Electronic Supplementary Information

A Fluorescent Probe with Ultra-Rapid Response to Nitric Oxide

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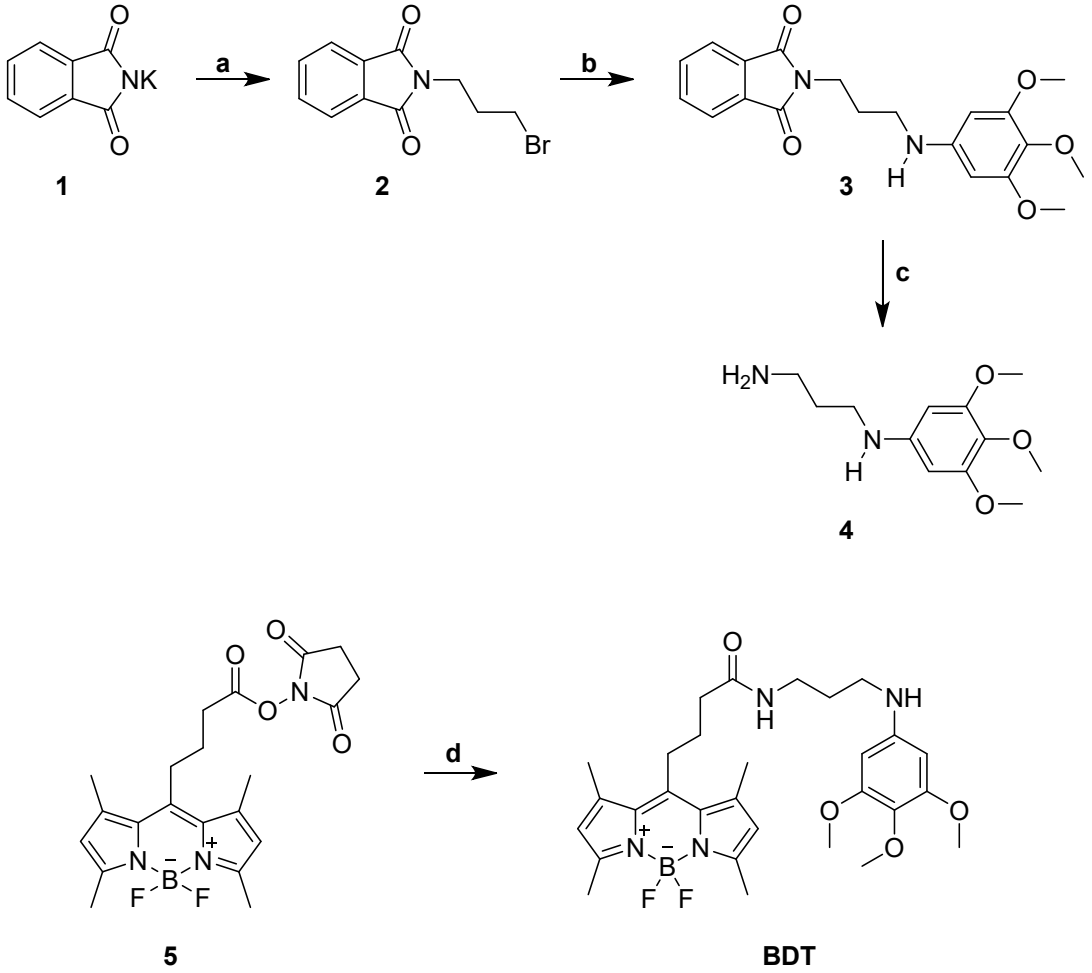
Synthesis and characterization

The synthetic steps involved in the preparation of **BDT** and of the authentic photoproduct **BDT-NO** are reported in Scheme S1 and Scheme S2, respectively and described in the following. Compound **5**[S1] was synthetized according to literature. All operations were carried out under a low intensity level of visible light.



Scheme S1. a) 1, 1,3- dibromopropane, acetone, reflux, 24 h; **b) 2**, 3,4,5-Trimethoxyaniline, Cs_2CO_3, KI, dry DMF, 70 °C, overnight; **c) 3**, hydrazine hydrate, ethanol, 60 °C, 4 h; **d) 4**, **5**, dry DCM, r.t., 24 h.

Synthesis of 3-Bromo-1-phthalimidepropane (2).

A mixture of potassium phthalimide **1** (2.2 mmol, 408 mg) and 1,3- dibromopropane (2.9 mmol, 586 mg) in 2.5 mL of acetone was refluxed for 24 hours. After filtering off the precipitated potassium bromide, the cake was washed with acetone. The solvent was evaporated, and the pure product **2** was obtained as a colorless oil (530 mg, 90 %). ^{1}H NMR (500 MHz, $CDCl_3$). δ 7.83 (m, 2H), 7.7 (m, 2H), 3.83 (t, 2H), 3.41 (t, 2H), 2.27 (quint, 2H).

Synthesis of 2-(3-((3,4,5-trimethoxyphenyl)amino)propyl)isoindoline-1,3-dione (3).

3,4,5-Trimethoxyaniline (4.9 mmol, 900 mg), cesium carbonate (4.9 mmol, 1.58 gr) and potassium iodide (9.8 mmol, 1.6 gr) were dissolved in dry DMF (20 mL). Then a solution of compound **2** (4.9 mmol, 1.3 gr) in dry DMF (6 mL) was added dropwise to the previous solution. The reaction mixture was stirred at 70 °C overnight. Then, reaction mixture was poured into ice cold water (100 mL) and it was extracted three times with DCM (3 x 100 mL). The organic phase was dried over sodium sulphate, filtered and the solvent evaporated under reduced pressure. Purification by flash chromatography eluting with Cyclohexane/Ethyl Acetate (Cy/ EtOAc, 50/50 v/v) gave compound **3** as a brown solid (300 mg, 16%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (m, 2H), 7.71 (m, 2H), 5.86 (s, 2H), 3.76 (m, 11H), 3.15 (t, J = 6.5 Hz, 2H), 1.97 (quint, J = 6.5 Hz, 2H).

