Supporting Information for:

Molecular Approach to Rationally Constructing Specific Fluorogenic Substrate for Detection of Acetylcholinesterase Activity in Live Cells, Mice Brains and Tissues

Xiaofeng Wu,[a,g] Jong Min An,[b,g] Jizhen Shang,[c,g] Eugene Huh,[d,g] Sujie Qi,[a] Eunhye Lee,[a] Haidong Li,[a] Gyoungmi Kim,[a] Huimin Ma,[c] Myung Sook Oh,[e]* Dokyoung Kim[b,f]* and Juyoung Yoon[a]*

[a]Department of Chemistry and Nanoscience, Ewha Womans University, Seoul 03760, Republic of Korea.
[b]Department of Biomedical Science, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea.
[c]Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China.
[d]Department of Medical Science of Meridian, College of Korean Medicine, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea.
[e]Department of Life and Nanopharmaceutical Sciences, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea.
[f]Department of Anatomy and Neurobiology, College of Medicine, Kyung Hee University, Seoul 02447, Republic of Korea.
[g]These authors contributed equally to this work.

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Experimental

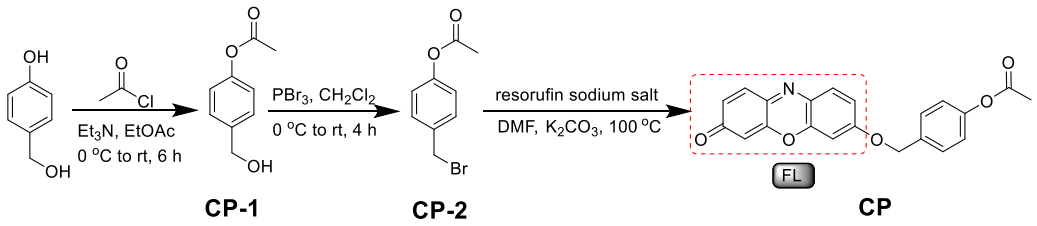
Apparatus and reagents

^{1}H-NMR, ^{13}C-NMR and ^{19}F-NMR spectra were recorded on a Bruker AM 300 spectrometer. Electrospray ionization mass spectra (ESI-MS) were recorded on a Shimadzu LC-MS 2010A instrument (Kyoto, Japan). High-resolution electrospray ionization mass spectra (HR-ESI-MS) were measured on an APEX IV FTMS instrument (Bruker, Daltonics). A Jeol JMS 700 high-resolution mass spectrometer was used to acquire fast atom bombardment (FAB) mass spectra at the Korea Basic Science Institute (Daegu). UV absorption spectra were obtained with Thermo Scientific Evolution 201 UV/VIS Spectrometer. Fluorescence spectra were obtained using JASCO FP8500. High-performed liquid chromatography with LC-20 AT solvent delivery unit, SPD-20 A UV-vis detector (Shimadzu, Japan). Fluorescence images were visualized by a confocal laser scanning microscope (CLSM, LSM-800, Carl Zeiss, Germany). Tissue images were visualized using fluorescence tissue imaging systems (FTIS, VISQUE® InVivo Elite, Vieworks Co. Ltd, Rep. of Korea) and two-photon microscopy (TPM, TCS SP5, Leica microsystem, Germany).

Vanillin, isobutyl chloroformate, 4-hydroxy-3-nitrobenzaldehyde, dimethylcarbamyl chloride, 3-bromo-4-hydroxybenzaldehyde, sodium azide, 4-hydroxy-3-nitrobenzaldehyde, triphenylphosphine, 3-chloro-4-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, 3-fluoro-4-hydroxybenzaldehyde, and 2-(diphenylphosphino)benzoic acid were purchased from Tokyo Chemical Industry Co., Ltd. Resorufin sodium salt, formaldehyde solution, paraoxon ethyl, palladium on carbon (wt%, 10%), hydrazine monohydrate, 4-hydroxybenzyl alcohol, sodium borohydride, sodium nitrite, acetyl chloride, iodomethane, hydrogen peroxide, tert-butyl hydroperoxide (TBHP), CORM-3 (CO donor), sodium hypochlorite, dimethylamine, potassium superoxide, dichloromethane-d_2 (CD_2Cl_2-d_2), KCl, $MgCl_2$, $ZnCl_2$, $NaNO_2$, $CaCl_2$, glucose, vitamin C, tyrosine, glutamic acid, cysteine, glycine, arginine, alanine, lysine, glutathione, urea, bovine serum albumin (BSA), human serum albumin (HSA), alkaline phosphatase (ALP), β-glucosidase, carboxyl esterase (CE), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), xanthine oxidase, apyrase, monoamine oxidase A (MAO-A), monoamine oxidase B (MAO-B), Sodium Nitroferricyanide (III) Dihydrate, tyrosinase from mushroom, dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF, anhydrous) were purchased from Sigma-Aldrich. Aluminium oxide (neutral) for column chromatography was obtained from EMD Millipore Corporation. Dulbecco's modified Eagle's media (DMEM) and fetal bovine serum (FBS) for cell culture were purchased from Hyclone (Utah, US). U87MG and HEK293 cells lines were purchased from Korean Cell Line Bank and N2A cell line was obtained from American Type Culture Collection. Penicillin-streptomycin for cell lines was purchased from Gibco Industries Inc (Auckland, NZ). Tribromoethanol and phosphate buffered saline were purchased from Sigma-Aldrich. The stock solutions (1.0 mM) of **CP** and **P1-P10** were prepared by dissolving requisite amounts of them in DMSO.

Syntheses of control probe (CP) and probes 1-10

Syntheses of CP



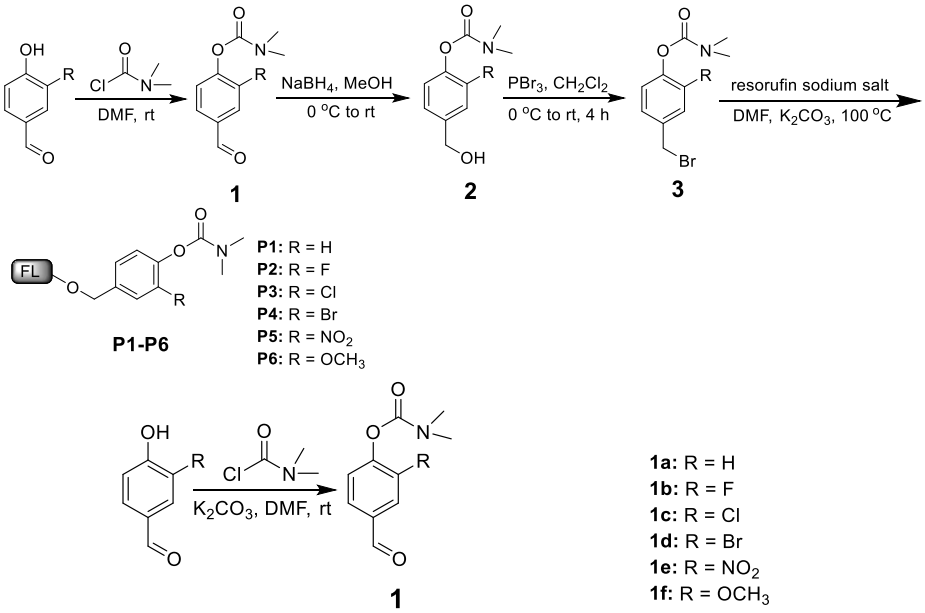
CP-1: A mixture of 4-hydroxybenzalcohol (0.49 g, 4.0 mmol), Et_3N (4.80 mmol, 0.65 mL) was added acetyl chloride (4.4 mmol, 0.31 mL) in 20 mL EtOAc, and then warmed to room temperature and stirred for 6 h. Then, the mixture was diluted with dichloromethane (20 mL), and washed three times with water (20 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation under reduced pressure, and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 0 to 10:1) as eluent, obtaining **CP-1** as a clear oil (0.47 g, yield 71%). The ^{1}H-NMR and ^{13}C-NMR spectra of **CP-1** are shown below in Figures S22 and S23, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 7.38-7.36 (m, 1H), 7.35-7.33 (m, 1H), 7.09-7.07 (m, 1H), 7.06-7.04 (m, 1H), 4.63 (s, 2H), 2.30 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 169.7, 150.0, 138.6, 128.1, 121.6, 64.5, 21.1. HR-ESI-MS: *m/z* calcd for **CP-1** ($C_9H_{10}NaO_3$, $[M]^+$), 189.0522; found, 189.0523.

CP-2: Compound **CP-1** (0.4 g, 2.41 mmol) was dissolved in 20 mL CH_2Cl_2 at 0 °C, and tribromophosphine (0.46 mL, 4.82 mmol) was subsequently added dropwise. After 30 mins, the resulting mixture was warmed to room temperature and stirred for 4 h. Then, the mixture was diluted with dichloromethane (20 mL), and washed three times with water (20 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified using silica gel chromatography with CH_2Cl_2 as eluent, obtaining **CP-2** as a clear oil (0.45 g, yield 82%). The ^{1}H-NMR and ^{13}C-NMR spectra of **CP-2** are shown below in Figures S24 and S25, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 7.44-7.43 (m, 1H), 7.41-7.40 (m, 1H), 7.11 (t, J = 2.64 Hz, 1H), 7.08 (t, J = 2.13 Hz, 1H), 4.50 (s, 2H), 2.31 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 169.3, 150.6, 135.4, 130.3, 122.0, 32.8, 32.1, 21.2. HR-EI-MS: *m/z* calcd for **CP-2** ($C_9H_9BrO_2$, $[M]^+$), 227.9586; found, 227.9585.

CP: To a solution of resorufin sodium salt (0.24 g, 1.0 mmol) in anhydrous DMF (10 mL), K_2CO_3 (0.28 g, 2.0 mmol) was added, followed by stirring at 100 °C for 10 min. Then, a solution of **CP-2** (0.23 g, 1.0 mmol) in DMF (2 mL) was added dropwise. After stirring at 100 °C for 12 h, the solution was cooled and diluted with CH_2Cl_2 (20 mL). The resulting solution was then washed with brine water (15 mL × 3). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation, and the residue was subjected to silica gel chromatography eluted with CH_2Cl_2, affording **CP** as an orange solid (0.25 g, yield 70%). The ^{1}H-NMR and ^{13}C-NMR spectra of **CP** are shown below in Figures

S26 and S27, respectively. ^{1}H-NMR (300 MHz, 298 K, CD_2Cl_2-d_2): δ 7.76 (d, J = 8.9 Hz, 1H), 7.54-7.49 (m, 2H), 7.45 (d, J = 9.8 Hz, 1H), 7.20-7.15 (m, 2H), 7.07-7.04 (m, 1H), 6.94 (d, J = 2.6 Hz, 1H), 6.82-6.78 (m, 1H), 6.29 (d, J = 2.0 Hz, 1H), 5.20 (s, 2H), 2.32 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, CD_2Cl_2-d_2): δ 185.9, 169.4, 162.5, 150.8, 149.8, 145.9, 145.6, 134.7,134.1, 133.3, 131.5, 128.8, 128.6, 122.0, 113.9, 106.5, 101.2, 70.2, 20.9. HR-ESI-MS: *m/z* calcd for **CP** ($C_{21}H_{16}NO_5$, $[M+H]^+$), 362.1023; found, 362.1023.

Syntheses of probes 1-6



1a: To a solution of p-Hydroxybenzaldehyde (0.61 g, 5.0 mmol) and K_2CO_3 (1.38 g, 10 mmol) in DMF (20 mL) was added Me_2NCOCl (0.92 mL, 10 mmol) at room temperature. The reaction mixture was stirred for 8 h at room temperature. After dilution with water (30 mL), the mixture was extracted with CH_2Cl_2 (30 mL×2), and the combined organic layer was washed with saturated Na_2CO_3 solution, evaporated and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 0 to 100:1) as eluent, obtaining **1a** as a clear oil (0.87 g, yield 90%). The ^{1}H-NMR and ^{13}C-NMR spectra of **1a** are shown below in Figures S28 and S29, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 9.56 (s, 1H), 7.90 (t, J = 2.41 Hz, 1H), 7.87 (t, J = 2.08, 1H), 7.31-7.29 (m, 1H), 7.28-7.26 (m, 1H), 3.10 (s, 3H), 3.01 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 191.1, 156.4, 153.8, 133.3, 131.1, 122.3, 36.7, 36.5. HR-ESI-MS: *m/z* calcd for **1a** ($C_{10}H_{11}NO_3$, $[M+H]^+$), 194.0811; found, 194.0812.

1b: **1b**, prepared similarly as **1a** using 3-fluoro-4-hydroxybenzaldehyde (0.56 g, 4.0 mmol), K_2CO_3 (1.1 g, 8.0 mmol), and Me_2NCOCl (0.73 mL, 8.0 mmol), was obtained as a clear oil (0.59 g, yield 70%). The ^{1}H-NMR and ^{13}C-NMR spectra of **1b** are shown below in Figures S30, S31 and S32, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 9.90 (d, J = 2.1 Hz, 1H), 7.66-7.74 (m, 1H), 7.63-7.60 (m, 1H), 7.39-7.34 (m, 1H), 3.10 (s, 3H), 2.99 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 190.0, 190.0, 156.5, 153.2, 152.9, 144.3, 144.1, 134.7, 134.6, 126.7, 126.6, 124.8, 116.6, 116.3, 36.9, 36.6. ^{19}F-NMR (300 MHz, 298 K, $CDCl_3$): δ -

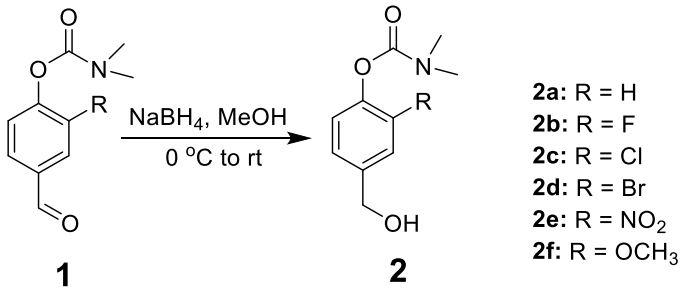
127.18, -127.19, -127.20, -127.22, -127.23, -127.30. HR-ESI-MS: *m/z* calcd for **1b** ($C_{10}H_{11}FNO_3$, $[M+H]^+$), 212.0717; found, 212.0718.

1c: **1c**, prepared similarly as **1a** using 3-chloro-4-hydroxybenzaldehyde (0.94 g, 6.0 mmol), K_2CO_3 (1.66 g, 12 mmol), and Me_2NCOCl (1.1 mL, 12 mmol), was obtained as a clear oil (1.14 g, yield 83%). The ^{1}H-NMR and ^{13}C-NMR spectra of **1c** are shown below in Figures S33 and S34, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 9.96 (d, J = 0.39 Hz, 1H), 7.98-7.97 (m, 1H), 7.83-7.80 (m, 1H), 7.47-7.45 (m, 1H), 3.19 (s, 3H), 3.06 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 189.9, 152.9, 152.4, 134.4, 131.1, 129.2, 128.4, 124.7, 36.9, 36.6. HR-EI-MS: *m/z* calcd for **1c** ($C_{10}H_{10}ClNO_3$, $[M]^+$), 237.0349; found, 227.0346.

1d: **1d**, prepared similarly as **1a** using 3-bromo-4-hydroxybenzaldehyde (1.0 g, 5.0 mmol), K_2CO_3 (1.38 g, 10 mmol), and Me_2NCOCl (0.92 mL, 10 mmol), was obtained as a clear oil (0.85 g, yield 63%). The ^{1}H-NMR and ^{13}C-NMR spectra of **1d** are shown below in Figures S35 and S36, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 9.92 (s, 1H), 8.11 (d, J = 1.92 Hz, 1H), 7.85-7.81 (m, 1H), 7.44 (d, J = 8.32 Hz, 1H), 3.18 (s, 3H), 3.04 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 189.8, 153.5, 152.8, 134.6, 134.3, 129.8, 124.6, 117.4, 36.9, 36.7. HR-EI-MS: *m/z* calcd for **1d** ($C_{10}H_{10}BrNO_3$, $[M]^+$), 270.9844; found, 270.9842.

1e: **1e**, prepared similarly as **1a** using 3-nitro-4-hydroxybenzaldehyde (0.84 g, 5.0 mmol), K_2CO_3 (1.38 g, 10 mmol), and Me_2NCOCl (0.92 mL, 10 mmol), was obtained as a clear oil (0.78 g, yield 65%). The ^{1}H-NMR and ^{13}C-NMR spectra of **1e** are shown below in Figures S37 and S38, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 10.03 (s, 1H), 8.53 (d, J = 2.01 Hz, 1H), 8.16-8.12 (m, 1H), 7.52 (d, J = 8.38 Hz, 1H), 3.15 (s, 3H), 3.06 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 188.8, 152.4, 149.3, 142.5, 134.2, 133.6, 127.1, 126.4. HR-EI-MS: *m/z* calcd for **1e** ($C_{10}H_{10}N_2O_5$, $[M]^+$), 238.0590; found, 235.0589.

1f: **1f**, prepared similarly as **1a** using vanillin (0.76 g, 5.0 mmol), K_2CO_3 (1.38 g, 10 mmol), and Me_2NCOCl (0.92 mL, 10 mmol), was obtained as a clear oil (0.48 g, yield 59%). The ^{1}H-NMR and ^{13}C-NMR spectra of **1f** are shown below in Figures S39 and S40, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 9.94 (s, 1H), 7.49-7.45 (m, 2H), 7.29-7.26 (m, 1H), 3.91 (s, 3H), 3.14 (s, 3H), 3.03 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 191.2, 153.8, 152.5, 145.9, 134.7, 124.8, 123.7, 110.7, 56.1, 36.8, 36.6. HR-EI-MS: *m/z* calcd for **1f** ($C_{11}H_{13}NO_4$, $[M]^+$), 223.0845; found, 223.0842.



2a: To a suspension of **1a** (0.6 g, 2.5 mmol) in CH_3OH (30 mL) at 0 °C, $NaBH_4$ (0.17 g, 5.0 mmol) was added slowly. After 30 min, the resulting suspension was stirred for 4 h at rt. Then,

the mixture was diluted with dichloromethane (20 mL), and washed three times with water (20 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation under reduced pressure, and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 100:1 to 50:1) as eluent, obtaining **2a** as a white solid (0.53 g, yield 88%). The ^{1}H-NMR and ^{13}C-NMR spectra of **2a** are shown below in Figures S41 and S42, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.41-7.36 (m, 2H), 7.14-7.10 (m, 2H), 5.39 (s, 1H), 3.34 (s, 2H), 3.04 (s, 3H), 3.01 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 151.7, 135.5, 127.9, 122.0, 102.7, 53.0, 36.7, 36.5. HR-EI-MS: *m/z* calcd for **2a** ($C_{10}H_{13}NO_3$, $[M]^+$), 195.0895; found, 195.0893.

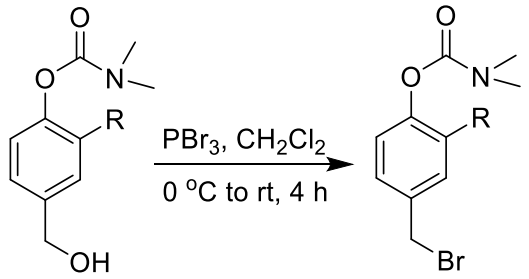
2b: **2b**, prepared similarly as **2a** using **1b** (0.42 g, 2.0 mmol) and $NaBH_4$ (0.15 g, 4.0 mmol), was obtained as a white solid (0.31 g, yield 73%). The ^{1}H-NMR, ^{13}C-NMR and ^{19}F-NMR spectra of **2b** are shown below in Figures S43, S44 and S45, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.25-7.18 (m, 2H), 7.14-7.11 (m, 1H), 5.36 (t, J = 5.7 Hz, 1H), 4.50 (d, J = 5.5 Hz, 2H), 3.06 (s, 3H), 2.92 (s, 3H). ^{13}C-NMR (75 MHz, 298K, DMSO-d_6): δ 155.9, 153.6, 152.7, 142.5, 137.5, 137.3, 124.5, 122.7, 122.7, 114.6, 114.4, 62.3, 36.9, 36.6. ^{19}F-NMR (300 MHz, 298 K, DMSO-d_6): δ -135.5, -135.5, -130.5, -130.6. HR-ESI-MS: *m/z* calcd for **2b** ($C_{10}H_{13}FNO_3$, $[M+H]^+$), 214.0874; found, 214.0875.

2c: **2c**, prepared similarly as **2a** using **1c** (0.91 g, 4.0 mmol) and $NaBH_4$ (0.30 g, 8.0 mmol), was obtained as a white solid (0.71 g, yield 71%). The ^{1}H-NMR and ^{13}C-NMR spectra of **2c** are shown below in Figures S46 and S47, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.46 (d, J = 1.5 Hz, 1H), 7.31-7.27 (m, 1H), 7.25 (d, J = 8.1 Hz, 1H), 5.37 (t, J= 5.8 Hz, 1H), 4.51 (t, J = 5.8 Hz, 2H), 3.08 (s, 3H), 2.93 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 153.6, 146.2, 142.2, 127.9, 126.4, 126.3, 124.6, 62.2, 36.9, 36.6. HR-EI-MS: *m/z* calcd for **2c** ($C_{10}H_{12}ClNO_3$, $[M]^+$), 229.0506; found, 229.0505.

2d: **2d**, prepared similarly as **2a** using **1d** (0.81 g, 3.0 mmol) and $NaBH_4$ (0.22 g, 6.0 mmol), was obtained as a white solid (0.56 g, yield 69%). The ^{1}H-NMR and ^{13}C-NMR spectra of **2d** are shown below in Figures S48 and S49, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.60 (d, J = 1.8 Hz, 1H), 7.34-7.30 (m, 1H), 7.23 (d, J = 8.4 Hz, 1H), 5.37 (t, J = 5.7 Hz, 1H), 4.50 (d, J = 5.7 Hz, 2H), 3.09 (s, 3H), 2.93 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 153.5, 147.5, 142.4, 130.9, 127.0, 124.6, 116.1, 62.1, 36.9, 36.6. HR-EI-MS: *m/z* calcd for **2d** ($C_{10}H_{12}BrNO_3$, $[M]^+$), 273.0002; found, 272.0001.

2e: **2e**, prepared similarly as **2a** using **1f** (0.71 g, 3.0 mmol) and $NaBH_4$ (0.22 g, 6.0 mmol), was obtained as a yellow oil (0.64 g, yield 90%). The ^{1}H-NMR and ^{13}C-NMR spectra of **2e** are shown below in Figures S50 and S51, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 7.90 (d, J = 2.1 Hz, 1H), 7.47-7.44 (m, 1H), 7.17 (d, J = 8.4 Hz, 1H), 4.51 (s, 2H), 3.53 (s, 1H), 3.09 (s, 3H), 2.96 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 153.5, 147.5, 142.4, 130.9, 127.0, 124.6, 116.1, 62.1, 36.9, 36.6. HR-EI-MS: *m/z* calcd for **2e** ($C_{10}H_{12}N_2O_5$, $[M]^+$), 240.0746; found, 240.0749.

2f: **2f**, prepared similarly as **2a** using **1f** (0.46 g, 2.0 mmol) and $NaBH_4$ (0.15 g, 4.0 mmol), was obtained as a white solid (0.32 g, yield 71%). The ^{1}H-NMR and ^{13}C-NMR spectra of **2f** are shown below in Figures S52 and S53, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 7.05 (d, J = 8.0, 1H), 6.99 (d, J = 1.88, 1H), 6.90-6.86 (m, 1H), 4.65 (d, J = 6.1, 2H), 3.83 (s, 3H), 3.14 (s, 3H), 3.02 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 155.0, 151.5, 139.9, 139.4, 122.9, 118.6, 110.9, 64.4, 55.8, 36.8, 36.5. HR-EI-MS: *m/z* calcd for **2f** ($C_{11}H_{15}NO_4$, $[M]^+$), 225.1001; found, 225.0998.



2 **3**

3a: R = H
3b: R = F
3c: R = Cl
3d: R = Br
3e: R = NO_2
3f: R = OCH_3

3a: To a solution of **2a** (0.49 g, 2.5 mmol) in dichloromethane (30 mL) at 0 °C, tribromophosphine (0.48 mL, 5.0 mmol) was added dropwise. The resulting reaction mixture was warmed up to room temperature and stirred for 4 h. Then, the mixture was diluted with dichloromethane (20 mL), and washed three times with water (20 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation under reduced pressure, and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 0 to 50:1) as eluent, obtaining **3a** as a clear oil (0.5 g, yield 78%). The ^{1}H-NMR and ^{13}C-NMR spectra of **3a** are shown below in Figures S54 and S55, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.47 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 8.7 Hz, 1H), 4.73 (s, 2H), 3.04 (s, 3H), 2.91 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 154.3, 151.6, 135.3, 130.8, 122.6, 36.8, 36.6, 34.5. HR-ESI-MS: *m/z* calcd for **3a** ($C_{10}H_{13}BrNO_2$, $[M+H]^+$), 258.0124; found, 258.0125.

3b: **3b**, prepared similarly as **3a** using **2b** (0.28 g, 1.3 mmol) and tribromophosphine (0.25 mL, 2.6 mmol), was obtained as a clear oil (0.27 g, 74%). The ^{1}H-NMR, ^{13}C-NMR and ^{19}F-NMR spectra of **3b** are shown below in Figures S56, S57 and S58, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 7.23-7.19 (m, 1H), 7.17-7.13 (m, 2H), 4.45 (s, 2H), 3.13 (s, 3H), 3.04 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 155.9, 153.6, 152.6, 139.1, 138.9, 136.4, 136.3, 124.9, 124.9, 124.4, 124.4, 117.4, 117.2, 36.9, 36.6, 32.0, 32.0. ^{19}F-NMR (300 MHz, 298 K, $CDCl_3$): δ -128.5. HR-EI-MS: *m/z* calcd for **3b** ($C_{10}H_{11}BrFNO_2$, $[M]^+$), 274.9957; found, 274.9956.

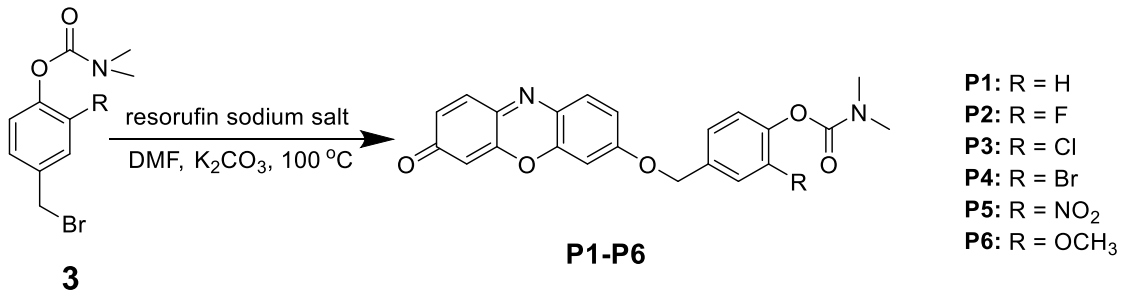
3c: **3c**, prepared similarly as **3a** using **2c** (0.65 g, 2.84 mmol) and tribromophosphine (0.55 mL, 5.68 mmol), was obtained as a clear oil (0.68 g, 83%). The ^{1}H-NMR and ^{13}C-NMR spectra of **3c** are shown below in Figures S59 and S60, respectively. ^{1}H-NMR (300 MHz, 298

K, $CDCl_3$): δ 7.48 (d, J = 2.13 Hz, 1H), 7.32-7.29 (m, 1H), 7.23 (d, J = 8.34 Hz, 1H), 4.45 (s, 2H), 3.17 (s, 3H), 3.05 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 153.6, 147.6, 136.2, 130.7, 128.3, 127.2, 124.4, 36.9, 36.6, 31.7. HR-EI-MS: *m/z* calcd for **3b** ($C_{10}H_{11}BrClNO_2$, $[M]^+$), 290.9662; found, 290.9661.

3d: **3d**, prepared similarly as **3a** using **2d** (0.5 g, 1.83 mmol) and tribromophosphine (3.52 mL, 3.66 mmol), was obtained as a clear oil (0.48 g, 79%). The ^{1}H-NMR and ^{13}C-NMR spectra of **3d** are shown below in Figures S61 and S62, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 7.64 (d, J = 2.19 Hz, 1H), 7.37-7.33 (m, 1H), 7.23 (d, J = 8.31 Hz, 1H), 4.47 (s, 2H), 3.17 (s, 3H), 3.05 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 153.5, 148.8, 136.4, 133.6, 129.0, 124.3, 116.4, 36.9, 36.6, 31.6. HR-EI-MS: *m/z* calcd for **3b** ($C_{10}H_{11}Br_2NO_2$, $[M]^+$), 334.9157; found, 334.7157.

3e: **3e**, prepared similarly as **3a** using **2e** (0.6 g, 2.5 mmol) and tribromophosphine (0.48 mL, 5.0 mmol), was obtained as a clear oil (0.63 g, 84%). The ^{1}H-NMR and ^{13}C-NMR spectra of **3e** are shown below in Figures S63 and S64, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 8.10 (d, J = 1.84 Hz, 1H), 7.68-7.65 (m, 1H), 7.32 (d, J = 8.4 Hz, 1H), 4.51 (s, 2H), 3.15 (s, 3H), 3.04 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 153.1, 144.8, 141.6, 136.0, 134.9, 126.0, 126.0, 37.0, 36.7, 30.7. HR-EI-MS: *m/z* calcd for **3e** ($C_{10}H_{11}BrN_2O_4$, $[M]^+$), 301.9902; found, 301.9901.

3f: **3f**, prepared similarly as **3a** using **2f** (0.28 g, 1.24 mmol) and tribromophosphine (0.24 mL, 2.49 mmol), was obtained as a clear oil (0.28 g, 78%). The ^{1}H-NMR and ^{13}C-NMR spectra of **3f** are shown below in Figures S65 and S66, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 7.05 (d, J = 7.96 Hz, 1H), 6.99 (d, J = 1.89 Hz, 1H), 6.98-6.94 (m, 1H), 4.49 (s, 2H), 3.85 (s, 3H), 3.07 (s, 6H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 154.5, 151.8, 140.6, 135.9, 123.4, 121.3, 113.1, 56.0, 36.7, 33.5. HR-EI-MS: *m/z* calcd for **3f** ($C_{11}H_{14}BrNO_3$, $[M]^+$), 287.0157; found, 287.0156.



P1: To a solution of resorufin sodium salt (0.29 g, 1.2 mmol) in anhydrous DMF (10 mL), K_2CO_3 (0.33 g, 2.4 mmol) was added, followed by stirring at 100 °C for 10 min. Then, a solution of **P1** (0.31 g, 1.2 mmol) in DMF (2 mL) was added dropwise. After stirring at 100 °C for 12 h, the solution was cooled and diluted with CH_2Cl_2 (20 mL). The resulting solution was then washed with brine water (15 mL × 3). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation, and the residue was

subjected to silica gel chromatography eluted with CH_2Cl_2:CH_3OH (v/v, 200:1 to 100:1), affording **P1** as an orange solid (0.24 g, yield 51%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P1** are shown below in Figures S67 and S68, respectively. ^{1}H-NMR (300 MHz, 298 K, CD_2Cl_2-d_2): δ 7.76 (d, J = 8.9 Hz, 1H), 7.51-7.46 (m, 2H), 7.46 (d, J = 9.8 Hz, 1H), 7.21-7.16 (m, 2H), 7.07 (m, 1H), 6.94 (d, J = 2.6 Hz, 1H), 6.82-6.78 (m, 1H), 6.29 (d, J = 2.0 Hz, 1H), 5.20 (s, 2H), 3.12 (s, 3H), 3.01 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, CD_2Cl_2-d_2): δ 185.9, 162.5, 154.5, 151.8, 149.8, 145.9, 145.6, 134.7, 134.1, 132.4, 131.5, 128.6, 128.5, 122.2, 114.0, 106.4, 101.0, 70.4, 36.5, 36.3. HR-ESI-MS: *m/z* calcd for **P1** ($C_{22}H_{19}N_2O_5$, $[M+H]^+$), 391.1288; found, 391.1289.

P2: **P2**, prepared similarly as **P1** using **3b** (0.20 g, 0.73 mmol), K_2CO_3 (0.2 g, 1.46 mmol), and resorufin sodium salt (0.18 g, 0.73 mmol), was obtained as an orange solid (0.18 g, yield 60%). The ^{1}H-NMR, ^{13}C-NMR and ^{19}F-NMR spectra of **P2** are shown below in Figures S69, S70 and S71, respectively. ^{1}H-NMR (300 MHz, 298 K, CD_2Cl_2-d_2): δ 7.77 (d, J = 8.88 Hz, 1H), 7.45 (d, J = 9.81 Hz, 1H), 7.33-7.29 (m, 1H), 7.27-7.25 (m, 2H), 7.07 (dd, J = 6.21, 2.7 Hz, 1H), 6.93 (d, J = 2.67 Hz, 1H), 6.82 (dd, J = 7.77, 2.07 Hz, 1H), 6.29 (d, J = 2.04 Hz, 1H), 5.20 (s, 2H), 3.14 (s, 3H), 3.01 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, CD_2Cl_2-d_2): δ 185.9, 162.2, 156.3, 153.5, 153.0, 149.8, 146.1, 145.6, 139.1, 139.0, 134.7, 134.6, 134.5, 134.1, 131.6, 128.6, 124.6, 123.2, 123.2, 115.7, 115.4, 113.9, 106.5, 101.1, 69.7, 36.7 36.4. ^{19}F-NMR (300 MHz, 298 K, CD_2Cl_2-d_2): δ -129.2, -129.2, -129.2, -129.2, -129.3, -129.3, -129.3. HR-ESI-MS: *m/z* calcd for **P2** ($C_{22}H_{18}FN_2O_5$, $[M+H]^+$), 409.1194; found, 409.1193.

P3: **P3**, prepared similarly as **P1** using **3c** (0.29 g, 1.0 mmol), K_2CO_3 (0.28 g, 2.0 mmol), and resorufin sodium salt (0.24 g, 1.0 mmol), was obtained as orange solid (0.2 g, yield 48%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P3** are shown below in Figures S72 and S73, respectively. ^{1}H-NMR (300 MHz, 298 K, CD_2Cl_2-d_2): δ 7.77 (d, J = 8.88 Hz, 1H), 7.59 (d, J = 1.98 Hz, 1H), 7.46 (d, J = 9.84 Hz, 1H), 7.41-7.39 (m, 1H), 7.31 (d, J = 8.31 Hz, 1H), 7.07 (dd, J = 6.24, 2.67 Hz, 1H), 6.93 (d, J = 2.67 Hz, 1H), 6.82 (dd, J = 7.77, 2.04 Hz, 1H), 6.29 (d, J = 2.04 Hz, 1H), 5.18 (s, 2H), 3.17 (s, 3H), 3.03 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, CD_2Cl_2-d_2): δ 185.9, 162.2, 153.5, 149.8, 147.8, 146.1, 145.6, 134.7, 134.3, 134.1, 131.6, 129.0, 128.6, 127.5, 126.7, 124.5, 113.9, 106.5, 101.1, 69.6, 36.6, 36.4. HR-ESI-MS: *m/z* calcd for **P3** ($C_{22}H_{18}ClN_2O_5$, $[M+H]^+$), 425.0899; found, 425.0898.

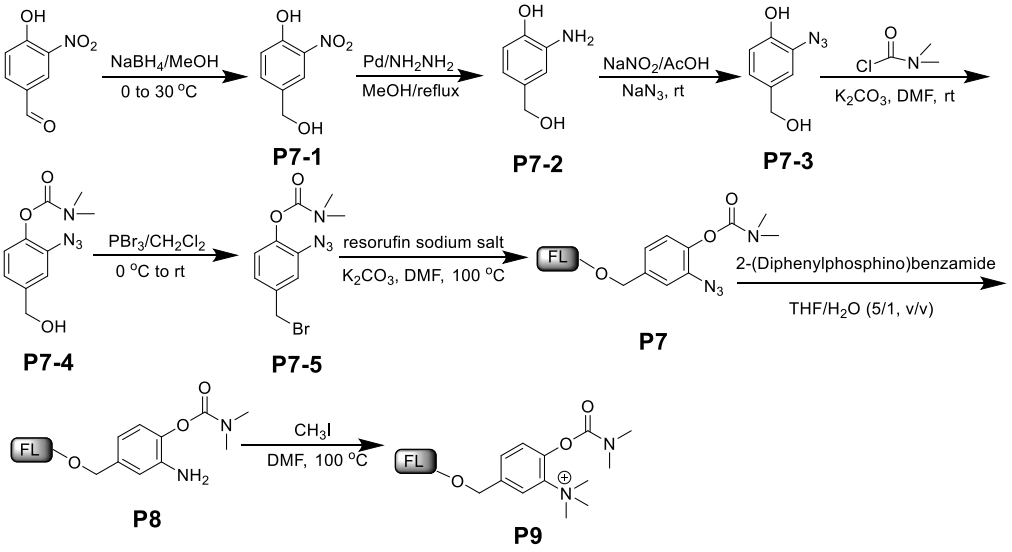
P4: **P4**, prepared similarly as **P1** using **3d** (0.34 g, 1.0 mmol), K_2CO_3 (0.28 g, 2.0 mmol), and resorufin sodium salt (0.24 g, 1.0 mmol), was obtained as an orange solid (0.24 g, yield 52%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P4** are shown below in Figures S74 and S75, respectively. ^{1}H-NMR (300 MHz, 298 K, CD_2Cl_2-d_2): δ 7.77 (d, J = 6.57 Hz, 1H), 7.74 (s, 1H), 7.47-7.43 (m, 2H), 7.30 (d, J = 8.31 Hz, 1H), 7.07 (dd, J = 6.24, 2.67 Hz, 1H), 6.93 (d, J = 2.64 Hz, 1H), 6.82 (dd, J = 7.77, 2.04 Hz, 1H), 6.29 (d, J = 2.04 Hz, 1H), 5.18 (s, 2H), 3.18 (s, 2H), 3.03 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, CD_2Cl_2-d_2): δ 185.9, 162.2, 153.4, 149.8, 149.0, 146.1, 145.6, 134.7, 134.5, 134.1, 132.0, 131.6, 128.6, 127.4, 124.4, 116.7, 113.9,

106.5, 101.1, 67.5, 36.6, 36.4. HR-ESI-MS: *m/z* calcd for **P4** ($C_{22}H_{18}BrN_2O_5$, $[M+H]^+$), 469.0934; found, 469.0932.

P5: **P5**, prepared similarly as **P1** using **3e** (0.48 g, 1.6 mmol), K_2CO_3 (0.44 g, 3.2 mmol), and resorufin sodium salt (0.39 g, 1.6 mmol), was obtained as an orange solid (0.32 g, yield 46%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P5** are shown below in Figures S76 and S77, respectively. ^{1}H-NMR (300 MHz, 298 K, CD_2Cl_2-d_2): δ 8.16-8.15 (m, 1H), 7.77-7.73 (m, 2H), 7.44 (d, J = 9.8 Hz, 1H), 7.39 (d, J = 8.3 Hz, 1H), 7.07-7.03 (m, 1H), 6.93 (d, J = 2.6 Hz, 1H), 6.81-6.77 (m, 1H), 6.28 (d, J = 2.0 Hz, 1H), 5.24 (s, 2H), 3.14 (s, 3H), 3.01 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, CD_2Cl_2-d_2): δ 185.9, 161.8, 153.0, 149.8, 146.3, 145.6, 144.8, 134.7, 134.2, 134.1, 133.1, 131.7, 128.8, 125.8, 124.3, 113.7, 106.6, 101.2, 69.0, 36.7, 36.4. HR-ESI-MS: *m/z* calcd for **P5** ($C_{22}H_{18}N_3O_7$, $[M+H]^+$), 436.1139; found, 436.1139.

P6: **P6**, prepared similarly as **P1** using **3f** (0.22 g, 0.77 mmol), K_2CO_3 (0.21 g, 1.54 mmol), and resorufin sodium salt (0.19 g, 0.77 mmol), was obtained as an orange solid (0.19 g, yield 58%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P6** are shown below in Figures S78 and S79, respectively. ^{1}H-NMR (300 MHz, 298 K, CD_2Cl_2-d_2): δ 7.73 (d, J = 8.9 Hz, 1H), 7.42 (d, J = 9.8 Hz, 1H), 7.10-7.7.06 (m, 2H), 7.05-7.01 (m, 2H), 6.91 (d, J = 2.6 Hz, 1H), 6.78-6.74 (m, 1H), 6.25 (d, J = 2.0 Hz, 1H), 5.15 (s, 2H), 3.84 (s, 3H), 3.09 (s, 3H), 2.97 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, CD_2Cl_2-d_2): δ 185.9, 162.5, 154.4, 152.1, 149.8, 145.9, 145.6, 140.8, 134.7, 134.1, 133.9, 131.5, 128.5, 123.5, 119.7, 114.0, 111.8, 106.4, 101.0, 70.6, 36.5, 36.4. HR-ESI-MS: *m/z* calcd for **P6** ($C_{23}H_{21}N_2O_6$, $[M+H]^+$), 421.1394; found, 412.1392.

Syntheses of Probes 7-9



P7-1: To a suspension of 4-Hydroxy-3-nitrobenzaldehyde (8.35 g, 50.0 mmol) in CH_3OH (30 mL) at 0 °C, $NaBH_4$ (0.34 g, 10.0 mmol) was added slowly. After 30 min, the resulting suspension was stirred for 4 h at rt. Then, the mixture was diluted with dichloromethane (20 mL), and washed three times with water (20 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation under reduced pressure,

and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 150:1 to 50:1) as eluent, obtaining **P7-1** as a yellow solid (4.73 g, yield 56%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P7-1** are shown below in Figures S80 and S81, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 10.80 (s, 1H), 7.83 (t, J = 1.2 Hz, 1H), 7.50 (m, 1H), 7.11 (d, J = 8.49 Hz, 1H), 5.31 (d, J = 2.7 Hz, 1H), 4.45 (s, 2H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 151.5, 136.6, 134.4, 134.2, 123.1, 119.4, 62.0. HR-EI-MS: *m/z* calcd for **P7-1** ($C_7H_7NO_4$, $[M]^+$), 169.0375; found, 169.0376.

