chemosensors

MDPI

Supplementary Material

Detection of a Nerve Agent Simulant by a Fluorescent Sensor Array

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Synthesis of probes

- Synthesis of RhBP

To a solution of Rhodamine B (0.552 g, 1.153 mmol) and DIPEA (0.23 mL, 1.32 mmol) in MeCN (100 mL) at 0° C under N_2, a solution of HBTU (0.5g, 1.32 mmol) was added. The mixture was stirred for 30 minutes at 0°, then an ice-cold solution of *N*-(2-hydroxyethyl)piperazine (0.162 mL, 1.32 mmol) in 15 mL of MeCN was added. Afterwards, the ice bath was removed, and the solvent was evaporated in a vacuum. The desired RhBP compound (yield 84%) was purified by column chromatography on silica gel using CH_2Cl_2/EtOH, 9.5:0.5 as the eluent. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68-7.57 (m, 2H), 7.55 (m, 1H), 7.34 (m, 1H), 7.27 (d, J = 9.5 Hz, 2H), 6.96 (dd, J_1 = 9.5 Hz, J_2 = 1.8 Hz, 2H), 6.77 (d, J = 1.8 Hz, 2H), 3.62 (m, 10H), 3.41 (m, 4 H), 2.50 (t, J = 5.5 Hz, 2H), 2.39 (m, 4 H), 1.33 (t, J = 8 Hz, 12 H).

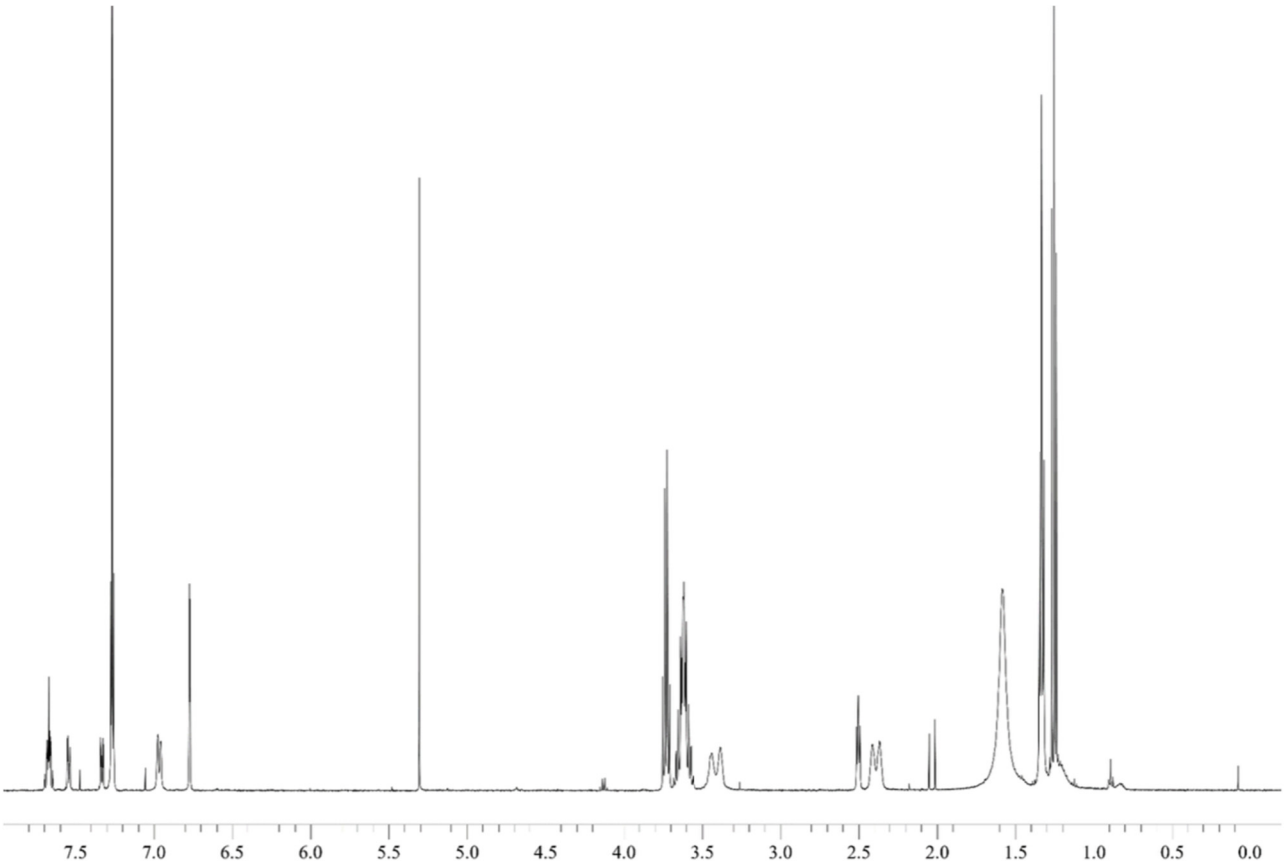

Figure S1. ^{1}H NMR spectrum of **RhBP** in $CDCl_3$.

- Synthesis of RhBM

To a solution of Rhodamine B (0.552 g, 1.153 mmol) and DIPEA (0.23 mL, 1.32 mmol) in MeCN (100 mL) at 0° C under N_2, a solution of HBTU (0.5 g, 1.32 mmol) was added. The mixture was stirred for 30 minutes at 0°, then an ice-cold solution of morpholine (0.113 mg, 1.153 mmol) in 15 mL of MeCN was added. Afterwards, the ice bath was removed, and the solvent was evaporated in a vacuum. The desired RhBM compound (yield 84%) was purified by column chromatography on silica gel using CH_2Cl_2/EtOH, 9.5:0.5 as the eluent. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (m, 2H), 7.55-7.52 (m, 1H), 7.34 (m, 1H), 7.27 (d, J = 12 Hz, 2H), 6.96 (dd, J_1 = 12 Hz, J_2 = 4 Hz, 2H), 6.76 (d, J = 4 Hz, 2H), 3.65–3.57 (q, J = 8 Hz, 8H), 3.49 (m, 2H), 3.40 (m, 2H), 1.31 (t, J = 8 Hz, 12 H).