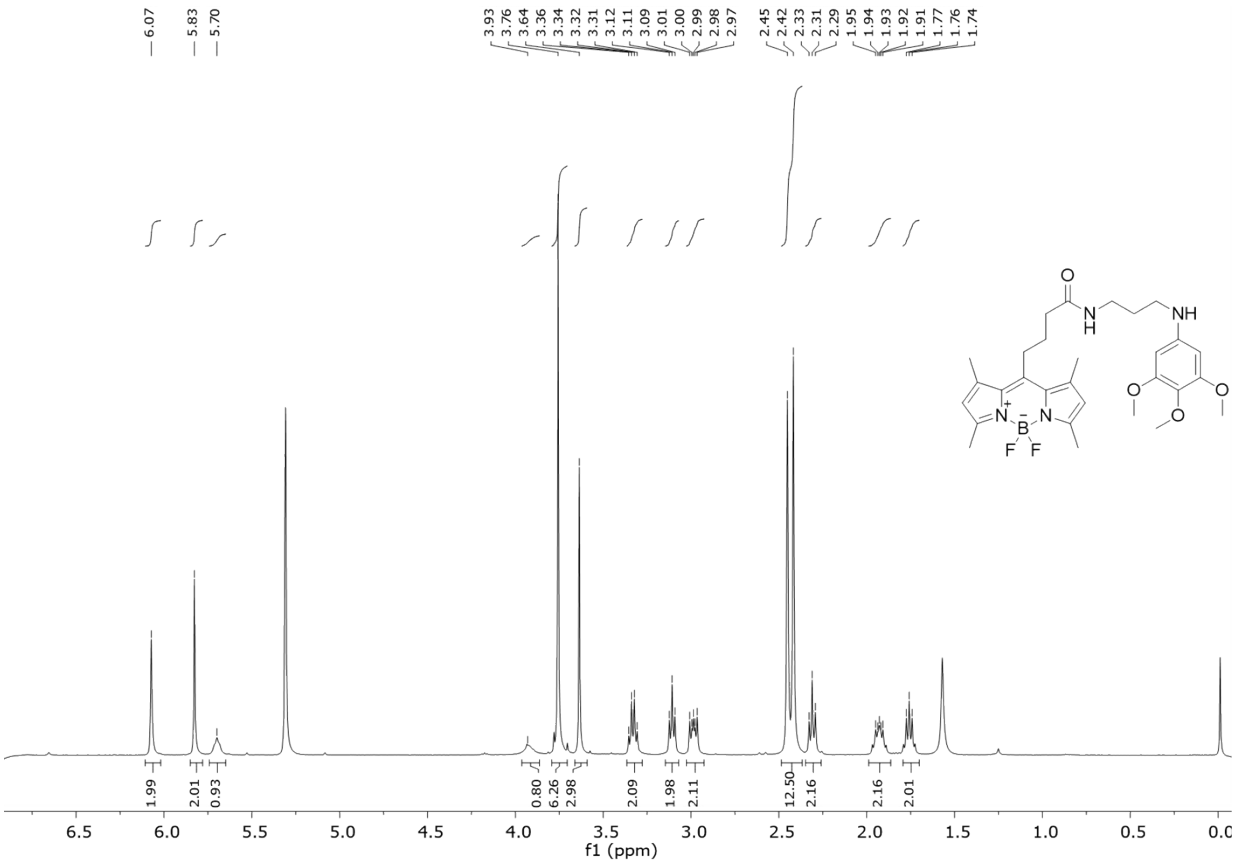
Synthesis of N^1-(3,4,5-trimethoxyphenyl)propane-1,3-diamine (4).

Compound **3** (0.7 mmol, 260 mg) was dissolved in ethanol (25 ml); hydrazine hydrate (17.5 mmol, 877 mg) was added and the reaction was stirred at 60 °C for 4 hours. Ethanol was evaporated under reduced pressure. Purification by flash chromatography eluting with DCM/MeOH/aqueous NH_3 (9/1/0.1) gave the target compound **4** as a red-brown solid (144 mg, 85%). ^{1}H NMR (500 MHz, CD_2Cl_2) δ 5.85 (s, 2H), 3.78 (s, 6H), 3.70 (s, 3H), 3.14 (t, J = 6.8 Hz, 2H), 2.82 (t, J = 6.7 Hz, 2H), 1.73 (quint, J = 6.7 Hz, 2H).