P7-2: To a solution of **P7-1** (4.2 g, 25.0 mmol) and 10% Pd/C (422 mg) in 30 mL ethanol was added hydrazine monohydrate (10 mL), the resulting mixture was then heated to reflux for 2h. The reaction solution was filtered to remove the Pd/C, and the collected solution was evaporated and subjected to silica gel chromatography using eluent (DCM: CH_3OH, v/v, 100:1 to 20:1) to afford **P7-2** as a brown solid (2.76 g, yield 80%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P7-2** are shown below in Figures S82 and S83, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 8.81 (s, 1H), 6.58 (m, 1H), 6.35 (m, 1H),4.82 (t, J = 5.7 Hz, 1H), 4.45 (s, 2H), 4.27 (d, J = 5.4 Hz, 2H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 143.3, 136.6, 133.9, 115.4, 114.3, 113.8, 63.7. HR-EI-MS: *m/z* calcd for **P7-2** ($C_7H_9NO_2$, $[M]^+$), 139.0633; found, 139.0631.

P7-3: To a stirred suspension of **P7-2** (2.5 g, 17.99 mmol) in AcOH (20 mL) at room temperature, $NaNO_2$ (1.37 g, 17.79 mmol) was gradually added. After 1 h, NaN_3 (1.75 g, 26.99 mmol) was added by portions, and the stirring was continued for 1.5 h. Then the reaction mixture was diluted with water (50 mL) and extracted using EtOAc (3×30 mL). The combined organic layers were washed with water (100 mL), dried with anhydrous Na_2SO_4, and the collected solution was evaporated under reduced pressure. The residue was purified by silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 100:1 to 50:1) as eluent, affording pale yellow oil (1.93 g, 65%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P7-3** are shown below in Figures S84 and S85, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 9.91 (s, 1H), 6.95 (m, 1H), 6.90 (d, J = 1.8 Hz, 1H), 6.83 (d, J = 8.13 Hz, 1H), 5.09 (t, J = 5.73 Hz, 1H), 4.37 (d, J = 5.55 Hz, 2H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 149.4, 134.7, 125.6, 124.8, 119.5, 116.5, 62.7. HR-EI-MS: *m/z* calcd for **P7-3** ($C_7H_7N_3O_2$, $[M]^+$), 165.0538; found, 165.0536.

P7-4: To a solution of **P7-3** (1.65 g, 10.0 mmol) and K_2CO_3 (2.07 g, 15 mmol) in DMF (20 mL) was added Me_2NCOCl (1.01 mL, 11 mmol) at room temperature. The reaction mixture was stirred for 8 h at room temperature. After dilution with water (30 mL), the mixture was extracted with CH_2Cl_2 (30 mL×2), and the combined organic layer was washed with saturated Na_2CO_3 solution, evaporated and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 0 to 100:1) as eluent, obtaining **P7-4** as gray solid (1.94 g, yield 82%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P7-4** are shown below in Figures S86 and S87,

respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.20 (s, 1H), 7.12 (d, J = 1.2 Hz, 2H), 5.34 (t, J = 5.4 Hz, 1H), 4.51 (d, J = 5.1 Hz, 2H), 3.05 (s, 3H), 2.91 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 154.0, 142.0, 141.6, 132.0, 124.5, 123.9, 118.3, 62.4. HR-EI-MS: *m/z* calcd for **P7-4** ($C_{10}H_{12}N_4O_3$, $[M]^+$), 236.0909; found, 236.0906.

P7-5: To a solution of **P7-4** (1.42 g, 6.0 mmol) in dichloromethane (30 mL) at 0 ^{o}C, tribromophosphine (1.15 mL, 12.0 mmol) was added dropwise. The resulting reaction mixture was warmed up to room temperature and stirred for 4 h. Then, the mixture was diluted with dichloromethane (20 mL), and washed three times with water (20 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation under reduced pressure to obtain **P7-5**, which was used directly for next step without further purification.

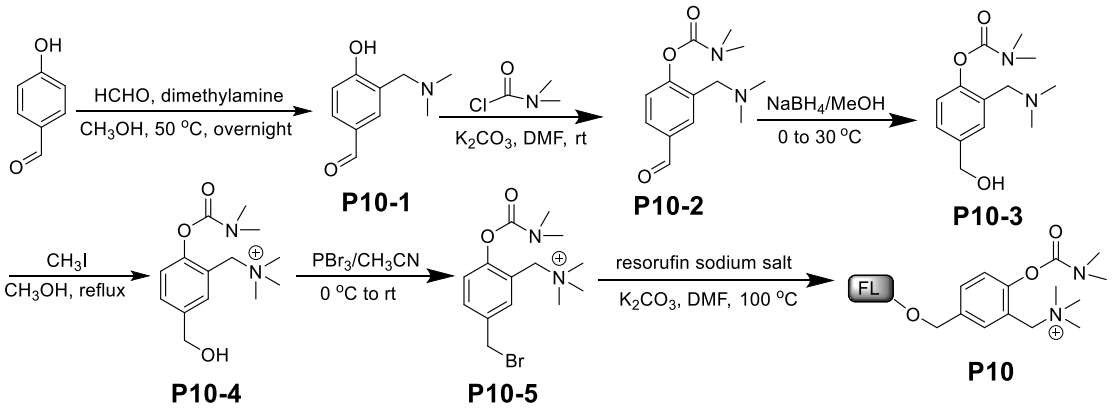
P7: To a solution of resorufin sodium salt (0.47 g, 2.0 mmol) in anhydrous DMF (10 mL), K_2CO_3 (0.55 g, 4.0 mmol) was added, followed by stirring at 100 ^{o}C for 10 min. Then, a solution of **P7-5** (0.60 g, 2.0 mmol) in DMF (2 mL) was added dropwise. After stirring at 100 ^{o}C for 12 h, the solution was cooled and diluted with CH_2Cl_2 (20 mL). The resulting solution was then washed with brine water (15 mL × 3). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation, and the residue was subjected to silica gel chromatography eluted with CH_2Cl_2:CH_3OH (v/v, 200:1 to 100:1), affording **P7** as an orange solid (0.39 g, yield 45%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P7** are shown below in Figures S88 and S89, respectively. ^{1}H-NMR (300 MHz, 298 K, CD_2Cl_2-d_2): δ 7.77 (d, J = 8.88 Hz, 1H), 7.46 (d, J = 9.84 Hz, 1H), 7.27 (m, 2H), 7.20 (m, 1H), 7.08(dd, J = 6.21, 2.67 Hz, 1H), 6.94 (d, J =2.64, 1H), 6.82 (dd, J = 7.77, 2.04 Hz, 1H), 6.29 (d, J = 2.04 Hz, 1H), 5.19 (s, 2H), 3.14 (s, 3H), 3.02 (s, 3H). 7.28-7.26 (m, 1H), 3.10 (s, 3H), 3.01 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, CD_2Cl_2-d_2): δ 185.9, 162.2, 153.8, 149.8, 146.0, 145.6, 143.1, 134.7, 134.3, 134.1, 133.1, 131.6, 128.6, 124.5, 124.4, 119.3, 113.9, 106.5, 101.1, 69.8, 36.7, 36.3. HR-EI-MS: *m/z* calcd for **P7** ($C_{22}H_{17}N_5O_5$, $[M]^+$), 431.1230; found, 431.1230.

P8: To a solution of **P7** (0.3 g, 0.7 mmol) in the mixture of THF-H_2O (10 mL, 9:1, v/v), the compound 2-(Diphenylphosphino)benzamide 2 (0.32 g, 1.05 mmol) was added. A mixture was stirred at room temperature for 0.5 h and evaporated in vacuo. The residue was purified by silica gel chromatography using the eluent (CH_2Cl_2:CH_3OH, v/v, 100:1 to 20:1), affording **P8** as an orange solid (0.20 g, yield 71%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P8** are shown below in Figures S90 and S91, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.80 (d, J = 8.85 Hz, 1H), 7.55 (d, J = 9.81 Hz, 1H), 7.17 (d, J = 2.52 Hz, 1H), 7.13 (dd, J = 6.24, 2.58 Hz, 1H), 6.87 (d, J = 8.1 Hz, 1H), 6.82-6.80 (m, 1H), 6.63 (dd, J = 6.06, 2.07 Hz, 1H), 6.28 (d, J = 2.07 Hz, 1H), 5.14 (s, 2H), 5.02 (s, 2H), 3.05 (s, 3H), 2.90 (s ,3H). ^{13}C-NMR

(75 MHz, 298 K, DMSO-d_6): δ 185.8, 162.9, 154.4, 150.2, 145.7, 145.6, 141.4, 137.9, 135.4, 133.7, 31.8, 128.4, 123.3, 115.7, 115.3, 114.9, 106.1, 101.6, 70.9, 37.0, 36.7. HR-EI-MS: *m/z* calcd for **P8** ($C_{22}H_{19}N_3O_5$, $[M]^+$), 405.1325; found, 405.1325.

P9: Compound **P8** (0.081 g, 0.2 mmol) was dissolved in 10 mL DMF, and CH_3I (0.25 mL, 4 mmol) was added slowly. Then, the resulting mixture was stirred at 100 °C. After 10 h, the solvent was removed under reduced pressure and the residue was subjected to neutral aluminium oxide with eluent (CH_2Cl_2:CH_3OH, v/v, 100:1 to 10:1), affording **P9** as an orange solid (0.011g, yield 9.6%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P9** are shown below in Figures S92 and S93, respectively. ^{1}H-NMR (300 MHz, 298 K, MeOD-d_4): δ 8.08 (d, J = 1.8 Hz, 1H), 7.85-7.79 (m, 2H), 7.58 (d, J = 9.81 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H), 7.21 (m, 2H), 6.88 (dd, J = 7.7, 2.1 Hz, 1H), 6.34 (d, J = 2.1 Hz, 1H), 5.37 (s, 2H), 3.79 (s, 9H), 3.27 (s, 3H), 3.11 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, MeOD-d_4): δ 187.0, 162.8, 153.0, 150.5, 145.7, 145.3, 143.9, 137.3, 135.3, 135.2, 133.3, 131.6, 130.8, 128.8, 127.1, 120.4, 114.3, 105.5, 100.9, 69.1, 36.1, 36.8. HR-ESI-MS: *m/z* calcd for **P9** ($C_{25}H_{26}N_3O_5^+$, $[M]^+$), 448.1867; found, 448.1866.

Syntheses of Probe 10



P10-1: A mixture of 4-hydroxybenzaldehyde (6.25 g, 50 mmol), 33% aqueous dimethylamine (6.88 g, 50 mmol) and 35% formaldehyde solution (5.1 g, 60 mmol) in 50 mL methanol was stirred overnight at 50 °C. Then, the solvent was removed under reduced pressure, and the residue was extracted with CH_2Cl_2 (15 mL×3). The collected organic phase was dried by anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography using CH_2Cl_2:CH_3OH (v/v, 100:1 to 10:1) as eluent to give **P10-1** as a yellow solid (3.85 g, 42%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P10-1** are shown below in Figures S94 and S95, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 9.76 (s, 1H), 7.70-7.65 (m, 2H), 6.90 (d, J = 8.1 Hz, 1H), 6.15 (s, 1H), 3.68 (s, 2H), 2.28 (s, 6H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 191.3, 164.6, 131.5, 131.3, 128.1, 123.8,

116.5, 60.0, 44.5. HR-EI-MS: *m/z* calcd for **P10-1** ($C_{10}H_{13}NO_2$, $[M]^+$), 179.0946; found, 179.0944.

P10-2: To a solution of **P10-1** (1.79 g, 10.0 mmol) and K_2CO_3 (2.76 g, 20 mmol) in DMF (20 mL) was added Me_2NCOCl (1.84 mL, 20 mmol) at room temperature. The reaction mixture was stirred for 12 h at room temperature. After dilution with water (30 mL), the mixture was extracted with CH_2Cl_2 (30 mL×2), and the combined organic layer was washed with saturated Na_2CO_3 solution, evaporated and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 0 to 100:1) as eluent, obtaining **P10-2** as a clear oil (2.17 g, yield 87%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P10-2** are shown below in Figures S96 and S97, respectively. ^{1}H-NMR (300 MHz, 298 K, $CDCl_3$): δ 9.91 (s, 1H), 7.91-7.90 (m, 1H), 7.75 (dd, J = 6.21, 2.13 Hz, 1H), 7.24 (d, J = 8.67 Hz, 1H), 3.38 (s, 12H), 3.08 (s, 3H), 2.96 (s, 3H), 2.19 (s, 6H). ^{13}C-NMR (75 MHz, 298 K, $CDCl_3$): δ 191.4, 154.8, 133.6, 132.7, 132.2, 129.0, 123.5, 57.8, 45.6, 36.8, 36.5. HR-EI-MS: *m/z* calcd for **P10-2** ($C_{13}H_{18}N_2O_3$, $[M]^+$), 250.1317; found, 250.1314.

P10-3: To a suspension of **P10-2** (1.75 g, 7.0 mmol) in CH_3OH (30 mL) at 0 °C, $NaBH_4$ (0.48 g, 14.0 mmol) was added slowly. After 30 min, the resulting suspension was stirred for 4 h at room temperature. Then, the mixture was diluted with dichloromethane (20 mL), and washed three times with water (20 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation under reduced pressure, and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 100:1 to 50:1) as eluent, obtaining **P10-3** as a yellow oil (1.53 g, yield 87%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P10-3** are shown below in Figures S98 and S99, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.34 (d, J = 1.98 Hz, 1H), 7.21 (dd, J = 6.03, 2.19 Hz, 1H),7.02 (d, J = 8.22 Hz, 1H), 5.21 (t, J = 5.76 Hz, 1H), 4.49 (d, J = 5.34 Hz, 2H), 3.28 (s, 2H), 3.07 (s, 3H), 2.91 (s, 3H), 2.13 (s, 6H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 154.4, 148.9, 139.8, 131.0, 128.8, 126.2, 122.9, 62.9, 58.0, 45.6, 36.8, 36.5. HR-ESI-MS: *m/z* calcd for **P10-3** ($C_{13}H_{21}N_2O_3$, $[M+H]^+$), 253.1547; found, 253.1547.

P10-4: To a solution of **P10-3** (1.0 g, 4.0 mmol) in CH_3OH (30 mL), CH_3I (2 mL, 32 mmol) was added dropwise. The resulting reaction mixture was refluxed for 12 h. Then, the solvent was removed, and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 100:1 to 10:1) as eluent, obtaining **P10-4** as a white solid (1.17 g, yield 75%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P10-4** are shown below in Figures S100 and S101, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.56 (d, J = 2.01 Hz, 1H), 7.50 (dd, J = 6.21, 2.16 Hz, 1H), 7.21 (d, J = 8.34 Hz, 1H), 5.33 (t, J = 5.64 Hz, 1H), 4.55 (s, 2H), 4.53 (s, 2H), 3.09 (s, 3H), 3.08 (s, 9H), 2.94 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 154.1, 150.5, 140.4, 132.7, 130.3, 124.0, 120.7, 62.4, 52.6, 49.1, 37.2, 36.9. HR-FAB-MS: *m/z* calcd for **P10-4** ($C_{14}H_{23}N_2O_3^+$, $[M]^+$), 267.1703; found, 267.1708.

P10-5: To a suspension of **P10-4** (0.99 g, 2.5 mmol) in CH_3CN (30 mL) at 0 ^oC, tribromophosphine (0.48 mL, 5.0 mmol) was added dropwise. The resulting reaction mixture was warmed up to room temperature and stirred for overnight. Then, the solvent was removed by evaporation under reduced pressure, and the residue was subjected to silica gel chromatography with CH_2Cl_2:CH_3OH (v/v, 100:1 to 10:1) as eluent, obtaining **P10-5** as a white solid (0.46 g, yield 40%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P10-5** are shown below in Figures S102 and S103, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.72 (d, J = 2.19 Hz, 1H), 7.65 (dd, J = 6.15, 2.28 Hz, 1H), 7.27 (d, J = 8.43 Hz, 1H), 4.77 (s, 2H), 4.55 (s, 2H), 3.09 (s, 3H), 3.08 (s, 9H), 2.94 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 153.9, 151.7, 136.0, 135.9, 133.1, 124.6, 121.5, 62.5, 52.6, 37.2, 36.9, 33.7. HR-ESI-MS: *m/z* calcd for **P10-5** ($C_{14}H_{22}BrN_2O_2^+$, $[M]^+$), 329.0859; found, 329.0857.

P10: To a solution of resorufin sodium salt (0.21 g, 0.9 mmol) in anhydrous DMF (15 mL), K_2CO_3 (0.25 g, 1.8 mmol) was added, followed by stirring at 100 ^oC for 10 min. Then, a solution of **P10-5** (0.41 g, 0.9 mmol) in DMF (2 mL) was added dropwise. After stirring at 100 ^oC for 12 h, the solution was cooled and diluted with CH_2Cl_2 (20 mL). The resulting solution was then washed with brine water (15 mL × 3). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation, and the residue was subjected to neutral aluminium oxide eluted with CH_2Cl_2:CH_3OH (v/v,100:1 to 10:1), affording **P10** as an orange solid (0.28 g, yield 52%). The ^{1}H-NMR and ^{13}C-NMR spectra of **P10** are shown below in Figures S104 and S105, respectively. ^{1}H-NMR (300 MHz, 298 K, DMSO-d_6): δ 7.82 (d, J = 8.88 Hz, 1H), 7.74 (d, J = 2.04 Hz, 1H), 7.70 (dd, J = 6.30, 2.07 Hz, 1H), 7.56 (d, J = 9.81 Hz, 1H), 7.34 (d, J = 8.37 Hz, 1H), 7.24 (d, J = 2.64 Hz, 1H), 7.17 (m, 1H), 6.82 (dd, J = 7.74, 2.07 Hz, 1H), 6.29 (d, J = 2.07 Hz, 1H), 5.35 (s, 2H), .4.55 (s, 2H), 3.09 (s, 3H), 3.06 (s, 9H), 2.95 (s, 3H). ^{13}C-NMR (75 MHz, 298 K, DMSO-d_6): δ 185.8, 162.5, 153.9, 151.7, 150.2, 145.9, 145.7, 135.4, 134.6, 134.3, 133.9, 131.9, 131.8, 128.5, 124.6, 121.3, 114.8, 106.2, 101.8, 69.8, 62.7, 52.6, 37.2, 36.8. HR-ESI-MS: *m/z* calcd for **P10** ($C_{26}H_{28}N_3O_5^+$, $[M]^+$), 462.2023; found, 462.2023.

Photophysical data

Fluorescence quantum yield (Φ) was measured using resorufin[1] as a standard according to the following equation:

$$\Phi_X = (\Phi_S \times A_S \times I_X \times \eta_X^2)/(A_X \times I_S \times \eta_S^2)$$

where A is the absorbance at the excitation wavelength (A is kept between 0.01 and 0.05), I is the integrated area of emission spectra, and η is the refractive index of the solvent. The subscripts S and X refer to the standard and unknown, respectively.

General procedure for spectroscopic analysis

Unless otherwise stated, all the fluorescence measurements were made according to the following procedure. In a 5-mL test tube, 10 μL of the stock solution of **P1**-**P10** and **CP**, and

appropriate volume of PBS (pH 7.4) were mixed, followed by addition of an appropriate volume of AChE and/or other substance solution. The mixed solution was adjusted to 2 mL with PBS. After incubation at 37 °C for 5 h for **P1**-**P10** and 0.5 h for **CP**, the reaction solution was transferred to a quartz cell of 1-cm optical length to measure fluorescence with $\lambda_{ex/em}$ = 550/582 nm (both excitation and emission slit widths were set to 5 nm). For absorbance measurements, 2 mL of the reaction solution was prepared and used. At the same time, a blank solution without AChE and/or other substance was prepared and measured under the same conditions for comparison. Data are expressed as mean ± standard deviation (SD) of three separate measurements.

Kinetic Parameters

Various concentrations of probe solution were mixed with CE (1 U/mL), BChE (5 U/mL) and AChE (5 U/mL), respectively in PBS buffer (pH 7.4) at 37 ^{o}C. After incubation, the fluorescence intensity was monitored. Then relative initial reaction velocity was calculated, plotted against the concentration of probes, and fitted to a Michaelis-Menten curve. The kinetic parameters were calculated by use of equations shown below: $V = V_{max}*[S] / (K_m + [S])$ and $k_{cat} = V_{max} / [E_0]$, where V is initial velocity, [S] is substrate concentration and $[E_0]$ is the enzyme concentration.

Preparation of ROS/RNS solution

Various ROS/RNS solutions were freshly prepared before use following the methods.[2]
The concentration of H_2O_2 solution was determined from the absorption at 240 nm (ε = 43.6 M^{-1} cm^{-1}). The concentration of ClO^- solution was determined by the absorption at 292 nm (ε = 350 M^{-1} cm^{-1}). TBHP was obtained commercially from Sigma-Aldrich. *Tert*-butoxy radical (TBO•) was prepared by Fenton reaction on mixing $FeSO_4$ with 10 equivalents of TBHP. Hydroxyl radical (•OH) was generated by Fenton reaction on mixing $FeSO_4$ with 10 equivalents of H_2O_2. Superoxide ($O_2^{\bullet-}$) is prepared from KO_2. Singlet oxygen ($^1O_2$) was generated by the addition of ClO^- and H_2O_2. The concentration of $ONOO^-$ solution was estimated by the absorption at 302 nm (ε = 1670 M^{-1} cm^{-1}). The source of NO_2^- was from $NaNO_2$. Nitric oxide (NO) was generated from SNP (Sodium Nitroferricyanide (III) Dihydrate). Carbon monoxide (CO) was generated from CORM3.

Preparation of cell lysate and activity assay of AChE in cell lysate

The cell lysates were obtained as following method. Cells were washed with PBS and collected using PBS (pH 7.4) containing 0.2 % (v/v) Triton X-100 and protease inhibitor. To extract cell lysates, cells were sonicated and incubated for 30 min in ice and centrifuged at 13000 rpm for 10 min at 4 ^{o}C and the supernatants were collected as cell lysates and stored at -80 ^{o}C. Protein concentration of lysates were calculated by Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). The activity of AChE in cell lysates were tested based on the Ellman method.[3]

Cytotoxicity assay

Cytotoxicity of **P10** was tested on cells (U87MG, N2A, and HEK293) using a Cell Counting Kit-8 (CCK-8, Dojindo Molecular Tech. Inc, Japan) assay according to the manufacturer's instructions. Each cell (5×10^3 cells per well) was seeded in 96-well plate and incubated for 24 h at 37 °C in a humidified 5% CO_2 incubator. The cells were then treated with serial dilution concentrations (2.5, 5.0, 10, 20 and 40 μM) in serum of the **P10**. The cell toxicity was measured after incubation for 6 h. Then, **P10** was removed by washing with PBS (3 times), followed by changing serum-free media. CCK-8 solution (10 μL, 10× working concentration) in serum-free media was added to each well of a 96-well plate, followed by incubation for 2 h. Then, the absorbance was measured at 450 nm using a microplate reader (Multiskan FC, Thermo Fisher, MA, USA). The percentage of cell cytotoxicity was calculated using the formula; Cell viability (%) = (Mean OD of sample × 100) / (Mean OD of the control group) (OD: optical density).

Cell imaging

Cells were grown on the glass-bottom culture dishes (MatTek CO.) in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin at 37 °C in a humidified 5% CO_2 incubator. Before use, the adherent cells were washed three times with FBS-free DMEM. For fluorescence imaging, the cells were incubated with 10 μM of **P10** in FBS-free DMEM at 37 °C for 5 h, and then washed twice with PBS to remove the free probe.

Mice brain and tissue imaging

Animals: 6-week-old male BALB/c nu/nu mice [Taconic, provided by Daehan Biolink Co., Ltd. (Eumseong, Republic of Korea)] were housed at an ambient temperature of 23 ± 1 °C and relative humidity of 60 ± 10% under a 12 h light/dark cycle and were allowed free access to water and food. All of the experiments performed with mice were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996 and protocols approved by the Institutional Animal Care and Use Committee of Kyung Hee University [KHUASP(SE)-19-002].

Stereotaxic surgery: mice were anesthetized with tribromoethanol (312.5 mg/kg, i.p.) and mounted in a stereotaxic apparatus [myNeuroLab (St. Louis, MO, USA)]. Each mouse received a unilateral injection of 100 μM of **P10** per 5 μL in PBS, according to the following coordinates: anteroposterior: -2.0 mm from bregma; mediolateral: 1.5 mm from bregma; and dorsoventral: −2.0 mm from the skull [Franklin, K. B. J. (2013). Paxinos and Franklin's The mouse brain in stereotaxic coordinates.]. The control of the flow of the injections was made by using electronic pump at 1 μL/min for 5 min and followed by 2 min with the needle in the injection site to avoid reflux. After surgery, mice were allowed to recover from anesthesia in a temperature-controlled chamber and then placed in the individual cages.

Fluorescence tissue imaging systems in brain:(1) *ex vivo* mouse brain: we investigated P10 ability in mouse brain tissue by using the fluorescence tissue imaging system, mice were arranged by 3 groups (n=3 per group) as follows. (A) PBS-treated group; (B) **P10**-treated group; (C) Paraoxon ethyl (P.O.) and P10 treated group. After brains were isolated, the intact brain was floating on PBS containing **P10** (100 μM) for 5 h incubation at 37 °C shaking incubator. In the case of P.O.-treated group, we pre-treated the paraoxon ethyl (200 μM) to brain for 3 h at 37 °C shaking incubator and the brain then was floating on PBS containing **P10** (100 μM) for 5 h incubation at 37 °C shaking incubator. Afterward, the brain was wash with PBS three times and then was fixed by 4% PFA for 12 h at 4°C. Then, the prepared samples were imaged by TPM. Detail condition for images (Lens zoom: 1.5 x; Focus: 63; Iris: F16.0; Filter: PE, Exposure time: 0.5 sec; Intensity low - high: 1330.9 - 4344.0). (2) in vivo imaging of brain: After unilateral injection of **P10** and 5-h incubation, the FTIS images were obtained. Detail condition for images (Lens zoom: 1.0 x; Focus: 110; Iris: F2.8; Filter: PE, Exposure time: 3.0 sec; Intensity low - high: 5123.9 - 39040.0).

Supporting Figures

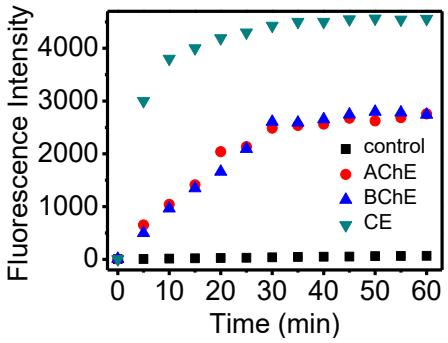


Fig. S1 Plots of fluorescence intensity vs. the reaction time of **CP** (5 μM) with CE, AChE and BChE (0.1 U/mL), respectively and control (5 μM **CP** in PBS buffer) without enzymes. The measurements were performed at 37 °C in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/582 nm. (excitation and emission slit widths were set to 5 nm and 2.5 nm, respectively).

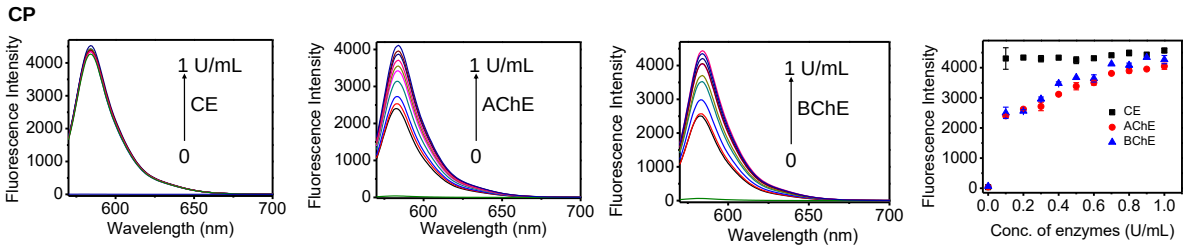


Fig. S2 Fluorescence responses of 5 μM of **CP** toward CE, BChE, and AChE at different concentrations (0-1 U/mL), respectively. The results are expressed as the mean ± SD (n = 3). The measurements were performed at 37 °C for 0.5 h in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/582 nm (excitation and emission slit widths were set to 5 nm and 2.5 nm, respectively).

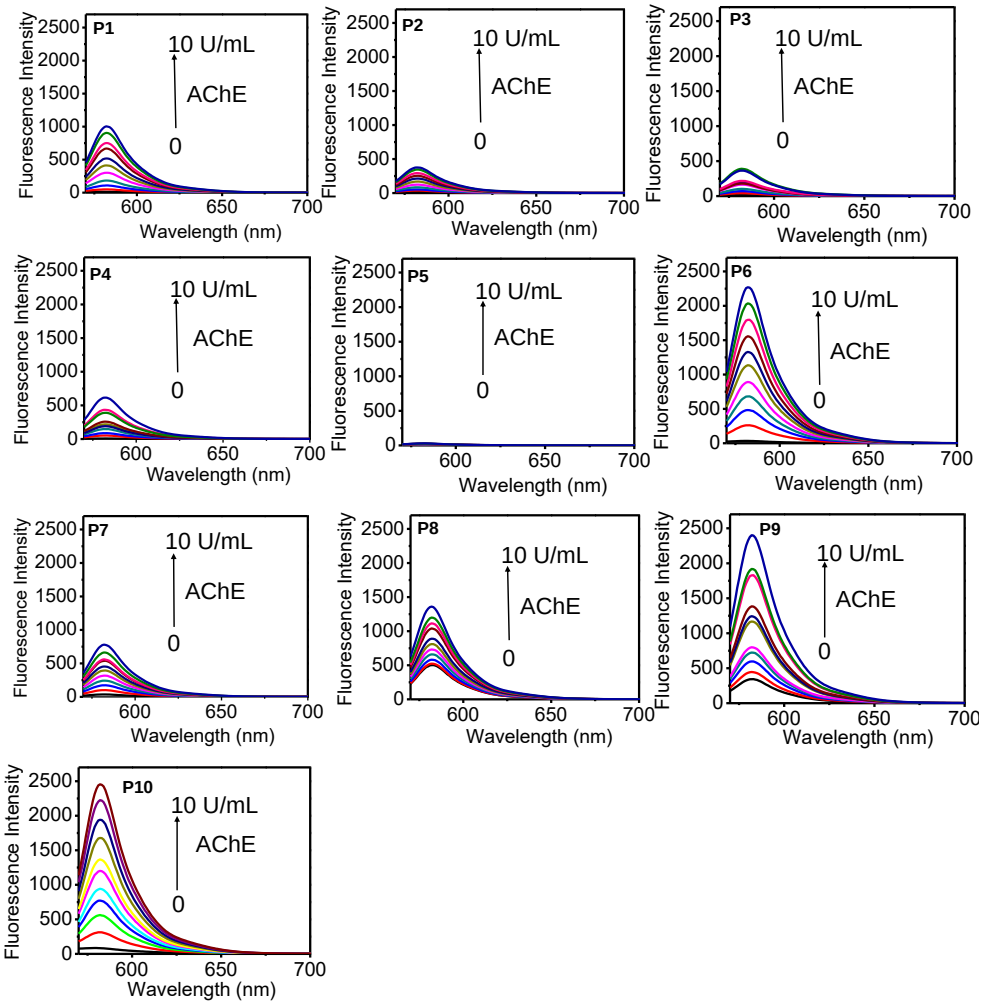
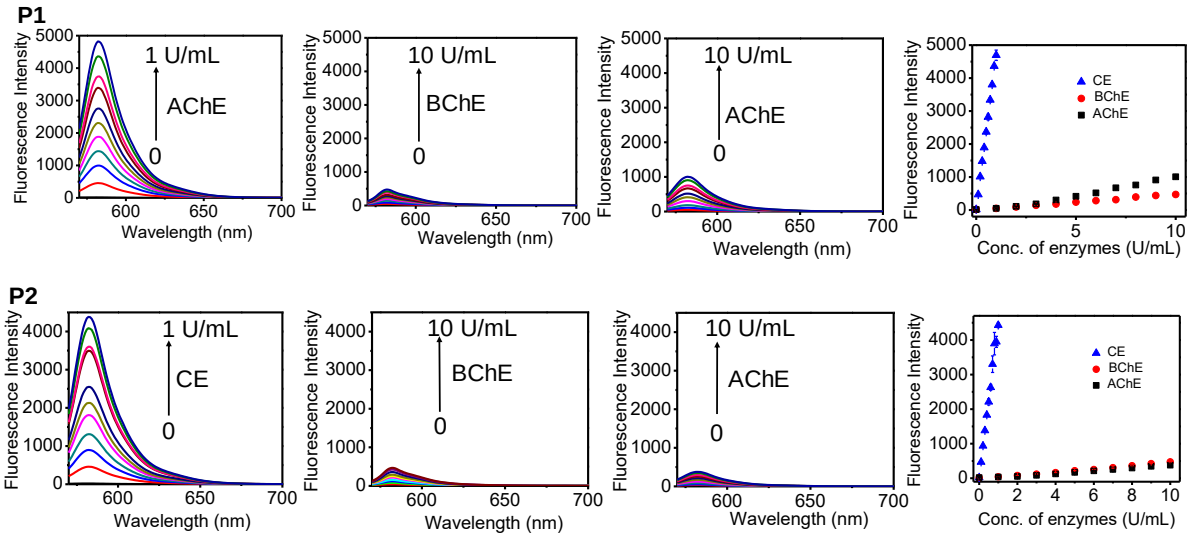
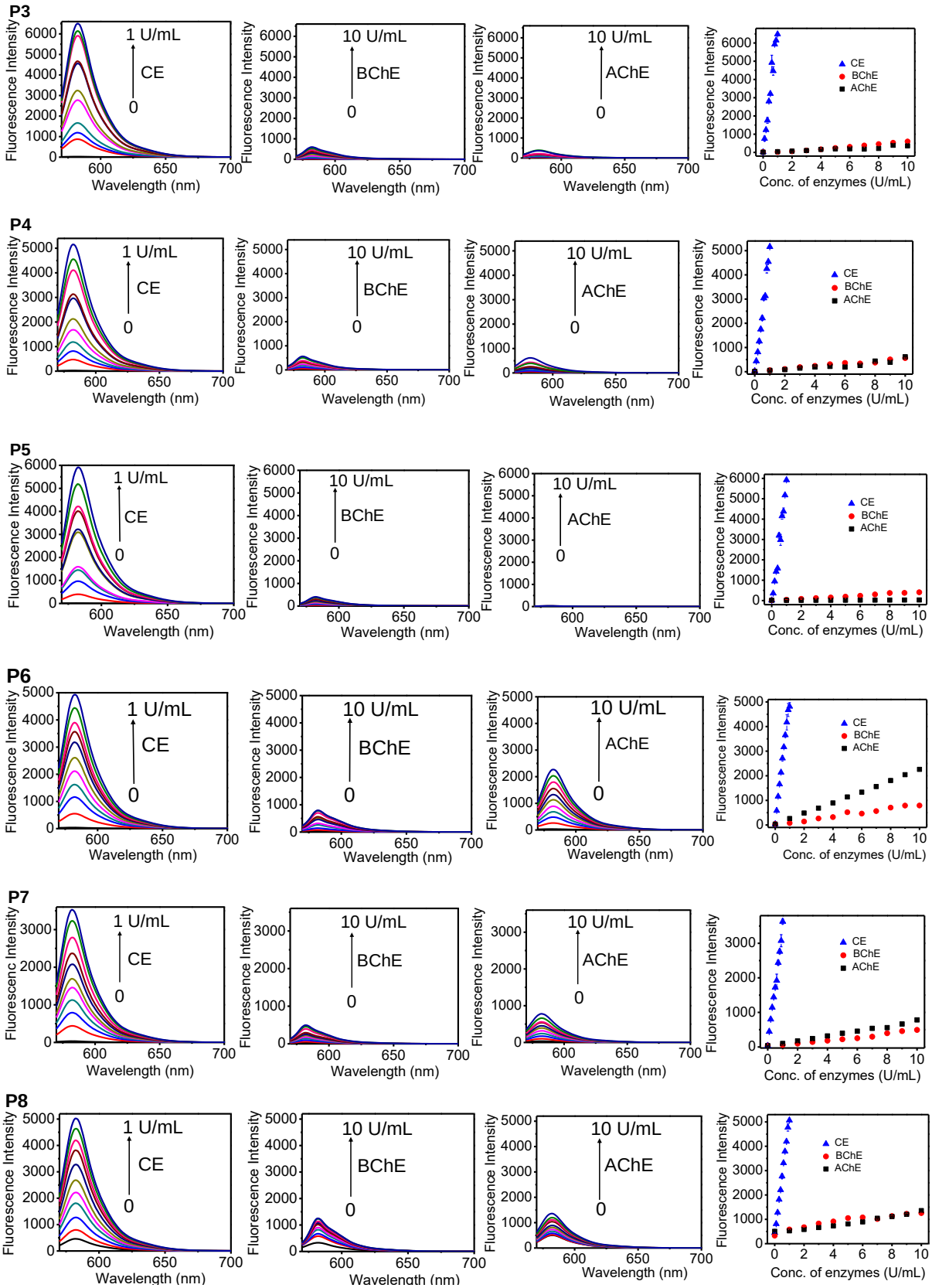


Fig. S3 Fluorescence responses of **P1-P10** (5 μM) toward AChE at different concentrations (0-10 U/mL), respectively. The results are expressed as the mean ± SD (n = 3). The measurements were performed at 37 °C for 5 h in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/582 nm.




P3
P4
P5
P6
P7
P8
1 U/mL
CE
0
10 U/mL
BChE
AChE
Fluorescence Intensity
Wavelength (nm)
Conc. of enzymes (U/mL)
CE
BChE
AChE

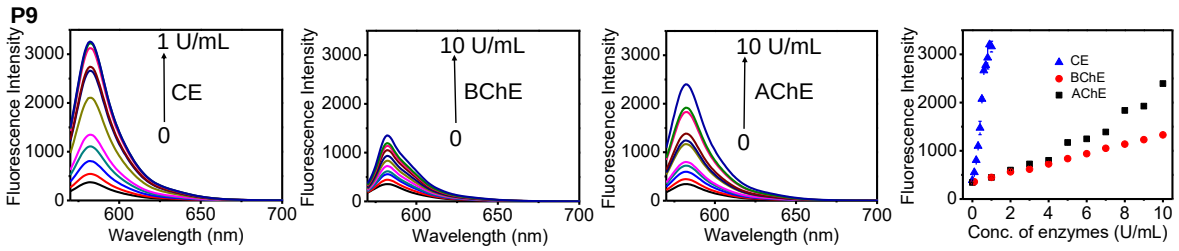


Fig. S4 Fluorescence responses of 5 μM of **P1-P9** toward CE (0-1 U/mL), BChE (0-10 U/mL), and AChE (0-10 U/mL), respectively. The results are expressed as the mean ± SD (n = 3). The measurements were performed at 37 °C for 5 h in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/582 nm.

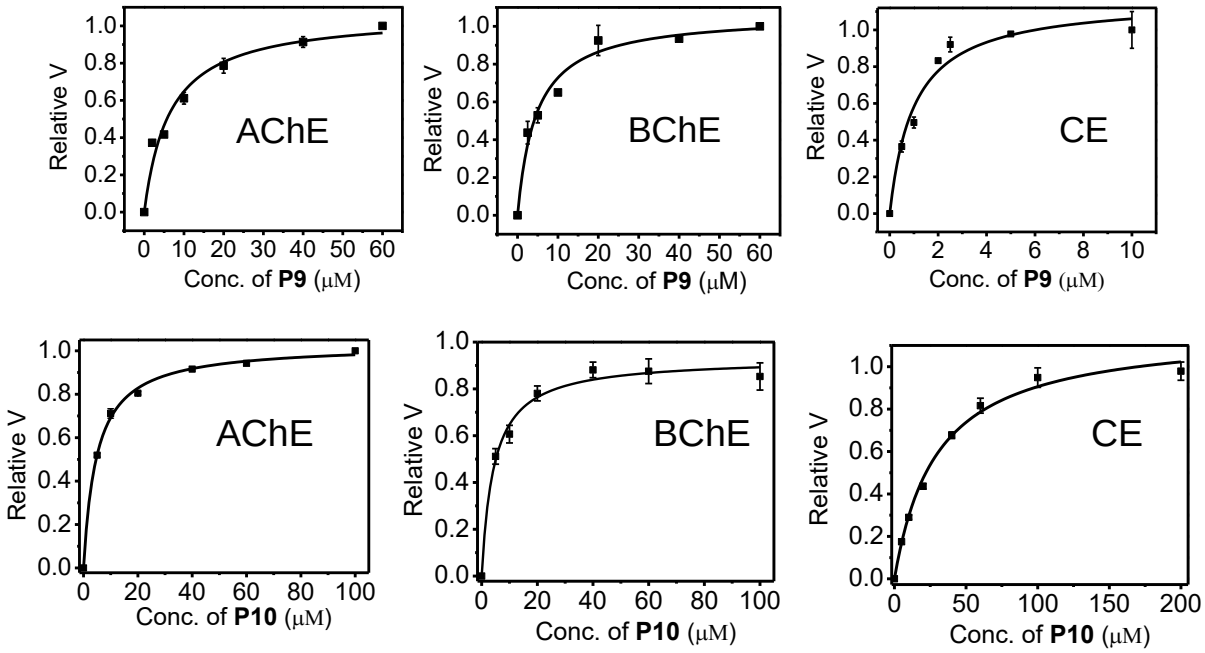


Fig. S5 Reaction kinetics of **P9** or **P10** with 5 U/mL of AChE (A), BChE (B), and CE (C). Relative reaction rate (*V*) of **P9** or **P10** in their corresponding concentration ranges. Data are expressed as the mean ± SD (n = 3). $\lambda_{ex/em}$ = 550/582 nm.