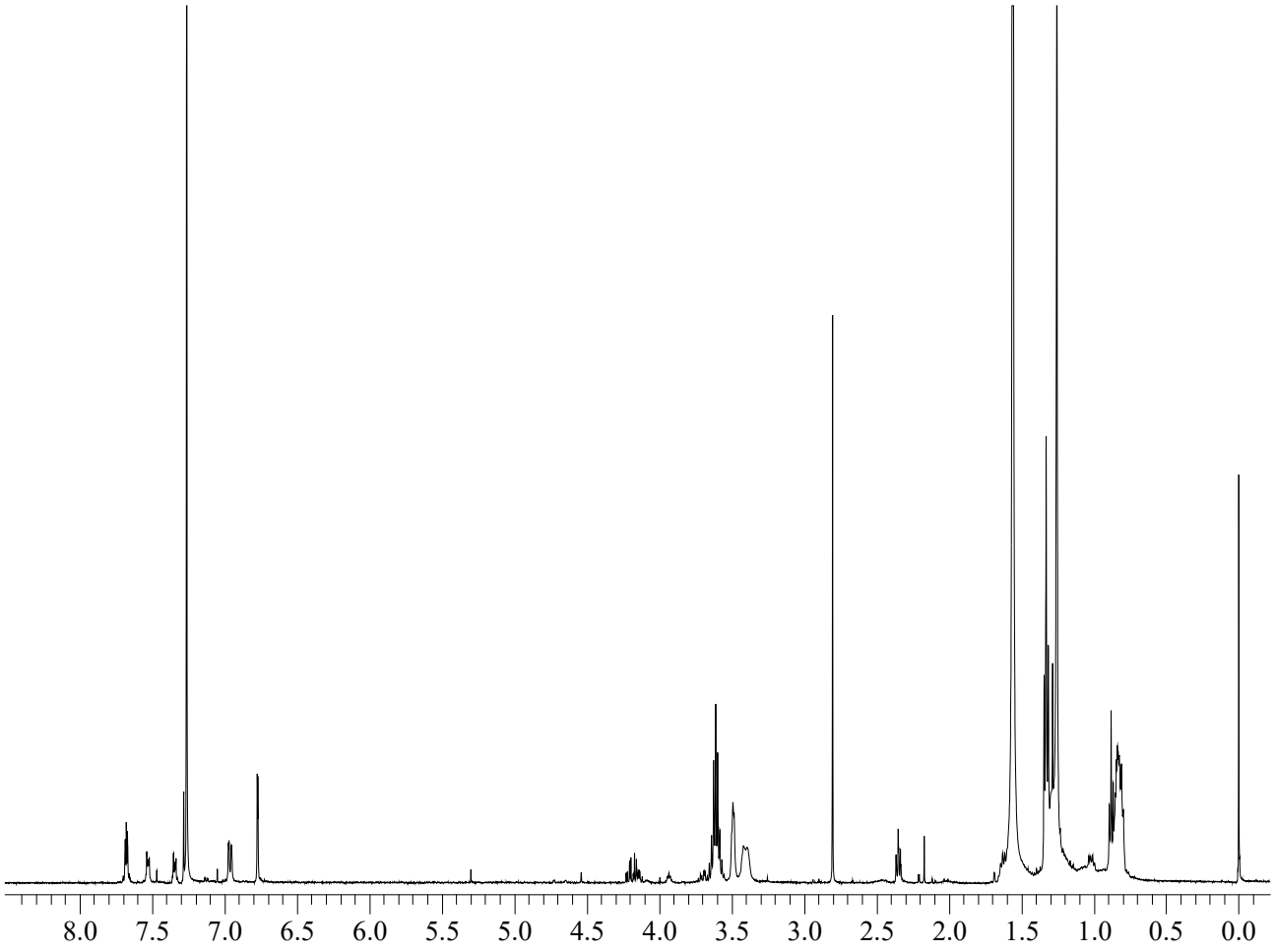

Figure S2. ^{1}H NMR spectrum of **RhBM** in $CDCl_3$.

- Synthesis of PBP

To a solution of 2,4-dimethyl-3-ethylpyrrole (1.19 mL, 8.8 mmol, 2 eq) in dry dichloromethane (250 mL), 0.42 mL of 4-pyridinecarboxaldehyde (4.4 mmol, 1 eq) was added, followed by a catalytic amount of trifluoroacetic acid. The mixture was stirred overnight under nitrogen and then concentrated to 50.0 mL under vacuum. Hence, 1.50 g of 2,3-dichloro-5,6-dicyanobenzoquinone (1.5 eq) were added, and the mixture was further stirred for 2 h. Triethylamine (6.0 mL) and $BF_3(OEt_2)$ (7.0 mL) were then added. After 2 h, the solvent was removed under vacuum, and the oily crude compound was redissolved in CH_2Cl_2 and washed with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The mixture was purified by silica flash column chromatography ($CHCl_3$) to yield PBP (22% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.90 (d, *J* = 6.0 Hz, 2H), 7.75 (d, *J* = 6.0 Hz, 2H), 2.53 (s, 6H), 2.30 (q, *J* = 7.5 Hz, 4H), 1.27 (s, 6H), 0.98 (t, *J* = 7.5 Hz, 6H).

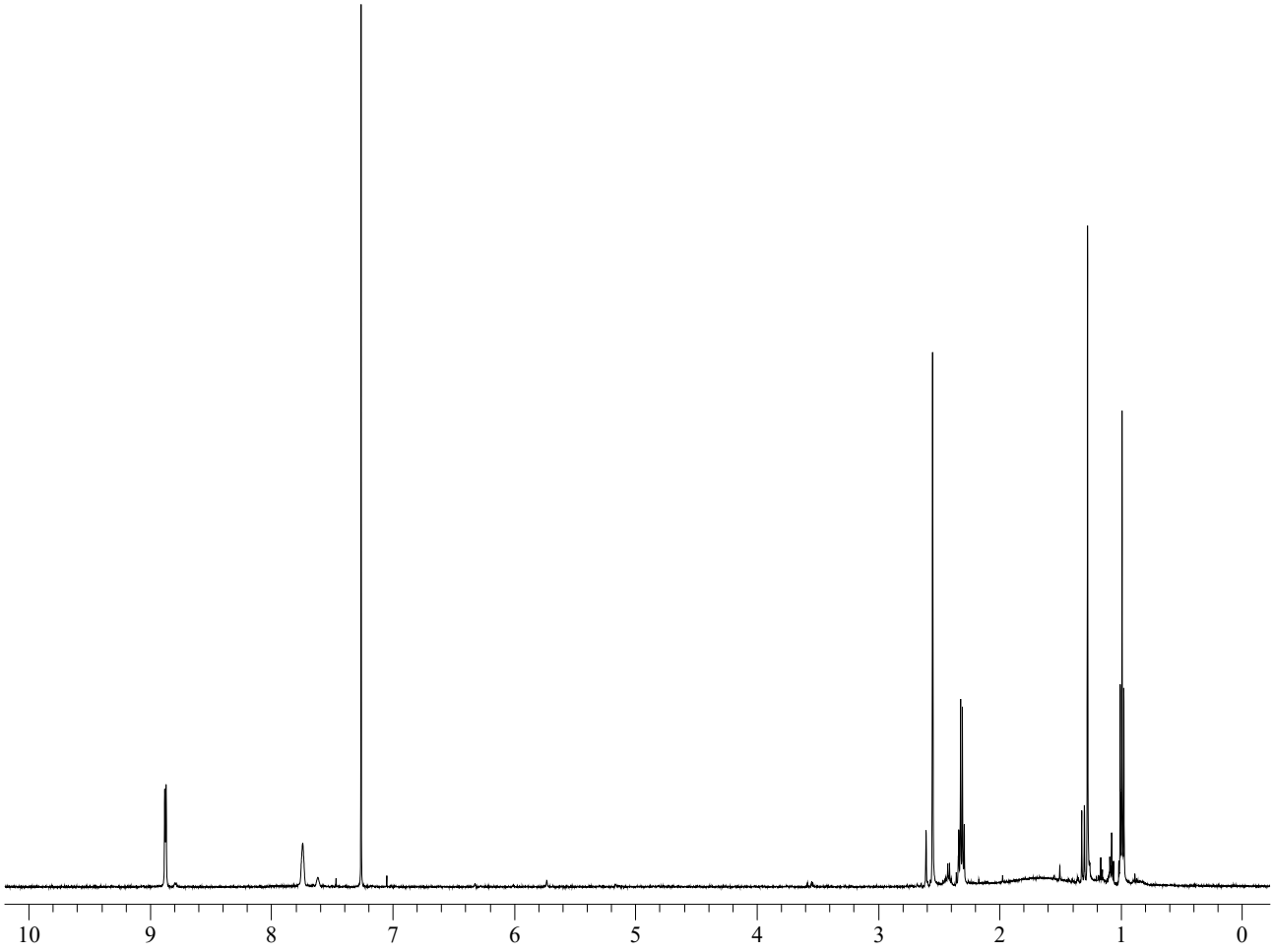

Figure S3. ^{1}H NMR spectrum of **PBP** in $CDCl_3$.

- Synthesis of MPB

2,4-dimethyl-4-ethyl pyrrole (2.38 mL, 17.6 mmol, 2 eq.) and 3- pyridinecarboxaldehyde (0.84 mL, 8.8 mmol, 1 eq.) was dissolved in dry CH_2Cl_2 (500 mL) under nitrogen atmosphere. A catalytic amount of trifluoracetic acid (TFA) was added, and the reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC (silica gel, CH_2Cl_2) following the total conversion of the starting aldehyde. Afterwards, 3 g (13.2 mmol, 1.5 eq.) of DDQ were added. The mixture was stirred at room temperature for 6 hours, then 12 mL of triethylamine and 14 mL of $BF_3(OEt_2)$ were added and stirred overnight at room temperature. The solvent was removed under vacuum, and the residue was redissolved in CH_2Cl_2 and filtered. The desired Bodipy MBP (yield 17%) was purified by chromatography (silica gel, CH_2Cl_2 100%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, J = 7.5 Hz, 6H), 1.28 (s, 6H), 2.30 (q, J = 7.8 Hz, 4H), 2.54 (s, 6H), 7.45 (dd, J_1 = 4.9 Hz, J_2 = 2.9 Hz, 2H), 7.64 (t of d, J_1 = 7.8 Hz, J_2 = 2.0 Hz, 1H), 8.56 (dd, J_1 = 2.0 Hz, J_2 = 1.0 Hz, 1H), 8.75 (dd, J_1 = 4.9 Hz, J_2 = 2.0 Hz, 1H) ppm.

