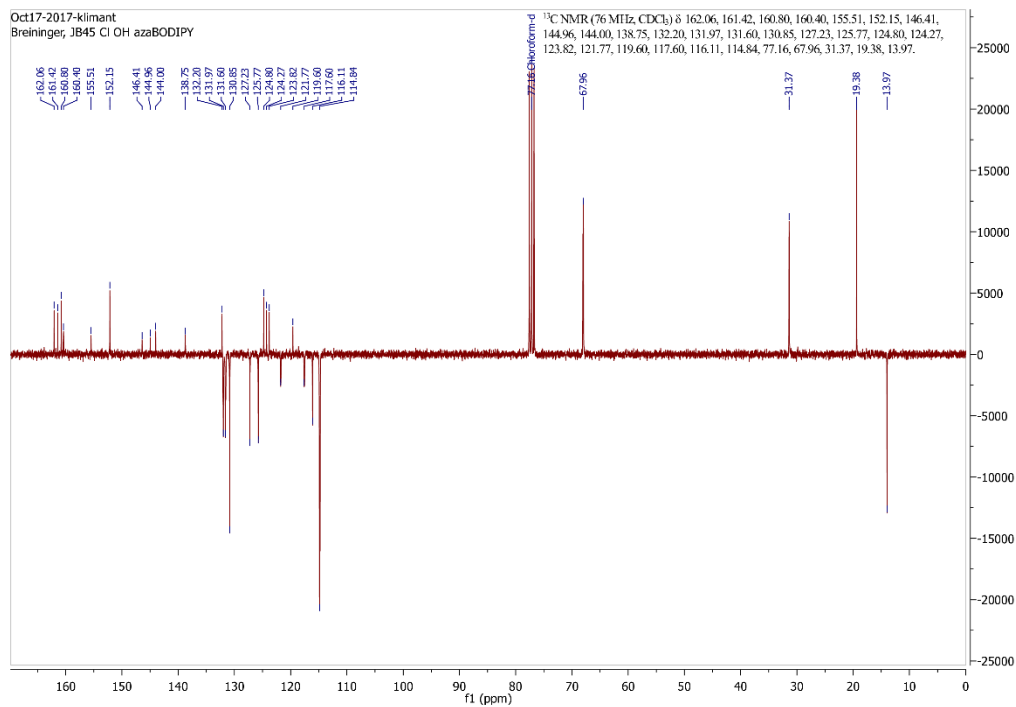


Figure S12: ^{13}C APT NMR spectrum of indicator **2**.