Table S1. Kinetic parameters of **P9** for AChE, BChE and CE.

enzyme	**P9**		
	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} M^{-1})
AChE	6.45±0.5	0.24±0.01	3.7×10^4
BChE	4.62±0.97	0.49±0.03	1.1×10^5
CE	1.01±0.26	6.0±0.008	5.9×10^6

Table S2. Kinetic parameters of **P10** for AChE, BChE and CE.

enzyme	P10		
	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}M^{-1}$)
AChE	4.87±0.35	0.26±0.004	5.3×10^4
BChE	4.37±0.72	0.1±0.003	2.3×10^4
CE	29.57±3.48	0.2±0.008	6.8×10^3

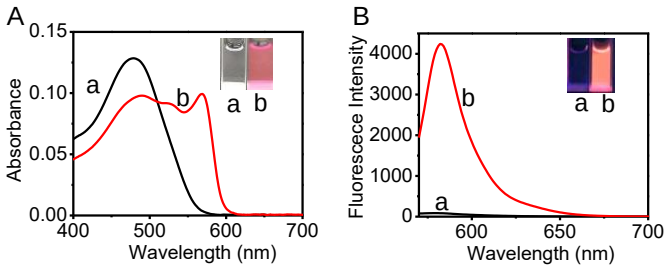


Fig. S6 Absorption and fluorescence spectra of **P10** (5 μM) before (a) and after reaction (b) with 20 U/mL of AChE. The inset shows the color change of the reactions. The measurements were performed at 37 °C for 5 h in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/582 nm.

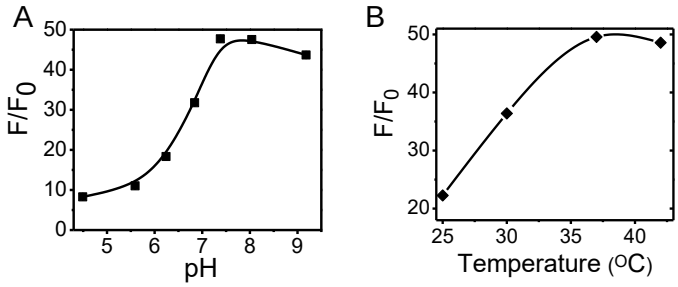


Fig. S7 Effects of (A) pH and (B) temperature on the fluorescence intensity of **P10** (5 μM) with and without AChE (20 U/mL). The results are expressed as the mean ± SD (n = 3). The measurements were performed at 37 °C for 5 h in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/582 nm.

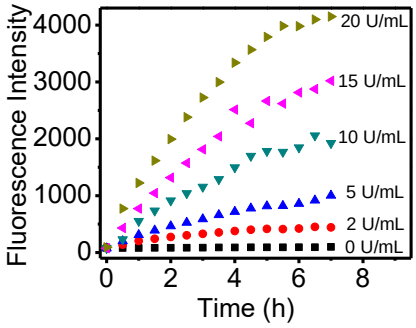


Fig. S8 Plots of fluorescence intensity vs. the reaction time of **P10** (5 μM) with varied concentrations of AChE (0-20 U/mL). The measurements were performed at 37 °C in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/582 nm.

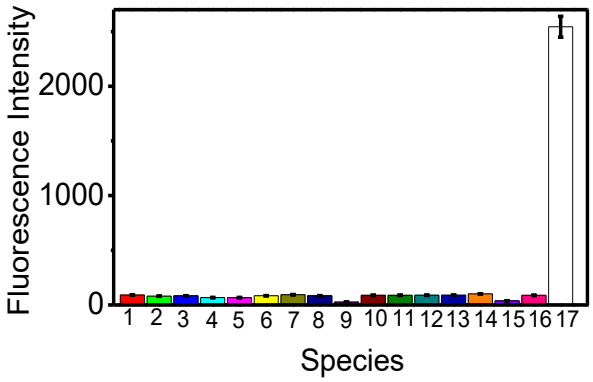


Fig. S9 Fluorescence responses of P**10** (5 μM) to various species: (1) blank; (2) KCl (150 mM); (3) $MgCl_2$ (2.5 mM); (4) $CaCl_2$ (2.5 mM); (5) $ZnCl_2$ (100 μM); (6) glucose (10 mM); (7) vitamin C (1 mM); (8) tyrosine (1 mM); (9) cysteine (1 mM); (10) glycine (1 mM); (11) glutamic acid (1 mM); (12) Argnine (1mM); (13) Alanine (1 mM); (14) lysine (1 mM); (15) glutathione (1 mM); (16) urea (20 mM); (17) AChE (10 U/mL). The results are expressed as the mean ± SD (n = 3). The measurements were performed at 37 °C for 5 h in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/582 nm.

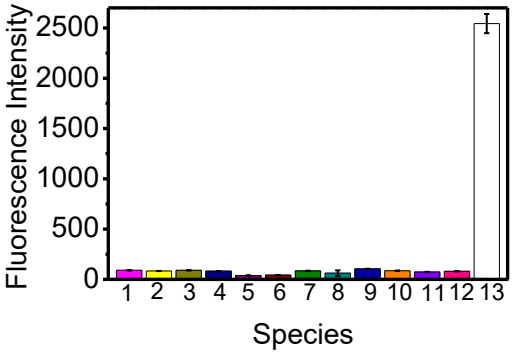


Fig. S10 Fluorescence responses of **P10** (5 μM) to various species: (1) blank; (2) H_2O_2 (10 μM); (3) ClO^- (10 μM); (4) TBHP (10 μM); (5) •OH (10 μM); (6) TBO• (10 μM); (7) $O2^{\bullet-}$ (10 μM); (8) $^1O_2$ (10 μM); (9) $ONOO^-$ (10 μM); (10) NO_2^- (10 μM); (11) CO (10 μM); (12) NO (10 μM); (13) AChE (10 U/mL). The results are expressed as the mean ± SD (n = 3). The measurements were performed at 37 °C for 5 h in PBS (pH 7.4) with $\lambda_{ex/em}$ = 550/582 nm.

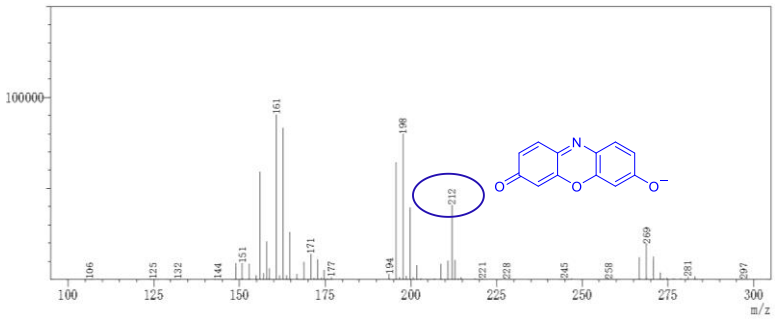


Fig. S11 ESI mass spectra of the reaction solution of **P10** with AChE. The peak at *m/z* = 212 reflects the generation of resorufin.

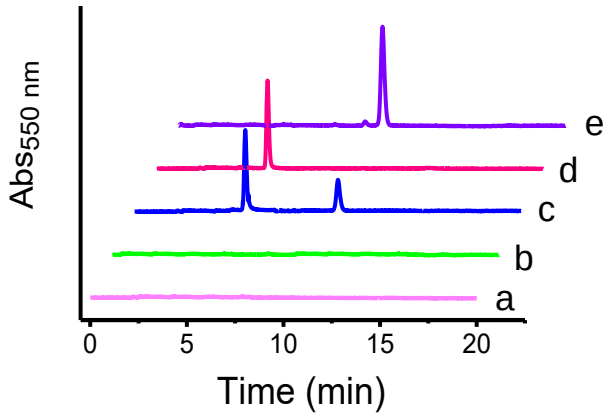


Fig. S12 HPLC kinetic profiles of different reaction systems. (a) Blank; (b) AChE solution; (c) the reaction solutions of **P10** (5 μM) with AChE (50 U/mL); (d) 5 μM **P10** (t_R = 5.73 min); (e) 5 μM resorufin (t_R = 10.52 min). Absorbance detection at 550 nm with methanol (flow rate, 0.55 mL/min) and water (flow rate, 0.45 mL/min) as eluents.

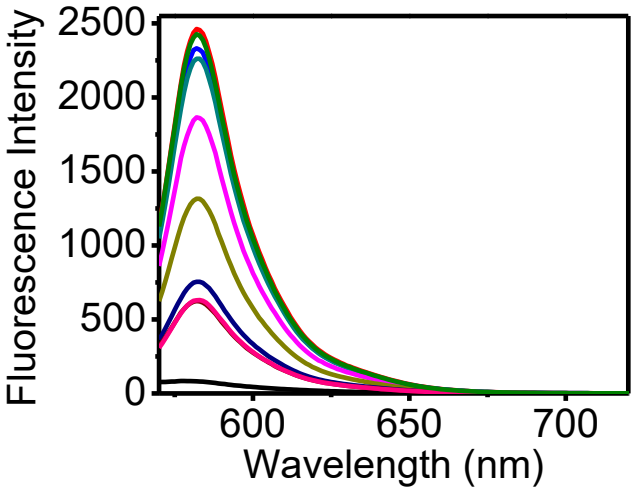


Fig. S13 Inhibitory activity of **P10** against paraoxon ethyl (10^{-9}-10^{-3} M). $\lambda_{ex/em}$ = 550/582 nm.

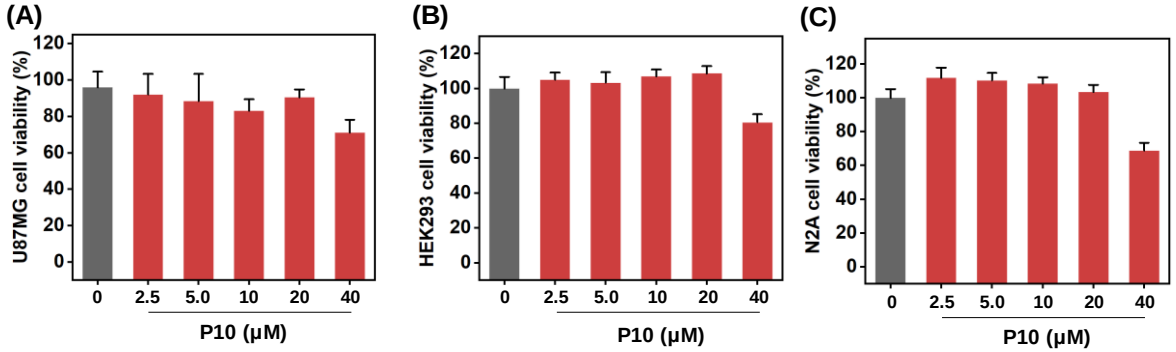


Fig. S14 The various cell viability for **P10** (0-40 μM) toward different cell lines. (A) U87MG cells, (B) HEK293 cells and (C) N2A cells. Each error bar represents mean ± SD.

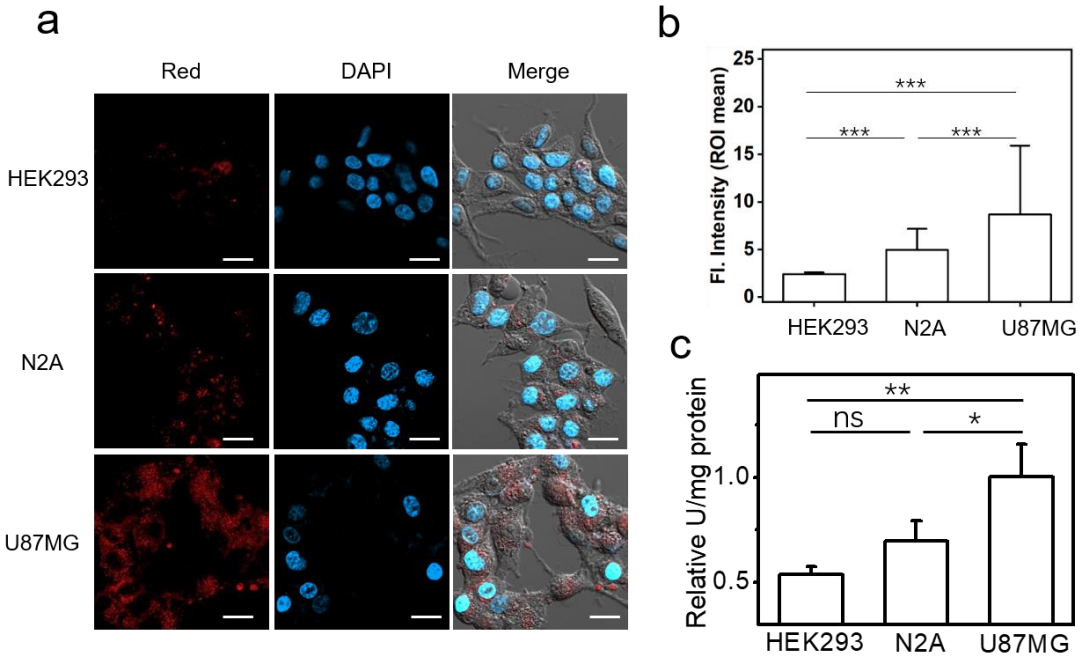


Fig. S15 Fluorescence images of HEK293 cells, N2A cells and U87MG cells after being treated with **P10** (10 μM) for 5 h at 37 °C. (a) The images of the first column and the second column were collected in the range of 571-700 nm for **P10** (ex. at 561 nm) and 415-550 nm for DAPI (ex. at 405 nm), respectively. The third column displays merged images. (b) Fluorescence intensity in each cell. (c) The activity of AChE in three cell lysates tested based on Ellman method. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Each error bar represents mean ± SD., vs HEK293, vs N2A, vs U87MG. (***$p < 0.001$). Intensity plotting was performed on Image J. Scale bar: 50 μm.

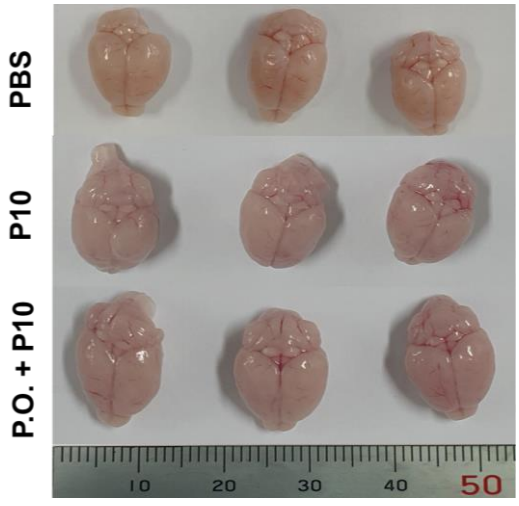


Fig. S16 The representative images of mice brain with naked eyes under different conditions. The number of mice for each group is 3 (n = 3).

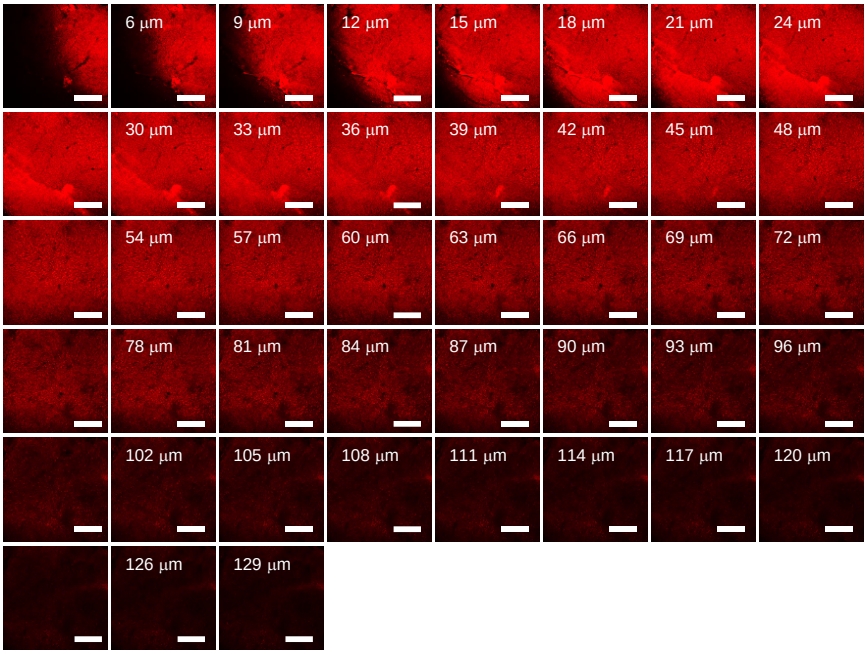


Fig. S17 Tissue images of lambda tissue derived from normal mouse brain at 3-129 μm depths by each interval of 3 μm as a step. Tissue after treatment of **P10** (100 μM) for 5 h at 37 ^{o}C. Scale bar: 200 μm. Two-photon mode: λ_{ex} = 1000 nm, λ_{em} = 565-675 nm.

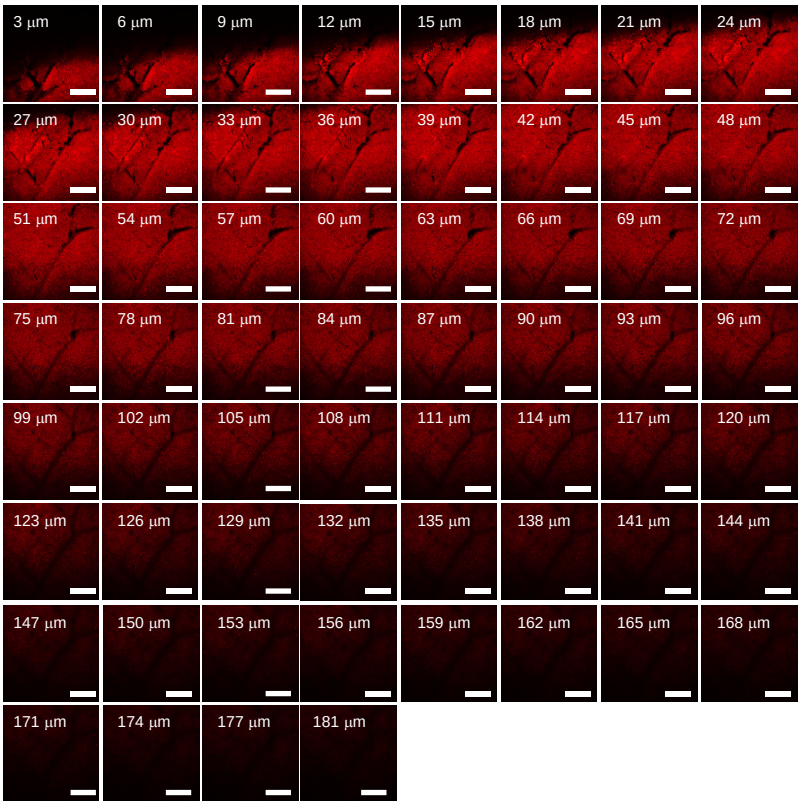


Fig. S18 Tissue images of left cerebral cortex colliculus derived from normal mouse brain at 3-181 μm depths by each interval of 3 μm as a step. Tissue after treatment of **P10** (100 μM) for 5 h at 37 ^{o}C. Scale bar: 200 μm. Two-photon mode: λ_{ex} = 1000 nm, λ_{em} = 565-675 nm.

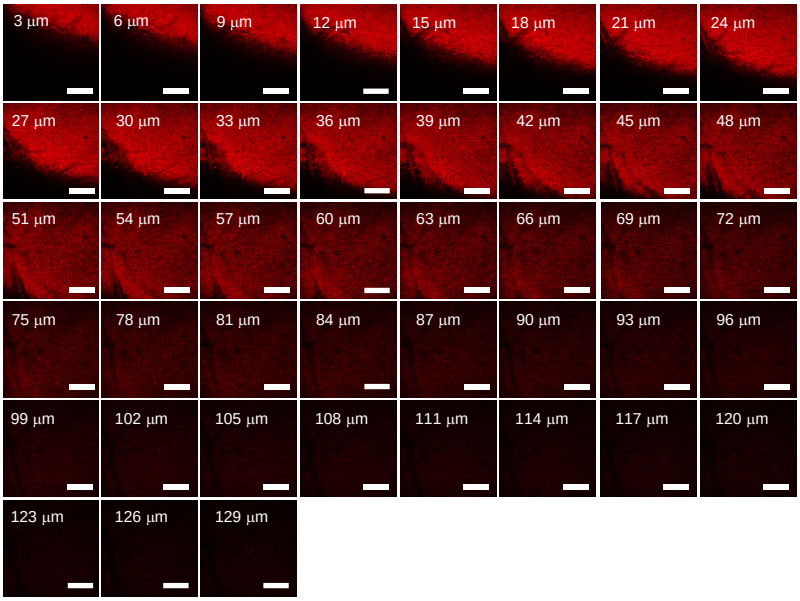


Fig. S19 Tissue images of right cerebral cortex colliculus derived from normal mouse brain at 3-129 μm depths by each interval of 3 μm as a step. Tissue after treatment of **P10** (100 μM) for 5 h at 37 °C. Scale bar: 200 μm. Two-photon mode: λ_{ex} = 1000 nm, λ_{em} = 565-675 nm.

Fig. S20 Tissue images of middle cortex derived from normal mouse brain at 3-241 μm depths by each interval of 3 μm as a step. Tissue after treatment of **P10** (100 μM) for 5 h at 37 °C. Scale bar: 200 μm. Two-photon mode: λ_{ex} = 1000 nm, λ_{em} = 565-675 nm.

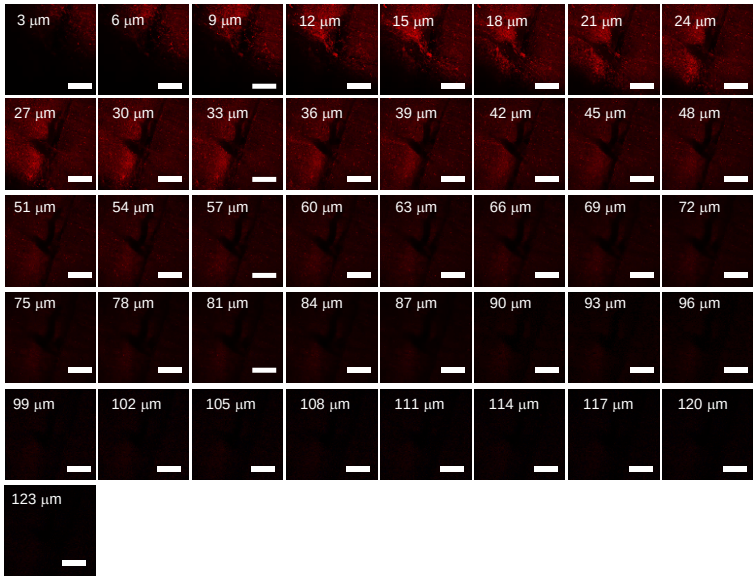


Fig. S21 Tissue images of inhibitor-treated lambda derived from normal mouse brain at 3-123 μm depths by each interval of 3 μm as a step. Tissue after treatment of **P10** (100 μM) for 5 h at 37 ºC. Scale bar: 200 μm. Two-photon mode: λ_{ex} = 1000 nm, λ_{em} = 565-675 nm.

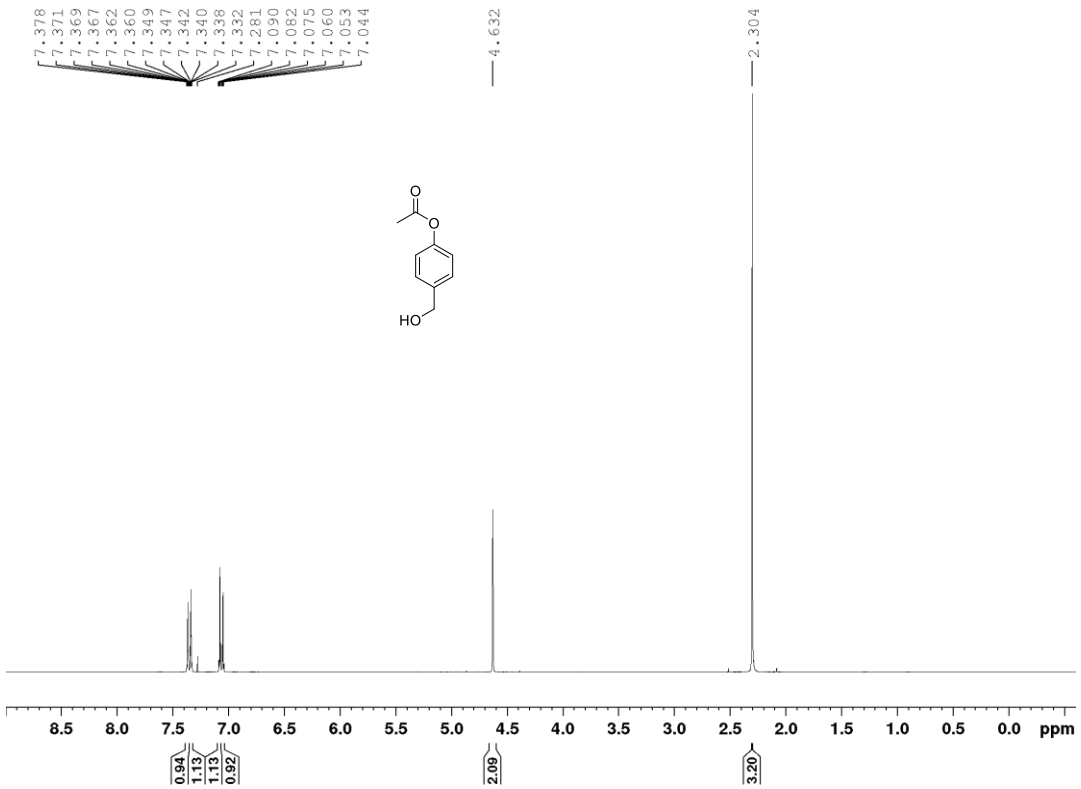


Fig. S22 ^{1}H-NMR spectrum of **CP-1** (300 MHz, 298 K, $CDCl_3$).

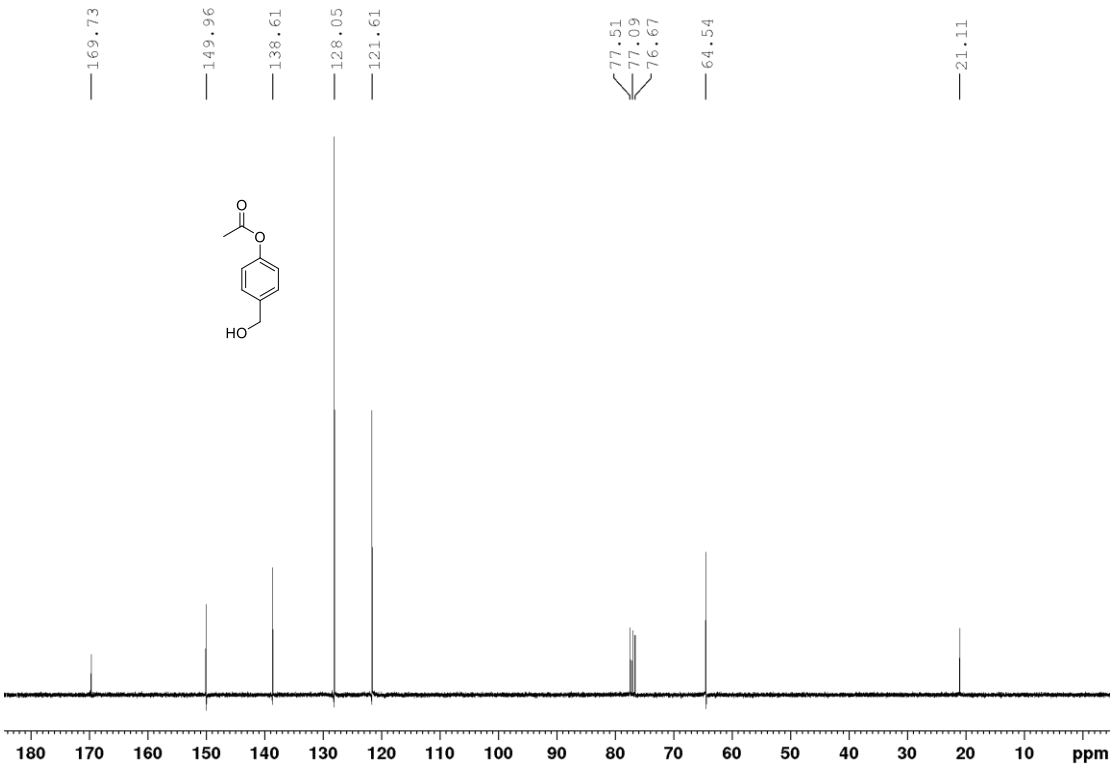


Fig. S23 ^{13}C-NMR spectrum of **CP-1** (75 MHz, 298 K, $CDCl_3$).

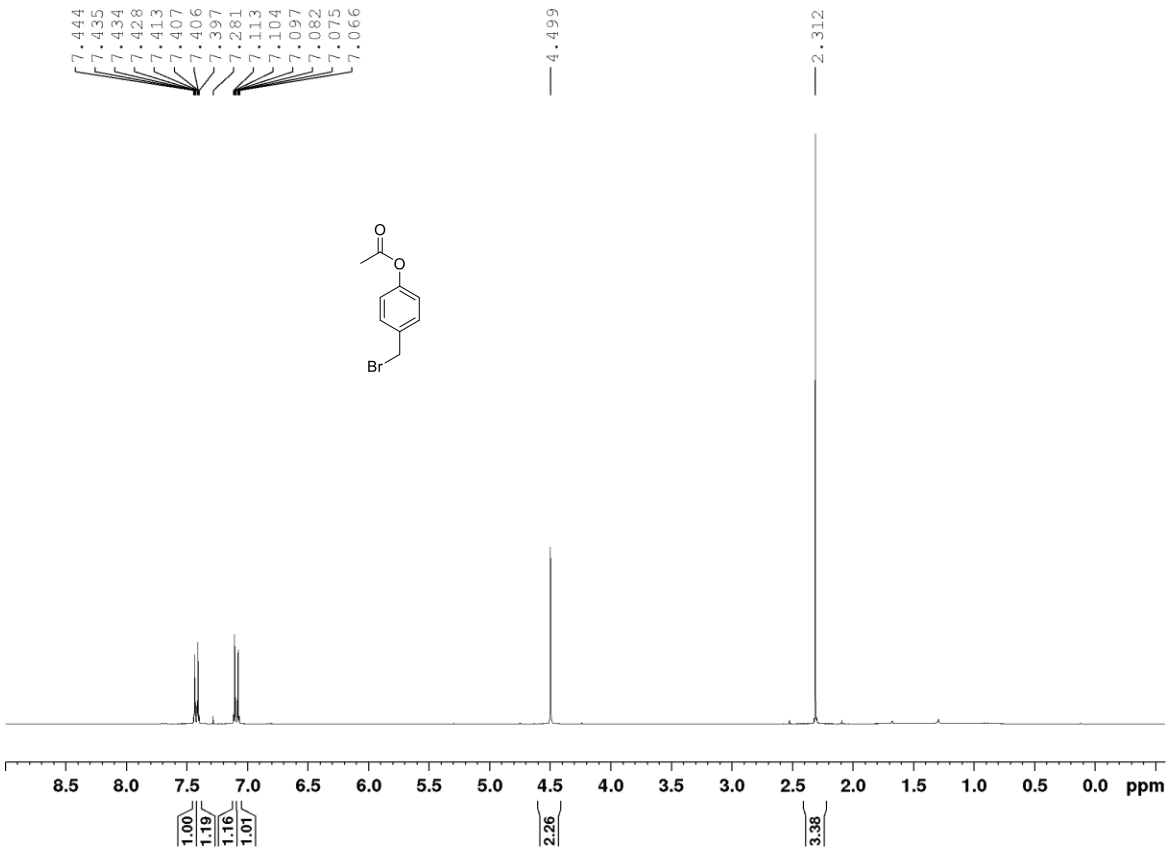


Fig. S24 ^{1}H-NMR spectrum of **CP-2** (300 MHz, 298 K, $CDCl_3$).

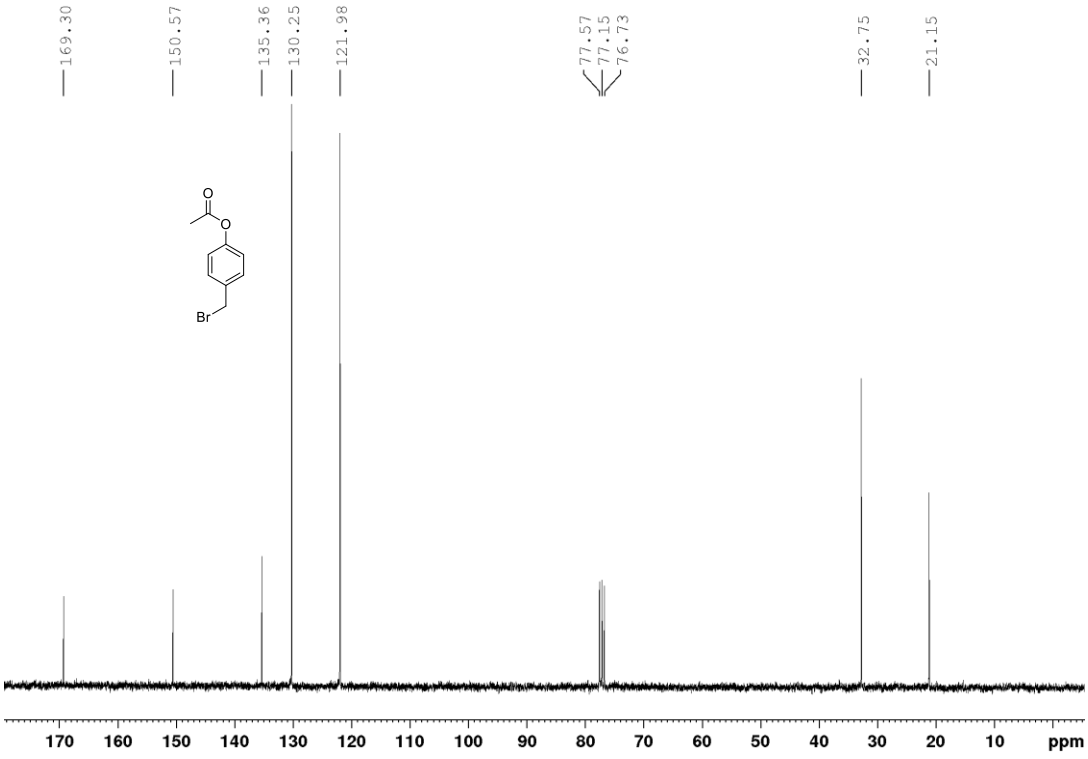


Fig. S25 ^{13}C-NMR spectrum of **CP-2** (75 MHz, 298 K, $CDCl_3$).

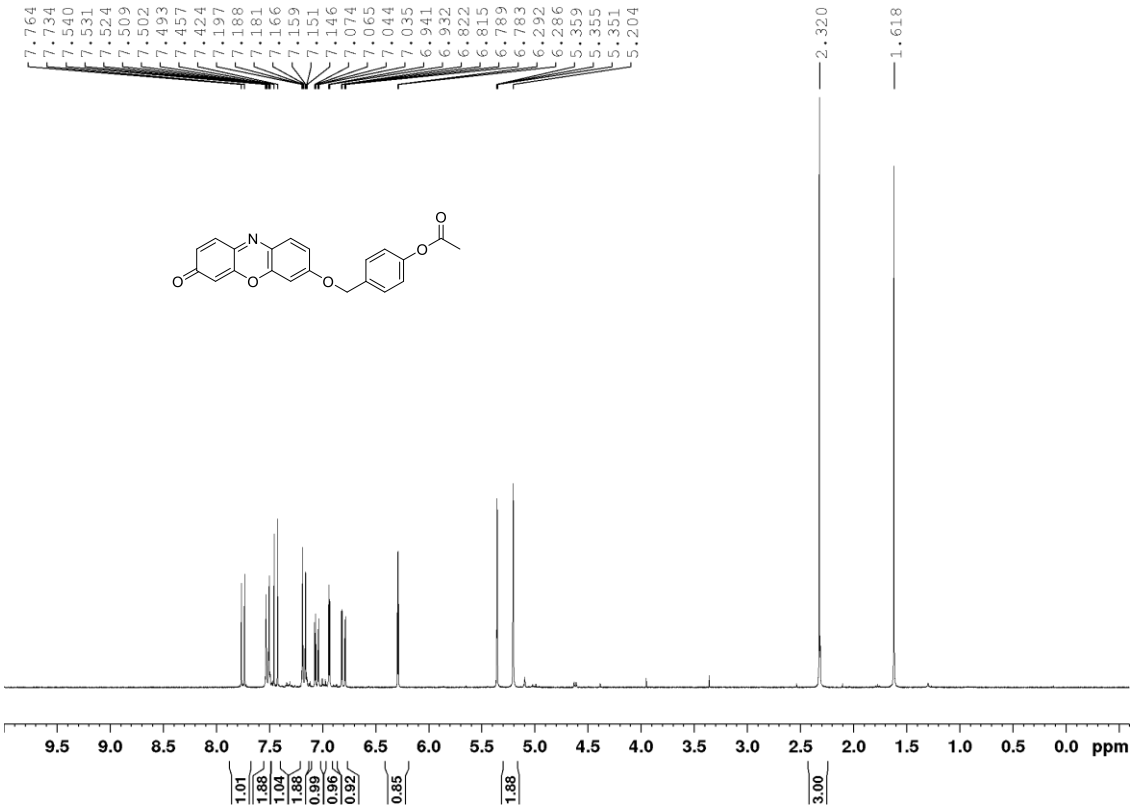


Fig. S26 ^{1}H-NMR spectrum of **CP** (300 MHz, 298 K, CD_2Cl_2-d_2).

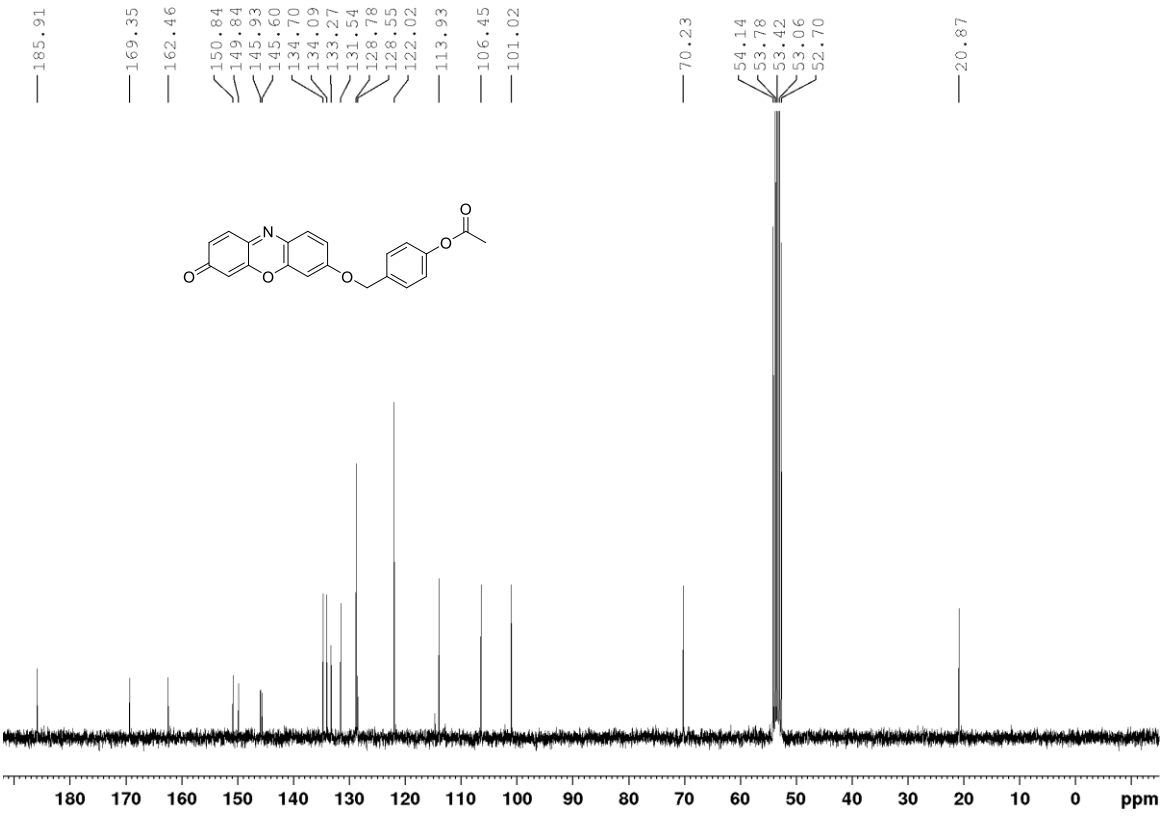


Fig. S27 ^{13}C-NMR spectrum of **CP** (75 MHz, 298 K, CD_2Cl_2-d_2).

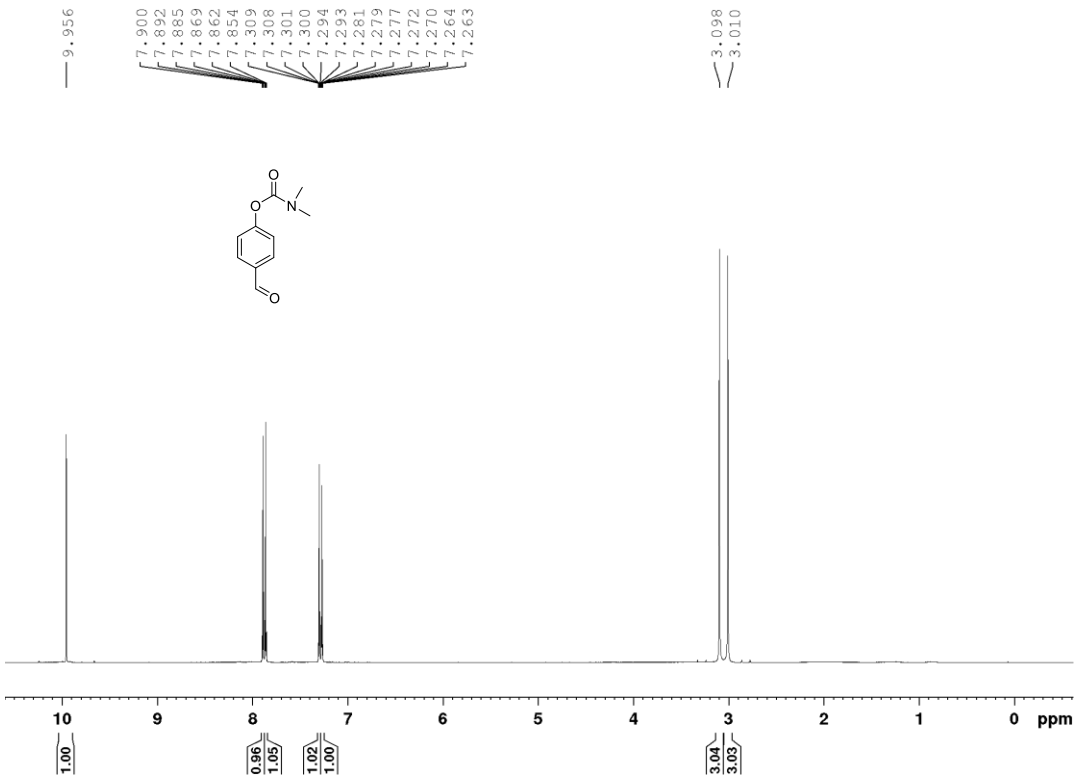


Fig. S28 ^{1}H-NMR spectrum of **1a** (300 MHz, 298 K, $CDCl_3$).