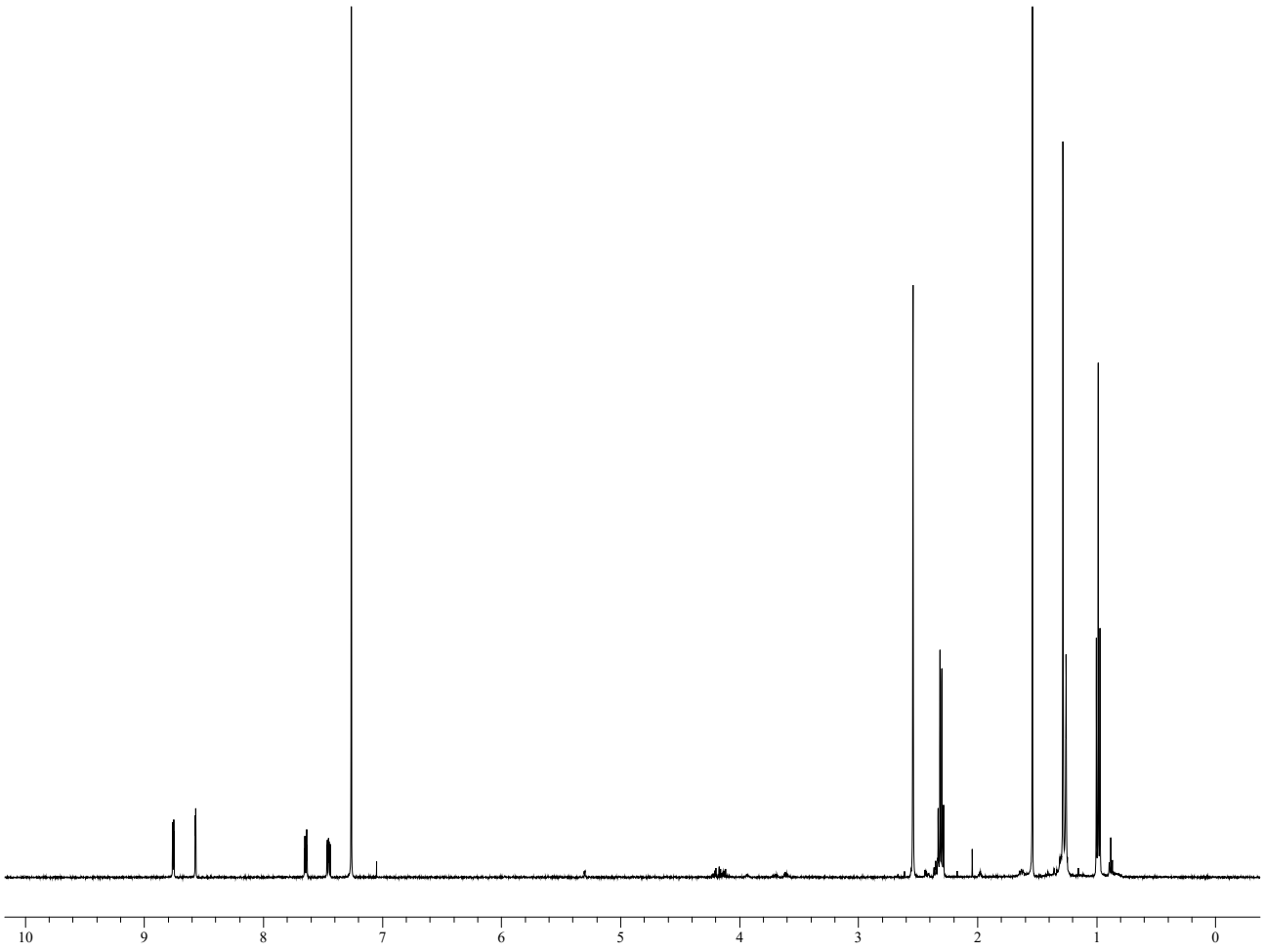

Figure S4. ^{1}H NMR spectrum of **MBP** in $CDCl_3$.

- Synthesis of OBP

To a solution of 2,4-dimethyl-3-ethylpyrrole (1.19 mL, 8.8 mmol, 2 eq) in 250 mL of dry dichloromethane, 0.42 mL of 2-pyridinecarboxaldehyde (4.4 mmol, 1 eq) and a catalytic amount of trifluoroacetic acid were added. After overnight stirring under nitrogen, the mixture was concentrated to 50.0 mL under reduced pressure, and 1.50 g of 2,3-dichloro-5,6-dicyanobenzoquinone (1.5 eq) was added and stirred for 2 h. Triethylamine (6.0 mL) and $BF_3(OEt_2)$ (7.0 mL) were then added to the mixture. After 2 h the mixture was concentrated under reduced pressure and washed with CH_2Cl_2 and water. The organic layer was dried with anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The mixture was purified by silica flash column chromatography ($CHCl_3$) to yield OBP (24% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.82 (d, *J* = 4.5 Hz, 1H), 7.90 (dt, *J* = 1.5, 7.5 Hz, 1H), 7.49 (m, 2H), 2.53 (s, 6H), 2.30 (q, *J* = 7.5 Hz, 4H), 1.21 (s, 6H), 0.98 (t, *J* = 7.5 Hz, 6H).

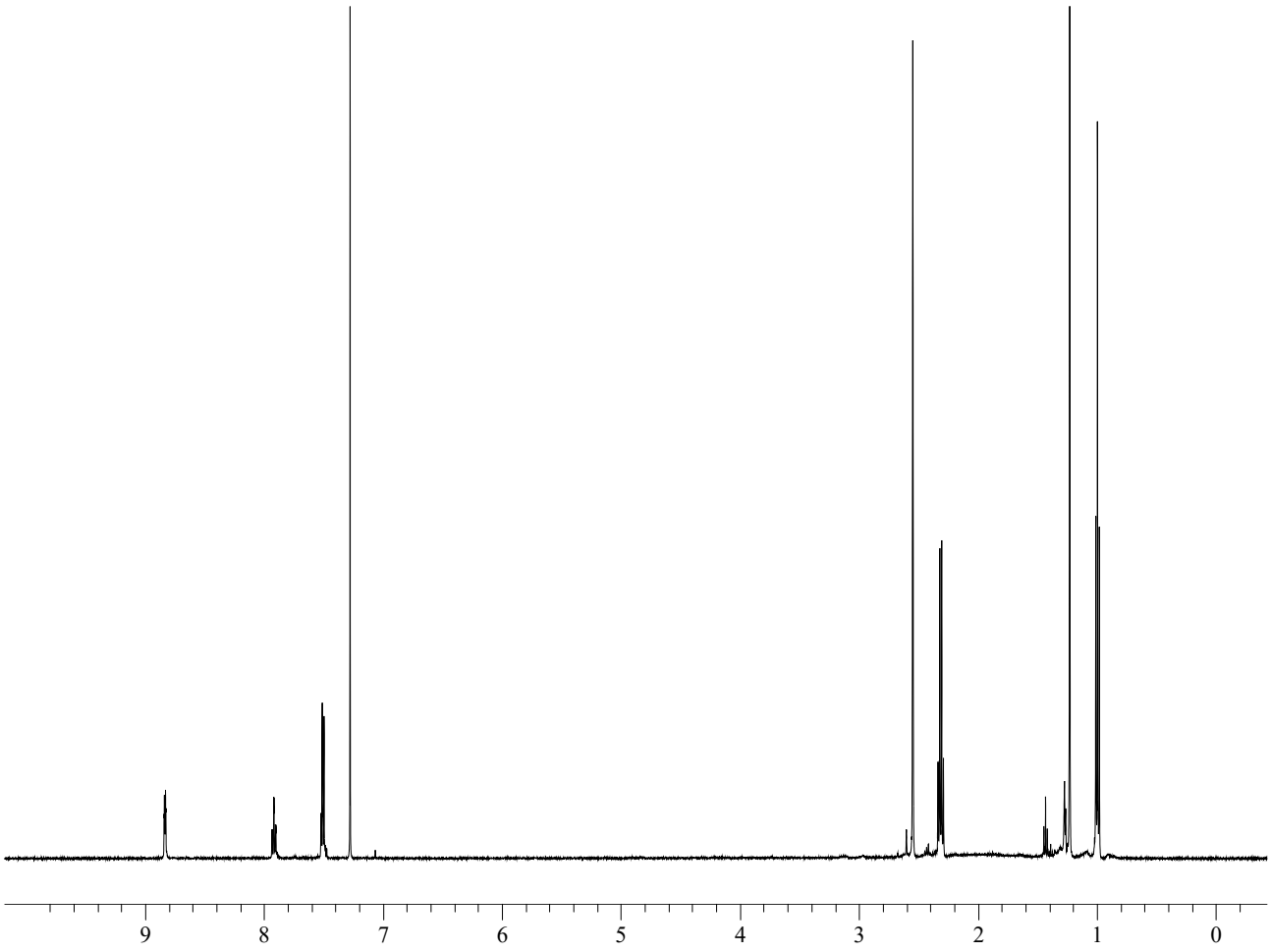

Figure S5. ^{1}H NMR spectrum of **OBP** in $CDCl_3$.