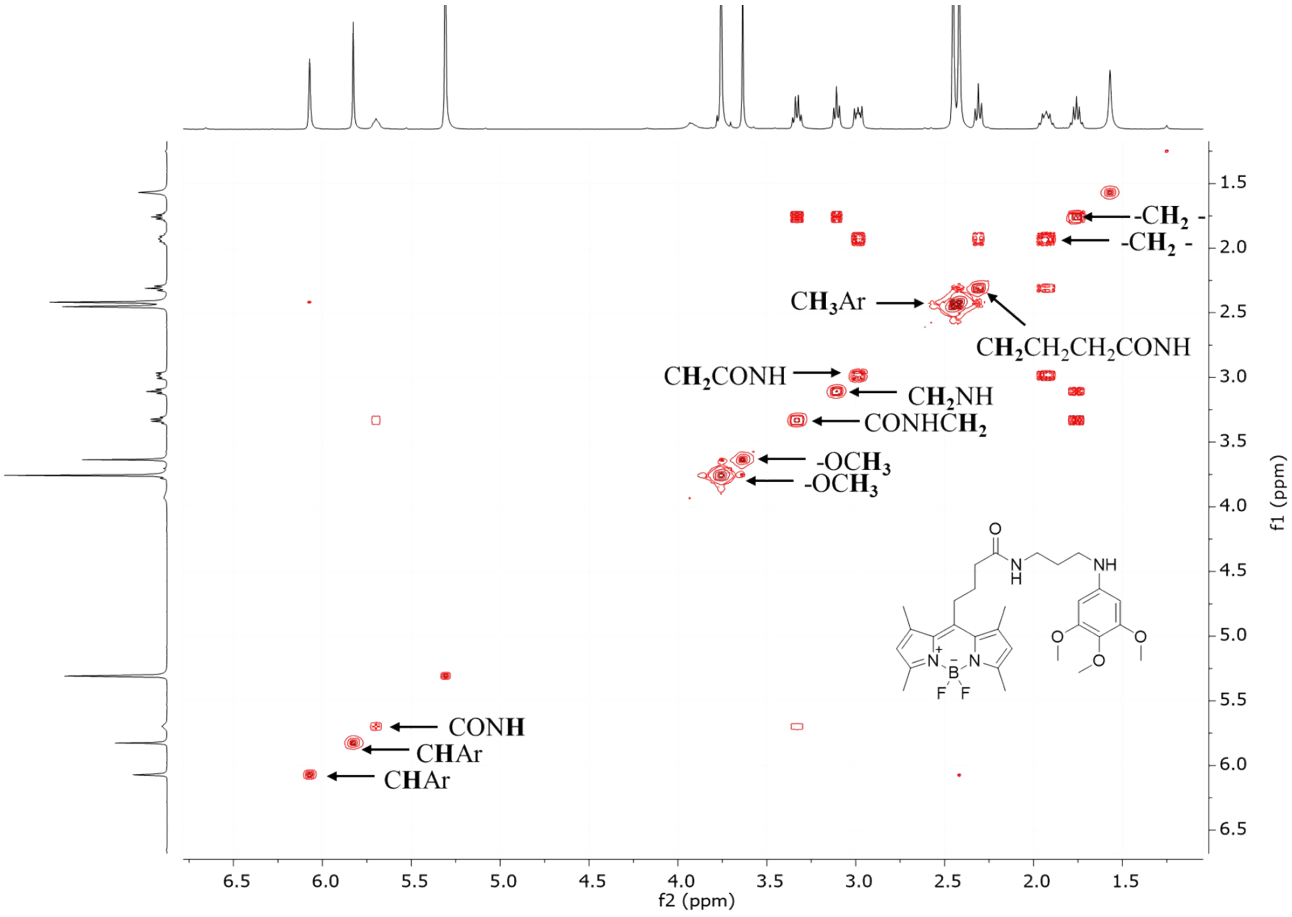
Synthesis of 5,5-difluoro-1,3,7,9-tetramethyl-10-(4-oxo-4-((3-((3,4,5-trimethoxyphenyl)amino)propyl) amino)butyl)-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide *(BDT)*

A solution of compound **5** (0.16 mmol, 60 mg) in dry DCM (10 mL) was added to a solution of compound **4** (0.16 mmol, 40 mg) in dry DCM (10 mL) stirred at room temperature and under N_2 atmosphere. The reaction was allowed to proceed at room temperature for 24 hours; then the obtained mixture was washed with H_2O (2 × 20 mL), dried over Na_2SO_4 and concentrated to dryness. Purification by flash chromatography eluting with DCM/EtOAc/aqueous NH_3 (75/25/0.1) gave **BDT** as an orange solid. (50 mg, 56%). ESI-MS m/z 555.3 $[M-H]^-$. ^{1}H NMR (500 MHz, CD_2Cl_2) δ 6.07 (s, 2H), 5.83 (s, 2H), 5.70 (s, 1H), 3.93 (bs, 1H), 3.76 (s, 6H), 3.64 (s, 3H), 3.33 (q, J = 6.5 Hz, 2H), 3.11 (t, J = 6.5 Hz, 2H), 3.03 – 2.91 (m, 2H), 2.45 (s, 6H), 2.42 (s, 6H), 2.31

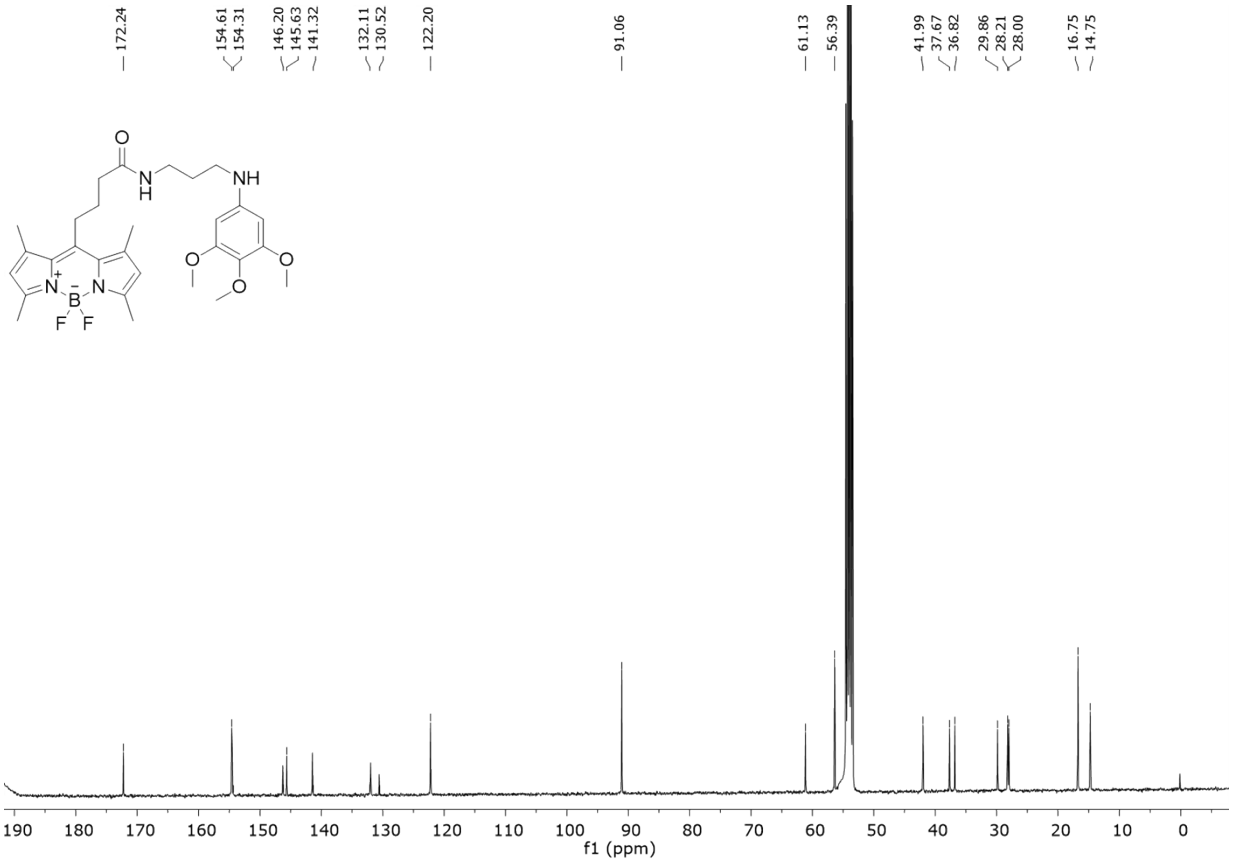
(t, *J* = 7.1 Hz, 2H), 1.93 (m, 2H), 1.76 (t, *J* = 6.6 Hz, 2H). ^{13}C NMR (125 MHz, CD_2Cl_2) δ 172.24 , 146.20 , 145.63 , 141.32 , 132.11 , 130.52 , 122.20 , 91.06 , 61.13 , 56.39 , 41.99 , 37.67 , 36.82 , 29.86 , 28.21 , 28.00 , 16.75 , 14.75 .