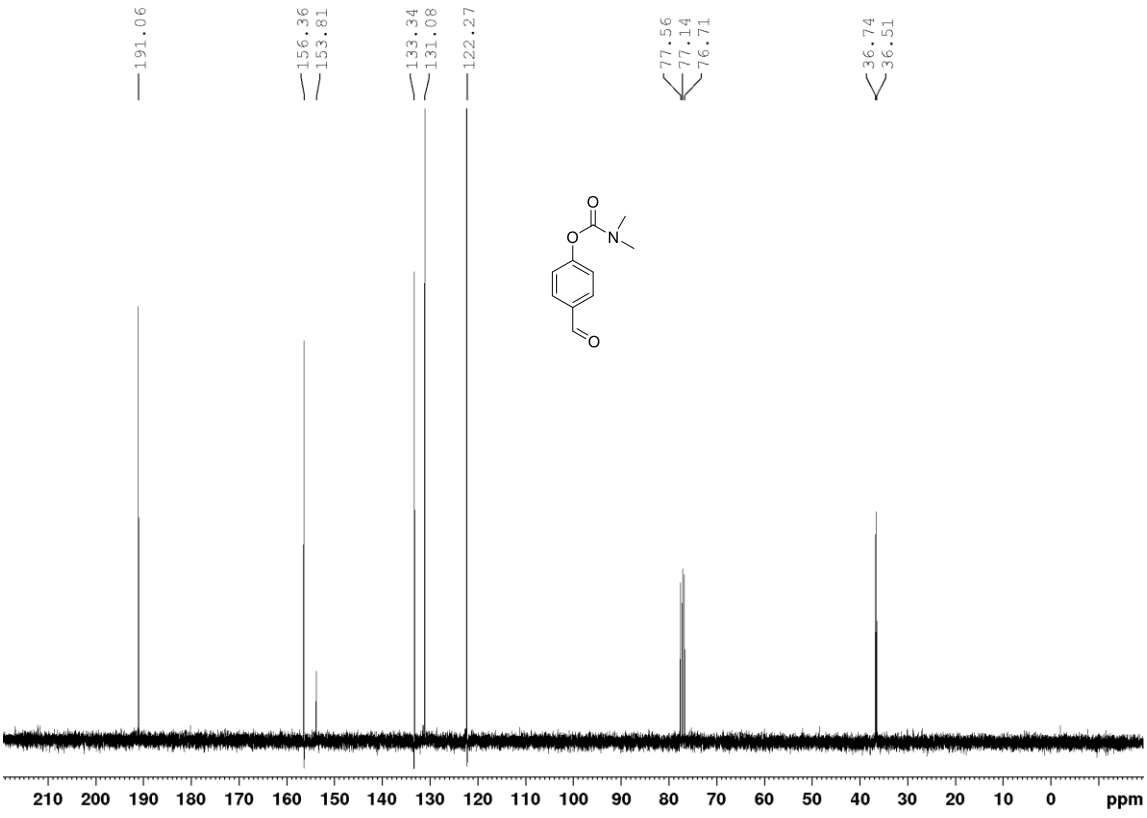


Fig. S29 ^{13}C-NMR spectrum of **1a** (75 MHz, 298 K, $CDCl_3$).

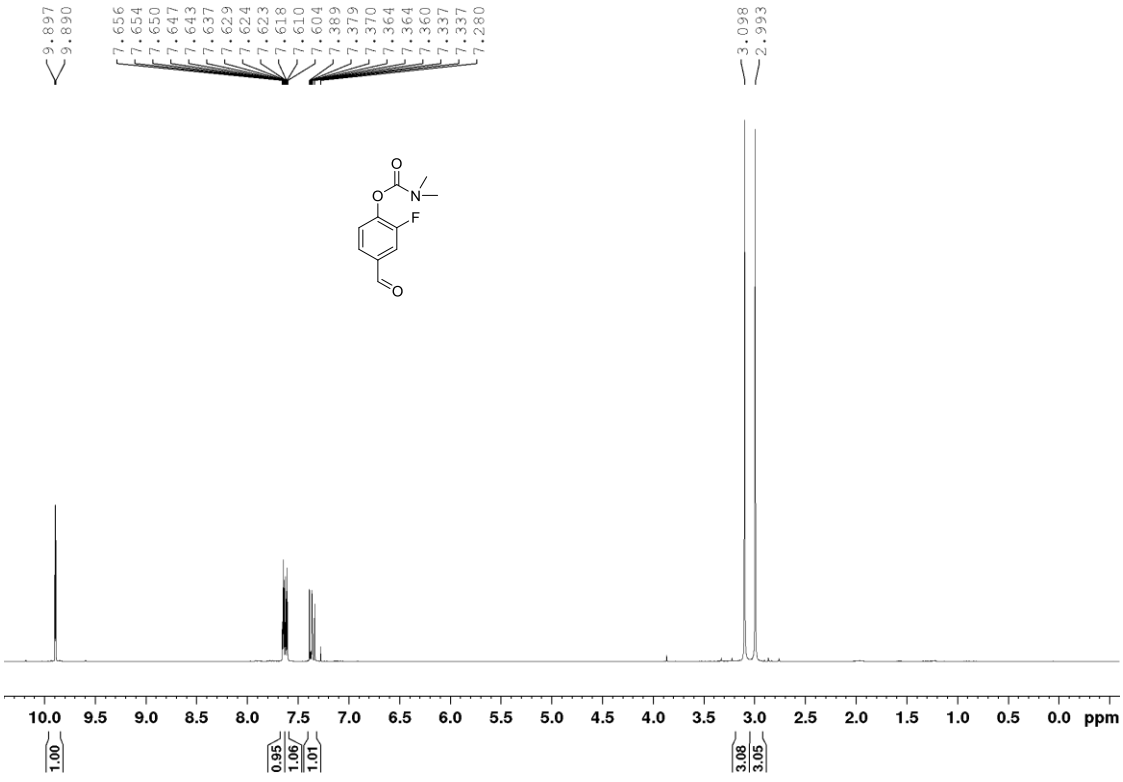


Fig. S30 ^{1}H-NMR spectrum of **1b** (300 MHz, 298 K, $CDCl_3$).

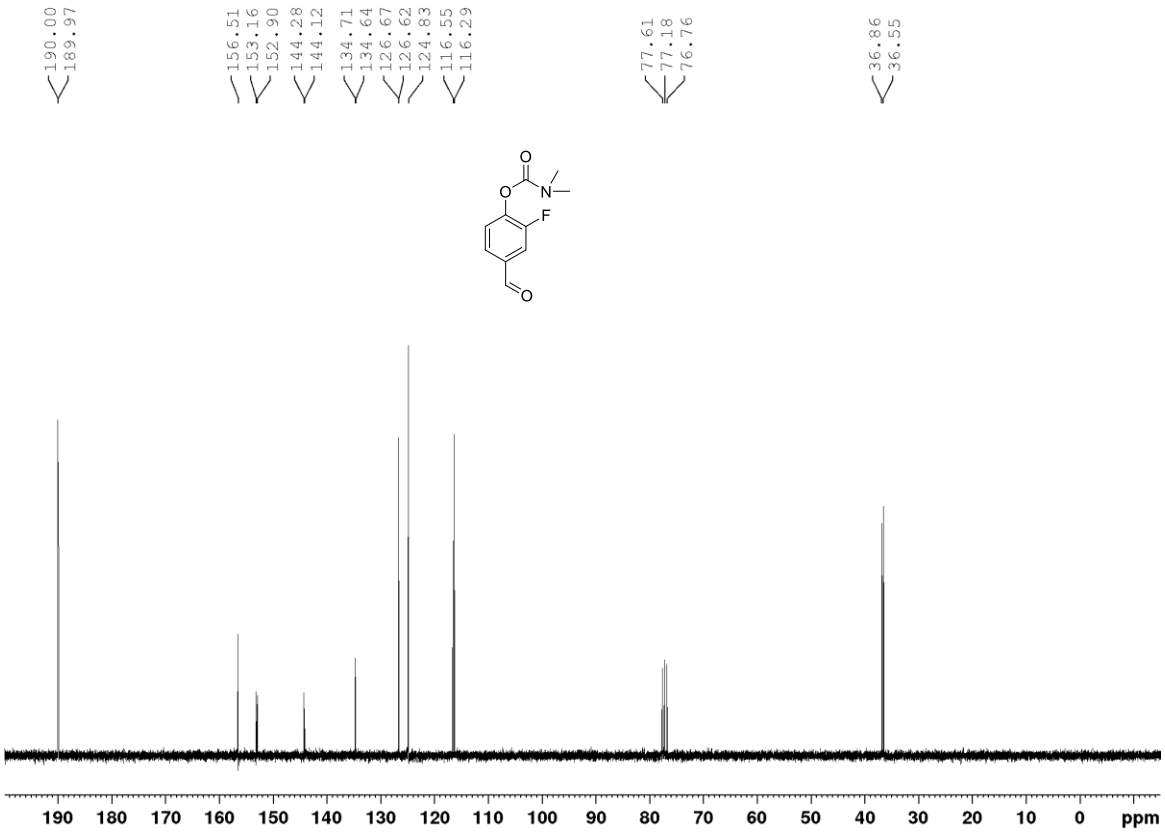


Fig. S31 ^{13}C-NMR spectrum of **1b** (75 MHz, 298 K, $CDCl_3$).

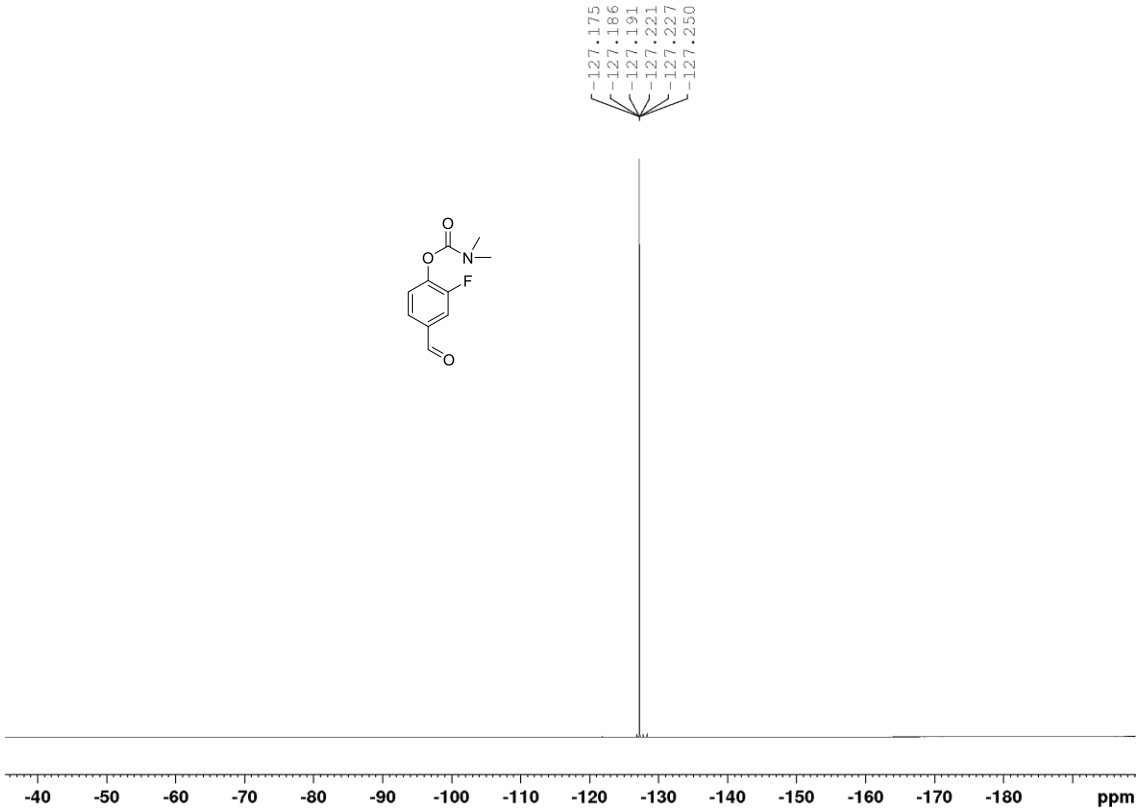


Fig. S32 ^{19}F-NMR spectrum of **1b** (300 MHz, 298 K, $CDCl_3$).

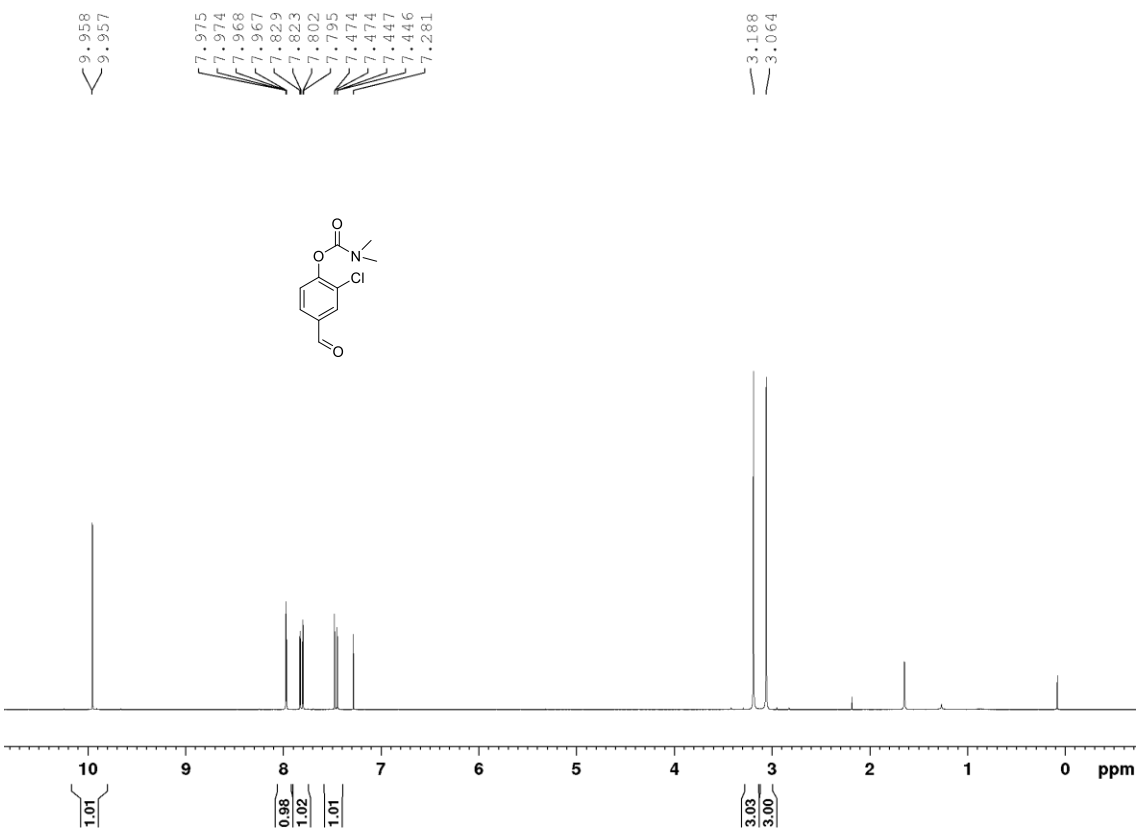


Fig. S33 ^{1}H-NMR spectrum of **1c** (300 MHz, 298 K, $CDCl_3$).

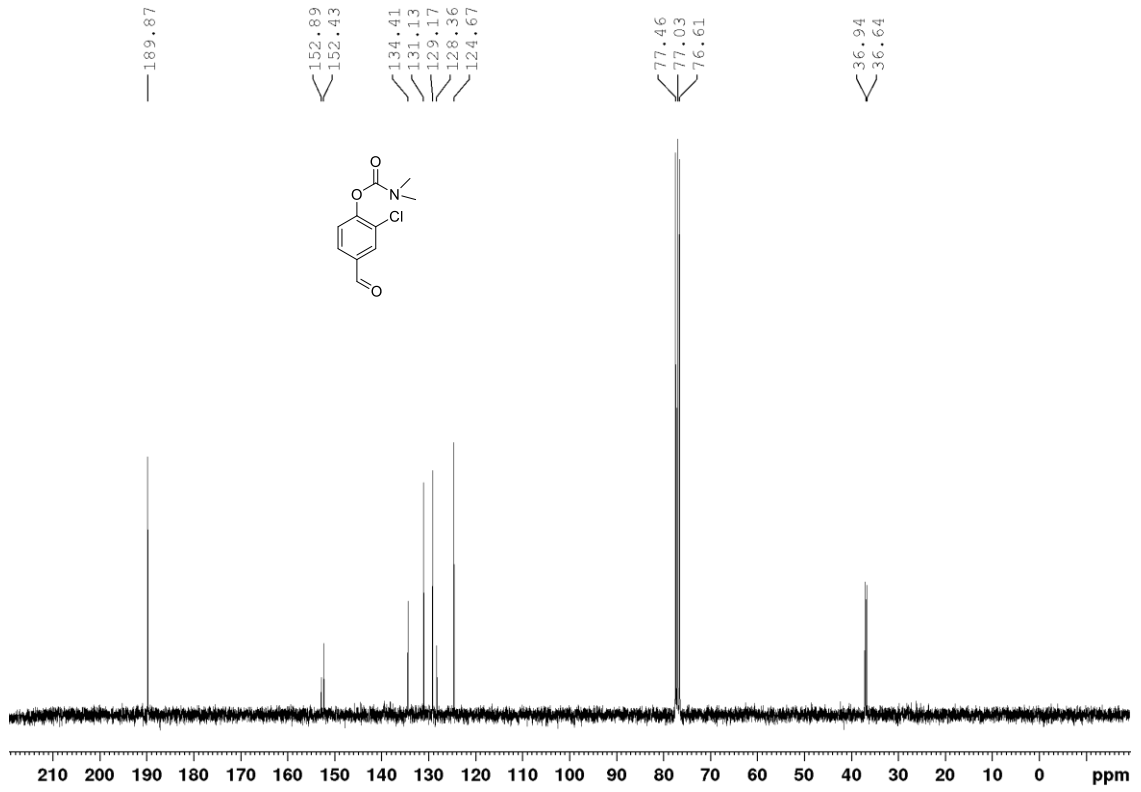


Fig. S34 ^{13}C-NMR spectrum of **1c** (75 MHz, 298 K, $CDCl_3$).

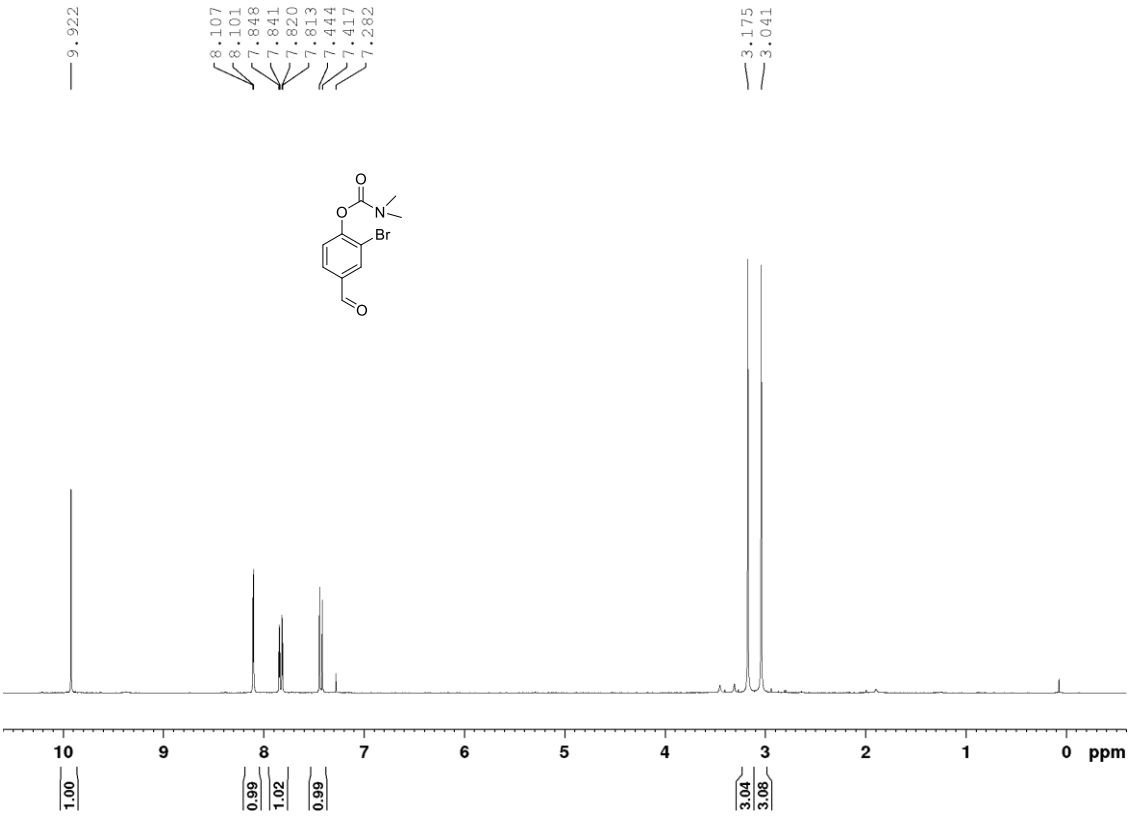


Fig. S35. ^{1}H-NMR spectrum of **1d** (300 MHz, 298 K, $CDCl_3$).

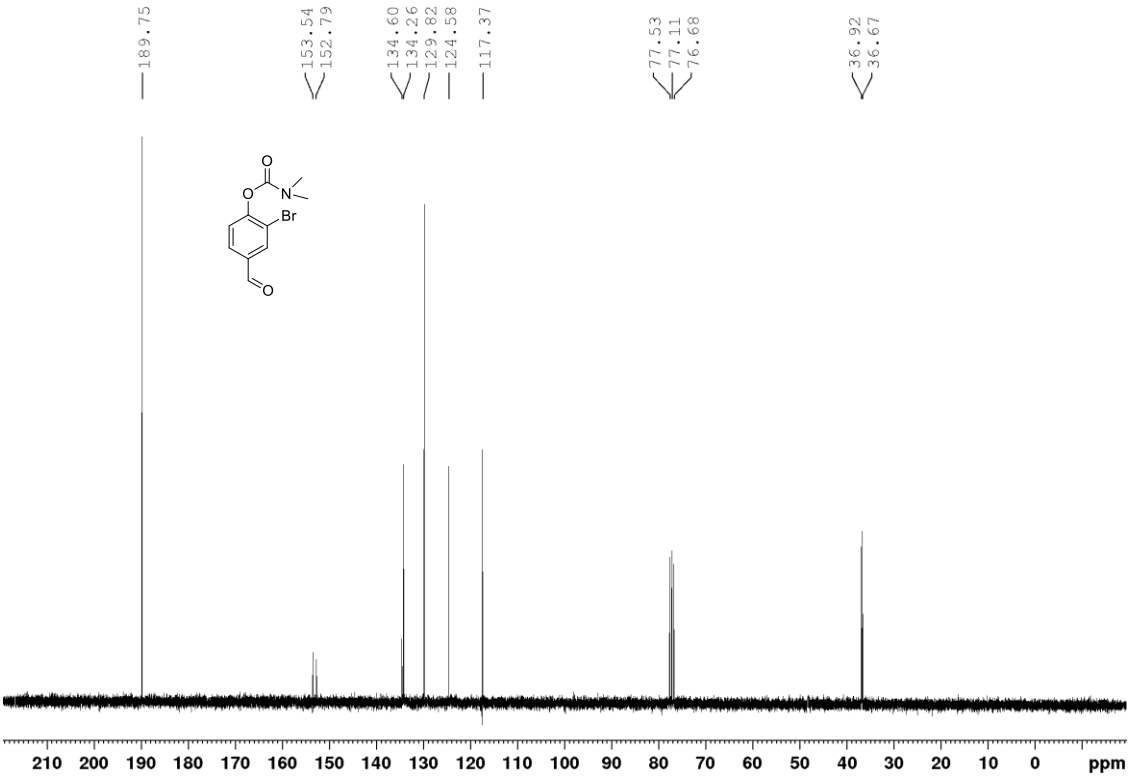


Fig. S36. ^{13}C-NMR spectrum of **1d** (75 MHz, 298 K, $CDCl_3$).

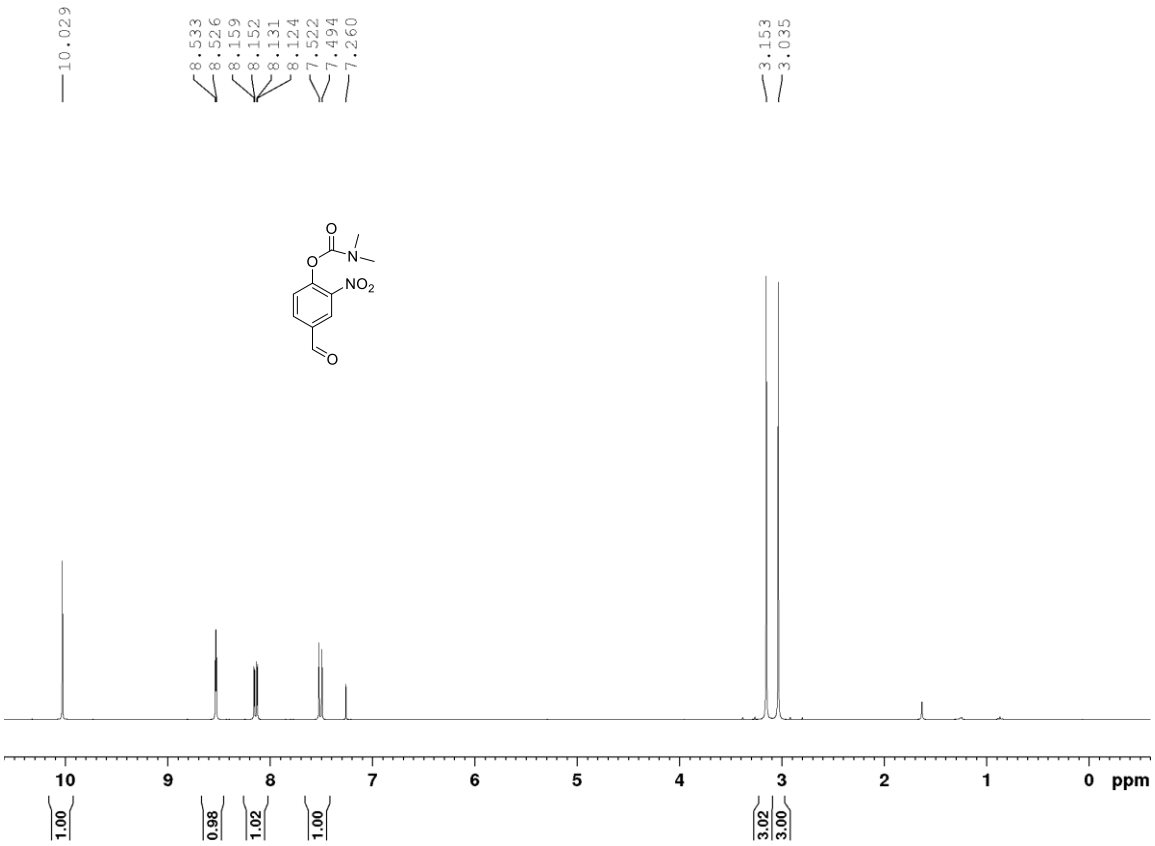


Fig. S37. ^{1}H-NMR spectrum of **1e** (300 MHz, 298 K, $CDCl_3$).

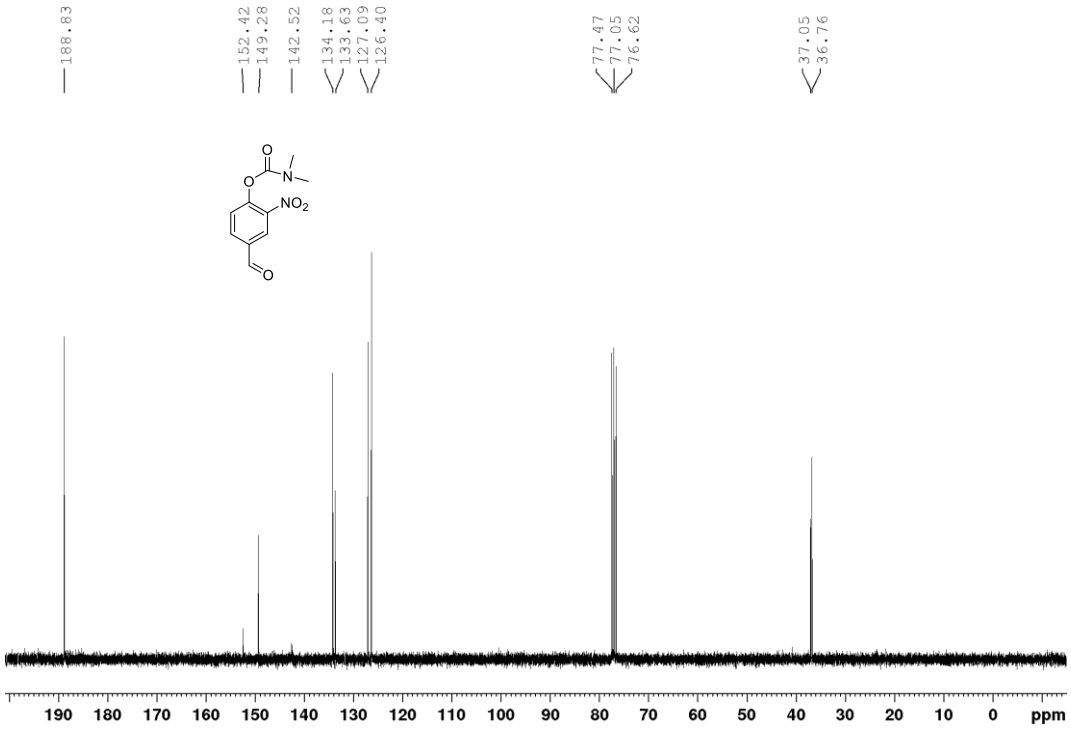


Fig. S38 ^{13}C-NMR spectrum of **1e** (75 MHz, 298 K, $CDCl_3$).

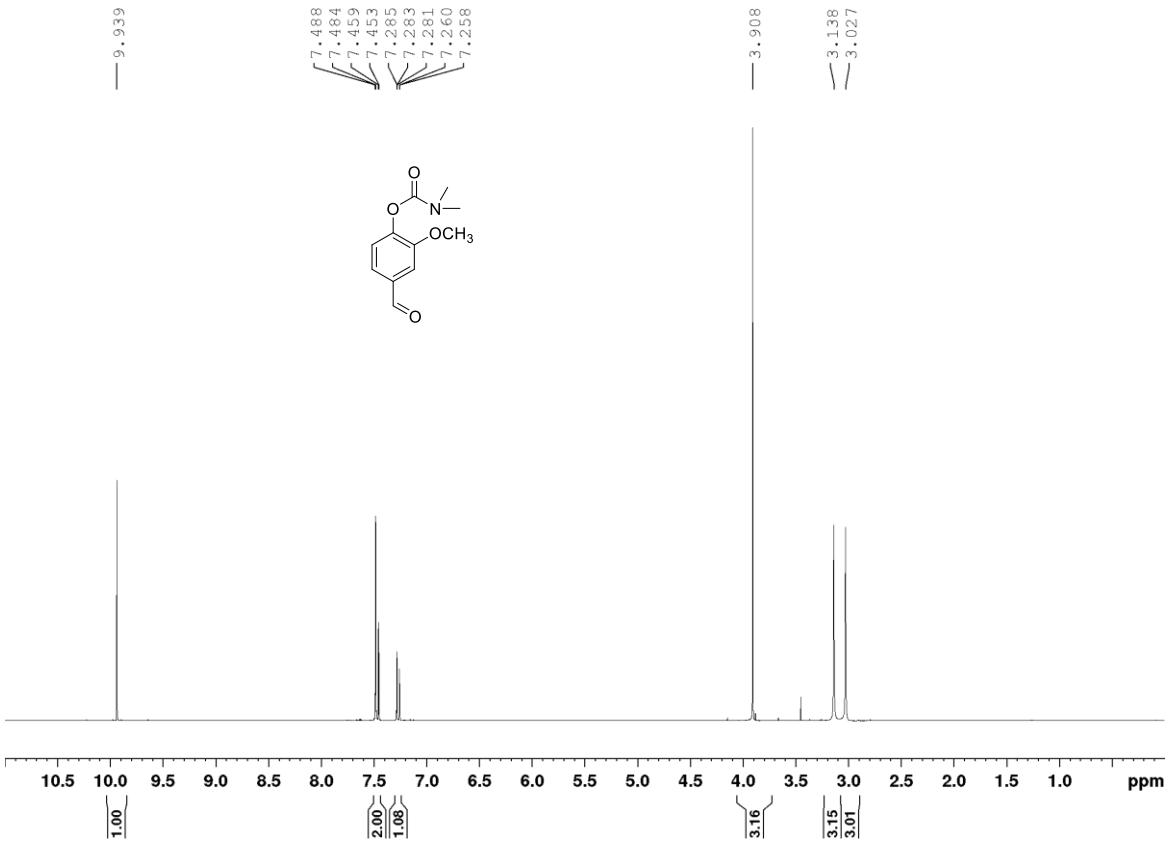


Fig. S39 ^{1}H-NMR spectrum of **1f** (300 MHz, 298 K, $CDCl_3$).

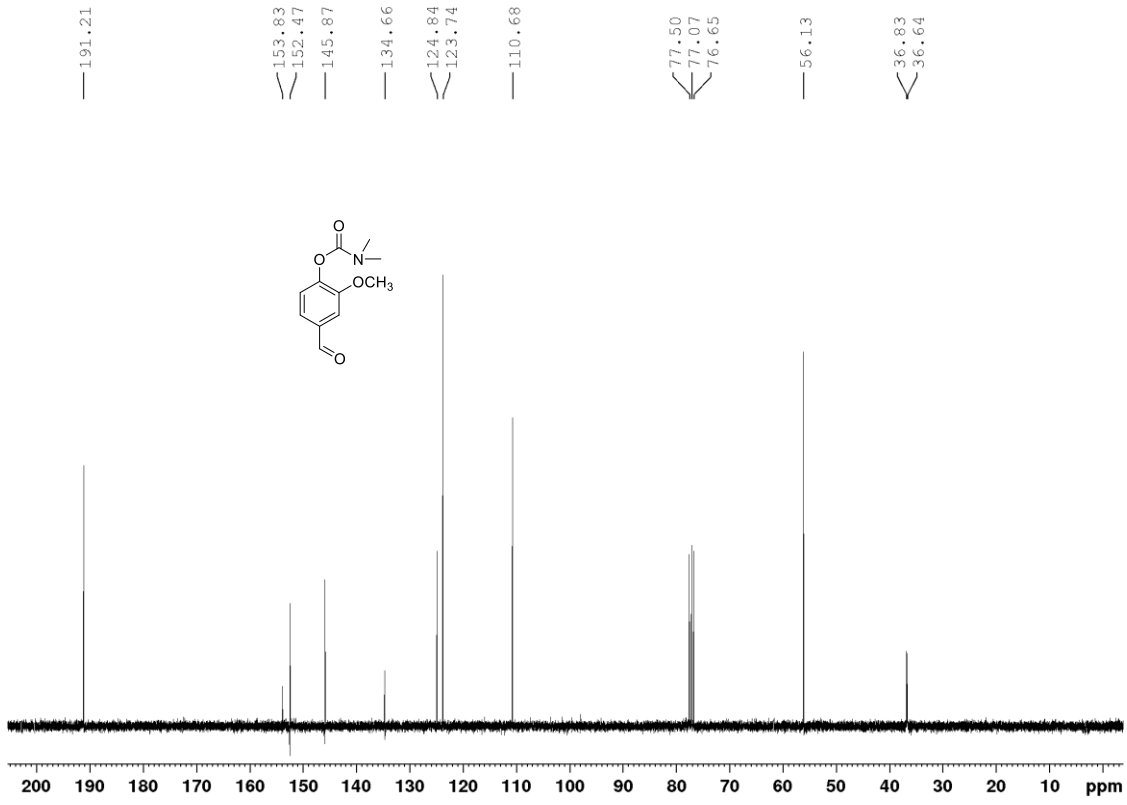


Fig. S40 ^{13}C-NMR spectrum of **1f** (75 MHz, 298 K, $CDCl_3$).

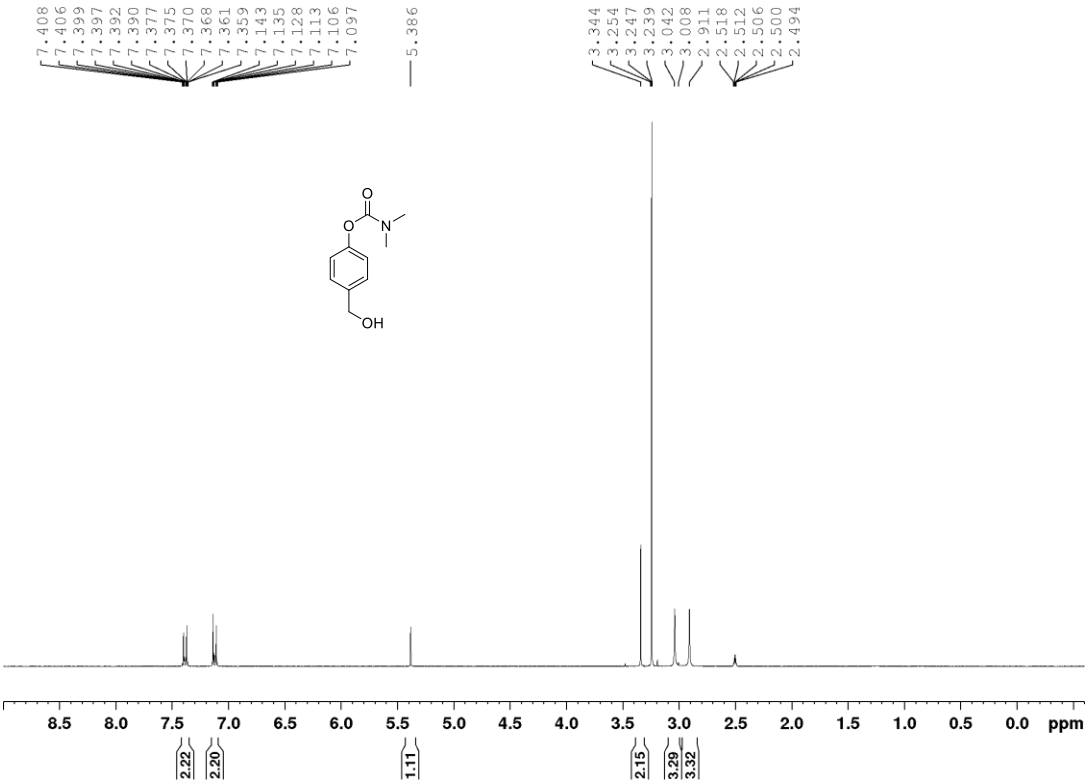


Fig. S41 ^{1}H-NMR spectrum of **2a** (300 MHz, 298 K, DMSO-d_6).

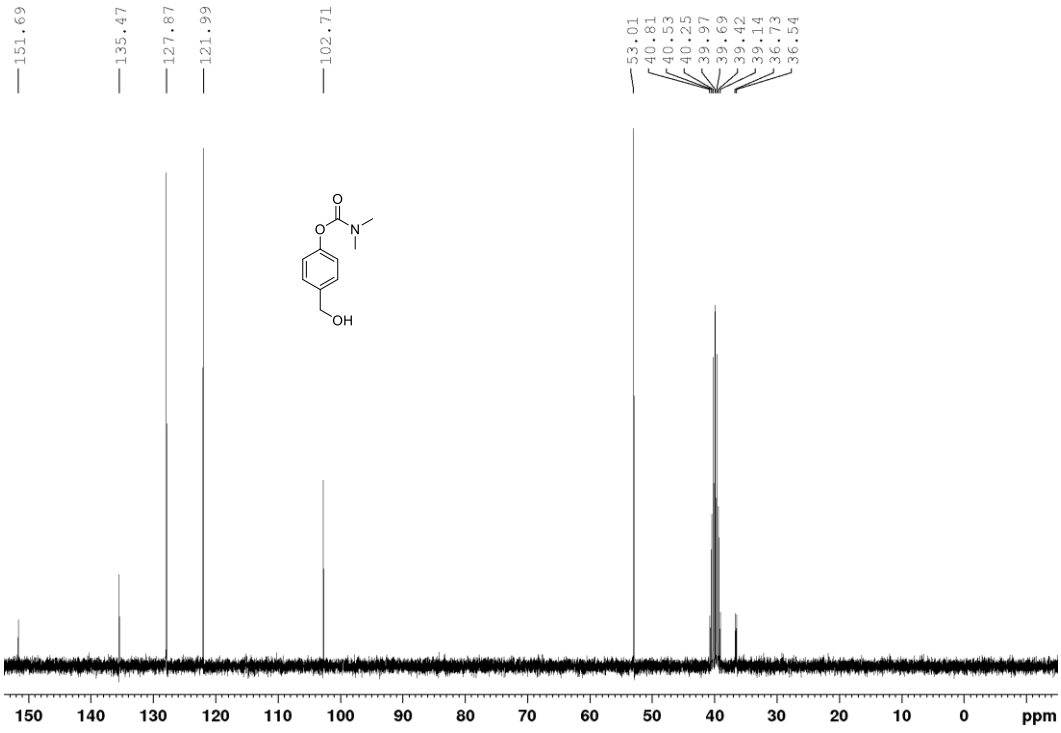


Fig. S42 ^{13}C-NMR spectrum of **2a** (75 MHz, 298 K, DMSO-d_6).