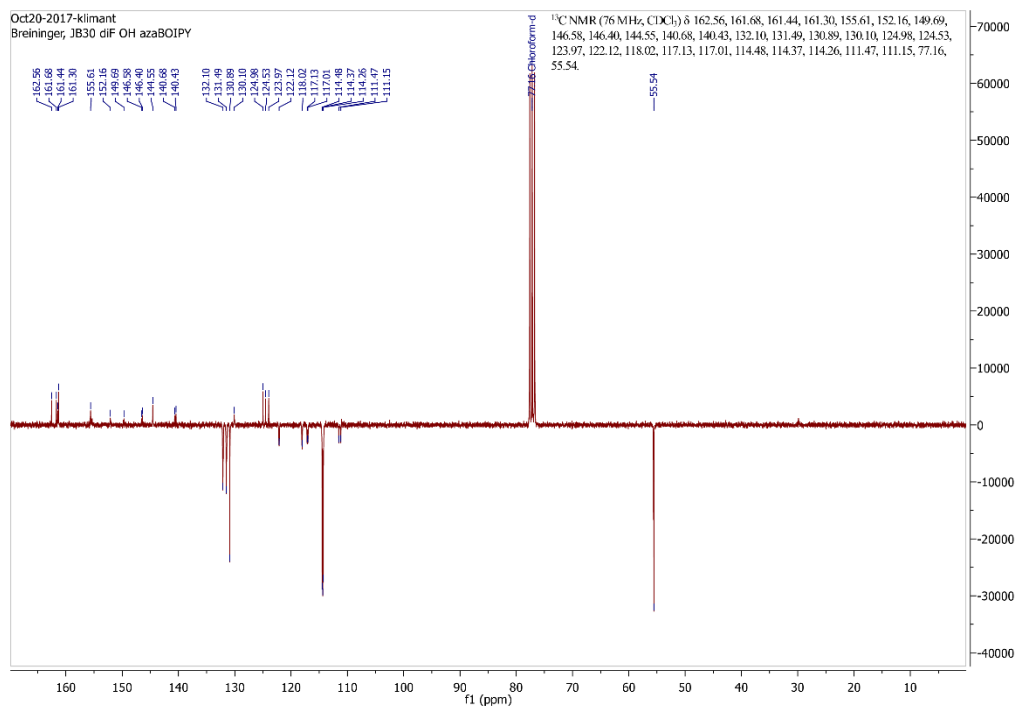


Figure S13: ^{13}C APT NMR spectrum of indicator **4**.

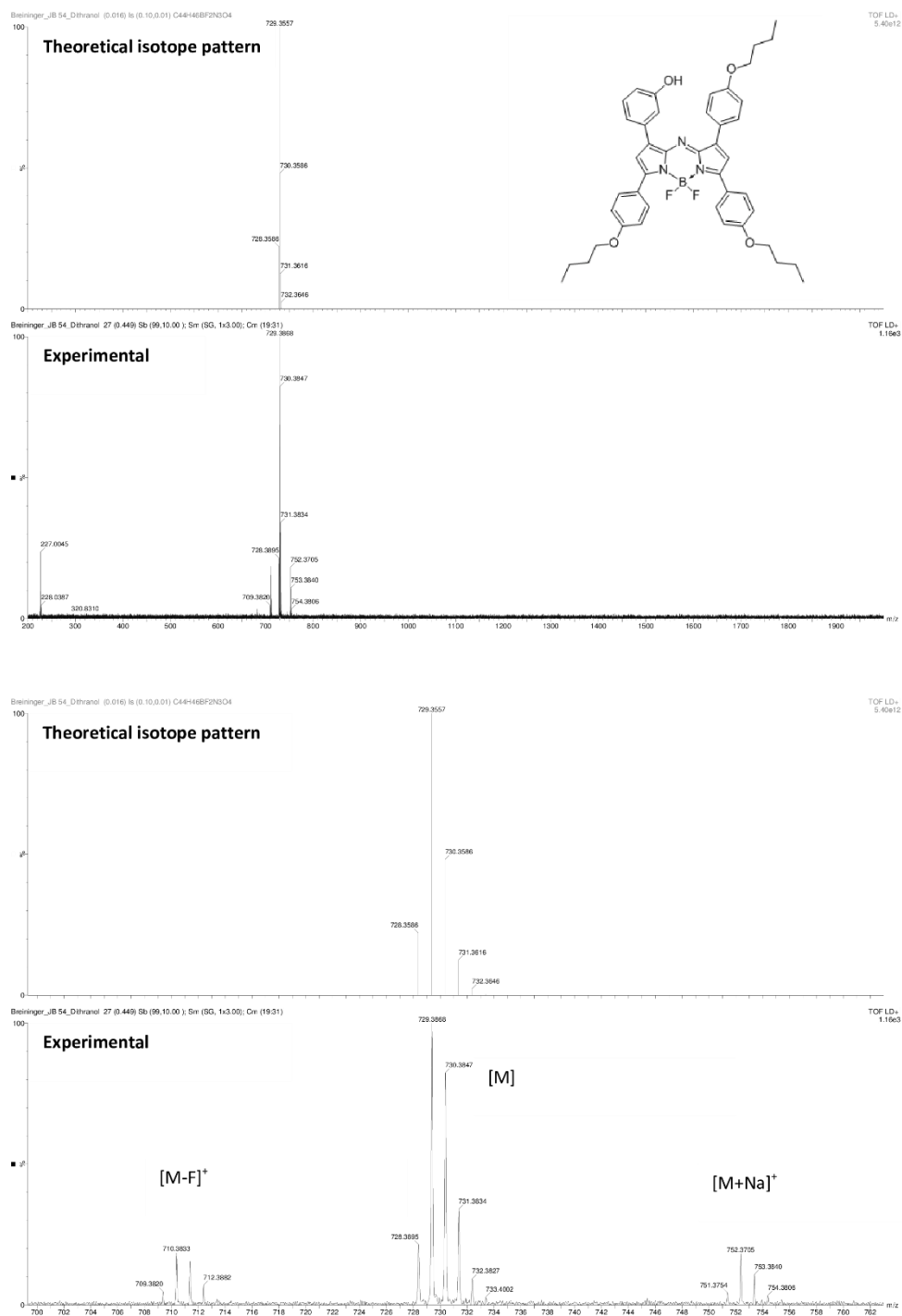


Figure S14: HR-MS (MALDI) spectrum of indicator 1.