- Synthesis of probe PBEP

An amount of 600 mg of PBP (1.57 mmol) was dissolved in dry CH_3CN (25 mL) under nitrogen. Then, 286 mg of dry K_2CO_3 were added, and the mixture was heated to 45° C. After 10 minutes, 10 mL of iodoethane were added, and the reaction mixture was stirred for 24 h. The total conversion of the starting reagents was monitored by TLC (Al_2O_3, CH_2Cl_2). At the end of the reaction, the solvent was removed under reduced pressure, and the PBEP (yield 95%) was purified by chromatography (CH_2Cl_2/CH_3OH 95/5). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.69 (d, 2H, J = 6.4 Hz), 8.05 (d, 2H, J = 6.1 Hz), 5.27 (q, 7.1 Hz, 2H), 2.55 (s, 6H), 2.31 (q, J = 7.5 Hz, 4H), 1,84 (t, J = 7.1 Hz, 3H), 1.34 (s, 6H), 0.99 (t, J = 7.5 Hz, 6H) ppm.

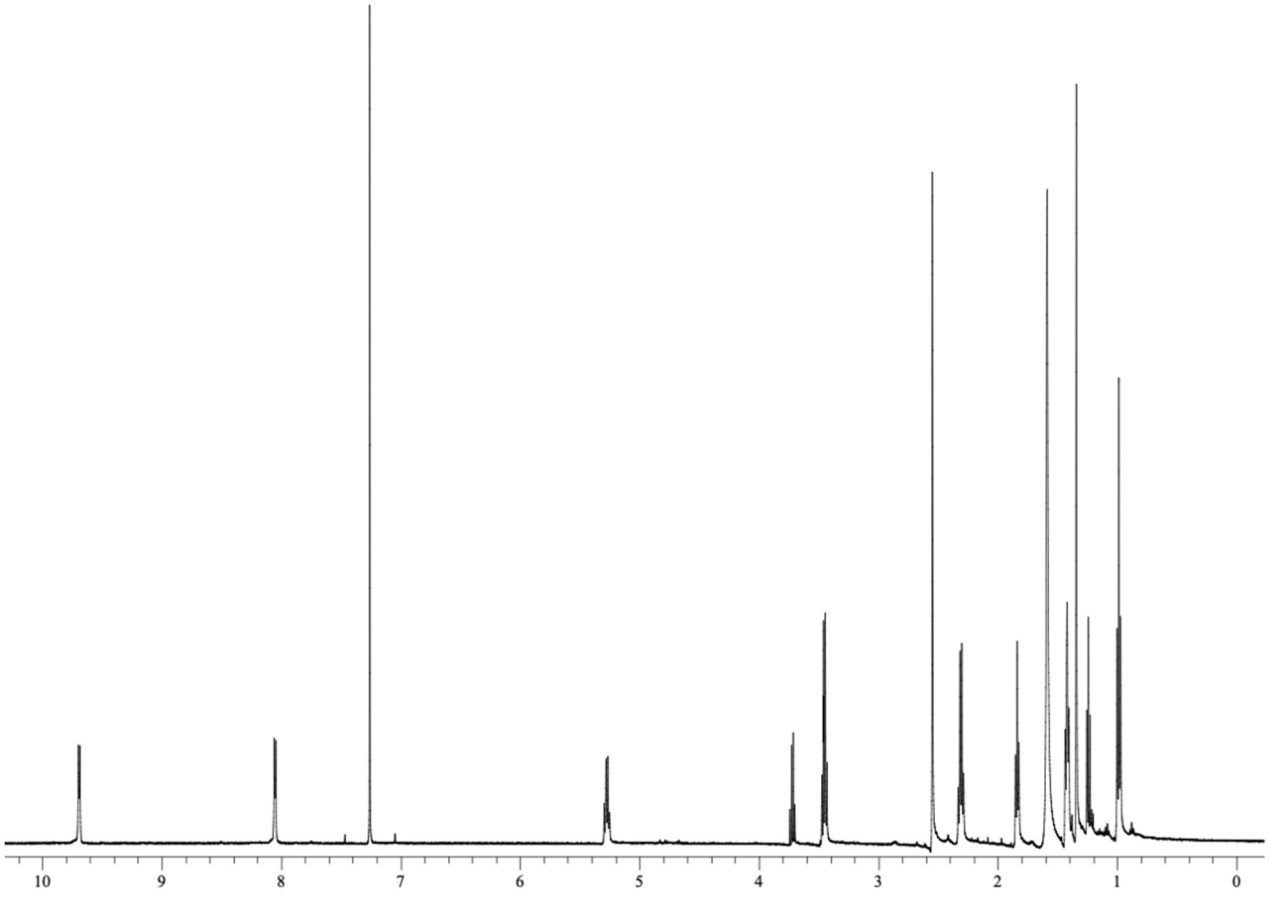

Figure S6. ^{1}H NMR spectrum of **PBEP** in $CDCl_3$.

- Synthesis of MBEP

An amount of 554 mg of MBP (1.45 mmol) were dissolved in dry CH_3CN (25 mL) under nitrogen. Then, 240 mg of dry K_2CO_3 were added, and the mixture was heated to 45° C. After 10 minutes, 10 mL of iodoethane were added, stirring for 24 h. The total conversion of the starting reagent was monitored by TLC (Al_2O_3, CH_2Cl_2). The solvent was evaporated under vacuum, and the MBEP (yield 95%) was purified by chromatography (Al_2O_3, CH_2Cl_2/CH_3OH, from 100/0 to 90/10). ^1H NMR (500 MHz, $CDCl_3$): δ 1.0 (t, J = 7.5 Hz, 6H), 1.34 (s, 6H), 1.8 (t, J = 7.3 Hz, 3H), 2.32 (q, J = 7.5 Hz, 4H), 2.56 (s, 6H), 5.17 (q, J = 7.3 Hz, 2H), 8.4 (m, 2H), 8.51 (d, J = 3.7 Hz, 1H), 10.35 (t, J = 6.1 Hz, 1H) ppm.