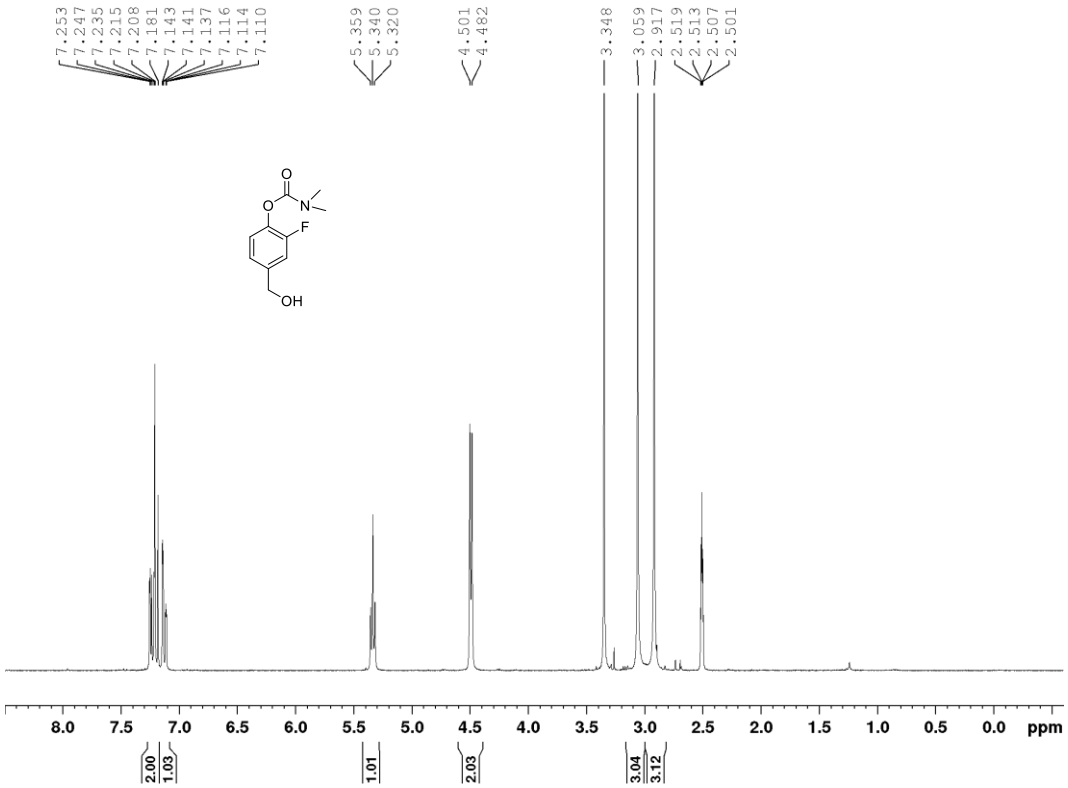


Fig. S43 ^{1}H-NMR spectrum of **2b** (300 MHz, 298 K, DMSO-d_6).

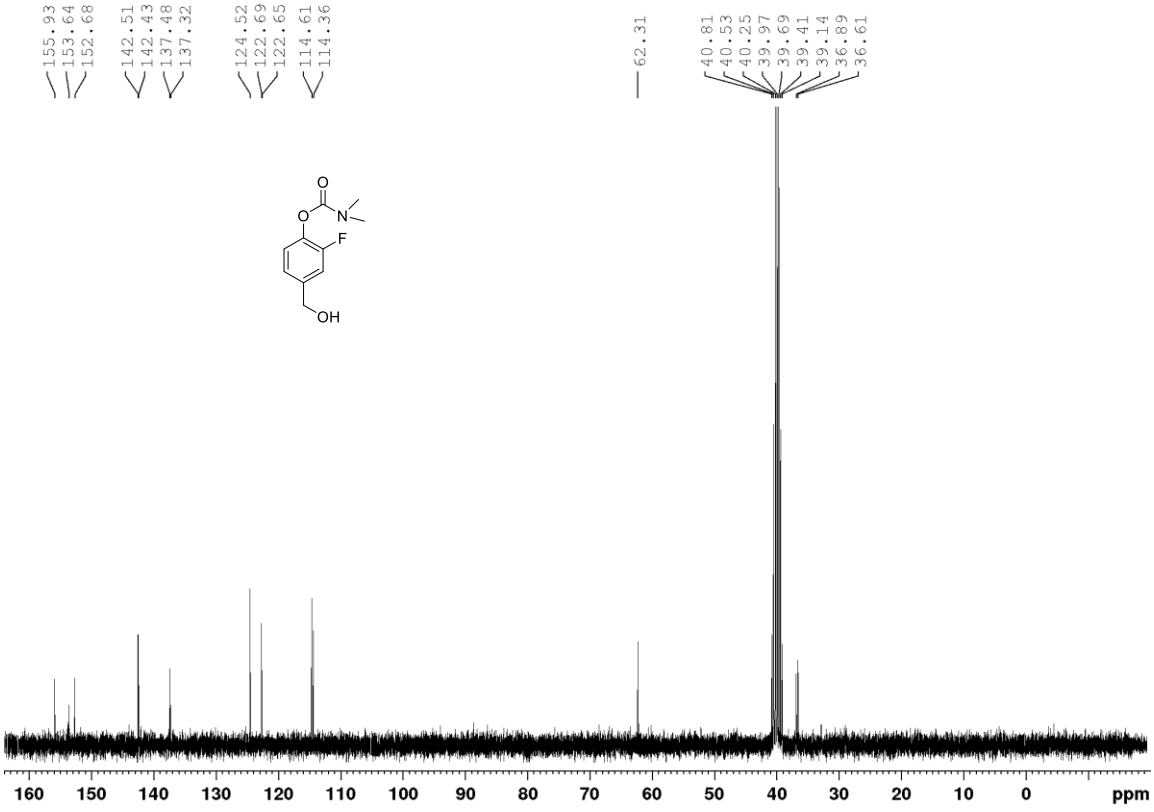


Fig. S44 ^{13}C-NMR spectrum of **2b** (75 MHz, 298 K, DMSO-d_6).

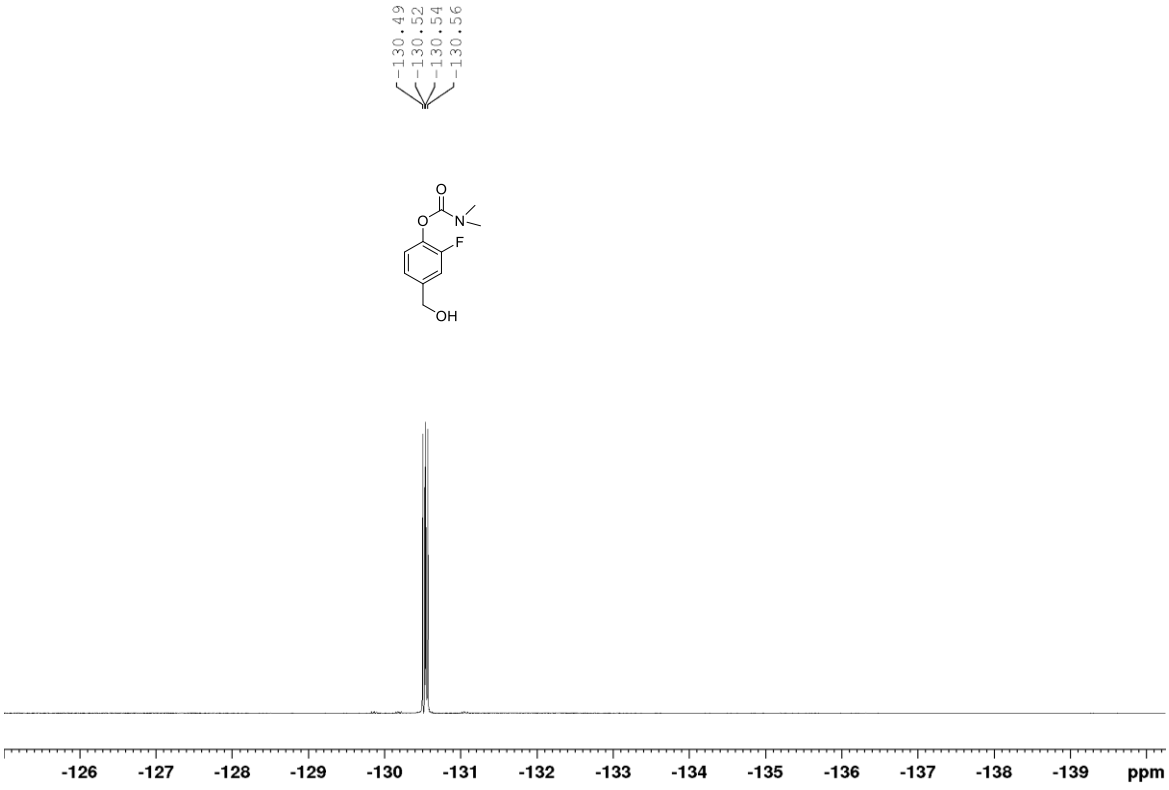


Fig. S45 ^{19}F-NMR spectrum of **2b** (300 MHz, 298 K, DMSO-d_6).

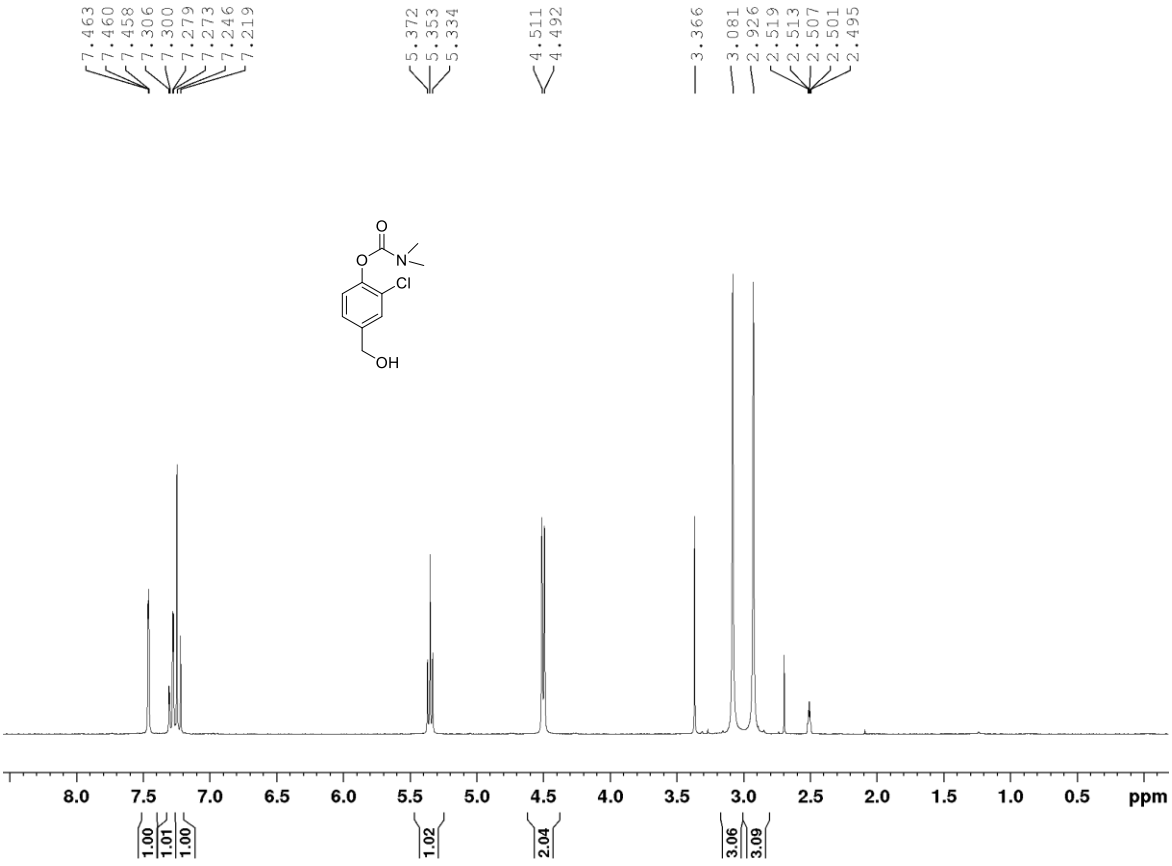


Fig. S46 ^{1}H-NMR spectrum of **2c** (300 MHz, 298 K, DMSO-d_6).

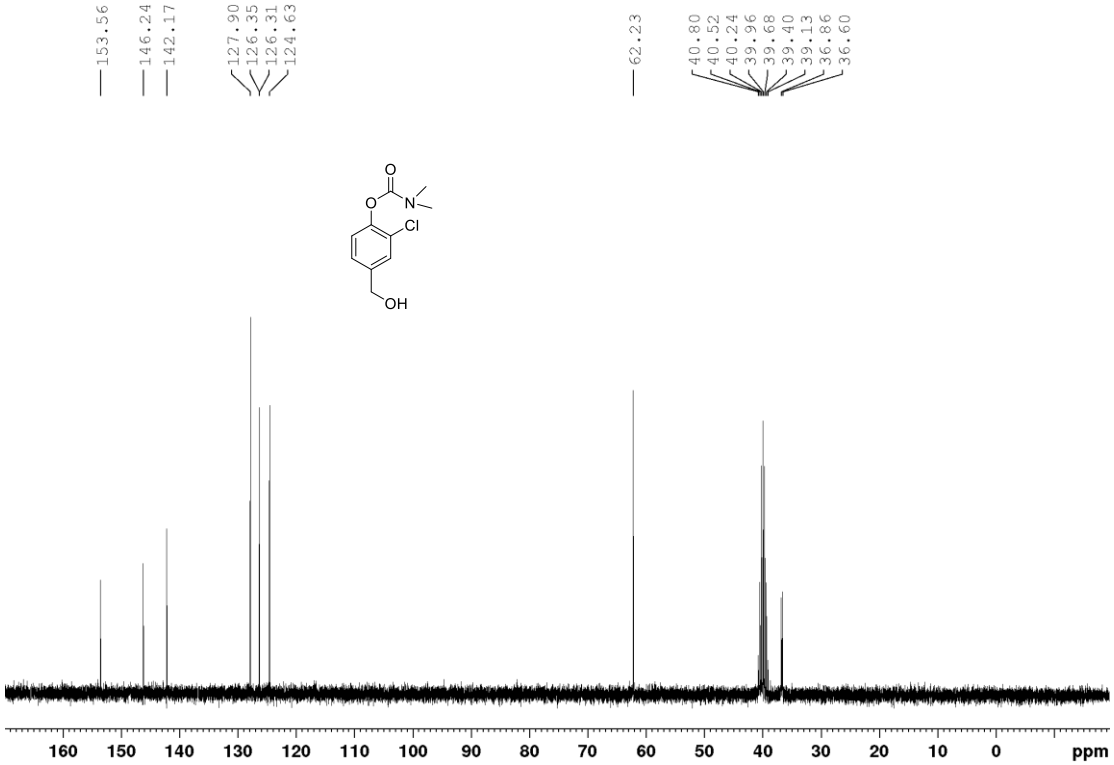


Fig. S47 ^{13}C-NMR spectrum of **2c** (75 MHz, 298 K, DMSO-d_6).

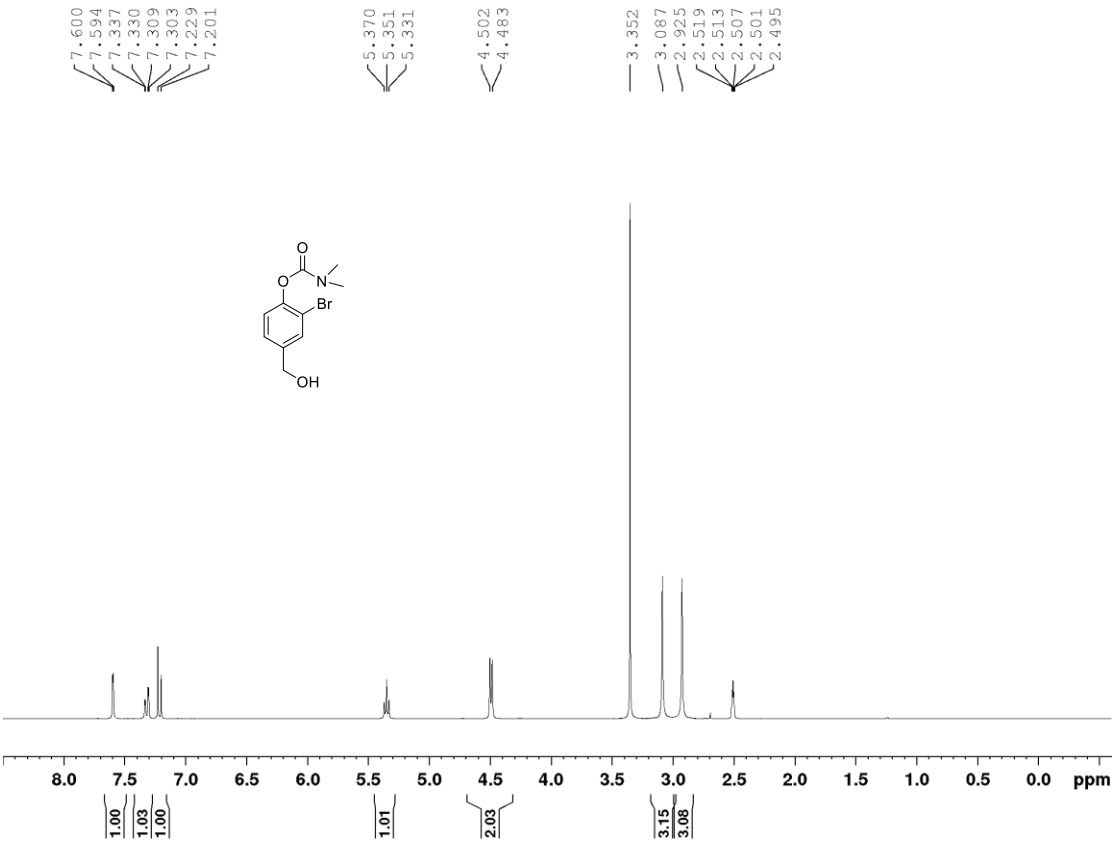


Fig. S48 ^{1}H-NMR spectrum of **2d** (300 MHz, 298 K, DMSO-d_6).

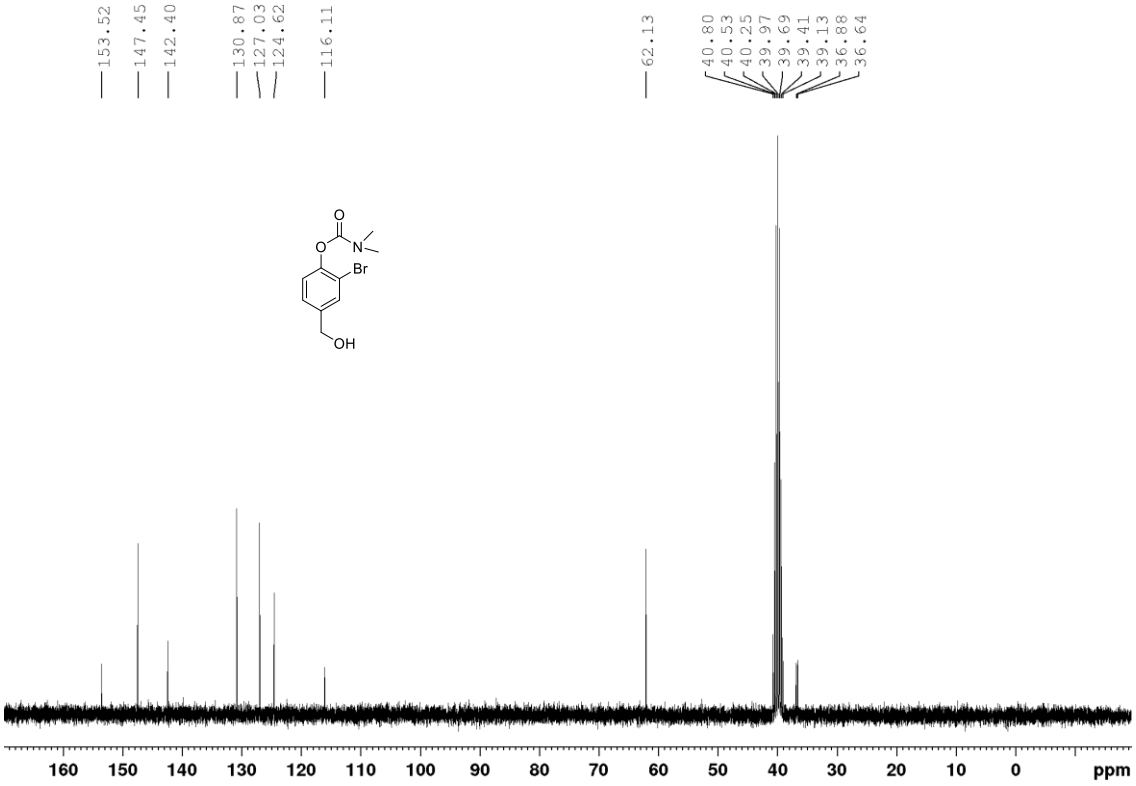


Fig. S49 ^{13}C-NMR spectrum of **2d** (75 MHz, 298 K, DMSO-d_6).

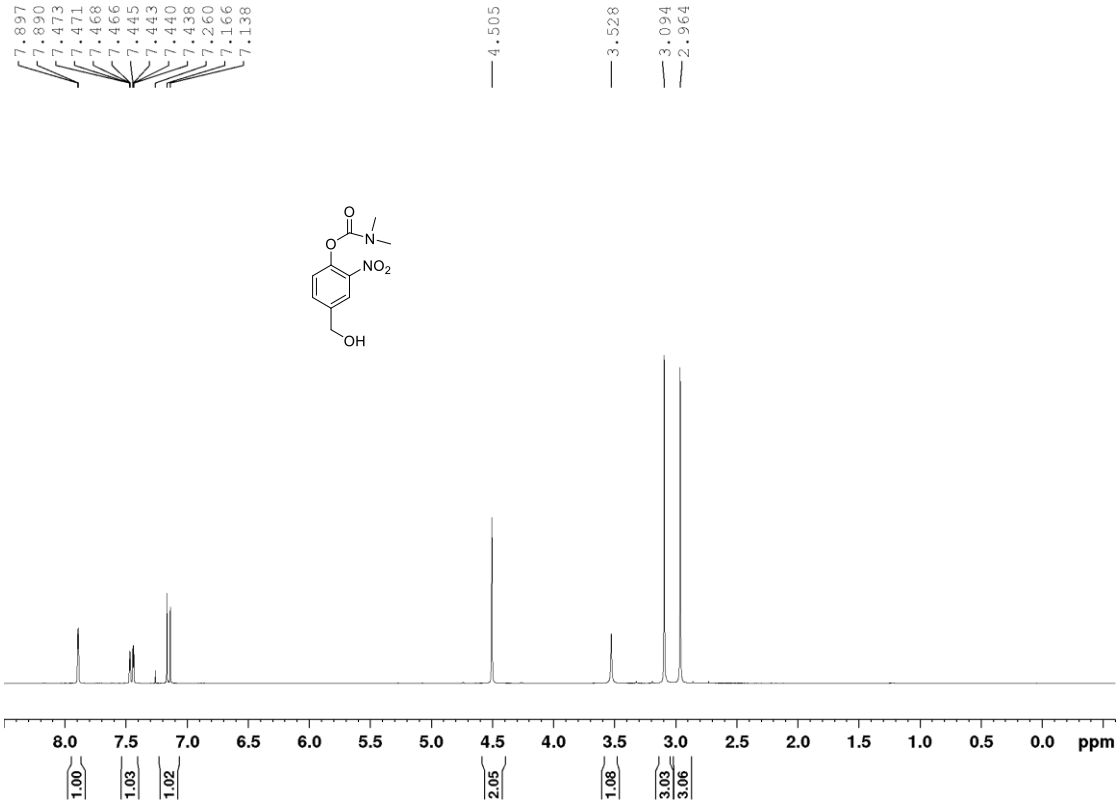


Fig S50 ^{1}H-NMR spectrum of **2e** (300 MHz, 298 K, $CDCl_3$).

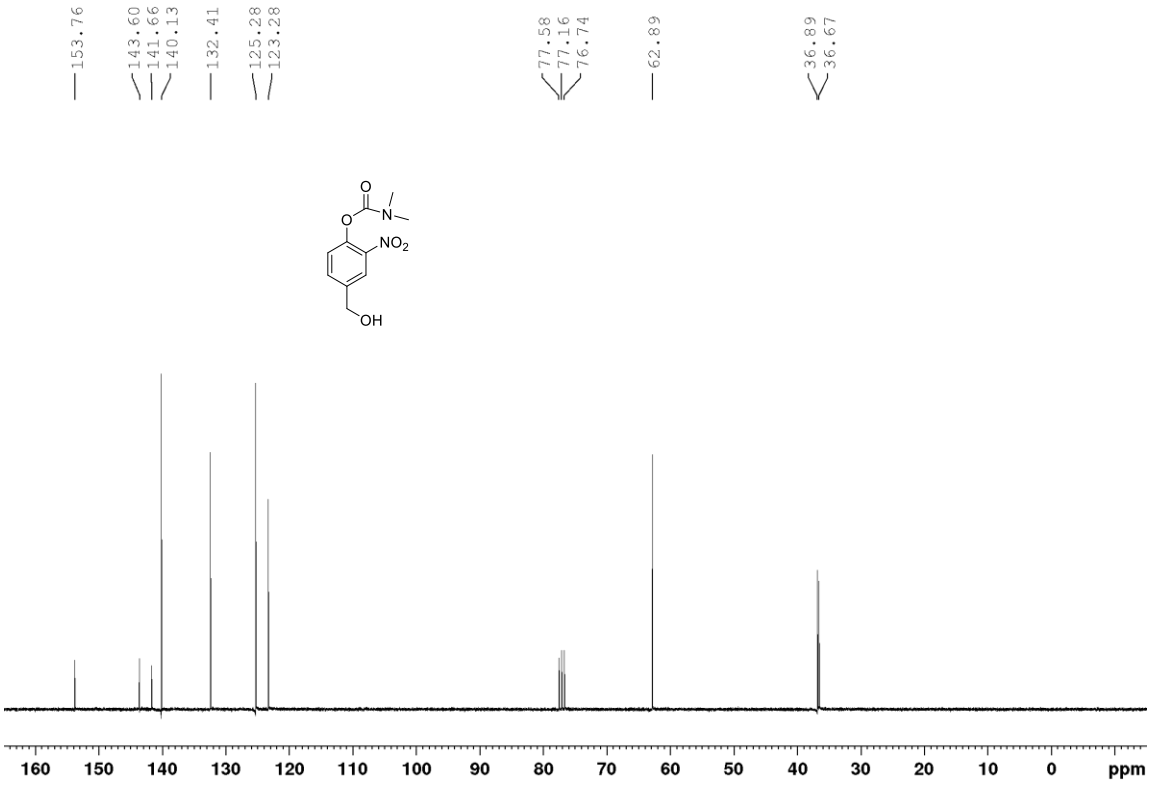


Fig. S51 ^{13}C-NMR spectrum of **2e** (75 MHz, 298 K, $CDCl_3$).

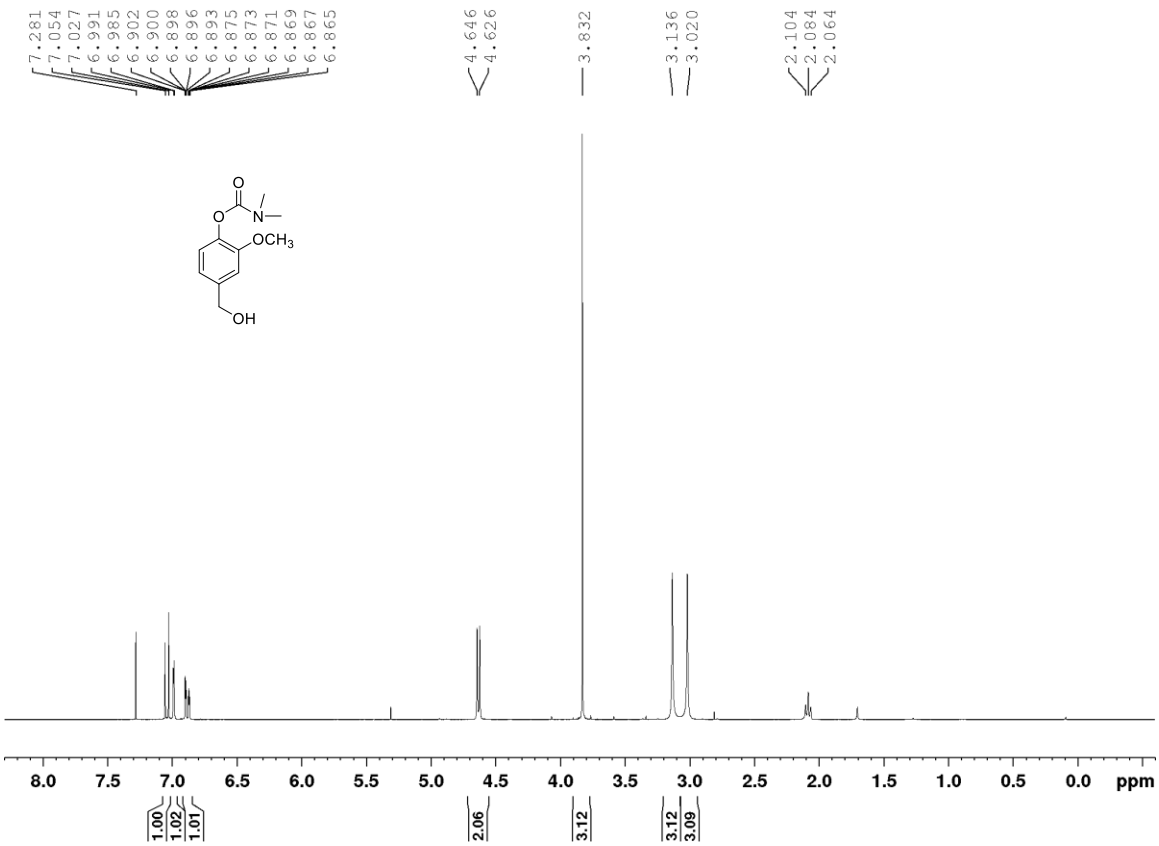


Fig. S52 ^{1}H-NMR spectrum of **2f** (300 MHz, 298 K, $CDCl_3$).

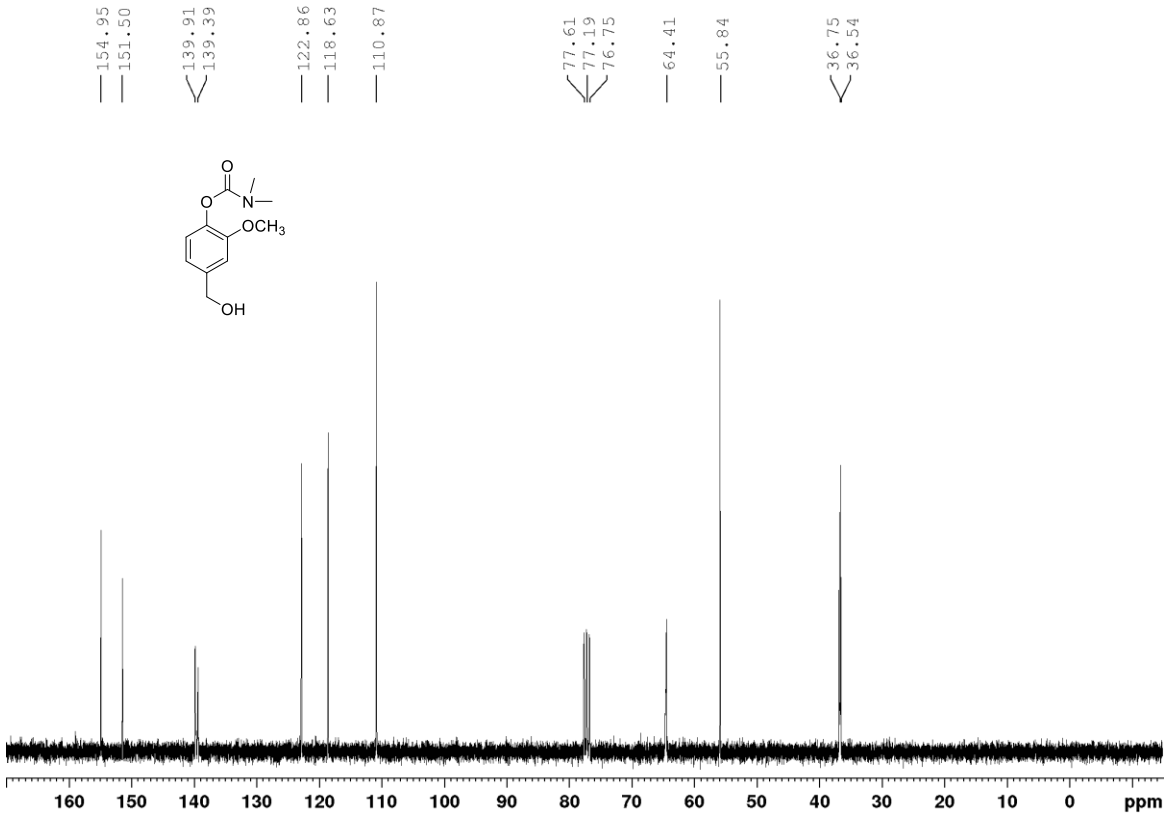


Fig. S53 ^{13}C-NMR spectrum of **2f** (75 MHz, 298 K, $CDCl_3$).

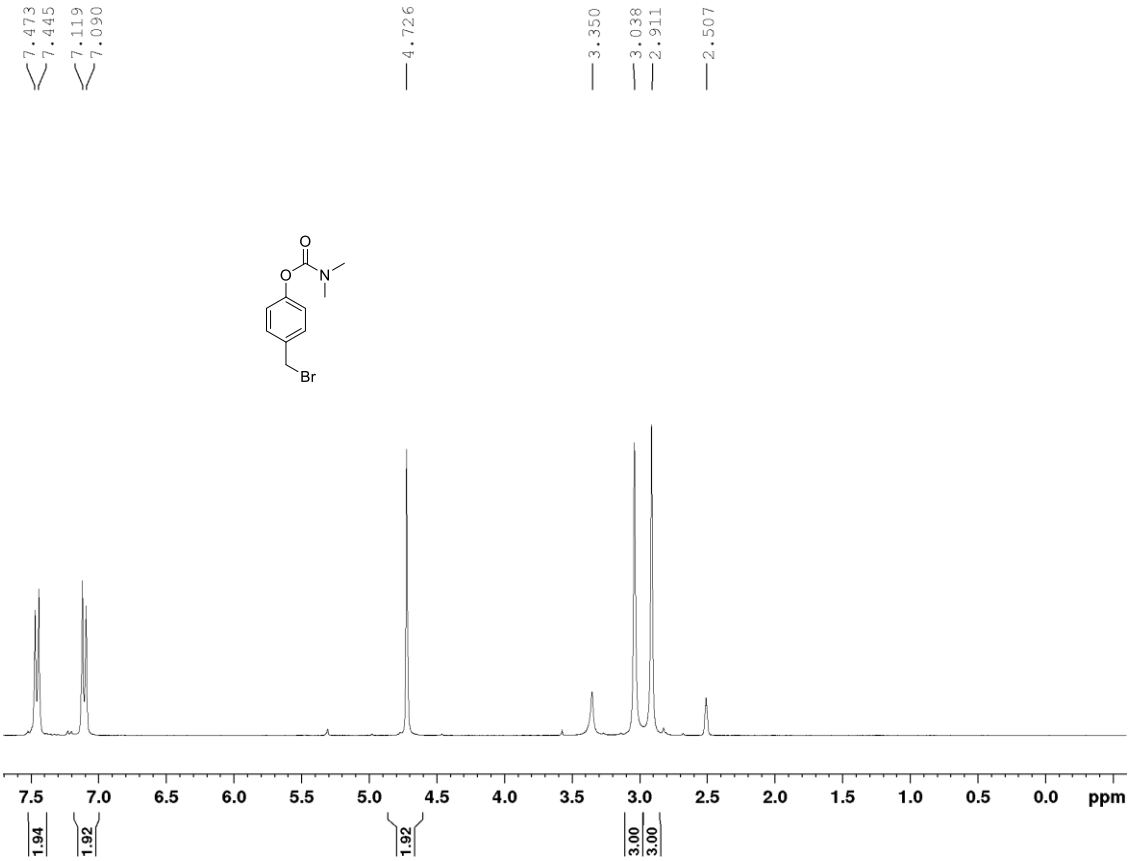


Fig. S54 ^{1}H-NMR spectrum of **3a** (300 MHz, 298 K, DMSO-d_6).

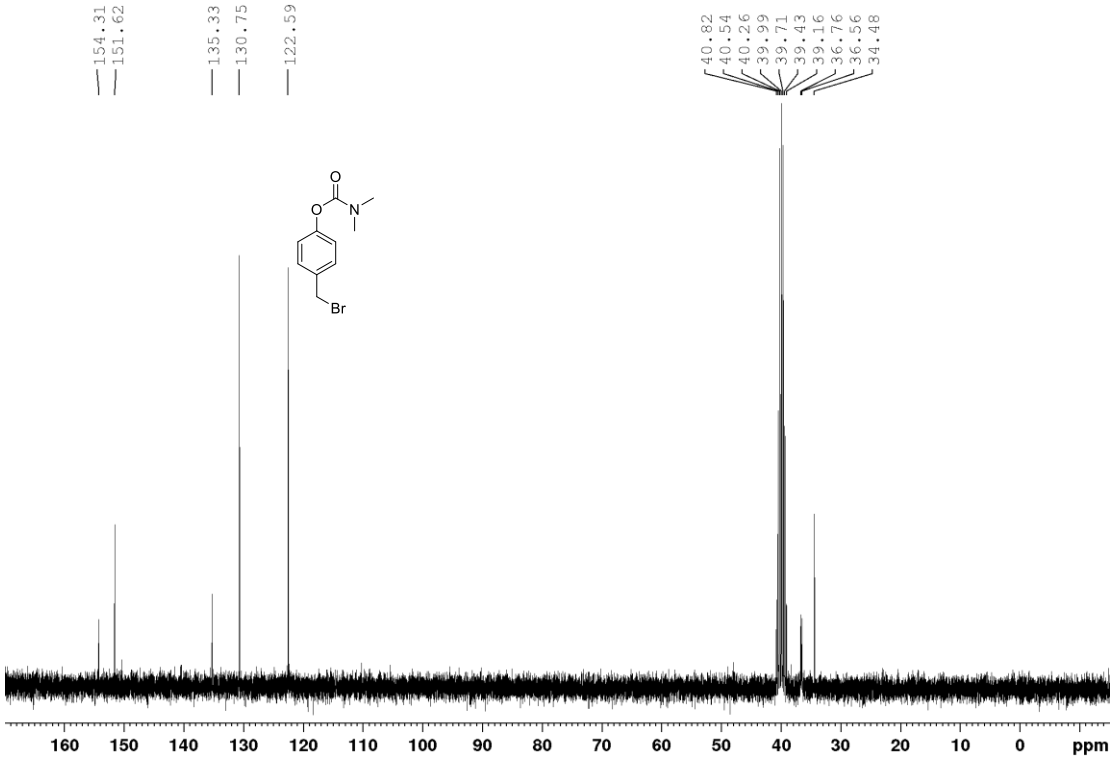


Fig. S55 ^{13}C-NMR spectrum of **3a** (75 MHz, 298 K, DMSO-d_6).

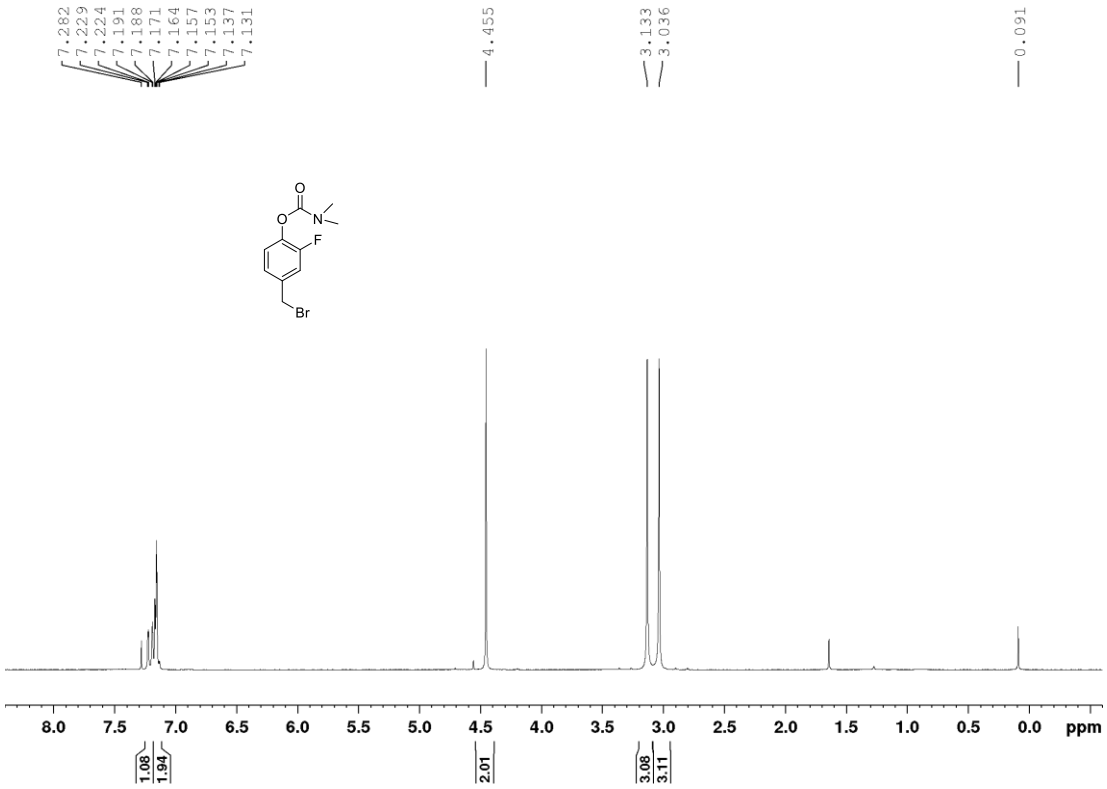


Fig. S56 ^{1}H-NMR spectrum of **3b** (300 MHz, 298 K, $CDCl_3$).