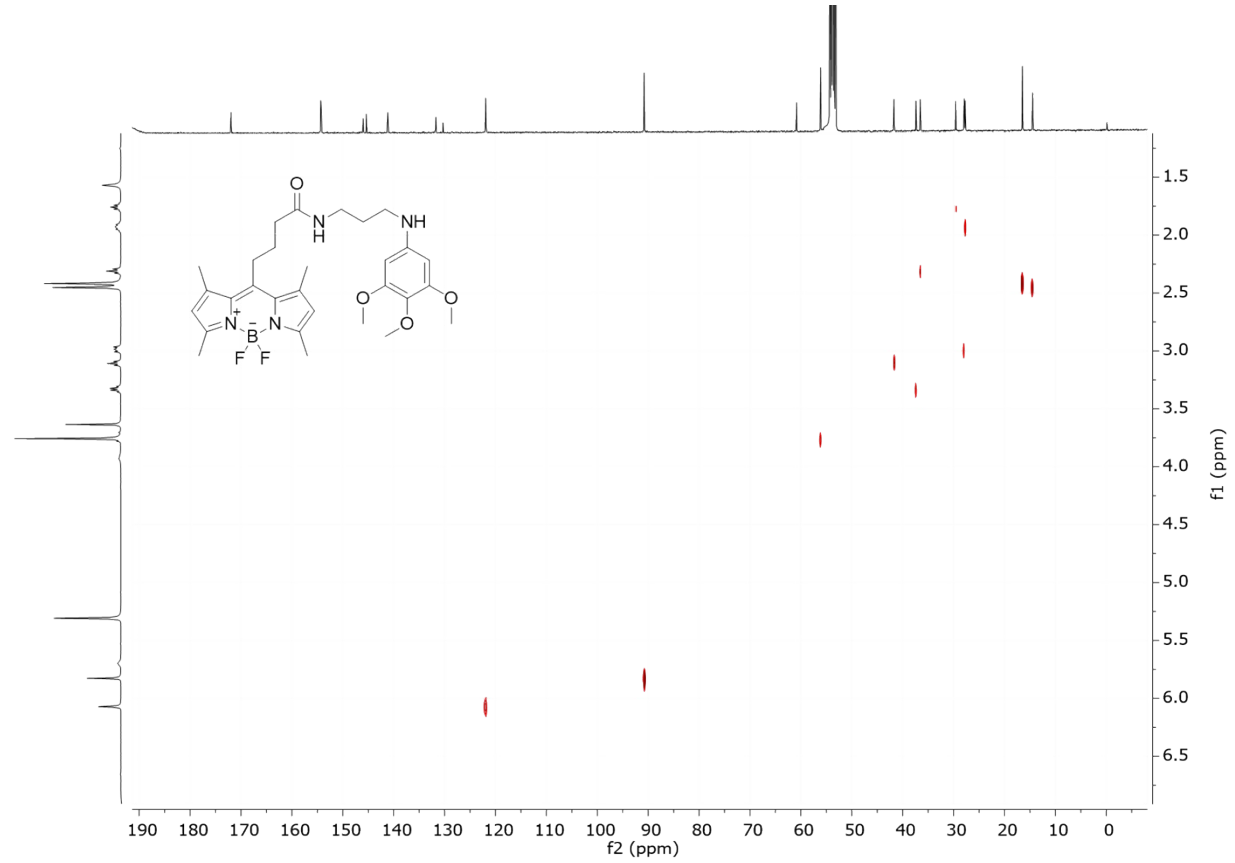
^{1}H NMR **BDT.**



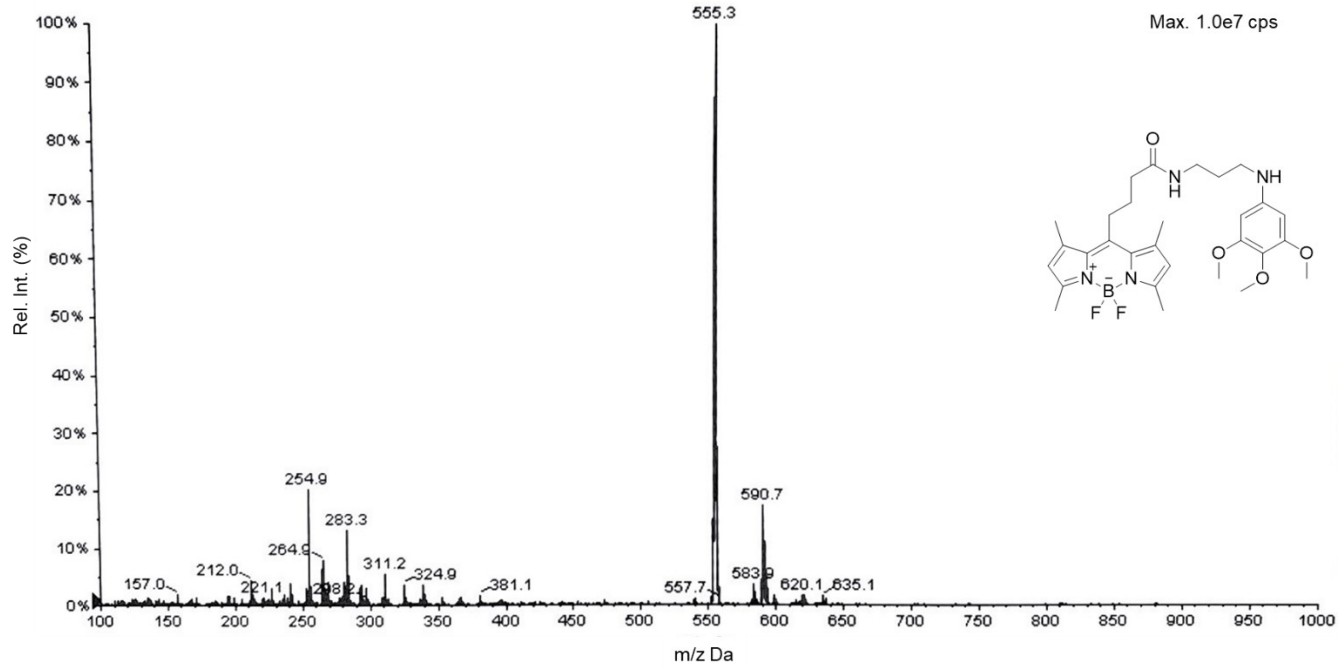
g COSY **BDT.** ^{1}H NMR (500 MHz, CD_2Cl_2) δ 6.07 (s, 2H, C**H**Ar), 5.83 (s, 2H, C**H**Ar), 5.70 (s, 1H, CON**H**), 3.93 (bs, 1H, CH_2N**H**), 3.76 (s, 6H, -OC**H$_3$**), 3.64 (s, 3H, -OC**H$_3$**), 3.33 (q, *J* = 6.5 Hz, 2H, CONHC**H$_2$**CH_2CH_2NH), 3.11 (t, *J* = 6.5 Hz, 2H, CH_2CH_2C**H$_2$**NH), 3.03 – 2.91 (m, 2H, CH_2CH_2C**H$_2$**CONH), 2.45 (s, 6H, C**H$_3$**Ar), 2.42 (s, 6H, C**H$_3$**Ar), 