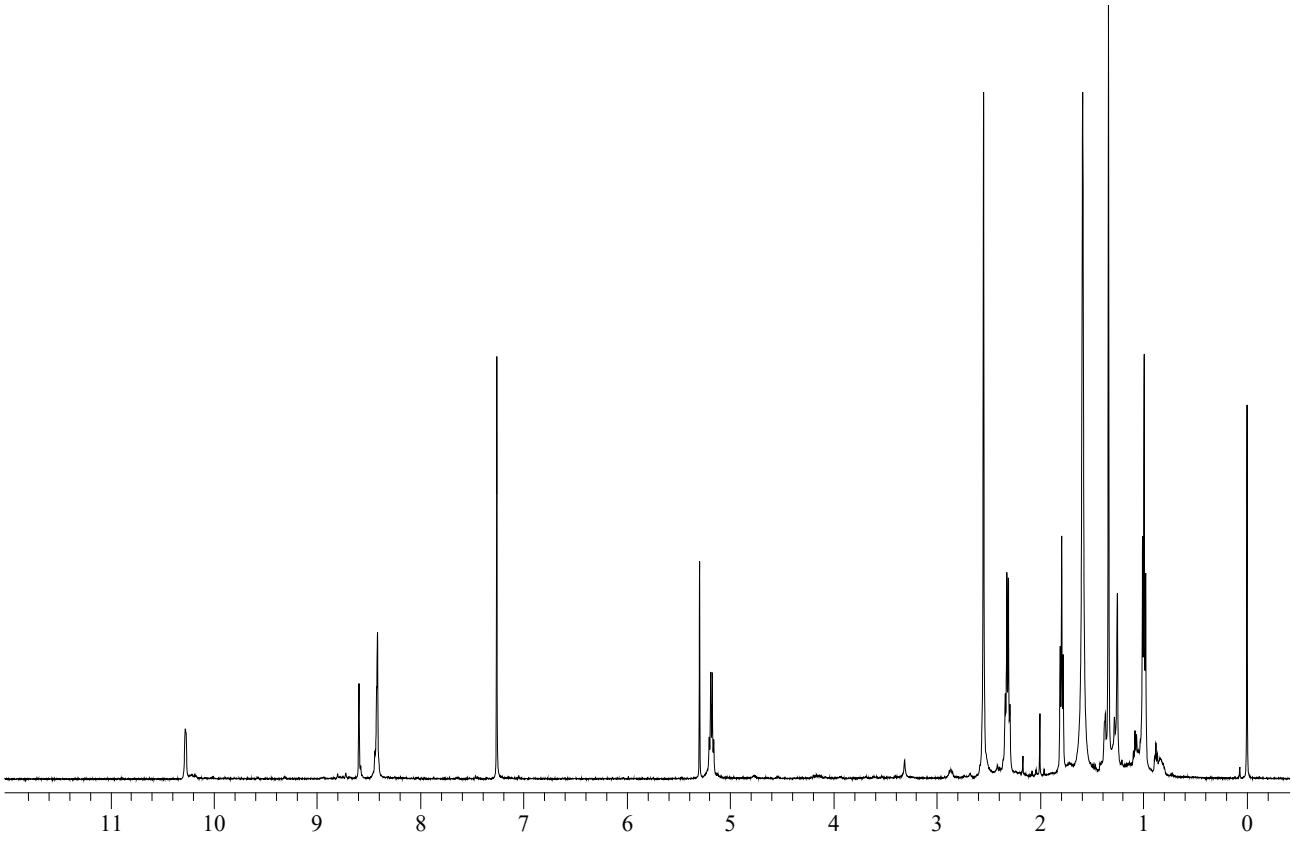

Figure S7. ^1H NMR spectrum of **MBEP** in $CDCl_3$.

- Synthesis of OBEP

To a solution of OBP (0.40 g, 1.5 mmol) in dry acetonitrile (25 mL), iodoethane (3.9 g, 20 mmol) was added under a nitrogen atmosphere. The mixture was then stirred at 50 °C for 2 days. Afterwards, the reaction mixture was cooled down to room temperature, and the solvent was removed under reduced pressure. The residue was purified by neutral aluminum oxide flash column chromatography (CH_2Cl_2/CH_3OH from 100/0 to 95/5), recovering the starting compound OBP (conv. 20%), and obtaining 98.1 mg of OBEP (61% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.76 (d, *J* = 6.5 Hz, 1H), 8.67 (d of t, *J* = 1.5, 7.5 Hz, 1H), 8.56 (t of d, *J* = 1.5, 7.5 Hz, 1H), 7.96 (dd, *J* = 1.5, 7.5 Hz, 1H), 4.99 (q, *J* = 7.5 Hz, 2H), 2.58 (s, 6H), 2.33 (q, *J* = 7.5 Hz, 4H), 1.67 (t, *J* = 7.5 Hz, 3H), 1.27 (s, 6H), 1.05 (t, *J* = 7.5 Hz, 6H).

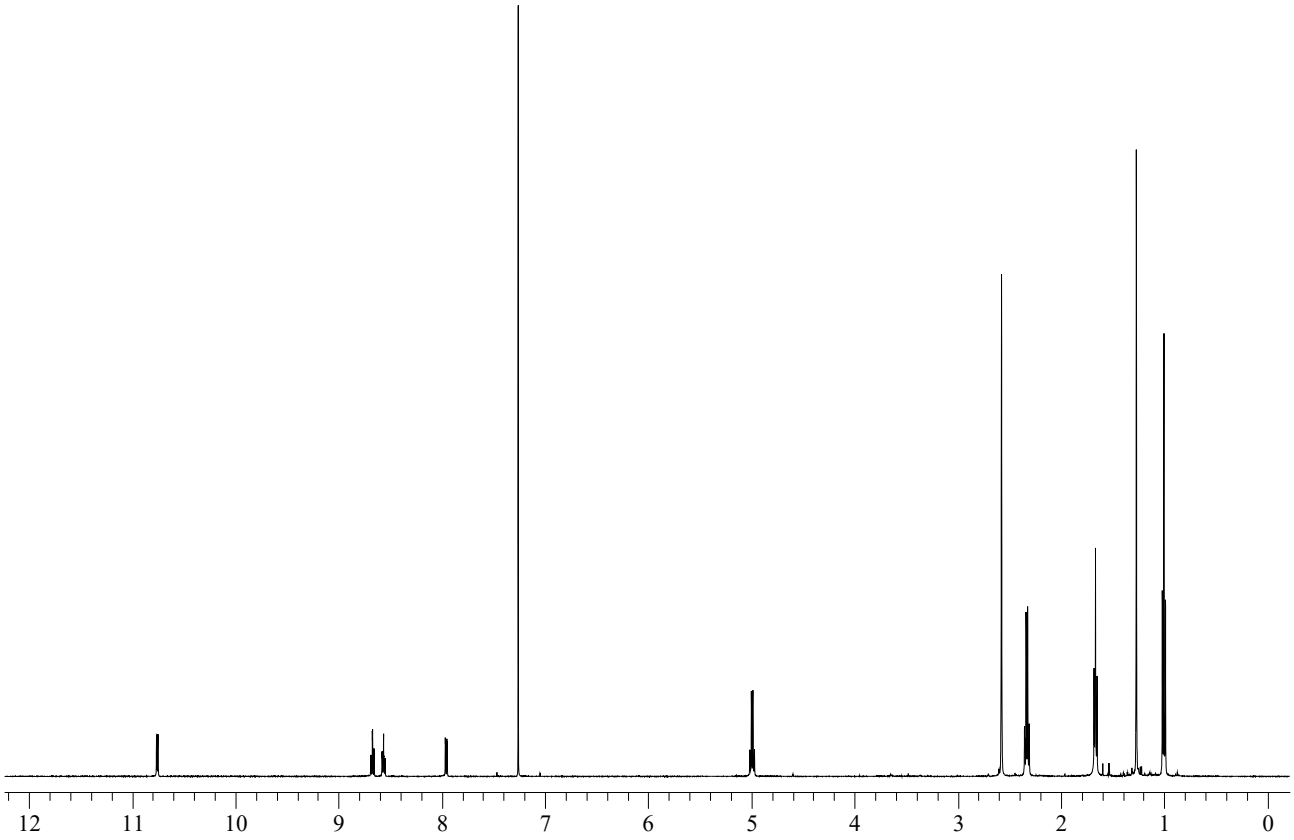

Figure S8. ^{1}H NMR spectrum of **OBEP** in $CDCl_3$.

- Synthesis of isobuthyl-Br-NO_2-naphthalimide

An amount of 1 g (3.1 mmol) of 4 Br-NO_2-anhydride was dissolved in 90 mL of absolute ethanol at 70 °C for 30 min. Afterward, the mixture was heated up to 50 °C, and 320 μL (3.1 mmol) of isobutyl amine in absolute ethanol (20 mL) were added dropwise. The reaction mixture was stirred at 50 °C for 18 h under nitrogen, monitoring the reaction by means of TLC (*n*-hexane/ethyl acetate 7:3). The solid precipitate was filtered, obtaining 620 mg (53% yield) of isobuthyl-Br-NO_2-naphthalimide. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.71 (d, *J* = 7.5 Hz, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 4.04 (d, *J* = 7.5 Hz, 2H), 2.23 (m, 1H), 0.98 (d, *J* = 6.5 Hz, 6H) ppm.