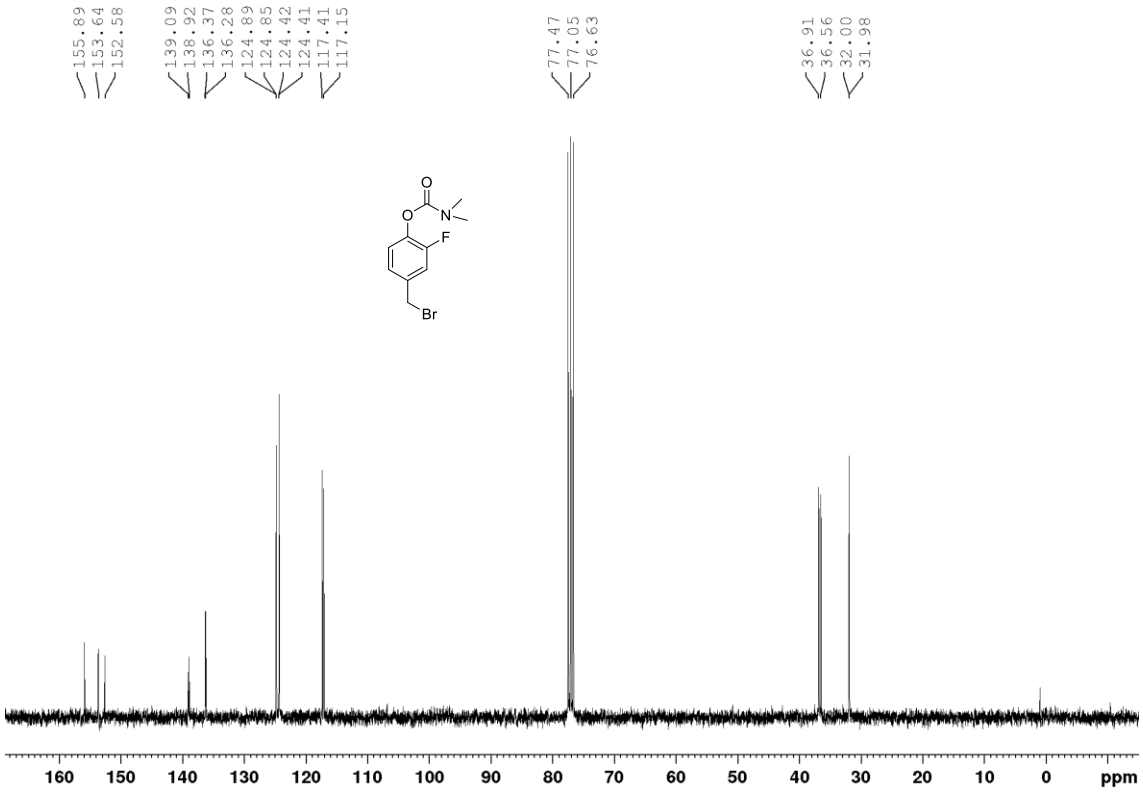


Fig. S57 ^{13}C-NMR spectrum of **3b** (75 MHz, 298 K, $CDCl_3$).

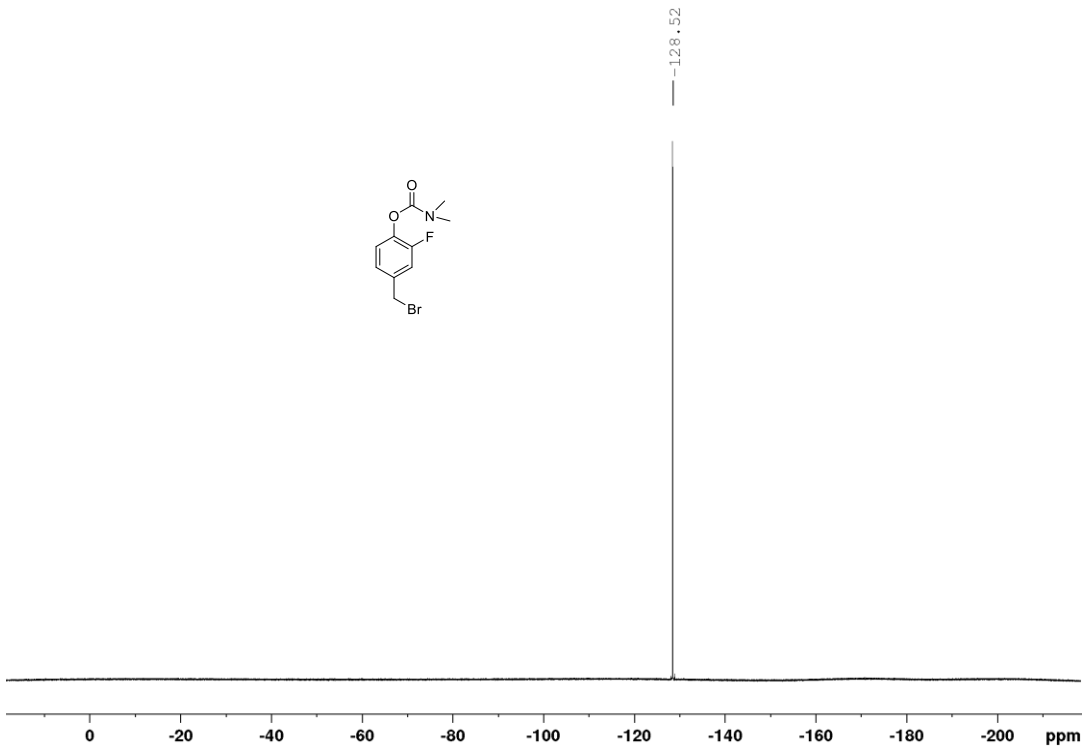


Fig. S58 ^{19}F-NMR spectrum of **3b** (300 MHz, 298 K, $CDCl_3$).

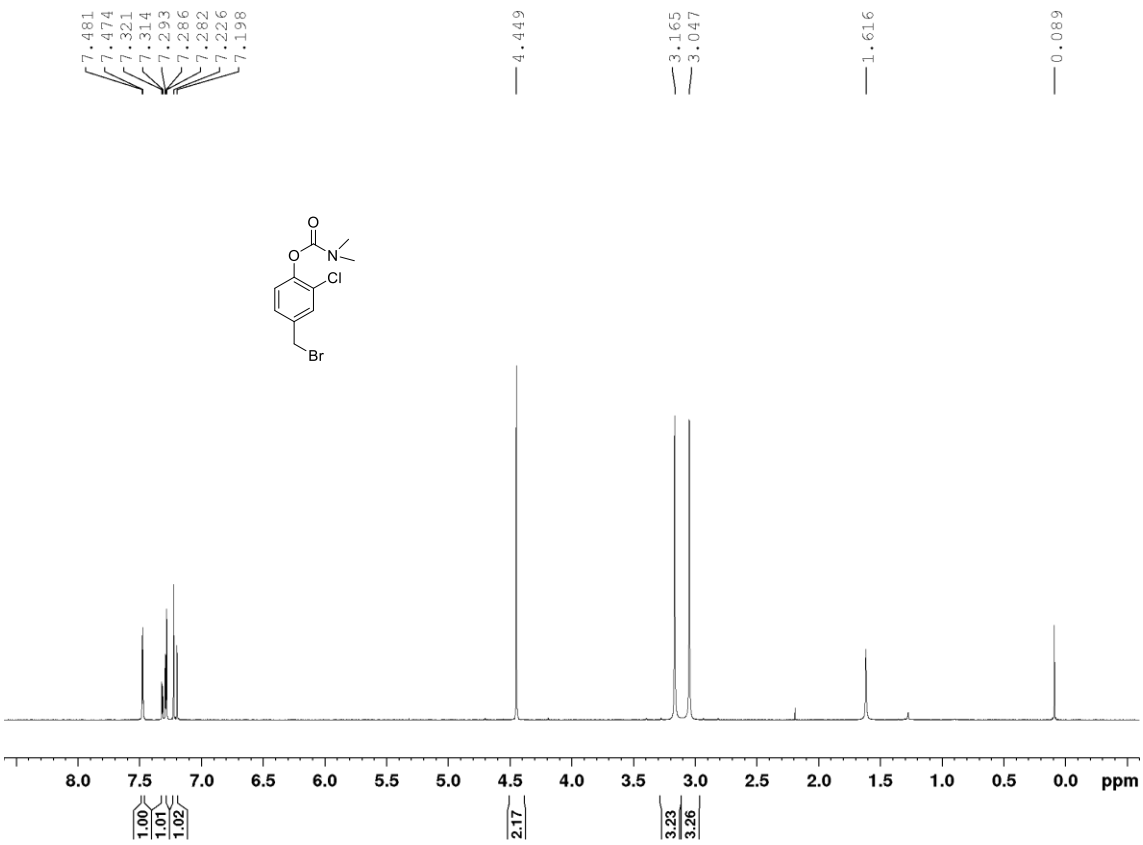


Fig. S59 ^{1}H-NMR spectrum of **3c** (300 MHz, 298 K, $CDCl_3$).

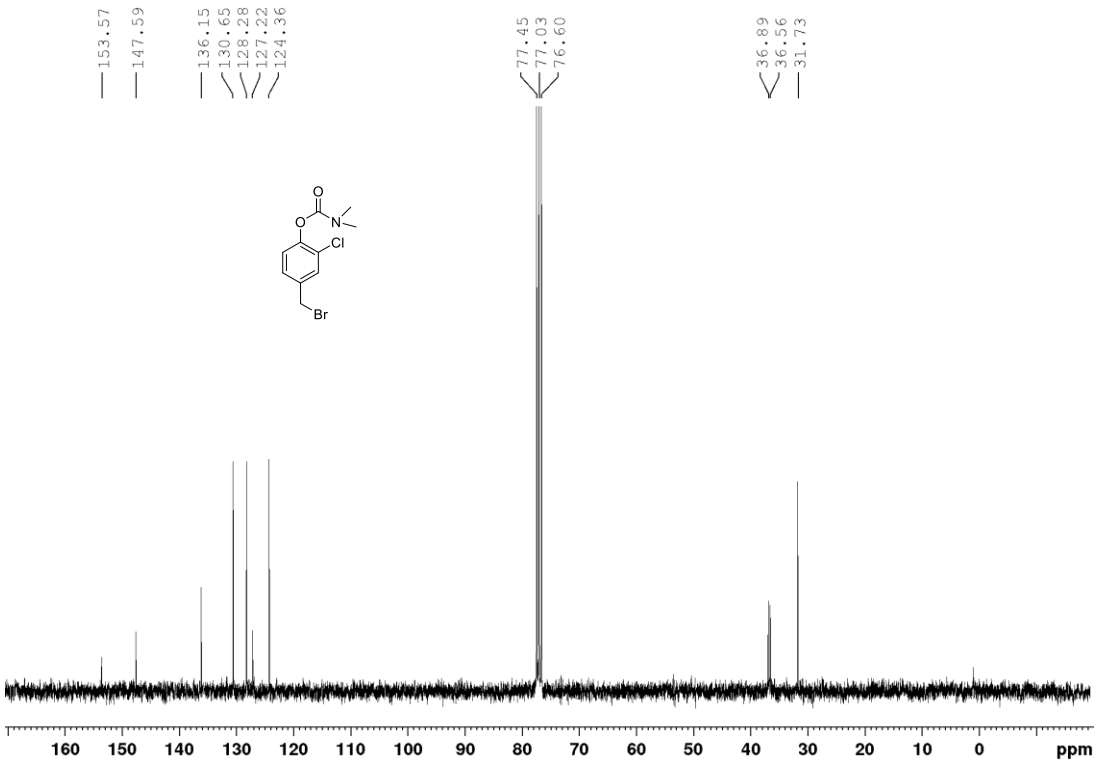


Fig. S60 ^{13}C-NMR spectrum of **3c** (75 MHz, 298 K, $CDCl_3$).

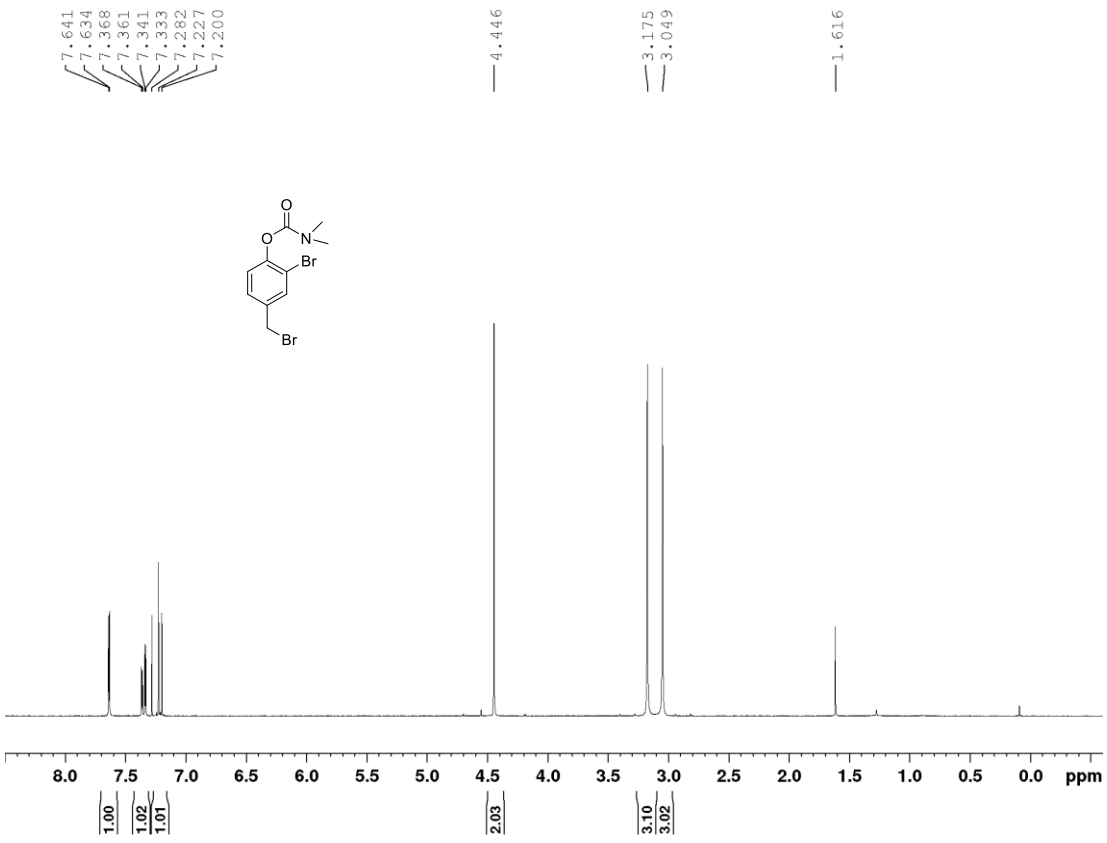


Fig. S61 ^{1}H-NMR spectrum of **3d** (300 MHz, 298 K, $CDCl_3$).

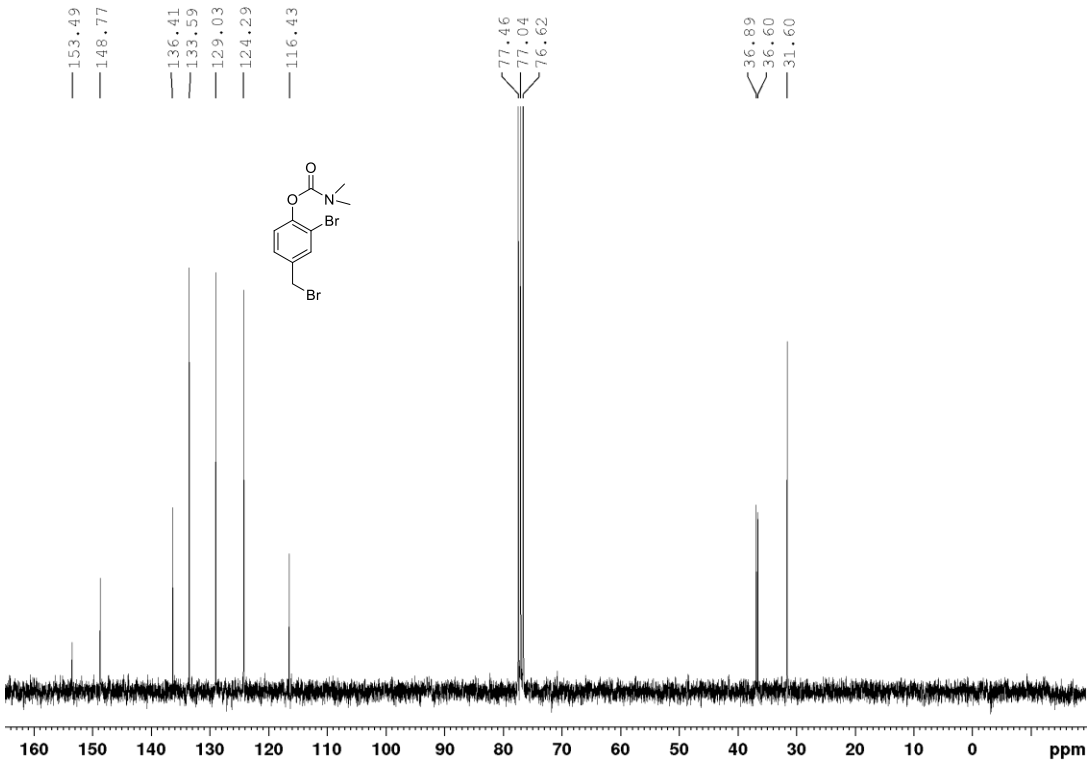


Fig. S62 ^{13}C-NMR spectrum of **3d** (75 MHz, 298 K, $CDCl_3$).

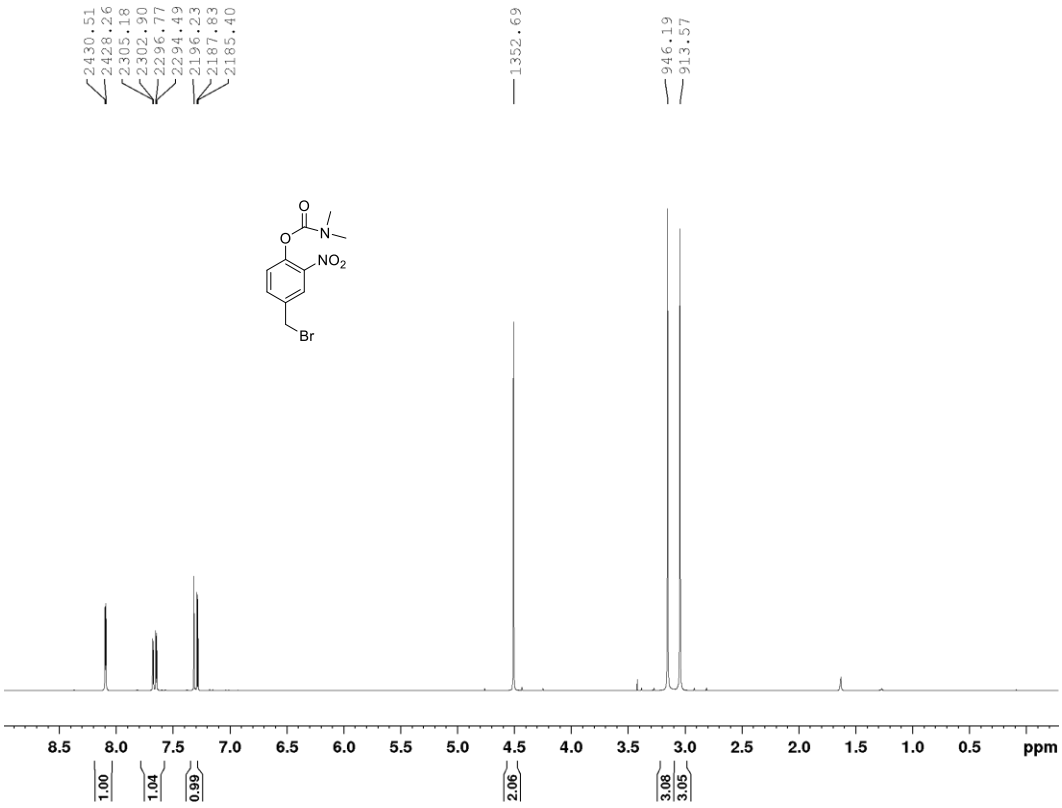


Fig. S63 ^{1}H-NMR spectrum of **3e** (300 MHz, 298 K, $CDCl_3$).

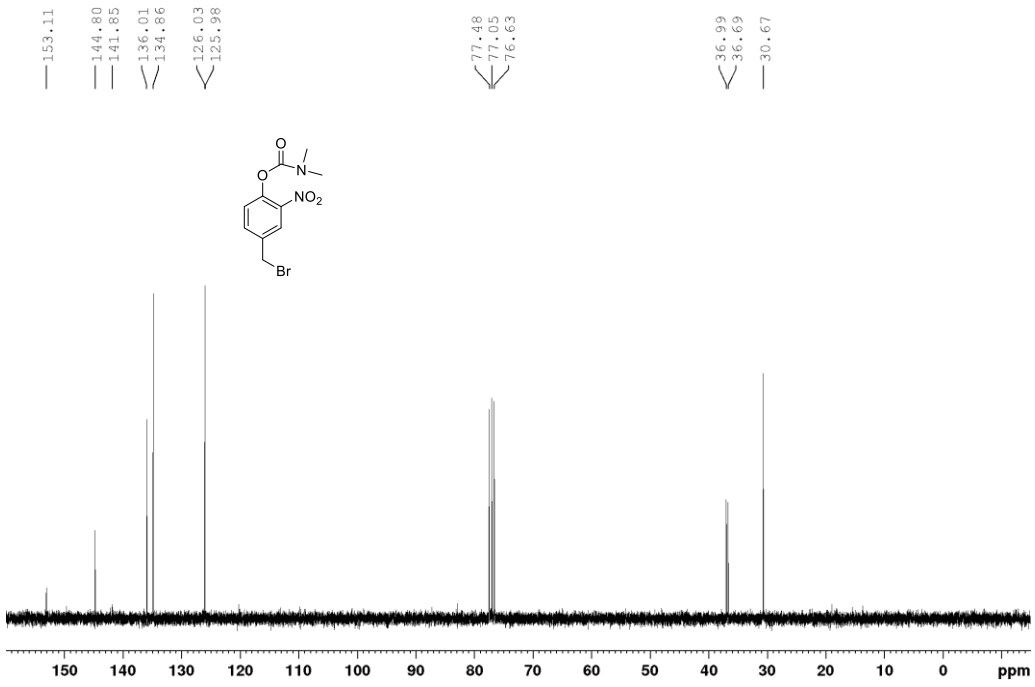


Fig. S64 ^{13}C-NMR spectrum of **3e** (75 MHz, 298 K, $CDCl_3$).

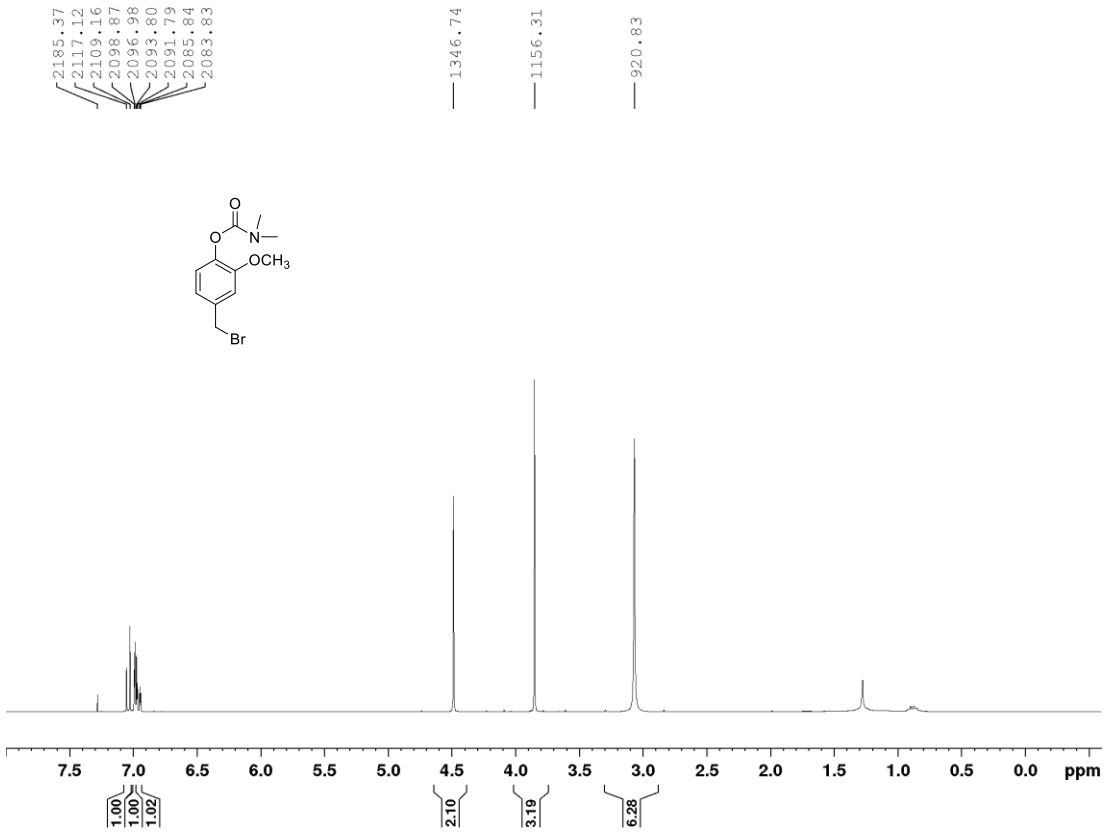


Fig. S65 ^{1}H-NMR spectrum of **3f** (300 MHz, 298 K, $CDCl_3$).

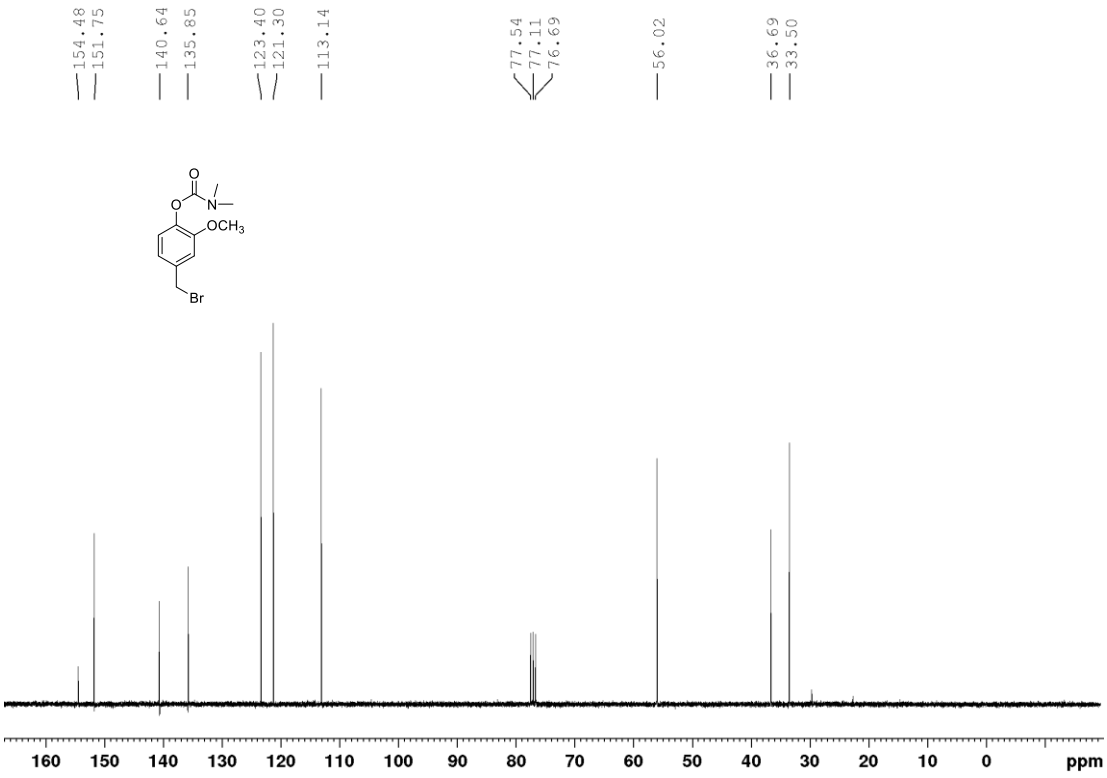


Fig. S66 ^{13}C-NMR spectrum of **3f** (75 MHz, 298 K, $CDCl_3$).

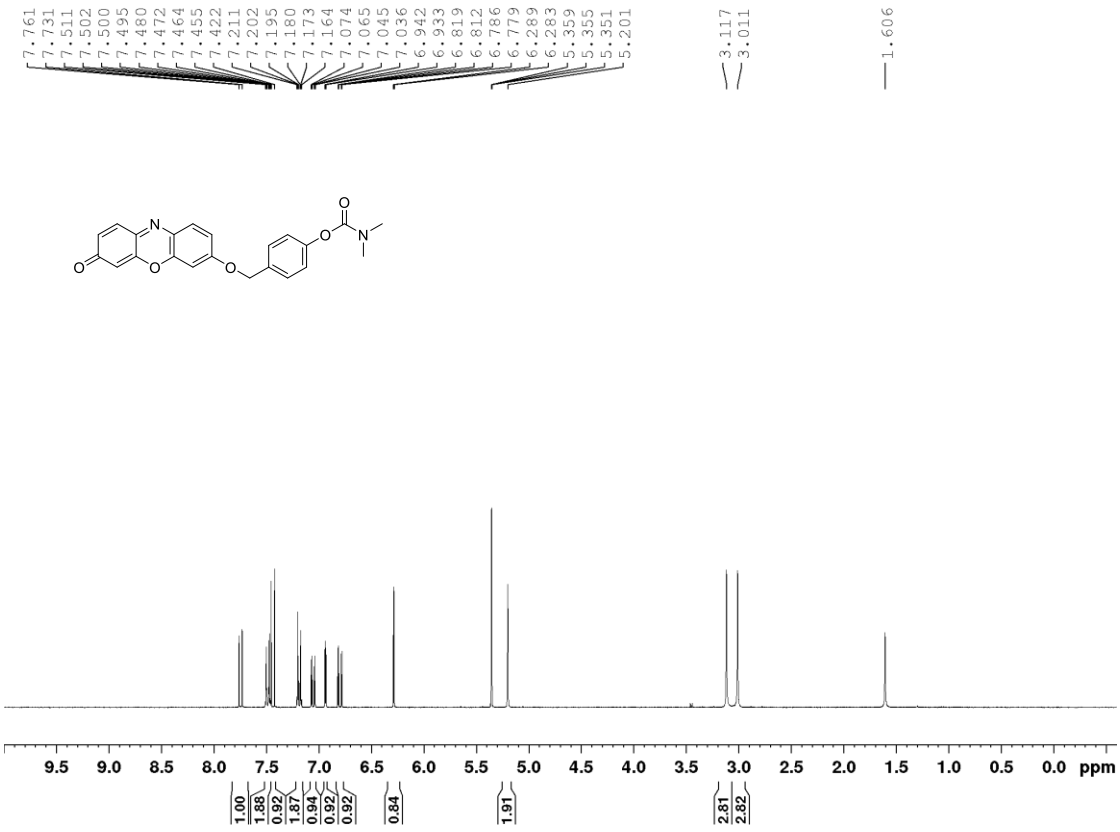


Fig. S67 ^{1}H-NMR spectrum of **P1** (300 MHz, 298 K, CD_2Cl_2-d_2).

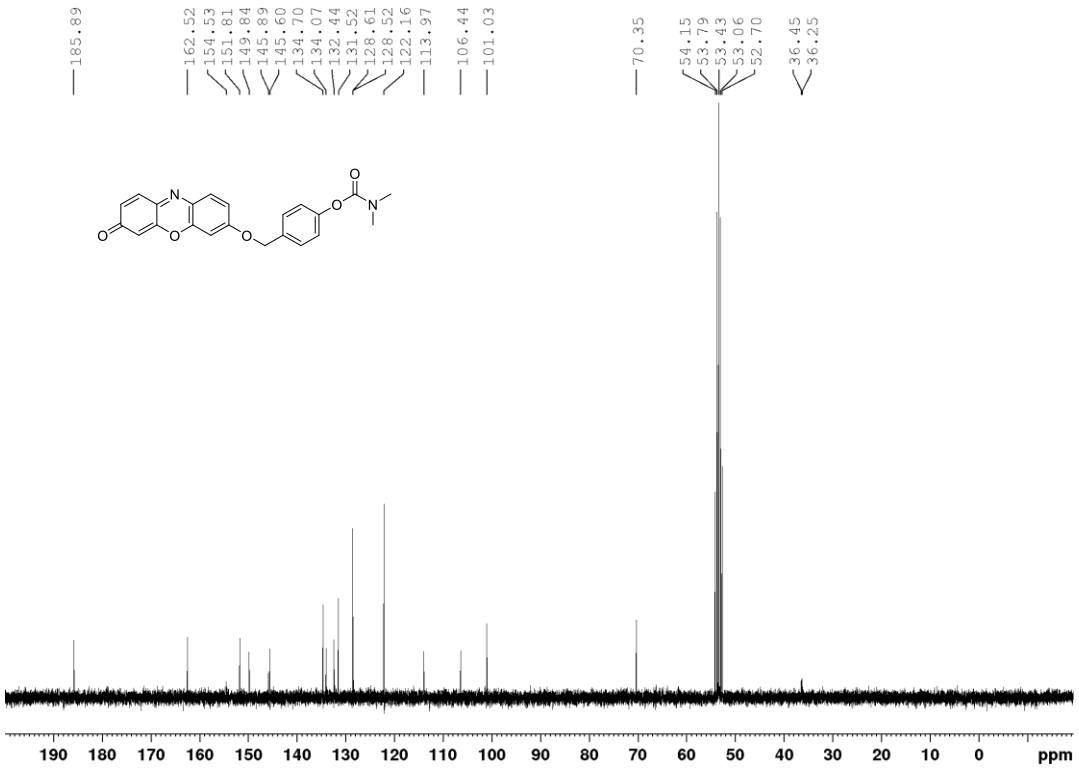


Fig. S68 ^{13}C-NMR spectrum of **P1** (75 MHz, 298 K, CD_2Cl_2-d_2).

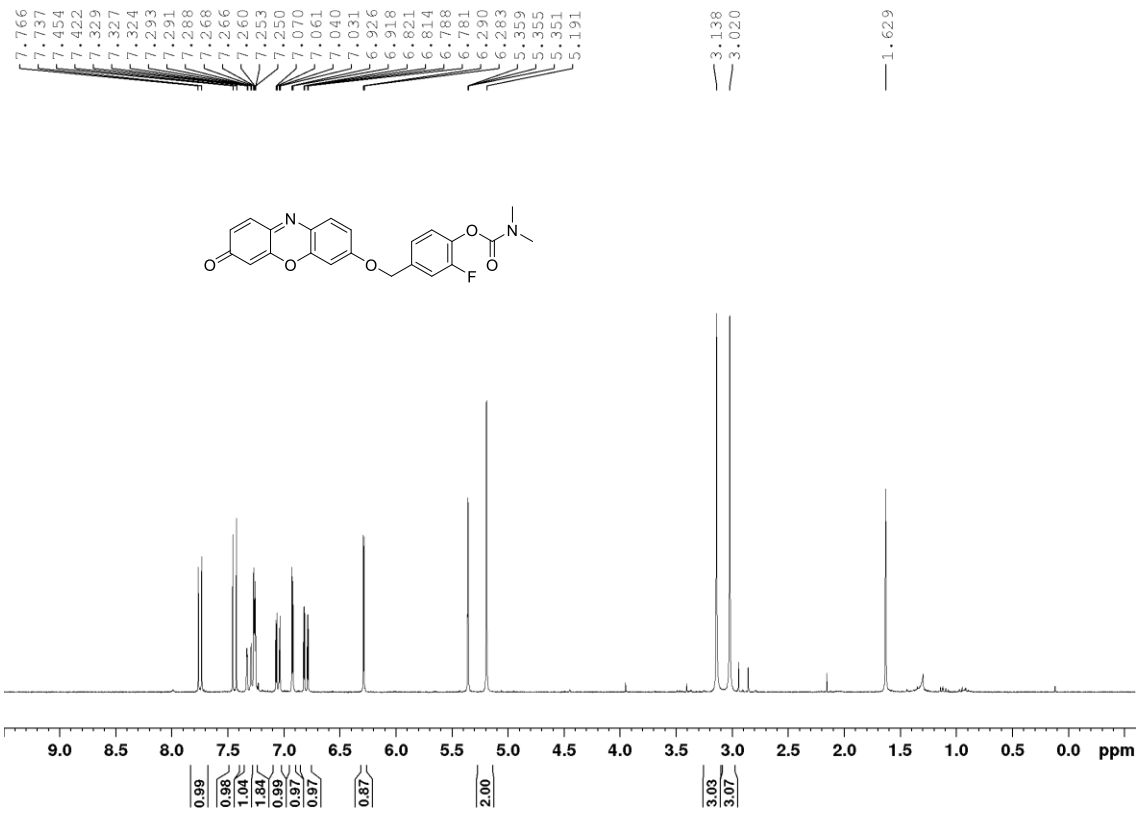


Fig. S69. ^{1}H-NMR spectrum of **P2** (300 MHz, 298 K, CD_2Cl_2-d_2).

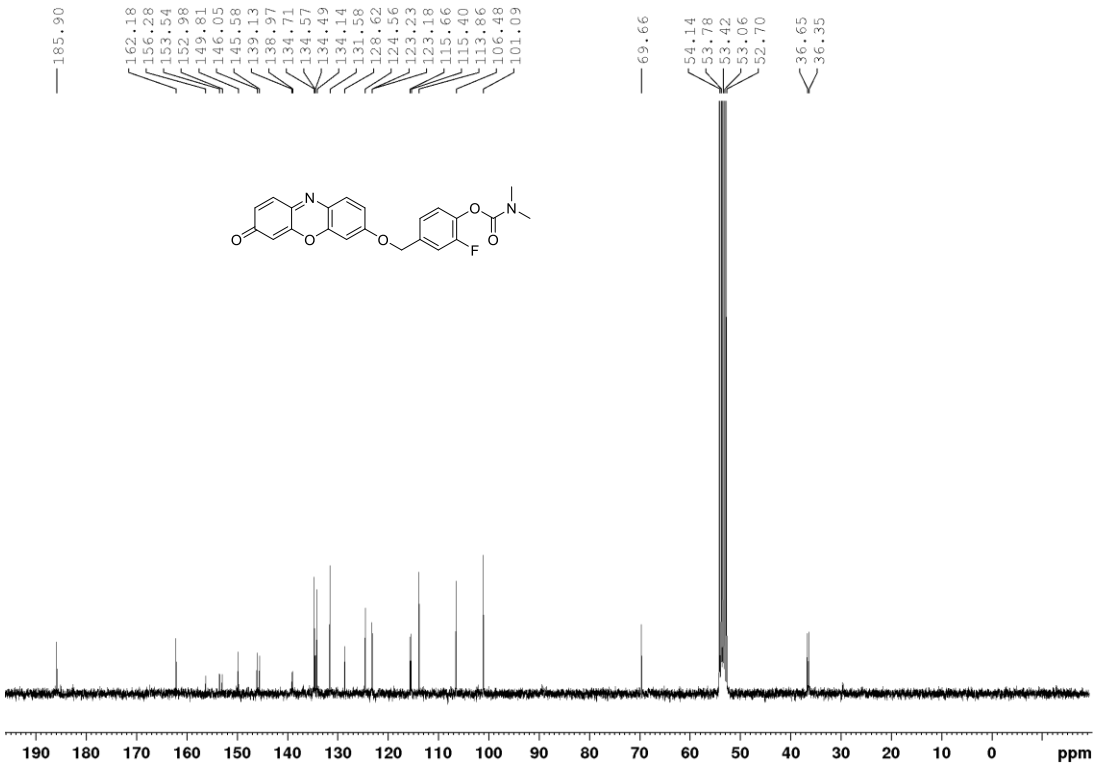


Fig. S70 ^{13}C-NMR spectrum of **P2** (75 MHz, 298 K, CD_2Cl_2-d_2).