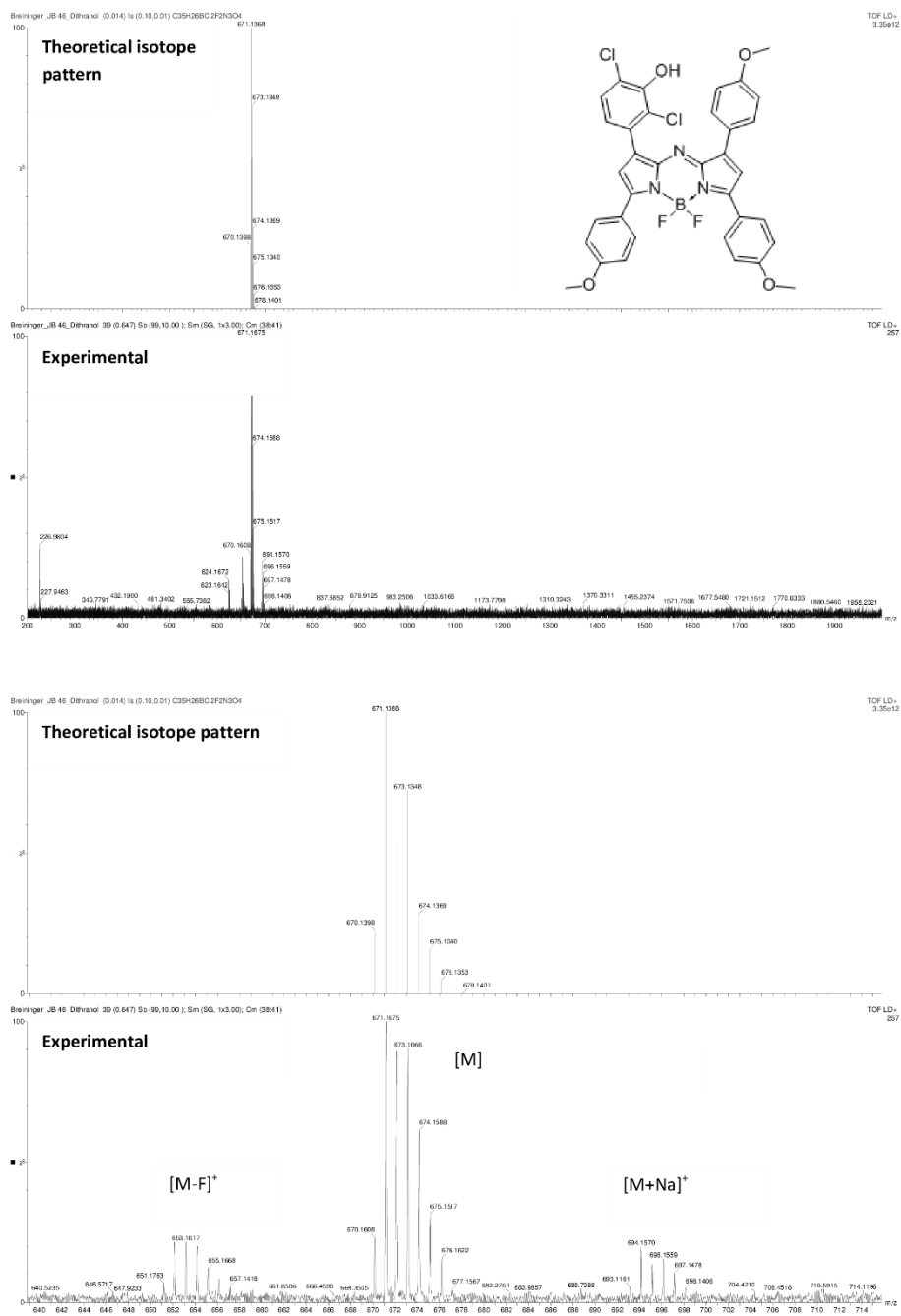


Figure S16: HR-MS (MALDI) spectrum of indicator **3**.