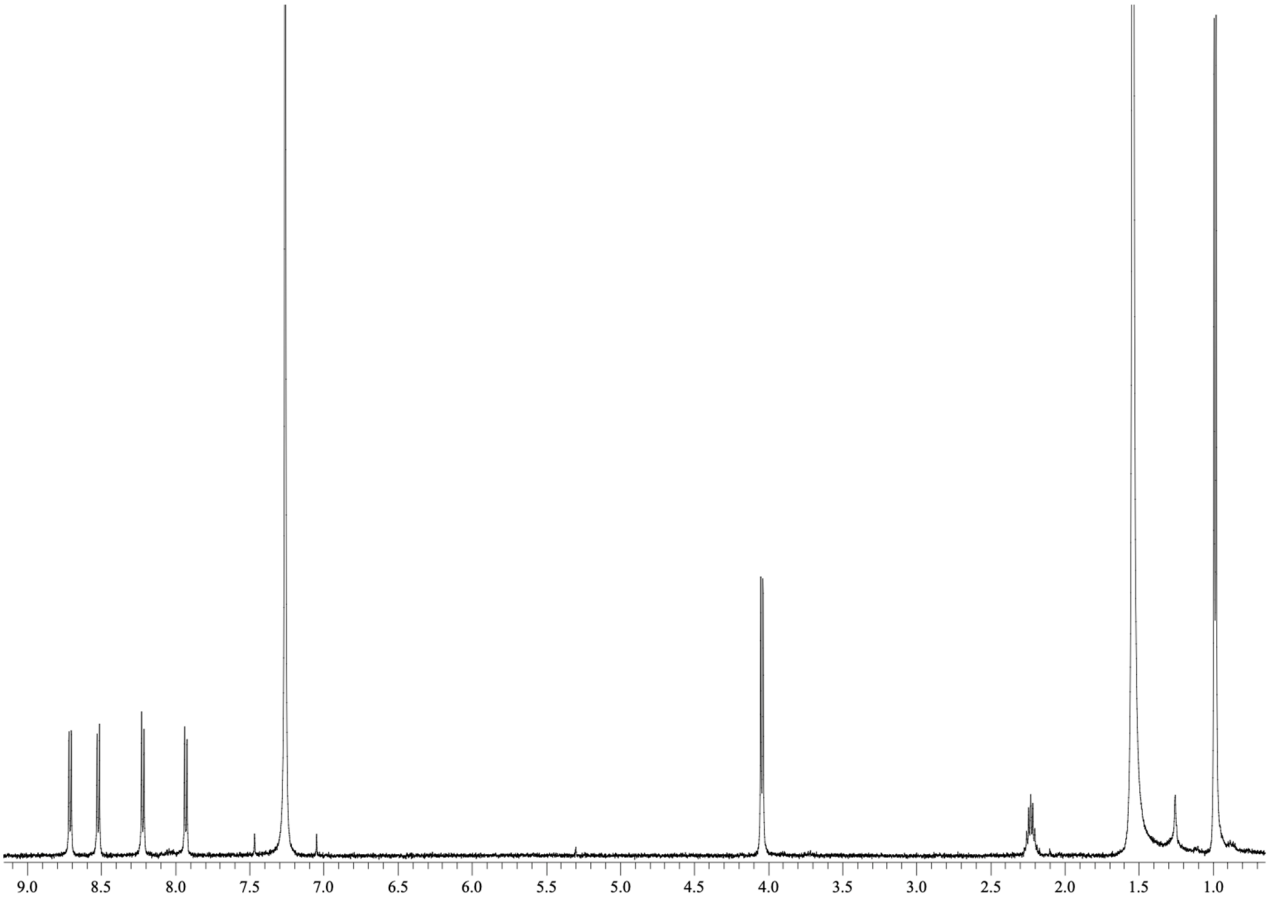

Figure S9. ^{1}H NMR spectrum of **isobuthyl-Br-NO_2-naphthalimide** in $CDCl_3$.

- Synthesis of Napht-1

An amount of 172 mg (0.46 mmol) of isobutyl-Br-NO_2-naphthalimide was dissolved in 20 mL of 2-methoxyethanol under nitrogen, and 10 equivalents of ethanolamine were added to the solution. The reaction mixture was stirred at reflux for 36 h. Napht-1 (47% yield) was obtained by removing the solvent under vacuum and after purification by flash chromatography (silica gel, $CHCl_3$/MeOH 9:1). ^{1}H NMR (500 MHz, acetone-d_6): δ 8.43 (d, J = 8.5 Hz, 2H), 6.74 (d, J = 8.5 Hz, 2H), 6.60 (t, J = 5.0 Hz, 2H), 4.05 (q, J = 5.0 Hz, 4H), 4.01 (d, J = 7.5 Hz, 2H), 3.42 (q, J = 5.0 Hz, 4H), 2.55 (br, 2H), 2.24 (m, 1H), 0.96 (d, J = 6.5 Hz, 6H) ppm.

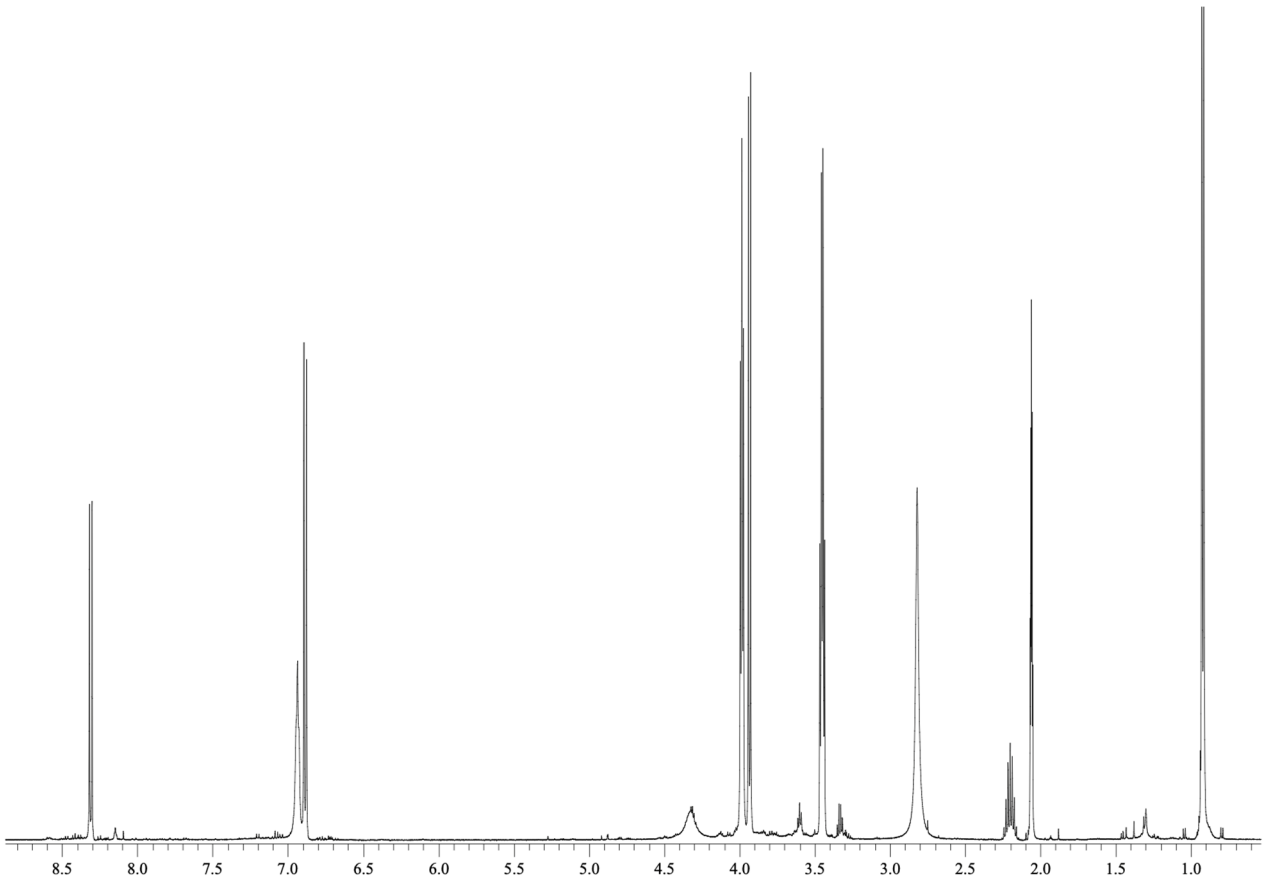

Figure S10. ^{1}H NMR spectrum of **Napht-1** in acetone-d_6.

- Synthesis of BDPy-CH_2Cl

Chloroacetyl chloride (642 μl, 8.1 mmol) and 2,4-dimethyl-3-ethylpyrrole (2.18 ml, 16.2 mmol) were added to dry dichloromethane (100 mL) under nitrogen. The reaction mixture was stirred at room temperature for 6 h under nitrogen, then triethylamine (6 mL, 43.2 mmol) was added to the residue, and the resulting mixture was stirred at room temperature for 30 min. After 30 minutes, boron trifluoride diethyl etherate (7.0 mL, 55.7 mmol) was added, and the reaction mixture was stirred for 12 h. The reaction was monitored by TLC (silica gel: *n*-hexane/CH_2Cl_2 6:4). The solvent was removed under reduced pressure, and BDPy-CH_2Cl was obtained after purification by column chromatography (yield 22%). ^{1}H NMR (500MHz, $CDCl_3$): δ 1.06 (t, 6H, J = 7.3 Hz), 2.41 (q, 4H, J = 7.3 Hz), 2.46 (s, 6H), 2.51 (s, 6H), 4.83 (s, 2H) ppm.