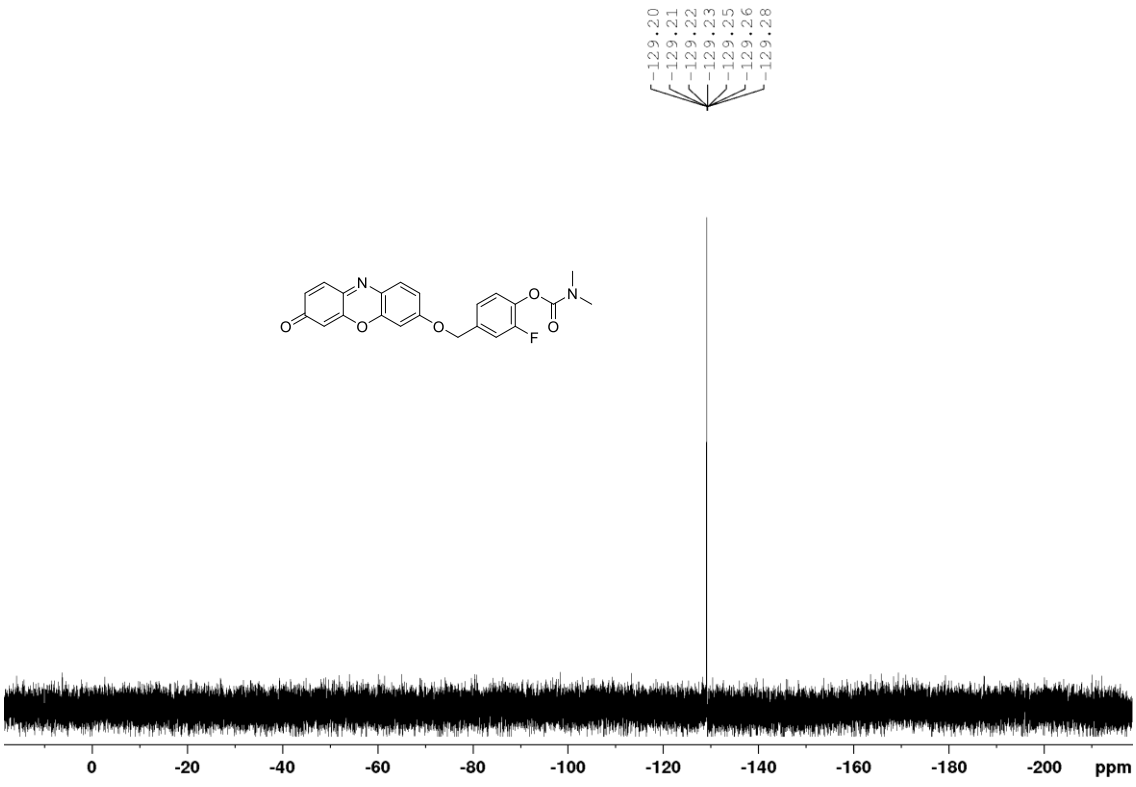


Fig. S71 ^{19}F-NMR spectrum of **P2** (300 MHz, 298 K, CD_2Cl_2-d_2).

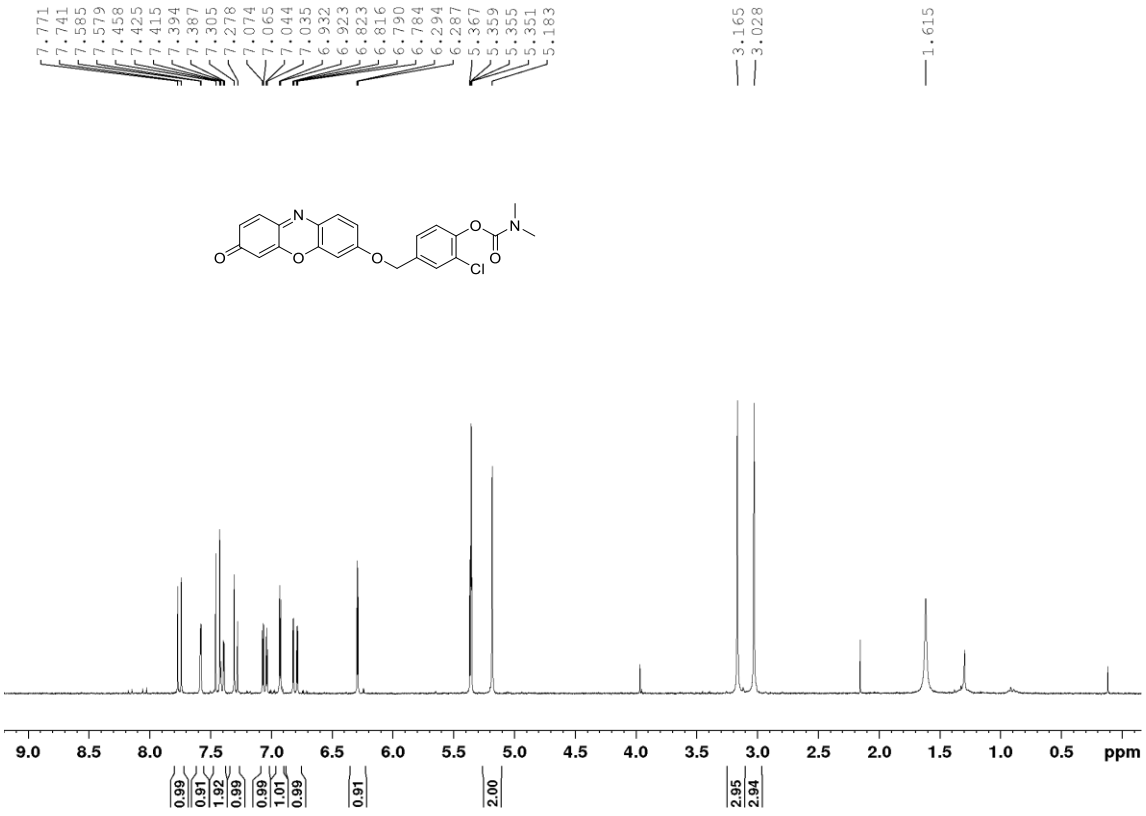


Fig. S72 ^{1}H-NMR spectrum of **P3** (300 MHz, 298 K, CD_2Cl_2-d_2).

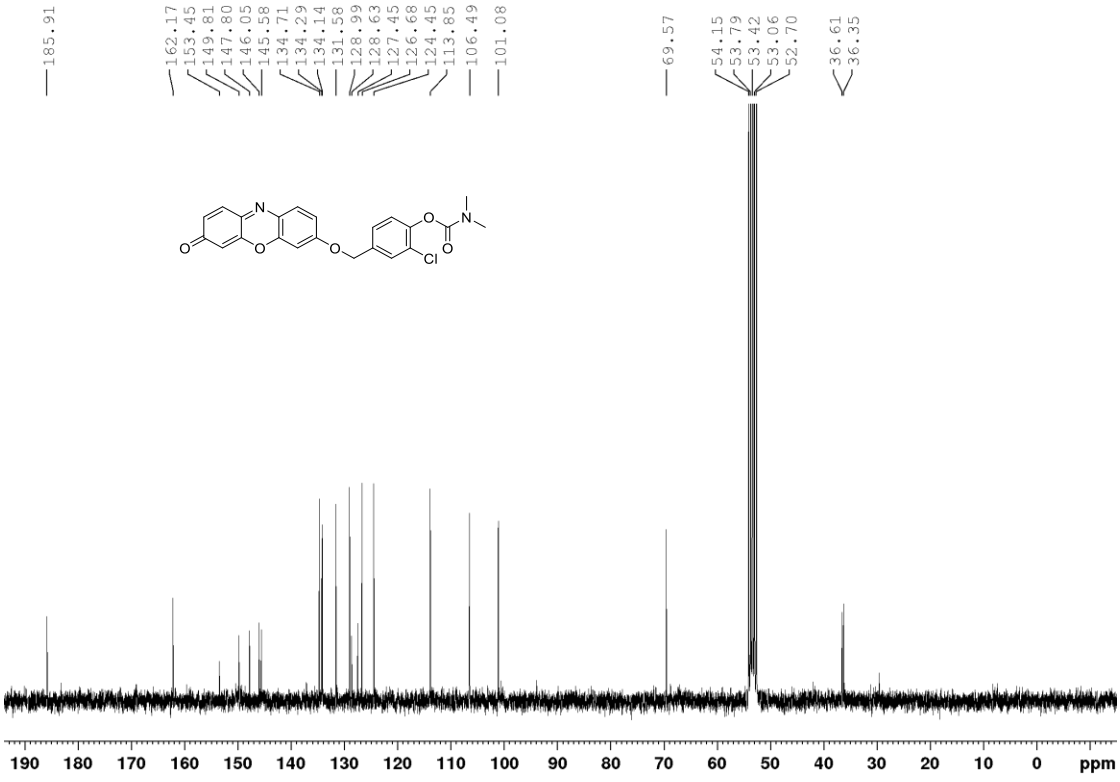


Fig. S73 ^{13}C-NMR spectrum of **P3** (75 MHz, 298 K, CD_2Cl_2-d_2).

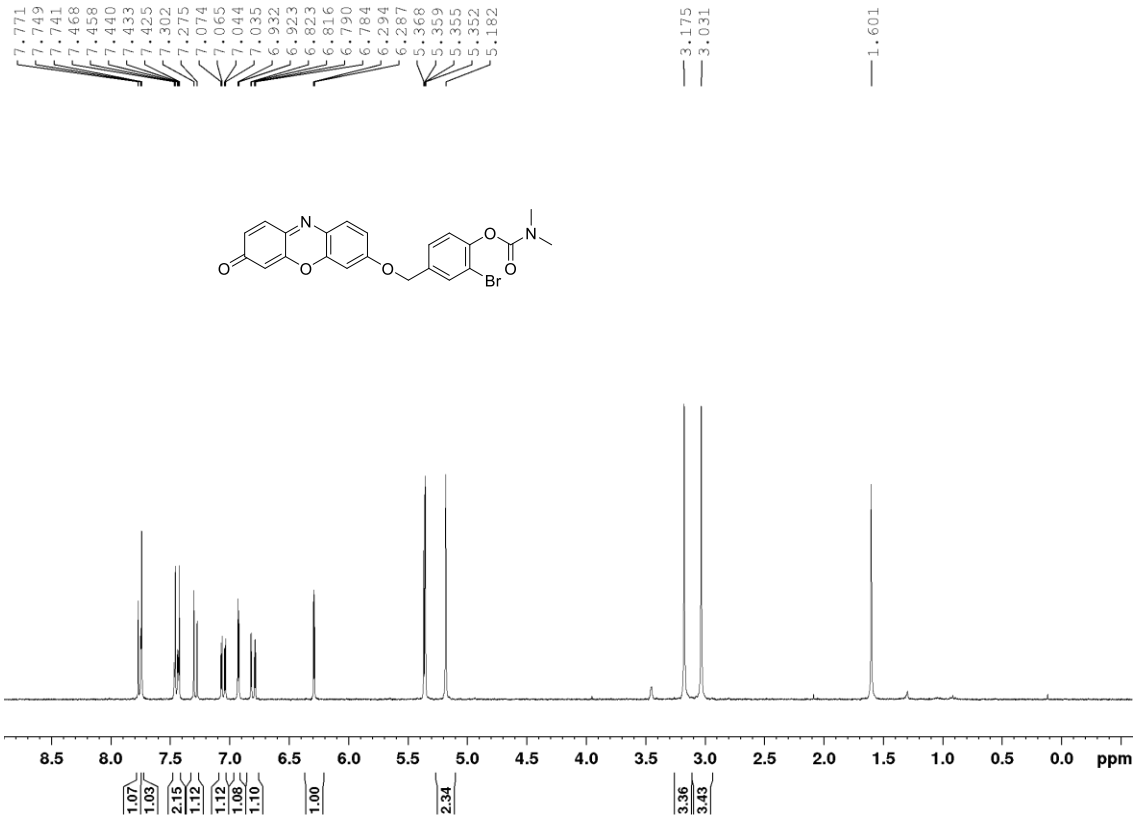


Fig. S74 ^{1}H-NMR spectrum of **P4** (300 MHz, 298 K, CD_2Cl_2-d_2).

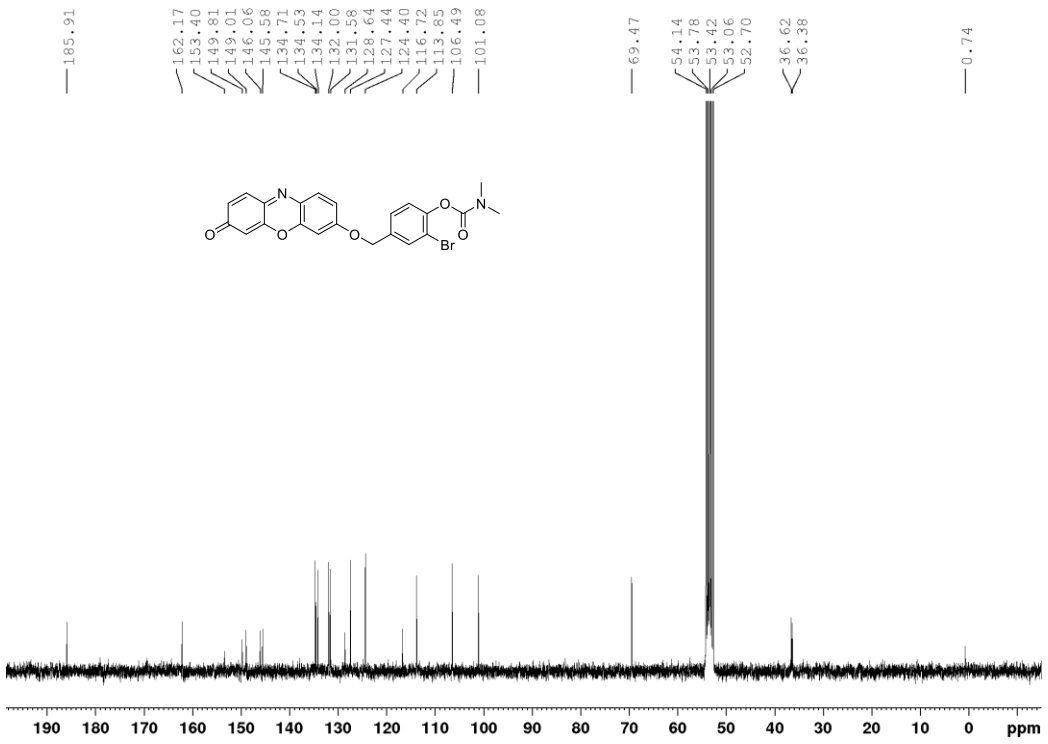


Fig. S75 ^{13}C-NMR spectrum of **P4** (75 MHz, 298 K, CD_2Cl_2-d_2).

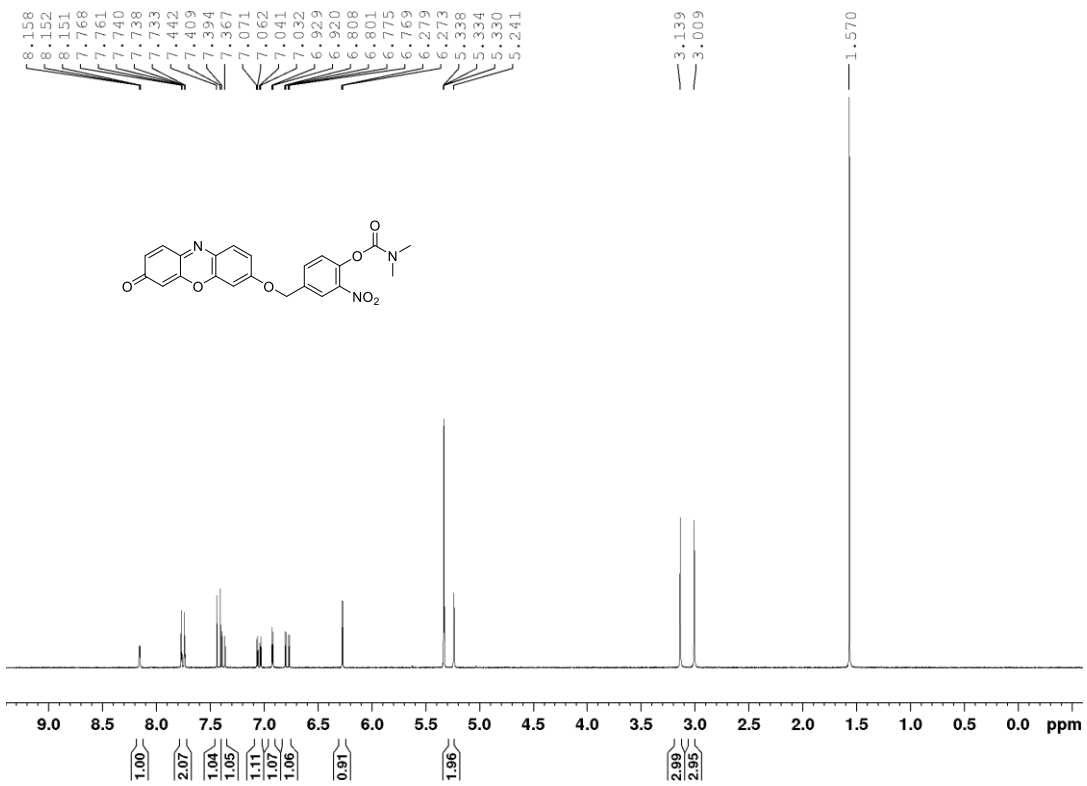


Fig. S76 ^{1}H-NMR spectrum of **P5** (300 MHz, 298 K, CD_2Cl_2-d_2).

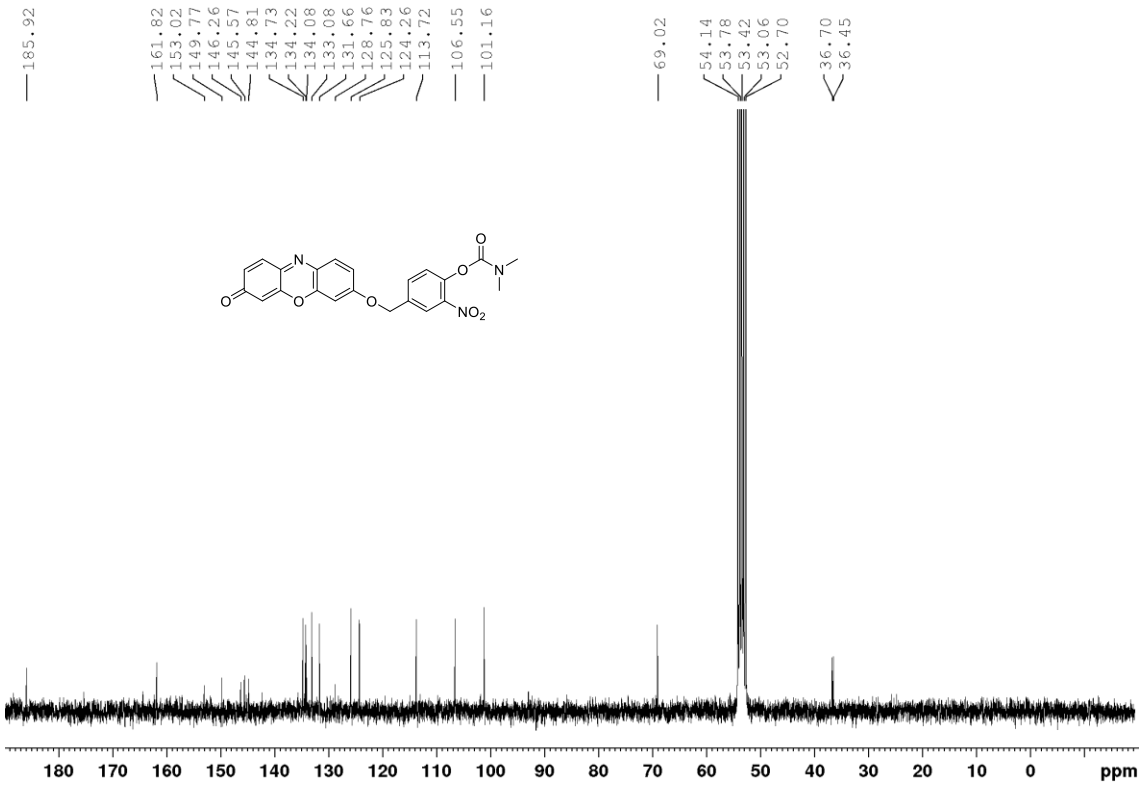


Fig. S77 ^{13}C-NMR spectrum of **P5** (75 MHz, 298 K, CD_2Cl_2-d_2).

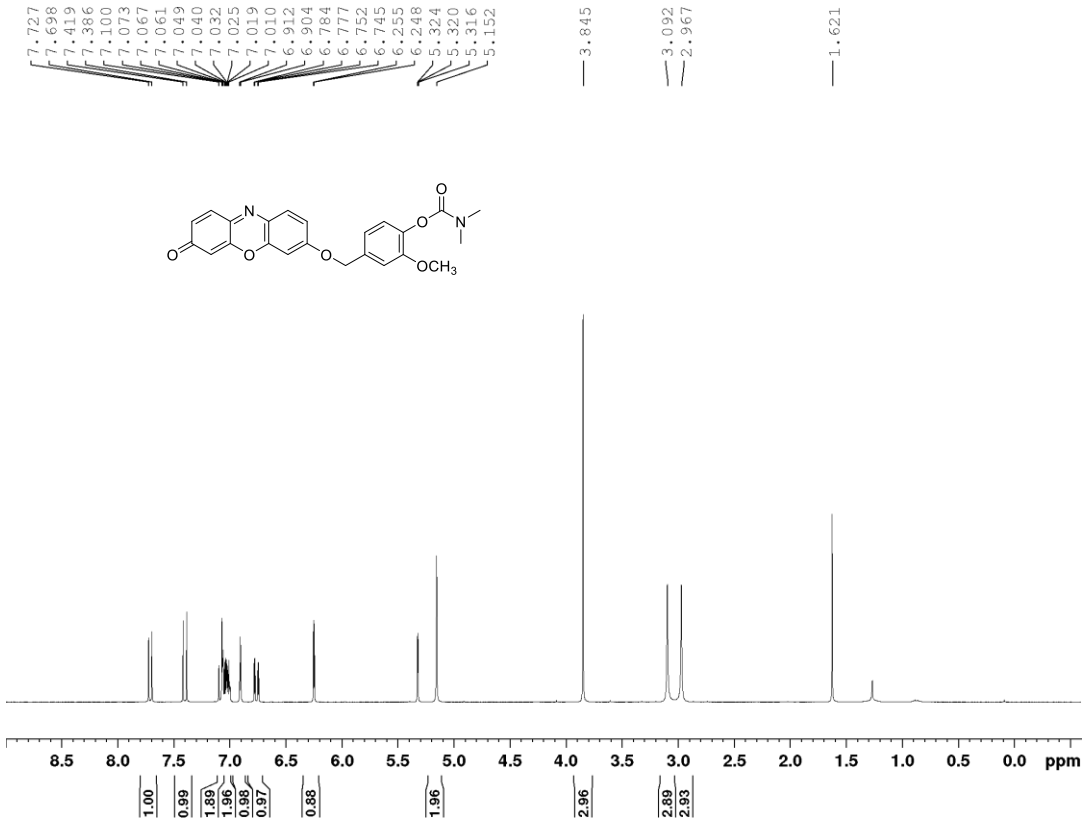


Fig. S78 ^{1}H-NMR spectrum of **P6** (300 MHz, 298 K, CD_2Cl_2-d_2).

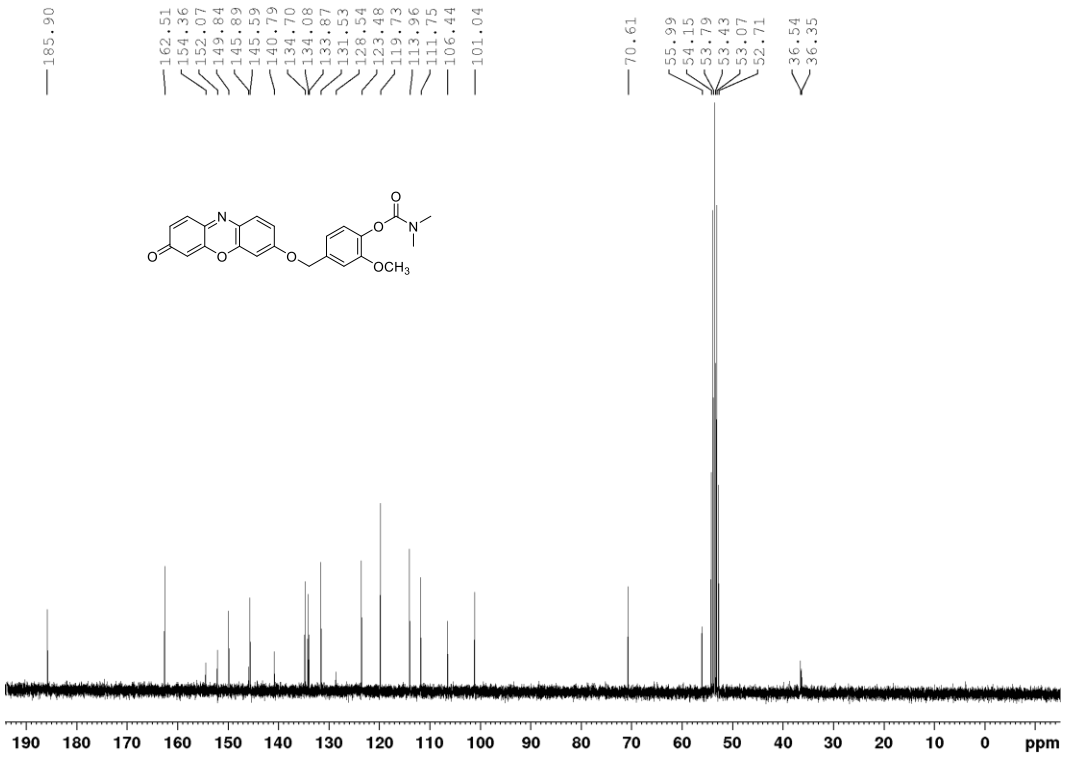


Fig. S79 ^{13}C-NMR spectrum of **P6** (75 MHz, 298 K, CD_2Cl_2-d_2).

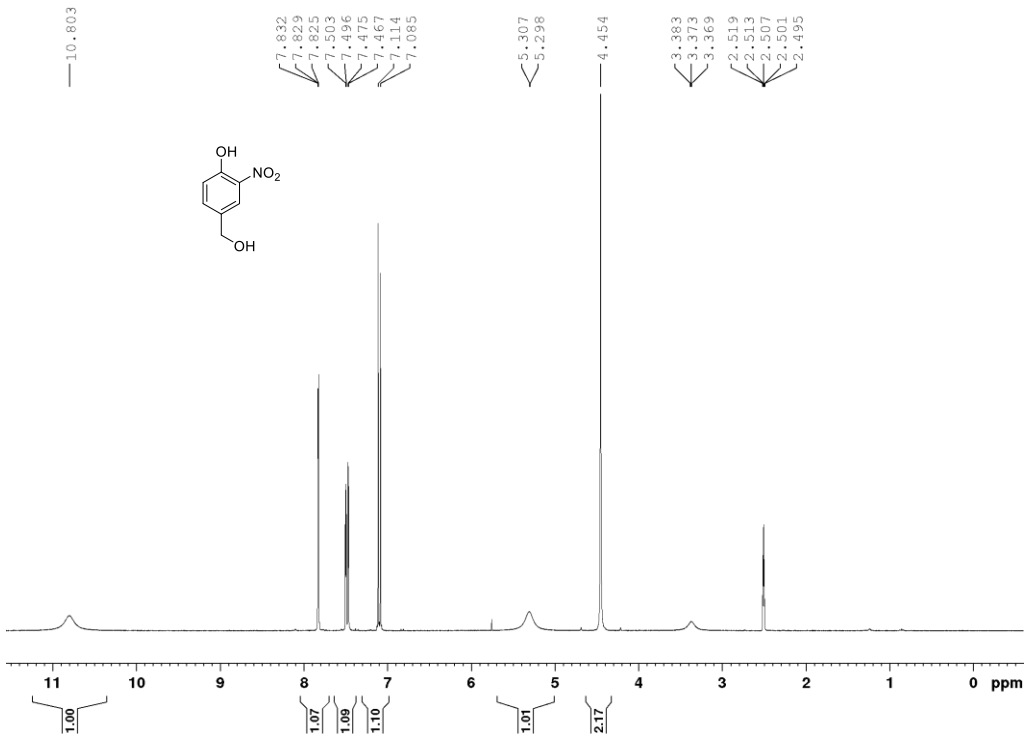


Fig. S80 ^{1}H-NMR spectrum of **P7-1** (300 MHz, 298 K, DMSO-d_6).

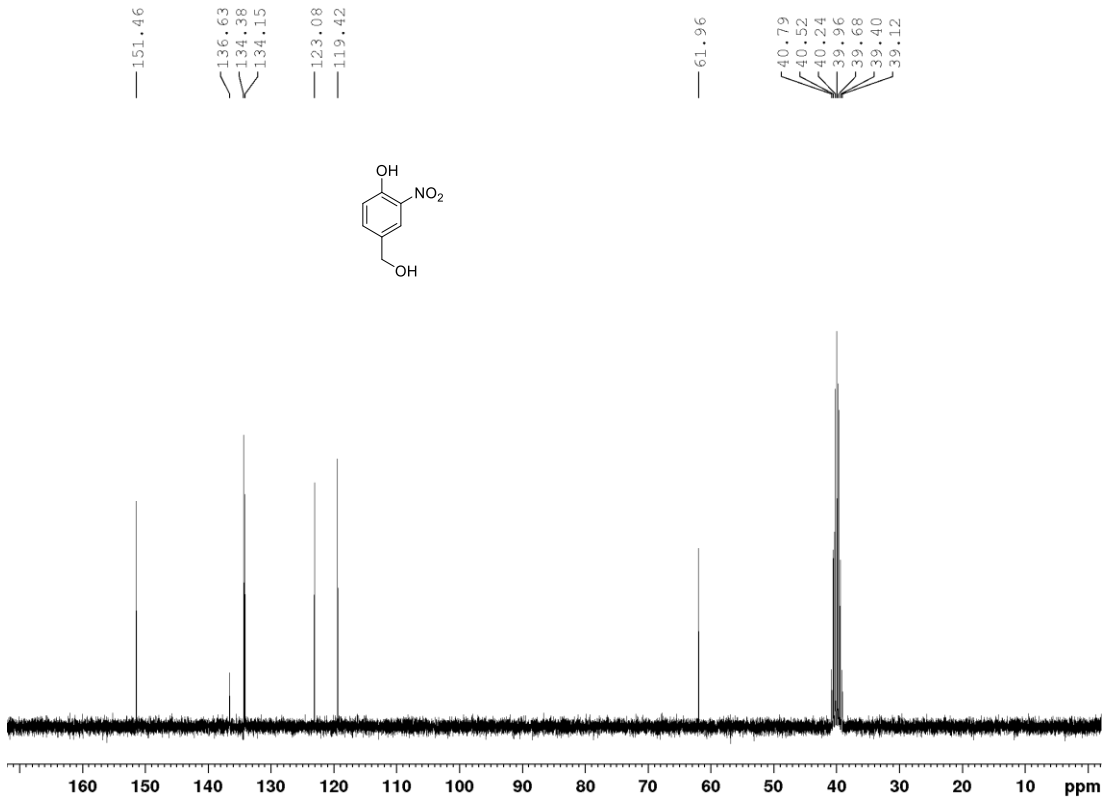


Fig. S81 ^{13}C-NMR spectrum of **P7-1** (75 MHz, 298 K, DMSO-d_6).

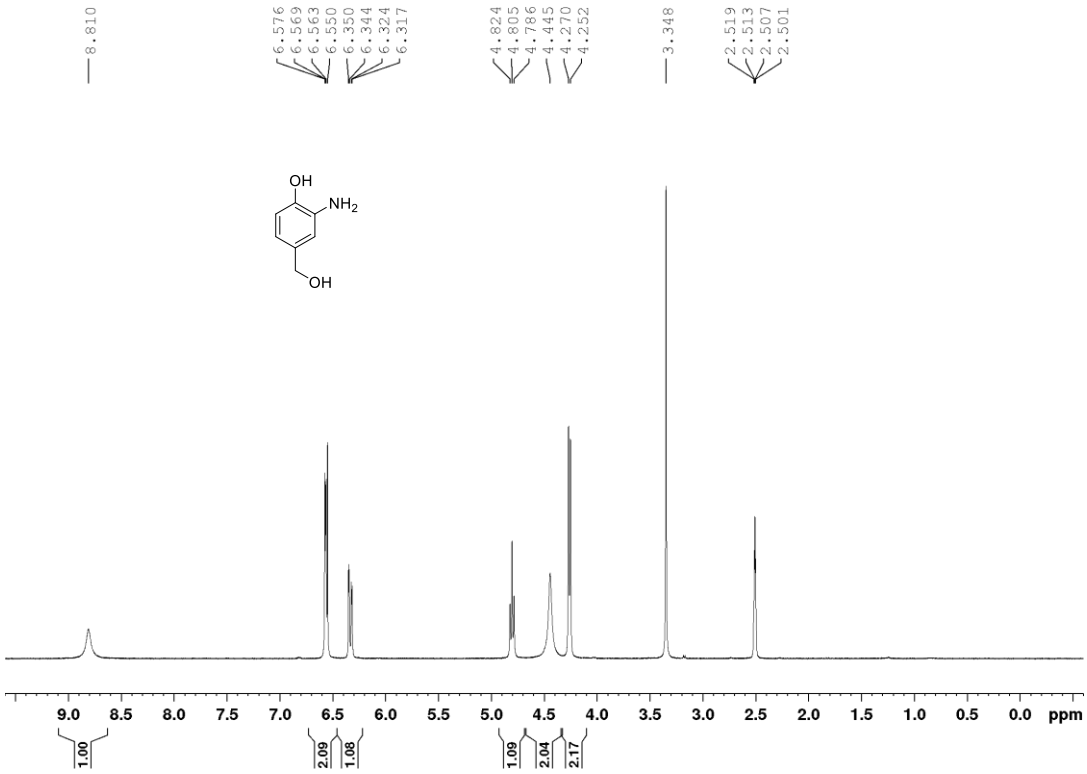


Fig. S82 ^{1}H-NMR spectrum of **P7-2** (300 MHz, 298 K, DMSO-d_6).

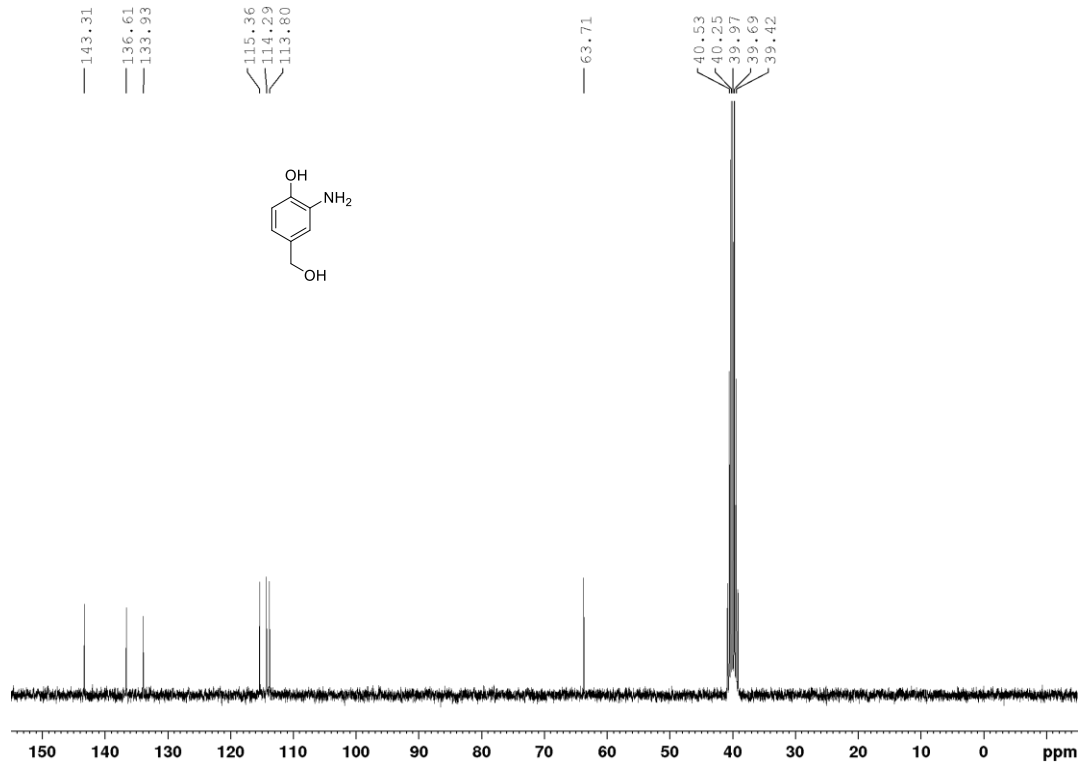


Fig. S83 ^{13}C-NMR spectrum of **P7-2** (75 MHz, 298 K, DMSO-d_6).

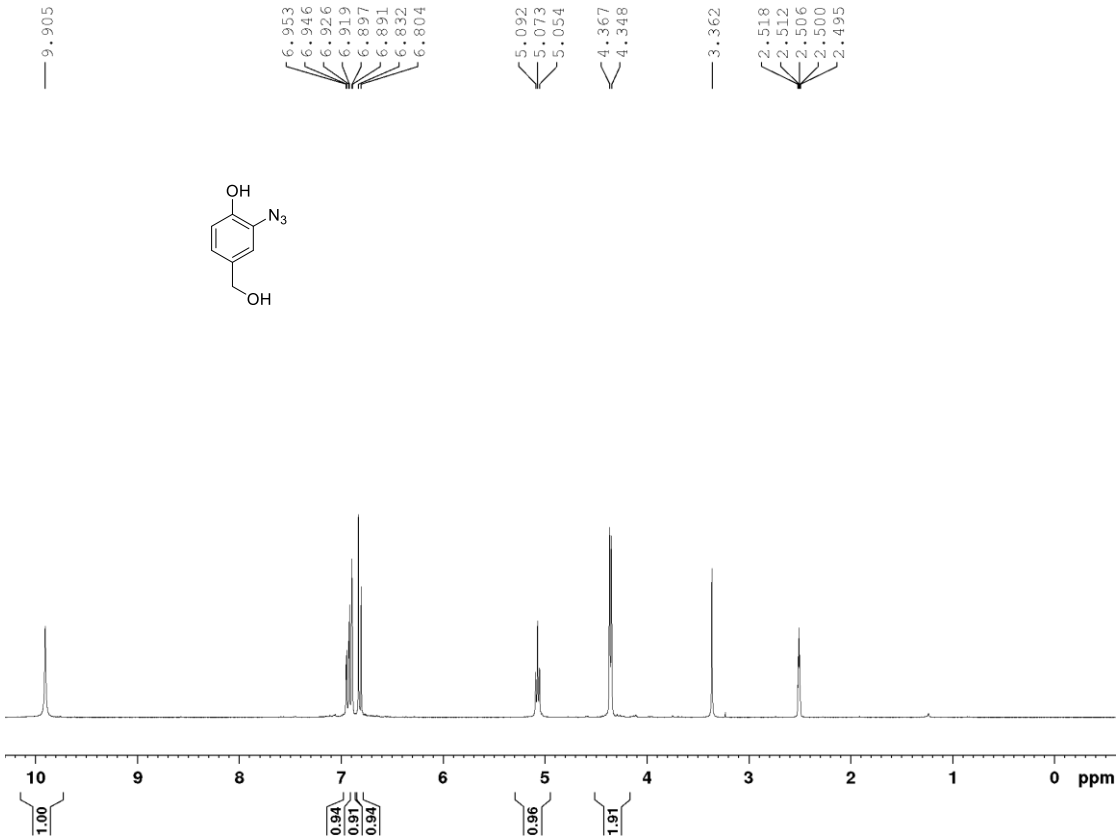


Fig. S84 ^{1}H-NMR spectrum of **P7-3** (300 MHz, 298 K, DMSO-d_6).

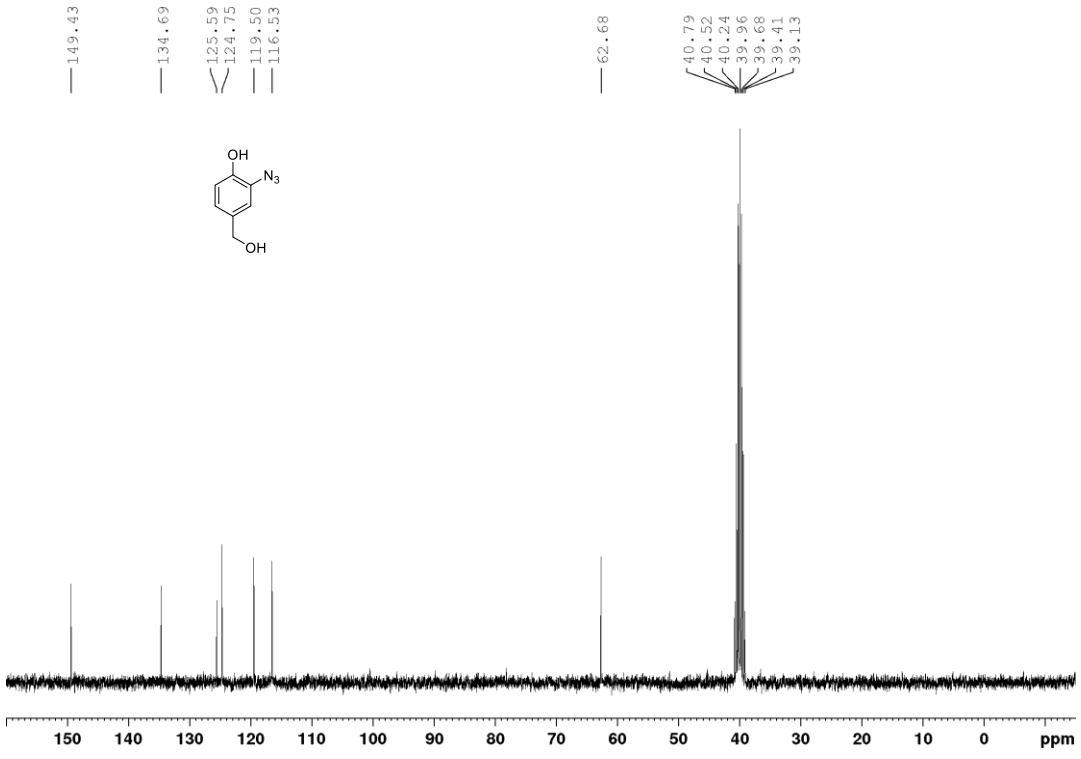


Fig. S85 ^{13}C-NMR spectrum of **P7-3** (75 MHz, 298 K, DMSO-d_6).