2.31 (t, *J* = 7.1 Hz, 2H, C**H$_2$**CH_2CH_2CONH), 1.93 (m, 2H, CH_2C**H$_2$**CH_2CONH), 1.76 (t, *J* = 6.6 Hz, 2H, CH_2C**H$_2$**CH_2NH).



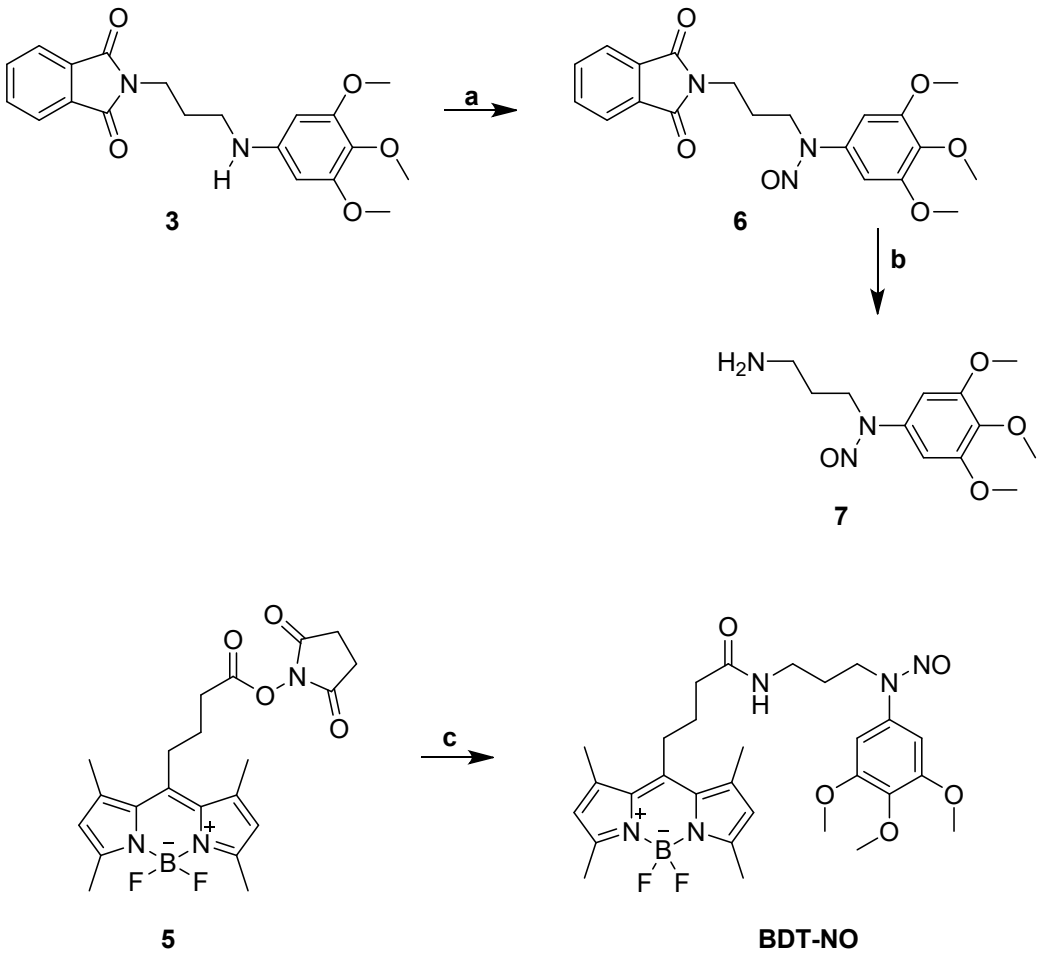
^{13}C NMR **BDT.**



HSQC **BDT**.



Mass spectrum of **BDT**.



Scheme S2. a) 3, $NaNO_2$, THF/CH_3COOH 2:1, 3 h, 0°C > r.t.; **b) 6**, hydrazine hydrate, ethanol, 60 °C, 4 h; **c) 5**, **7**, dry DCM, r.t., 24 h.

Synthesis of N-(3-(1,3-dioxoisoindolin-2-yl)propyl)-N-(3,4,5-trimethoxyphenyl)nitrous amide (6).