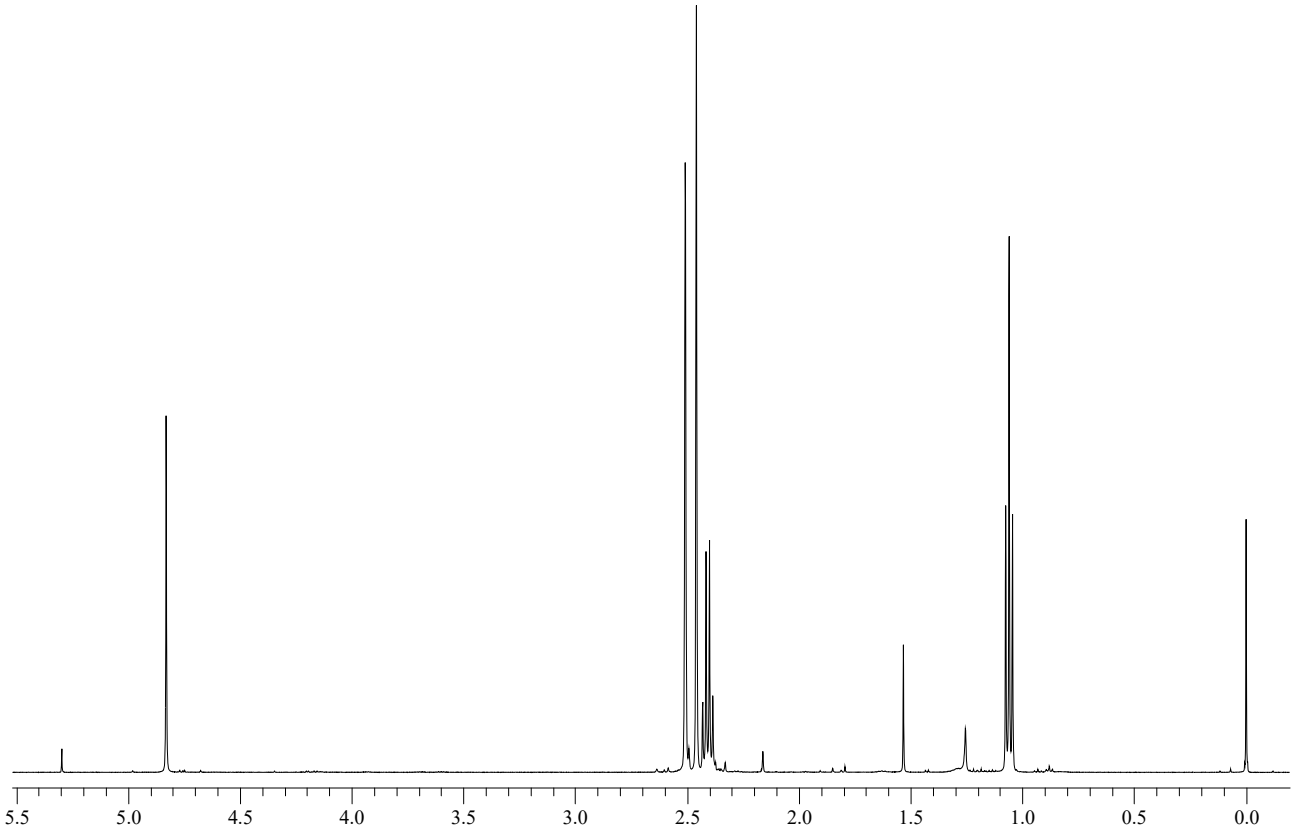

Figure S11. ^{1}H NMR spectrum of **BDPy-CH_2Cl** in $CDCl_3$.

- Synthesis of BDPy-AE

To a solution of BDPy-CH_2Cl (311.4 mg, 0.883 mmol) in acetonitrile dry (50mL), 1.23 mL of triethylamine and 0.266 mL of ethanolamine (4.41 mmol) were added, and the reaction mixture was stirred at 80 °C for 4 h under nitrogen. The reaction was monitored by TLC (silica gel: *n*-hexane/CH_2Cl_2 6:4). After the conversion, the solvent was removed under vacuum, and BDPy-AE was obtained after purification by column chromatography (yield 49%). ^{1}H NMR (500 MHz; $CDCl_3$): δ 1.05 (t, *J* = 7.5 Hz, 3H), 1.10 (t, *J* = 7.5 Hz, 3H), 2.36 (s, 6H), 2.41 (q, *J* = 7.5 Hz, 2H), 2.49 (q, *J* = 7.5 Hz, 2H), 2.64 (s, 6H), 2.87 (t, *J* = 5.0 Hz, 2H), 3.65 (t, *J* = 5.0 Hz, 2H), 3.96 (s, 2H) ppm.

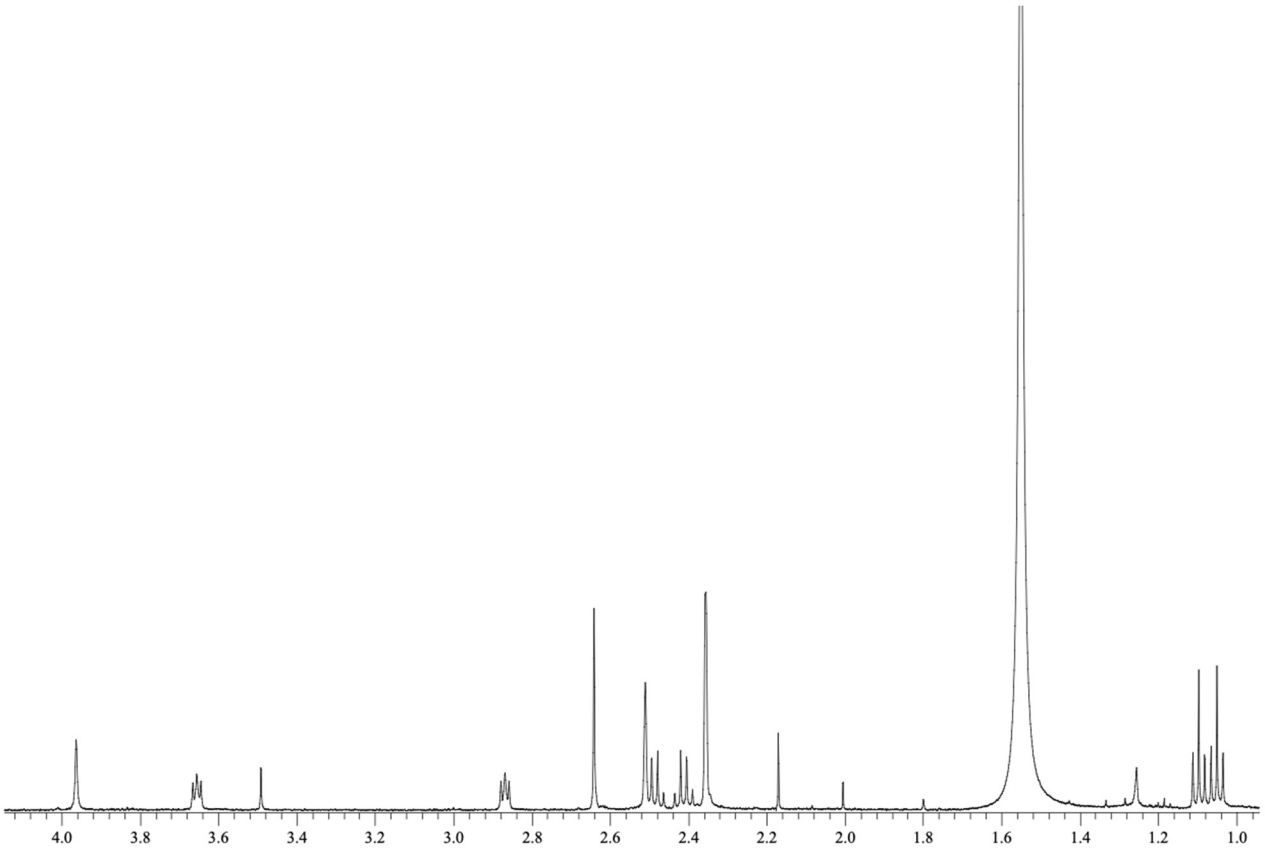

Figure S12. ^{1}H NMR spectrum of **BDPy-AE** in $CDCl_3$.