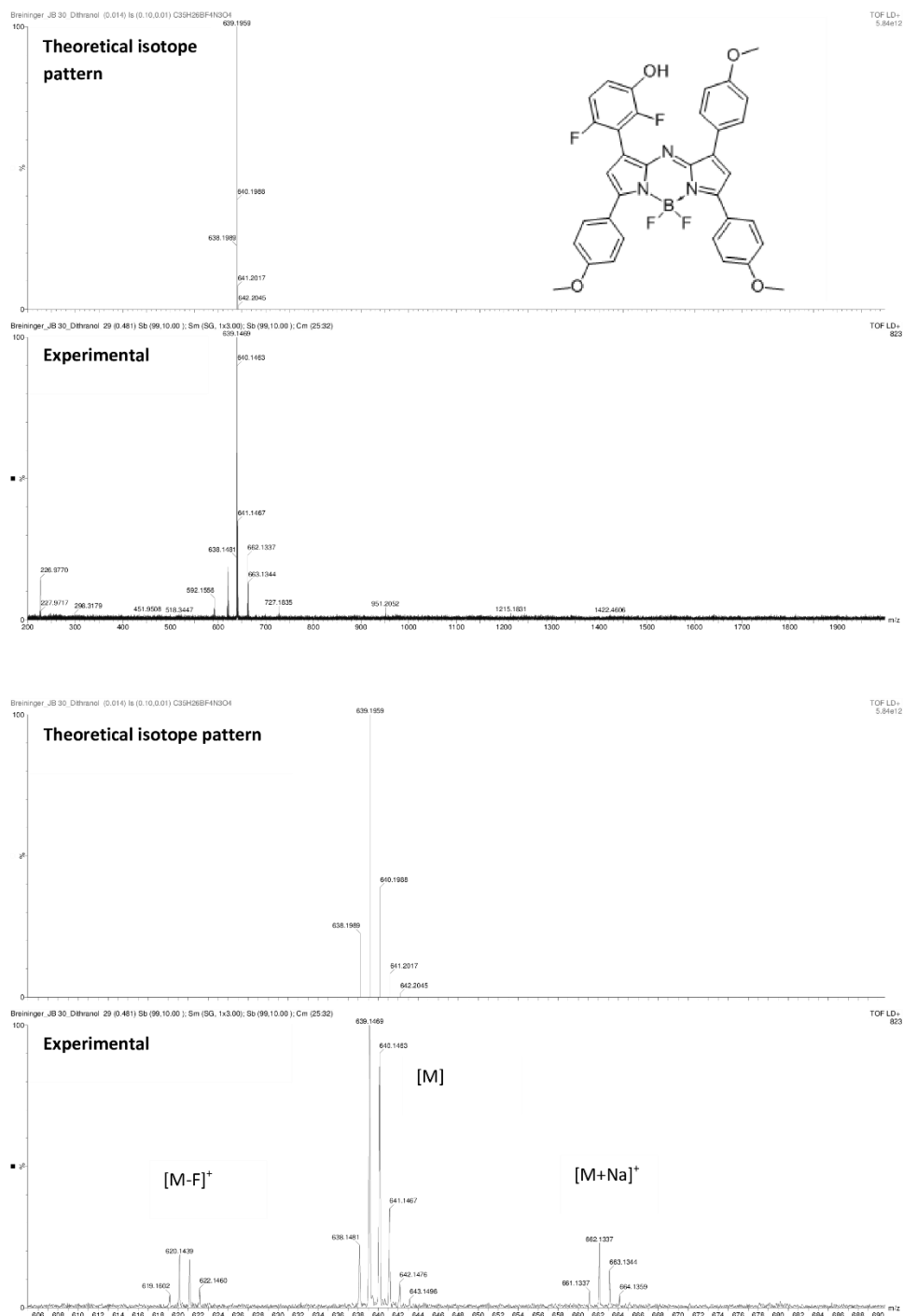


Figure S17: HR-MS (MALDI) spectrum of indicator 4.