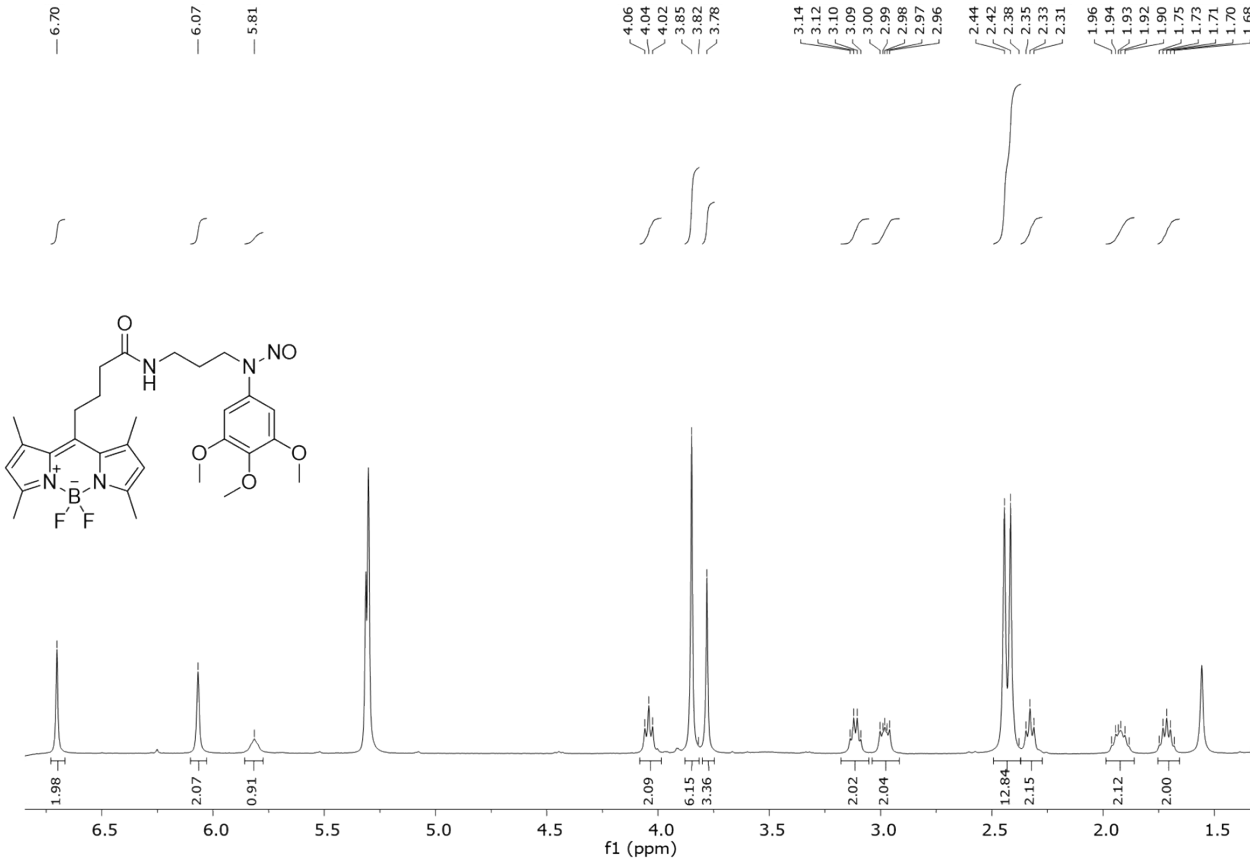
To a solution of compound **3** (0.135 mmol, 50 mg) in THF/CH_3COOH (2/1 v/v; 3 mL) cooled at 0 °C with an ice bath, sodium nitrite (0.135 mmol, 9.3 mg) was added; the reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 2 hours. The obtained mixture was diluted with DCM and washed with saturated sodium bicarbonate solution (3 × 20 mL), dried over Na_2SO_4 and concentrated to dryness. Purification of the residue by flash chromatography, using Cy/EtOAc (50/50 v/v) as the eluent, gave compound **6** as a dark yellow oil (50 mg, resa 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 – 7.79 (m, 2H), 7.76 – 7.64 (m, 2H), 6.69 (s, 2H), 4.16 – 3.92 (m, 3H), 3.87 (s, 6H), 3.85 (s, 3H), 3.69 (t, J = 7.0 Hz, 2H), 2.03 – 1.82 (m, 2H).

Synthesis of N-(3-aminopropyl)-N-(3,4,5-trimethoxyphenyl)nitrous amide (7)