- *Synthesis of BDPy-Di-AE*

An amount of 294 mg (0.833 mmol) of BDPy-CH_2Cl was dissolved in 15 mL of dry acetonitrile. To this solution, 1.16 mL of triethylamine and 402 μL (4.17 mmol) of *N*,*N*-diethanolamine were added. The reaction mixture was stirred at 80 °C under nitrogen for 16 h and monitored by TLC (silica gel: *n*-hexane/CH_2Cl_2 6:4). Then the solvent was evaporated under a vacuum, and BDPy-Di-AE was obtained after column chromatography (yield 49%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.04 (t, *J* = 7.5 Hz, 6H), 2.40 (q, *J* = 7.5 Hz, 4H), 2.44 (s, 6H), 2.50 (s, 6H), 2.88 (t, *J* = 5 Hz, 4H), 3.60 (t, *J* = 5 Hz, 4H), 4.13 (s, 2H) ppm.

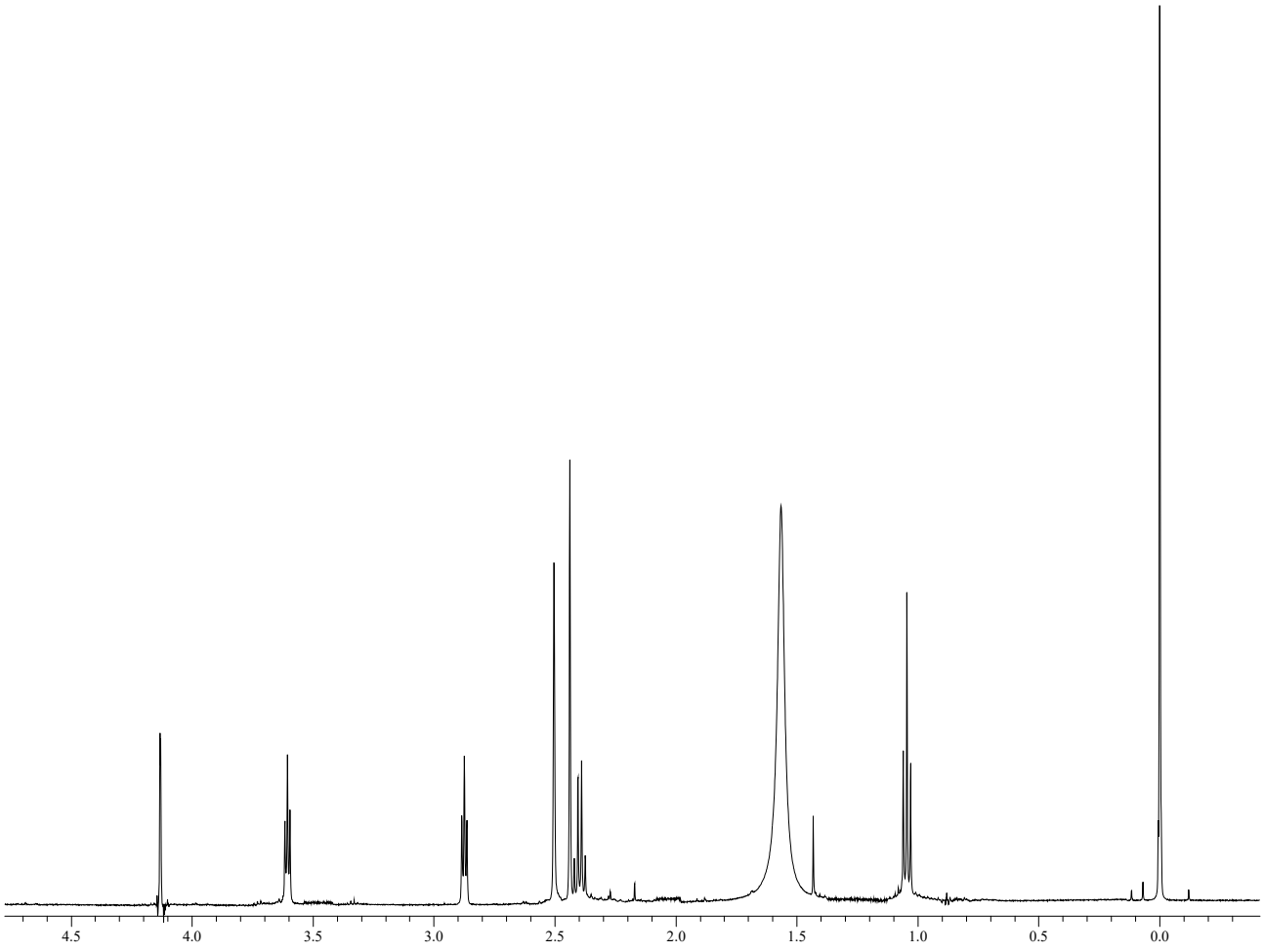

Figure S13. ^{1}H NMR spectrum of **BDPy-Di-AE** in $CDCl_3$.

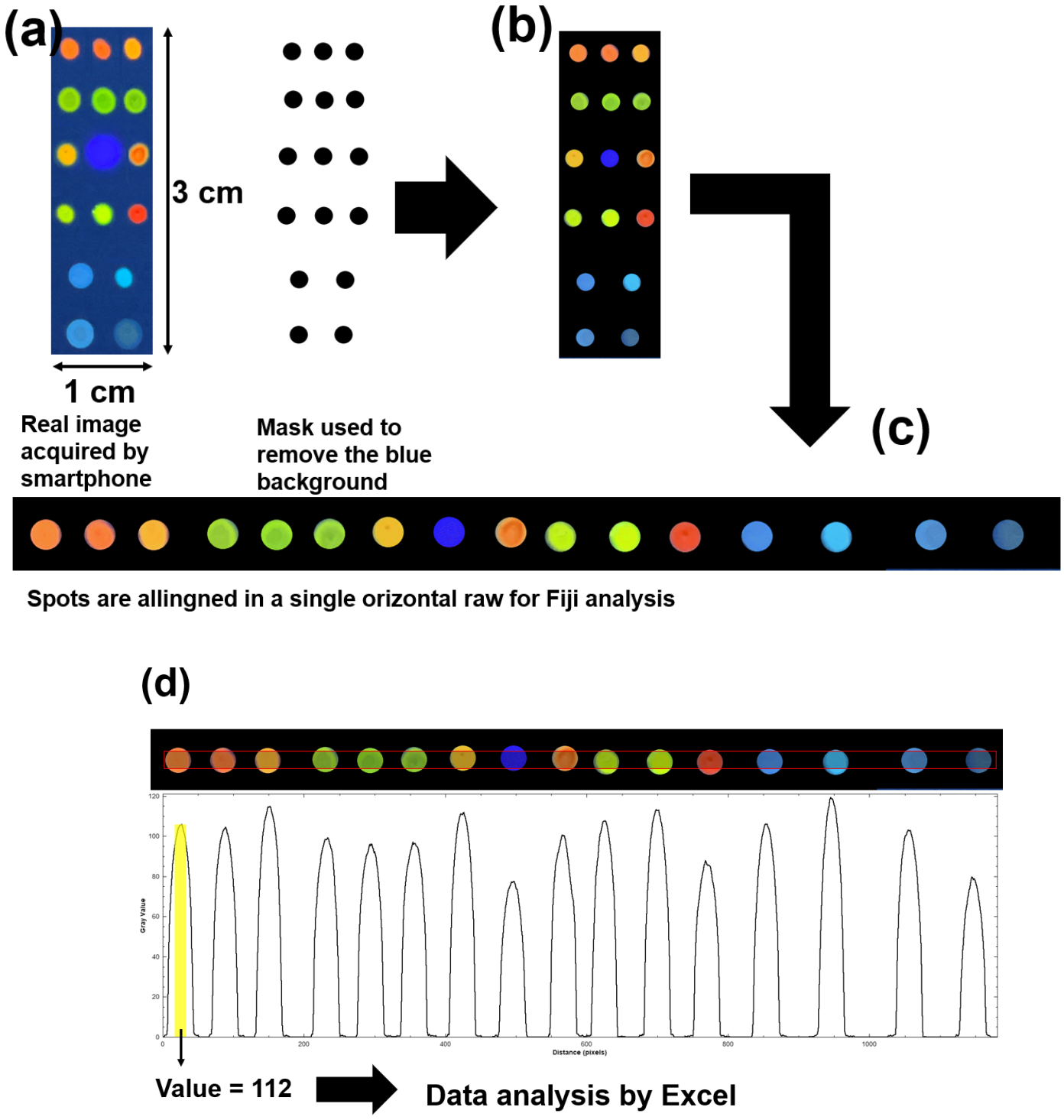

Figure S14. Representation of analysis setup. (**a**): real image acquired by smartphone (left) and section of image extracted from the jpeg file (right); (**b**) results of the subtraction performed in (a) in which noise has been deleted; (**c**) spots alignment performed with PowerPoint (Office 365); (**d**) Fiji analysis of the aligned spots.