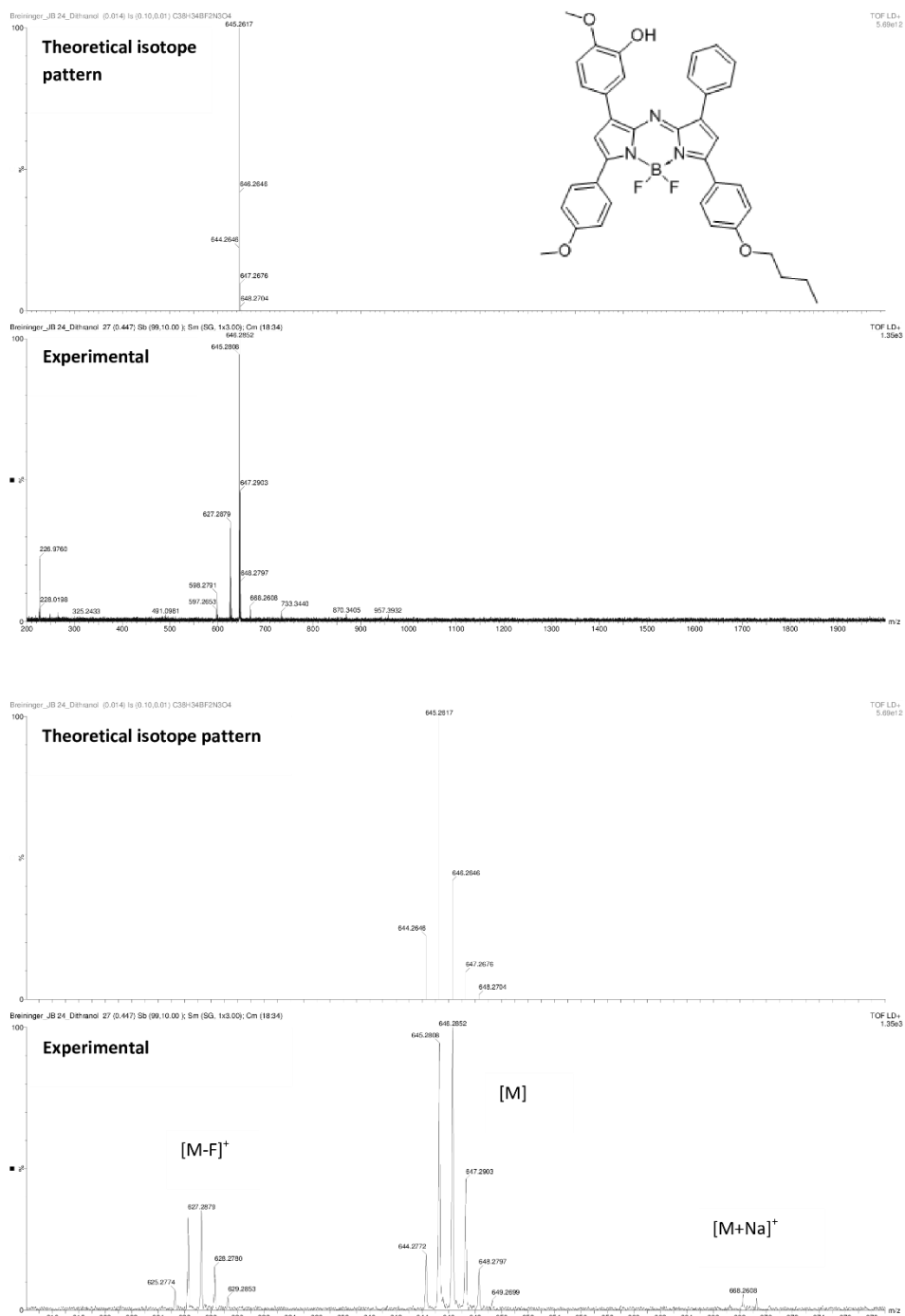


Figure S18: HR-MS (MALDI) spectrum of indicator **5**.