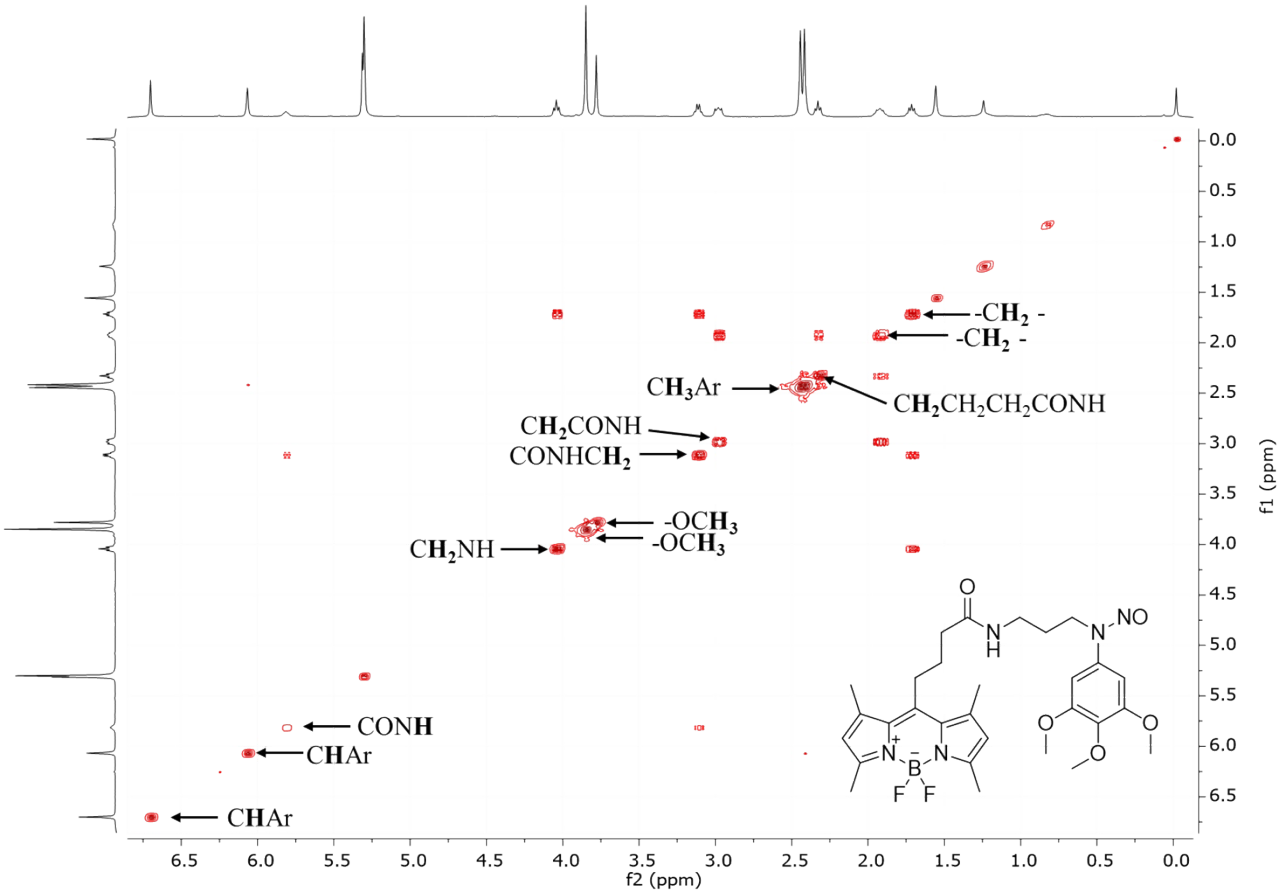
Compound **6** (0.125 mmol, 50 mg) was dissolved in ethanol (5 ml); hydrazine hydrate (3.125 mmol, 156 mg) was added and the reaction was stirred at 60 °C for 2 hours. Ethanol was evaporated under reduced pressure. Purification by flash chromatography eluting with DCM/MeOH/aqueous NH_3 (9/1/0.1) gave the target compound **7** as a dark yellow oil (30 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.79 (s, 2H), 4.18 – 4.05 (m, 2H), 3.89 (s, 6H), 3.86 (s, 3H), 2.68 (t, J = 6.6 Hz, 2H), 2.16 – 2.06 (m, 2H), 1.72 (quint, J = 6.6 Hz, 2H).

5,5-difluoro-1,3,7,9-tetramethyl-10-(4-((3-(nitroso(3,4,5-trimethoxyphenyl)amino)propyl)amino)-4-oxobutyl)-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide *(BDT-NO)*

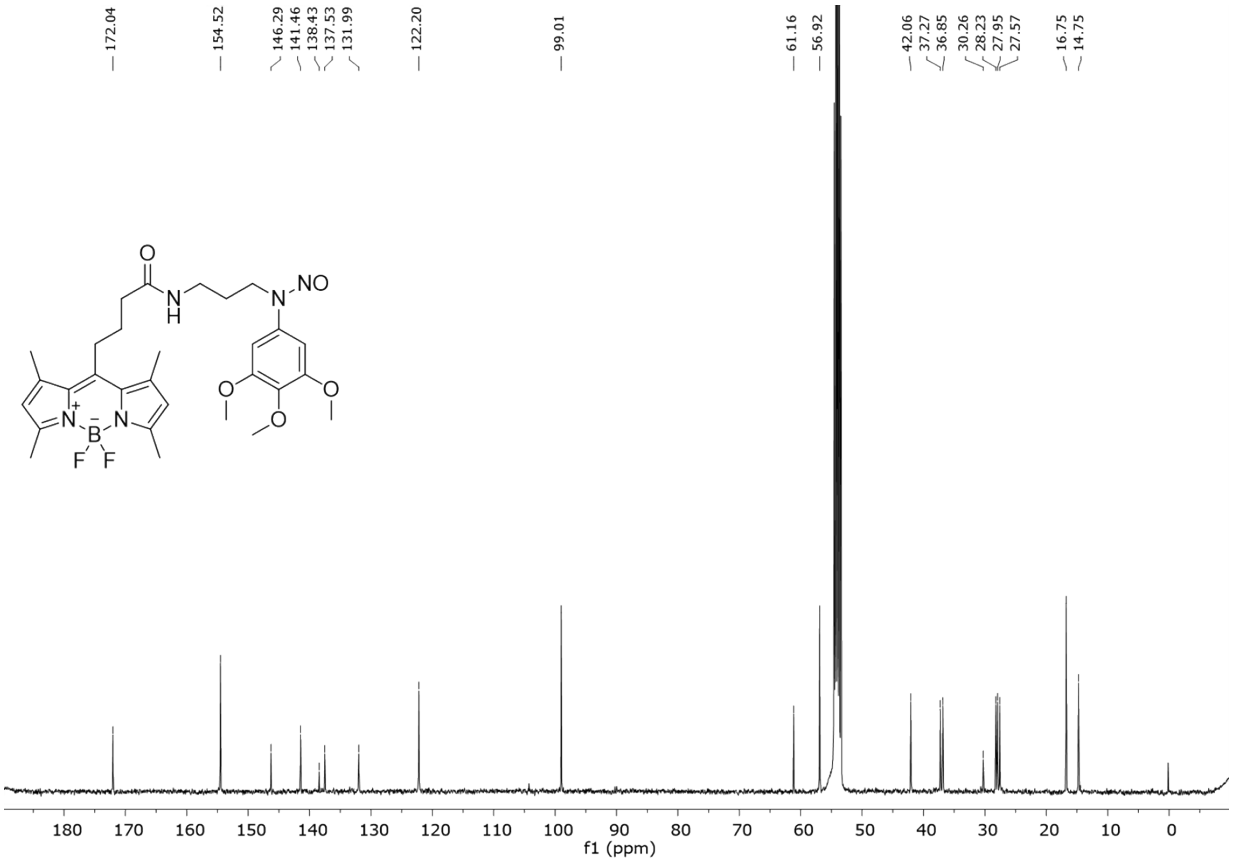
A solution of compound **5** (0.297 mmol, 112 mg) in dry DCM (10 mL) was added to a solution of compound **7** (0.297 mmol, 80 mg) in dry DCM (30 mL) stirred at room temperature and under N_2 atmosphere. The reaction was allowed to proceed at room temperature for 24 hours; then the obtained mixture was washed with H_2O (2 × 20 mL), dried over Na_2SO_4 and concentrated to dryness. Purification by flash chromatography eluting with DCM/EtOAc (50/50) gave **BDT-NO** as an orange solid. (50 mg, 56%). ESI-MS m/z 584.0 $[M-H]^-$. ^{1}H NMR (500 MHz, CD_2Cl_2) δ 6.70 (s, 2H), 6.07 (s, 2H), 5.81 (bs, 1H), 4.04 (t, *J* = 6.9 Hz, 2H), 3.85 (s, 6H), 3.78 (s, 3H), 3.11 (q, *J* = 6.4 Hz, 2H), 3.03 – 2.92 (m, 2H), 2.44 (s, 6H), 2.42 (s, 6H), 2.33 (t, *J* = 7.2 Hz, 2H), 1.92 (dq, *J* = 15.4, 7.4 Hz, 2H), 1.71 (p, *J* = 6.8 Hz, 2H). ^{13}C NMR (125 MHz, CD_2Cl_2) δ 172.04 , 154.52 , 146.29 , 141.46 , 138.43 , 137.53 , 131.99 , 122.20 , 99.01 , 61.16 , 56.92 , 42.06 , 37.27 , 36.85 , 30.26 , 28.23 , 27.95 , 27.57 , 16.75 , 14.75 .



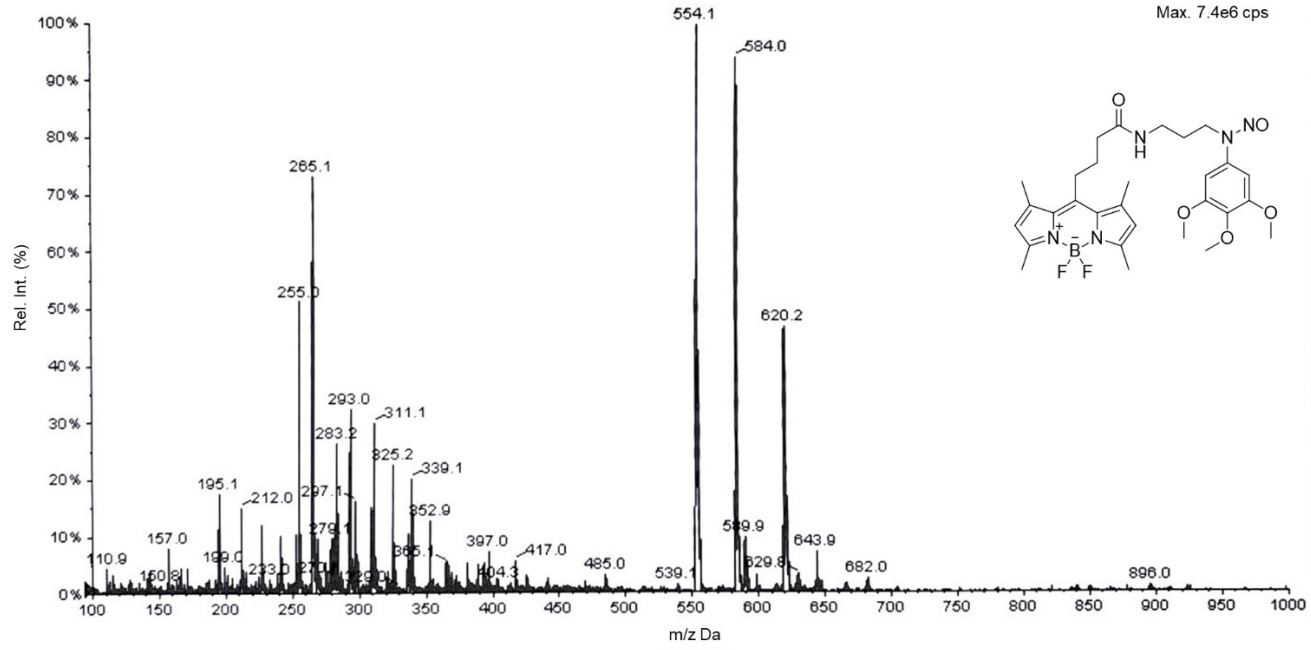
^{1}H NMR **BDT-NO.**



g COSY **BDT-NO.** ^{1}H NMR (500 MHz, CD_2Cl_2) δ 6.70 (s, 2H, C**H**Ar), 6.07 (s, 2H, C**H**Ar), 5.81 (bs, 1H, CON**H**), 4.04 (t, *J* = 6.9 Hz, 2H, $CH_2CH_2C\mathbf{H_2}NH$), 3.85 (s, 6H, -OC**H$_3$**), 3.78 (s, 3H, -OC**H$_3$**), 3.11 (q, *J* = 6.4 Hz, 2H, CONHC**H$_2$**CH_2CH_2NH), 3.03 – 2.92 (m, 2H, $CH_2CH_2C\mathbf{H_2}CONH$), 2.44 (s, 6H, C**H$_3$**Ar), 2.42 (s, 6H, C**H$_3$**Ar), 2.33 (t, *J* = 7.2 Hz, 2H, C**H$_2$**CH_2CH_2CONH), 1.92 (dq, *J* = 15.4, 7.4 Hz, 2H, $CH_2C\mathbf{H_2}CH_2CONH$), 1.71 (p, *J* = 6.8 Hz, 2H, $CH_2C\mathbf{H_2}CH_2NH$).



^{13}C NMR **BDT-NO.**



Mass spectrum of **BDT-NO**.

S1. D. Wang, J. Fan, X. Gao, B. Wang, S. Sun, X. Peng, *J. Org. Chem.,* **2009**, *74*, 7675–7683.