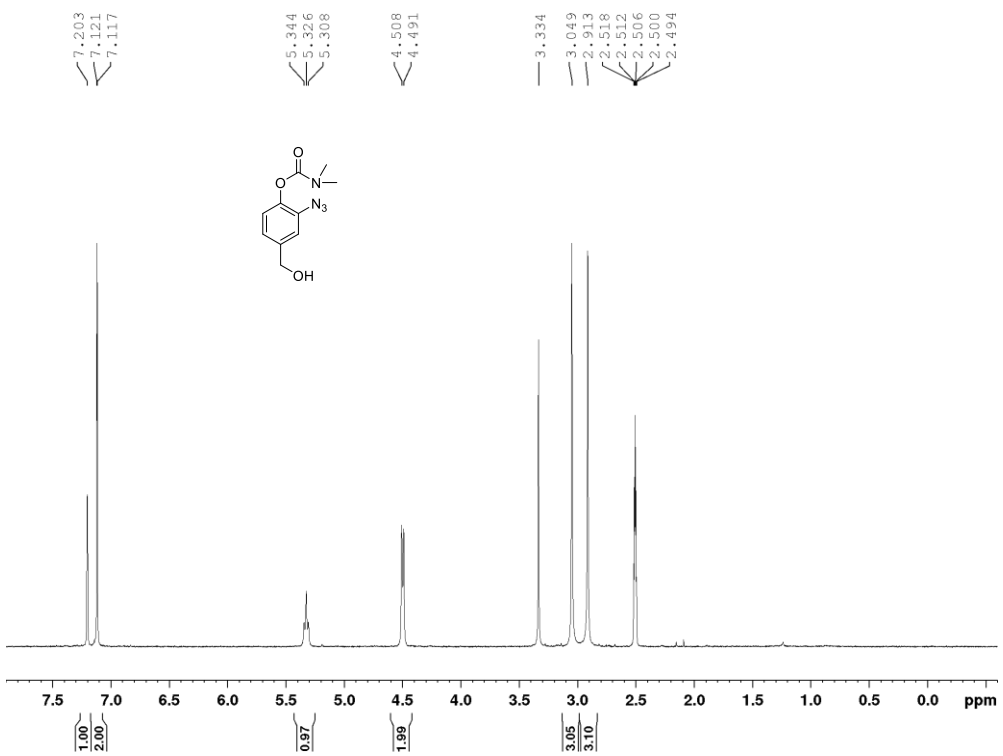


Fig. S86 ^{1}H-NMR spectrum of **P7-4** (300 MHz, 298 K, DMSO-d_6).

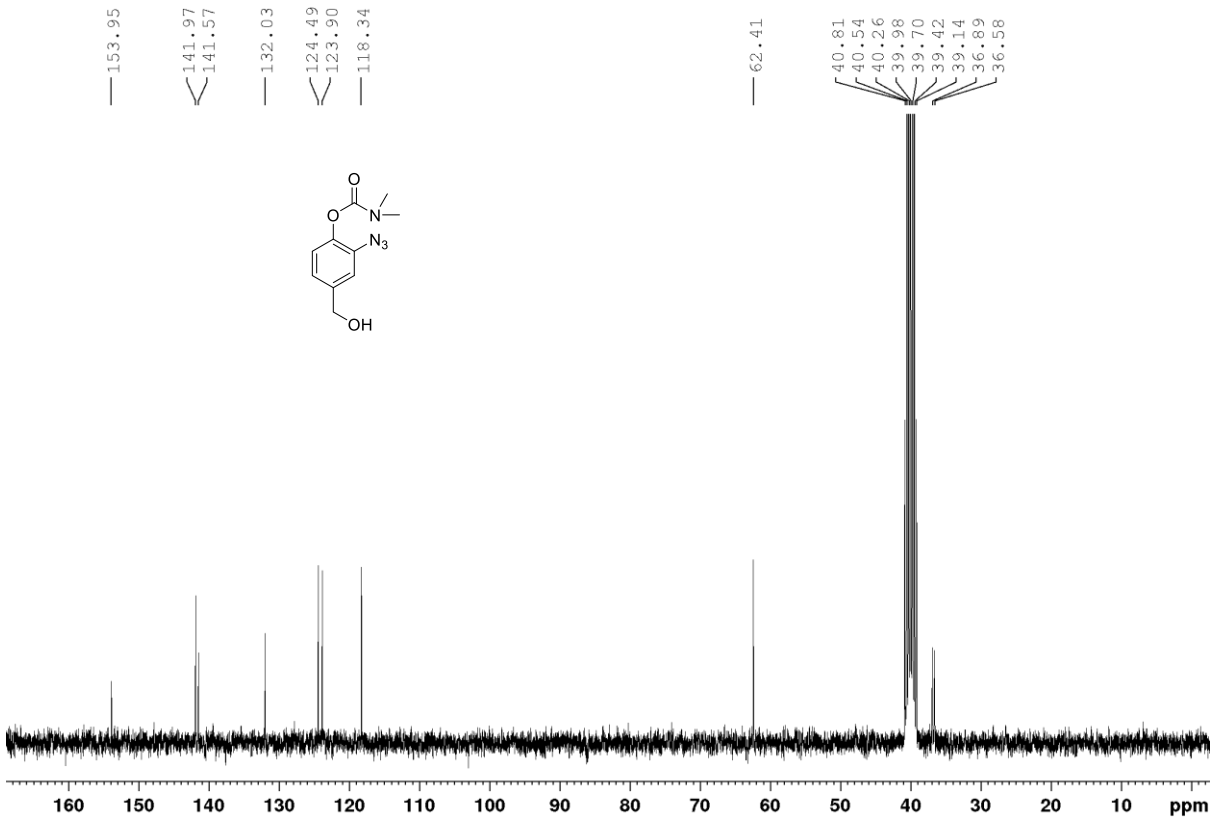


Fig. S87 ^{13}C-NMR spectrum of **P7-4** (75 MHz, 298 K, DMSO-d_6).

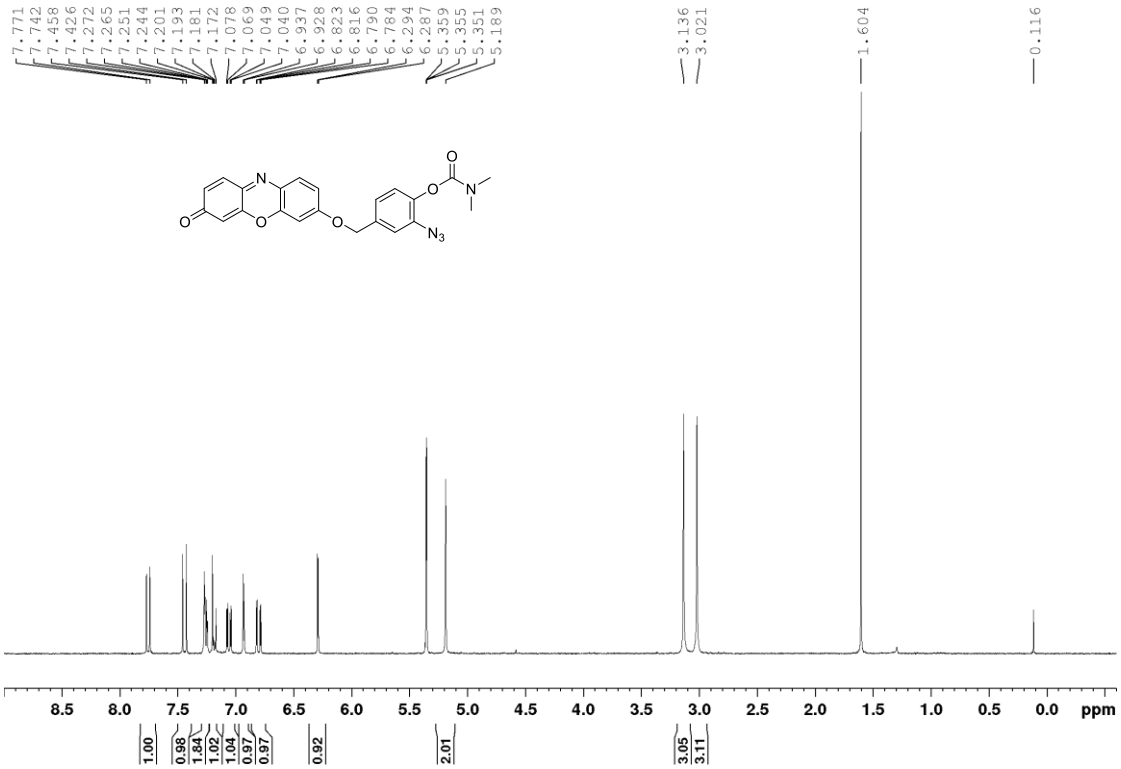


Fig. S88 ^{1}H-NMR spectrum of **P7** (300 MHz, 298 K, CD_2Cl-d_2).

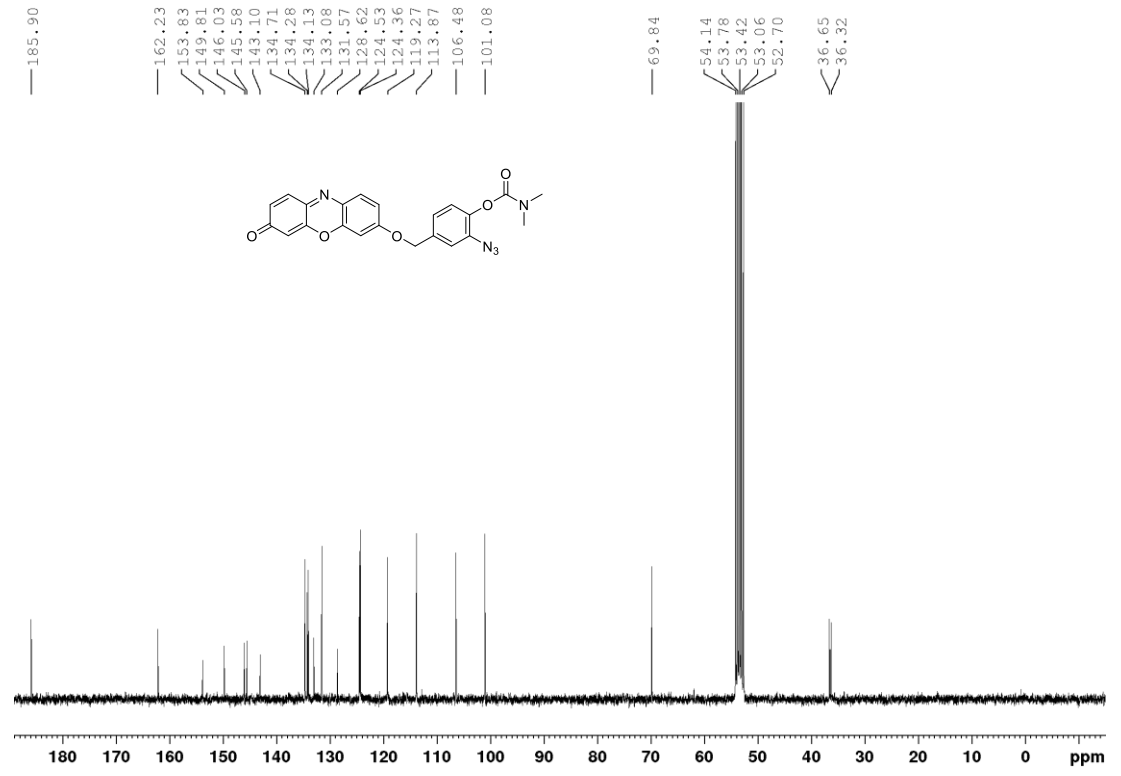


Fig. S89 ^{13}C-NMR spectrum of **P7** (75 MHz, 298 K, CD_2Cl-d_2).

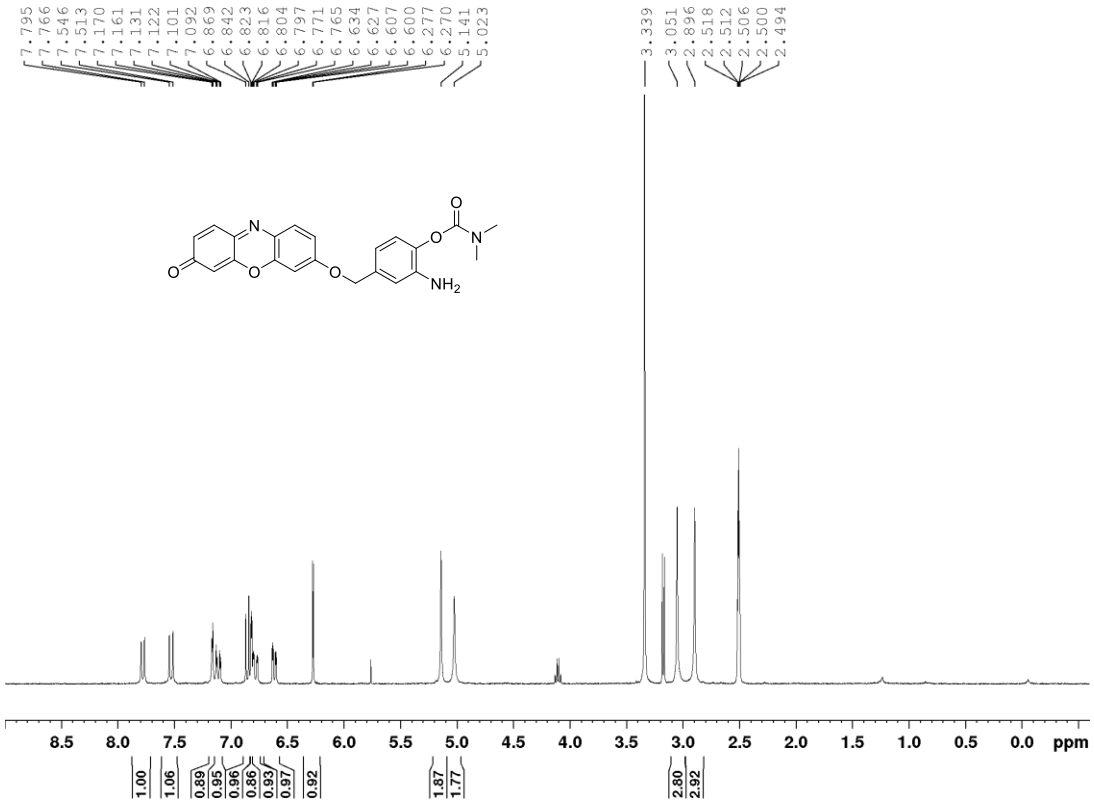


Fig. S90 ^{1}H-NMR spectrum of **P8** (300 MHz, 298 K, DMSO-d_6).

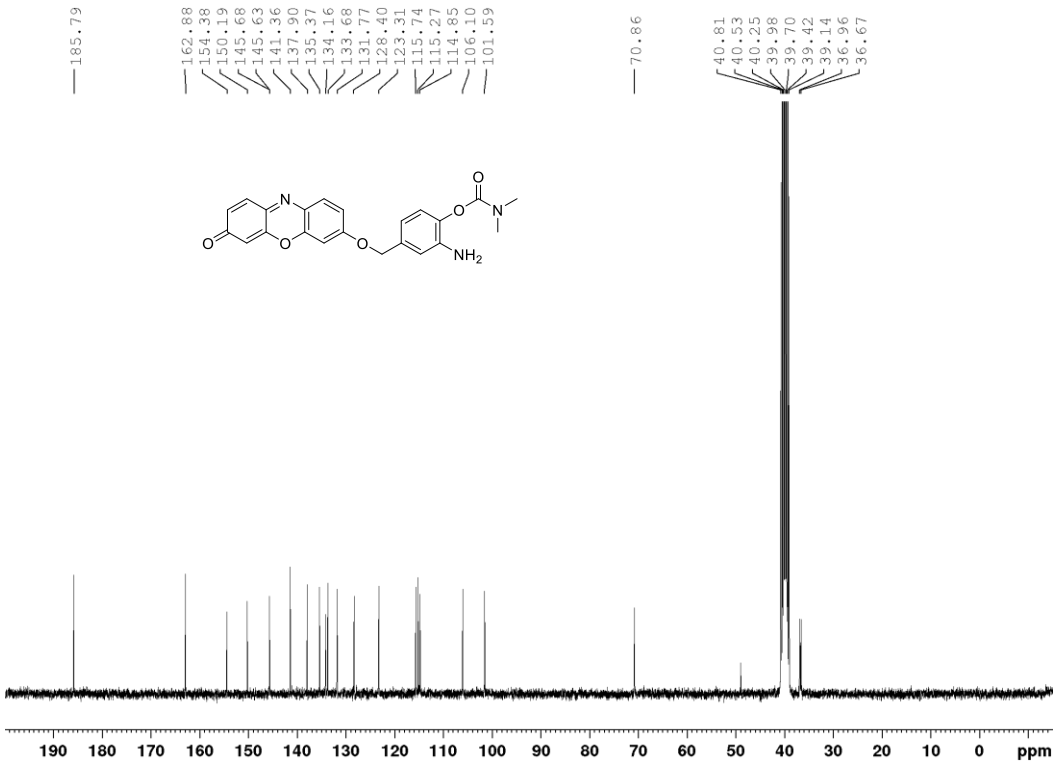


Fig. S91 ^{13}C-NMR spectrum of **P8** (75 MHz, 298 K, DMSO-d_6).

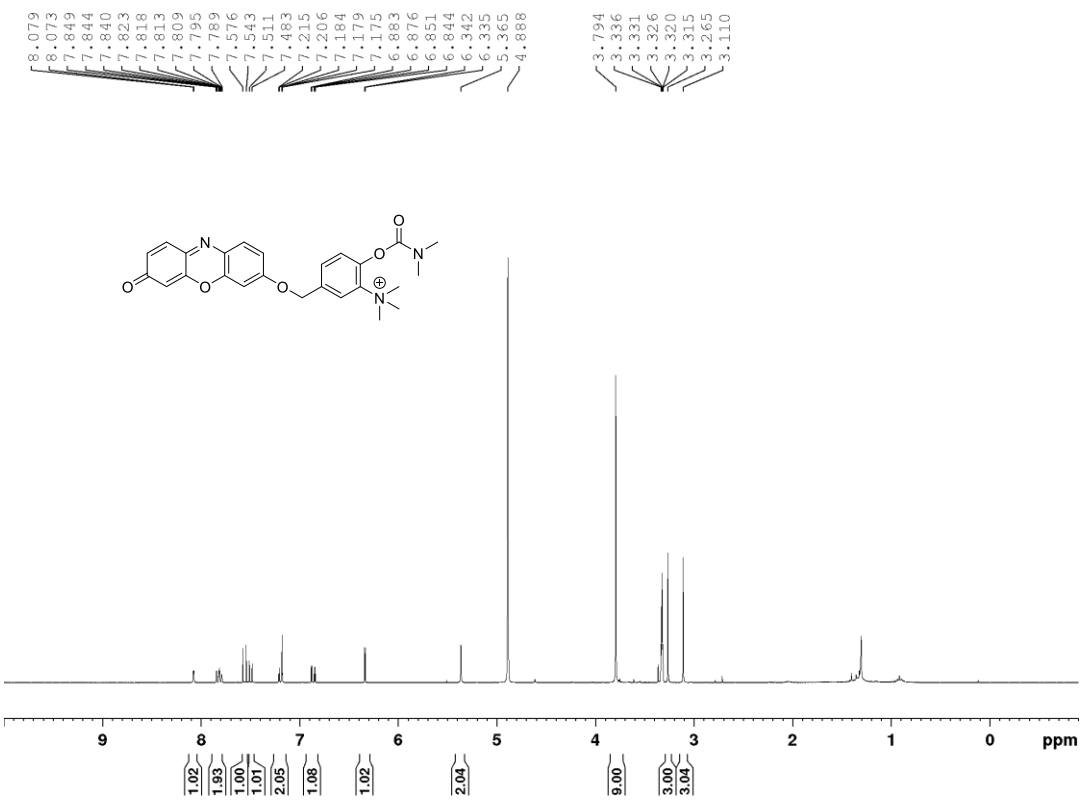


Fig. S92 ^{1}H-NMR spectrum of **P9** (300 MHz, 298 K, MeOD-d_4).

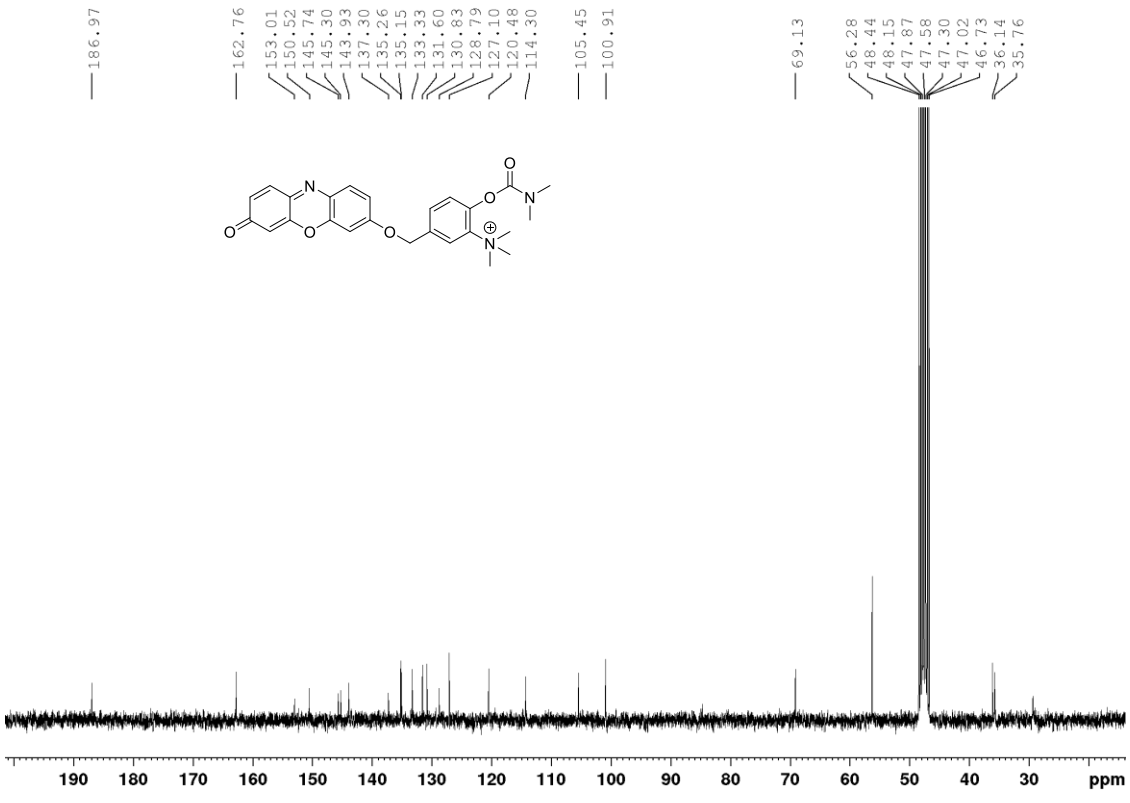


Fig. S93 ^{13}C-NMR spectrum of **P9** (75 MHz, 298 K, MeOD-d_4).

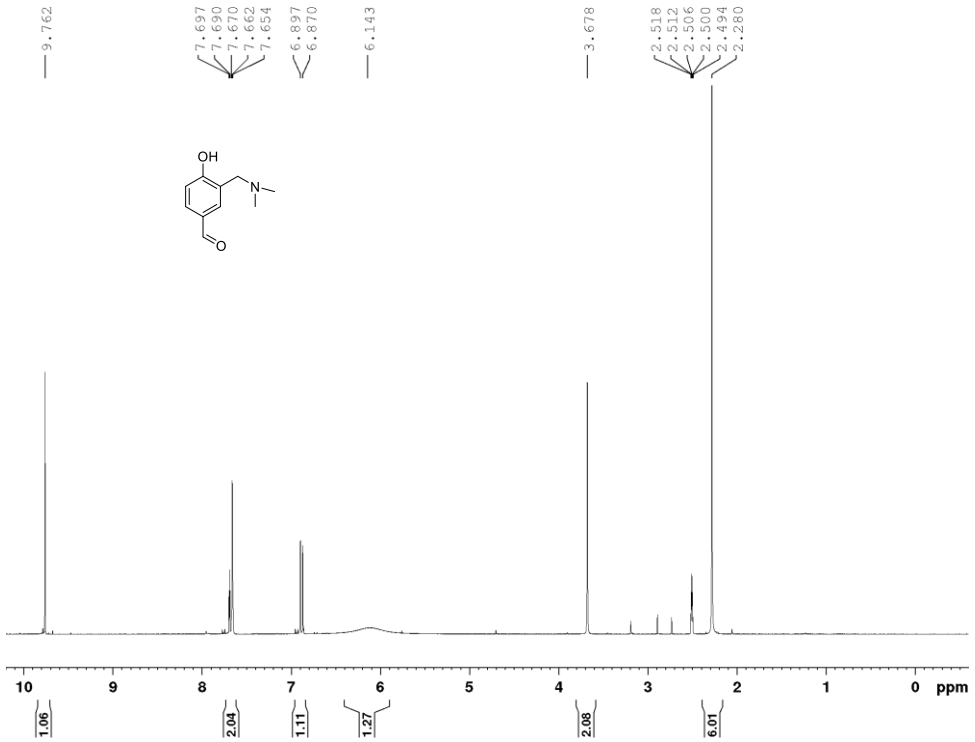


Fig. S94 ^{1}H-NMR spectrum of **P10-1** (300 MHz, 298 K, DMSO-d_6).

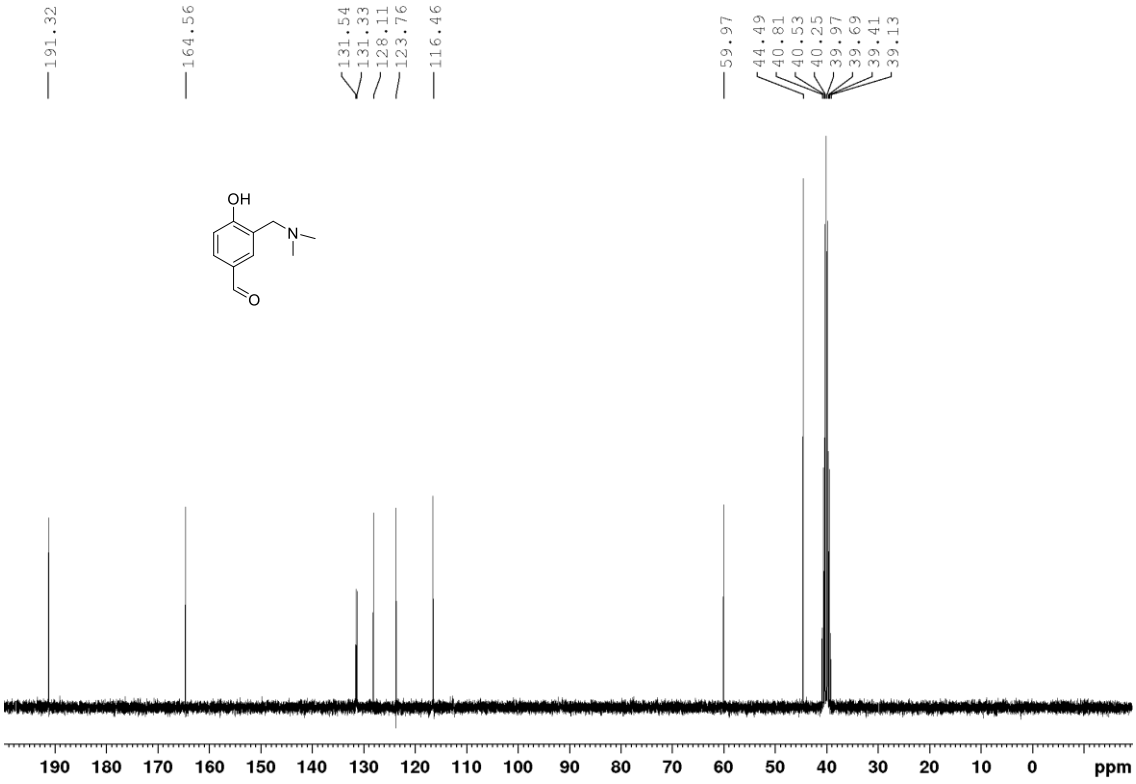


Fig. S95 ^{13}C-NMR spectrum of **P10-1** (75 MHz, 298 K, DMSO-d_6).

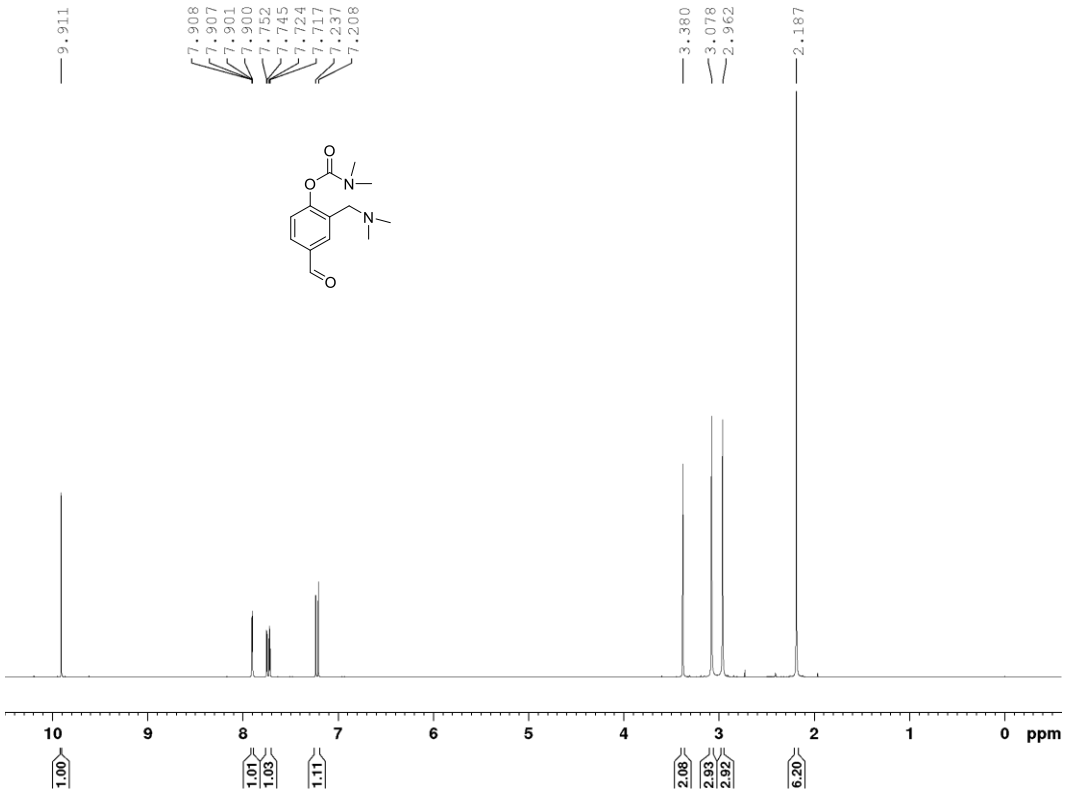


Fig. S96 ^{1}H-NMR spectrum of **P10-2** (300 MHz, 298 K, $CDCl_3$).

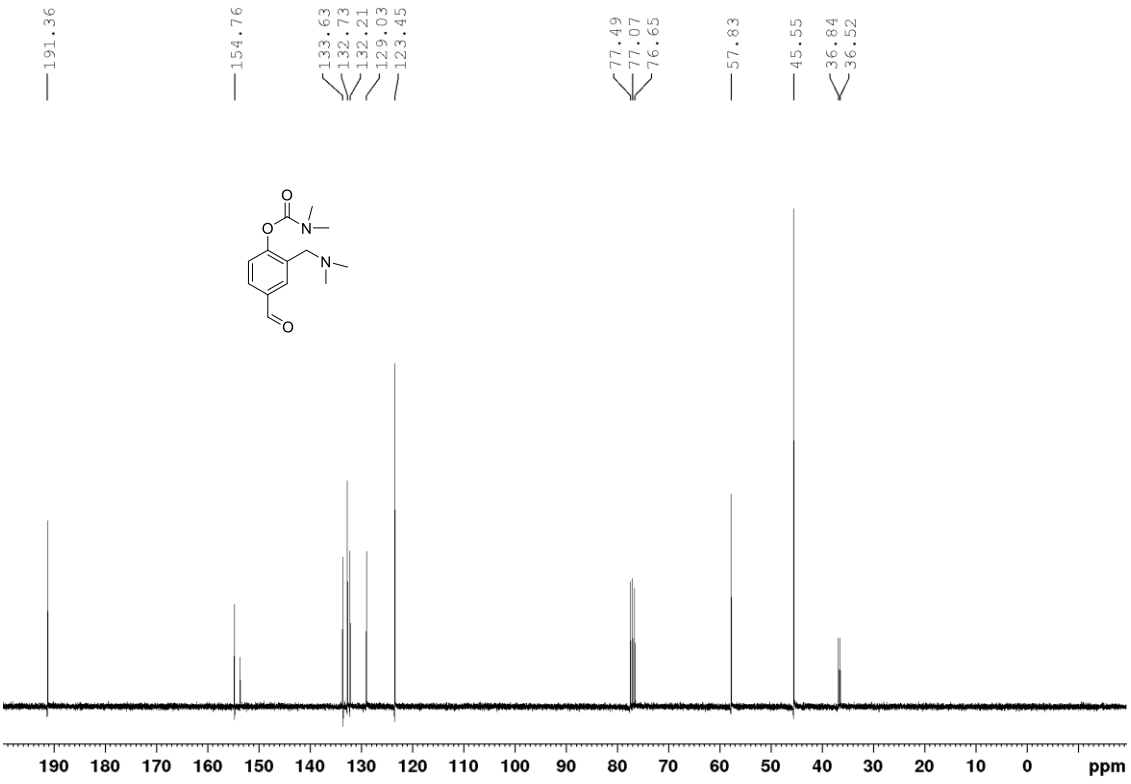


Fig. S97 ^{13}C-NMR spectrum of **P10-2** (75 MHz, 298 K, $CDCl_3$).

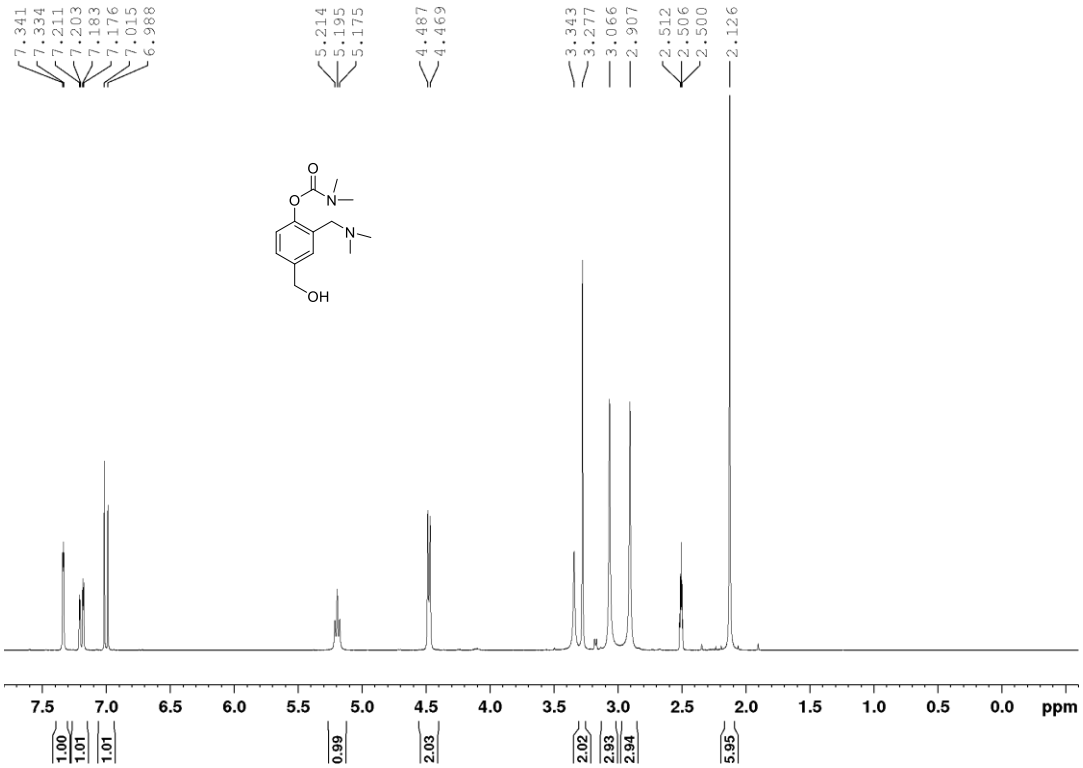


Fig. S98 ^{1}H-NMR spectrum of **P10-3** (300 MHz, 298 K, DMSO-d_6).

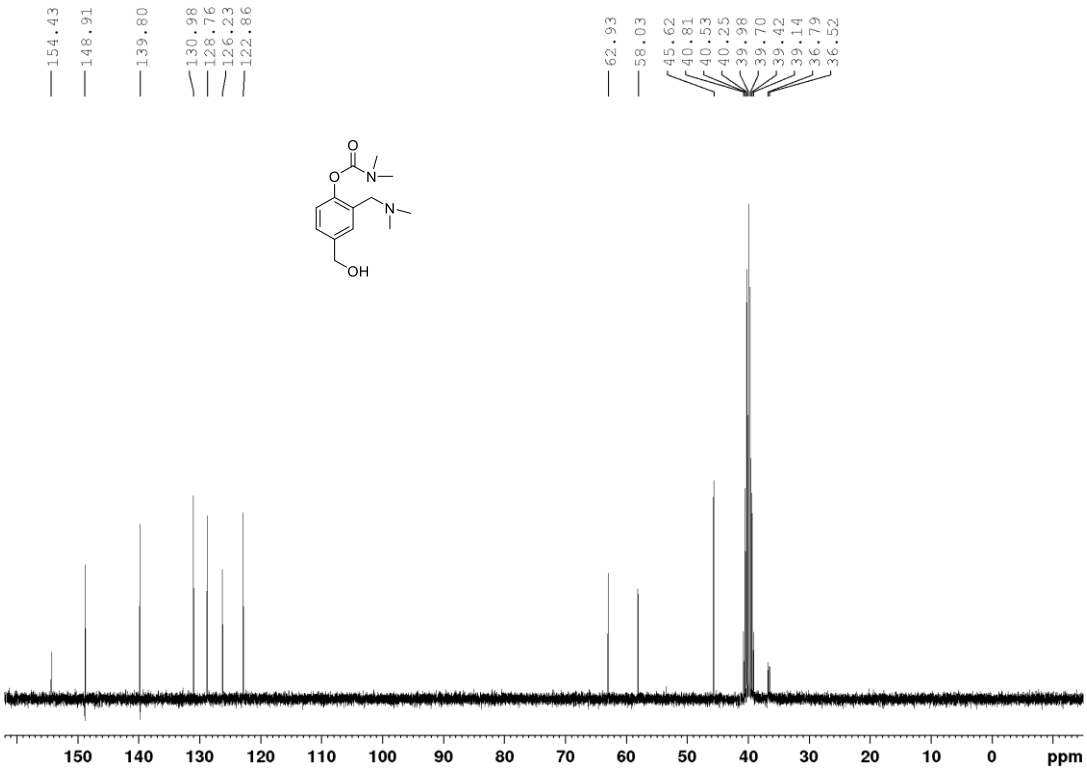


Fig. S99 ^{13}C-NMR spectrum of **P10-3** (75MHz, 298 K, DMSO-d_6).

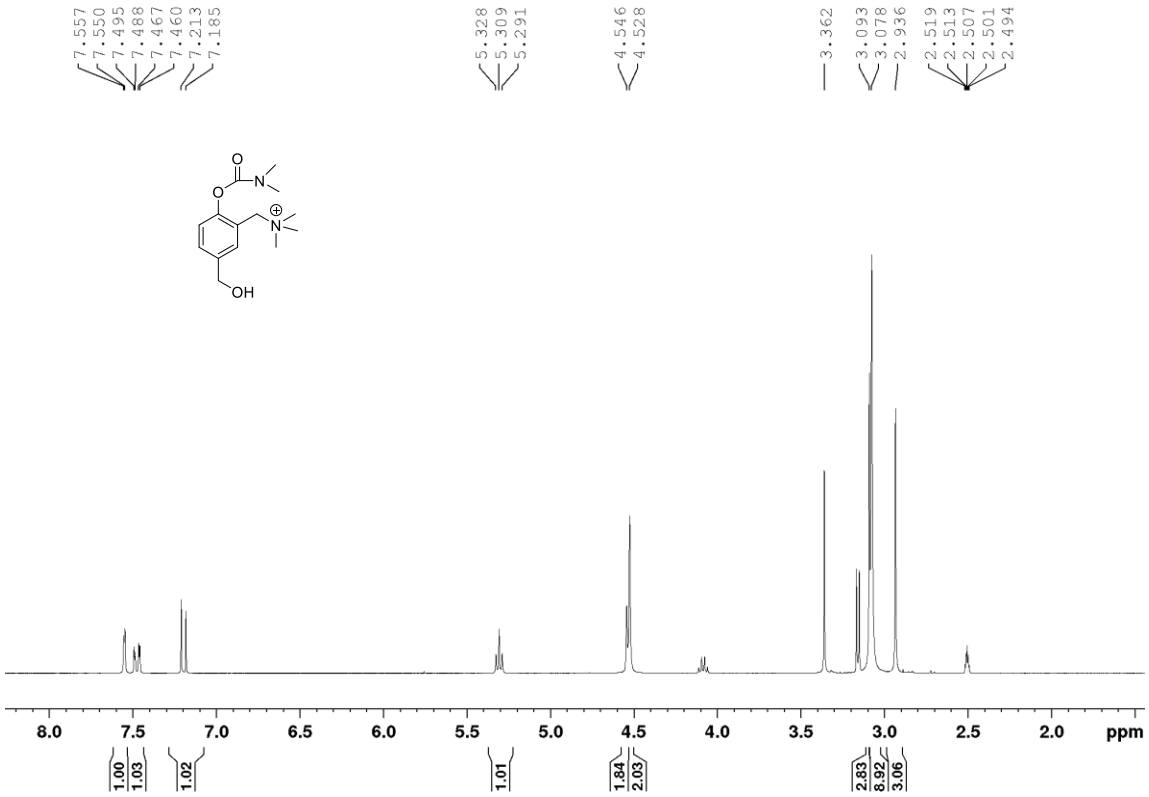


Fig. S100 ^{1}H-NMR spectrum of **P10-4** (300 MHz, 298 K, DMSO-d_6).

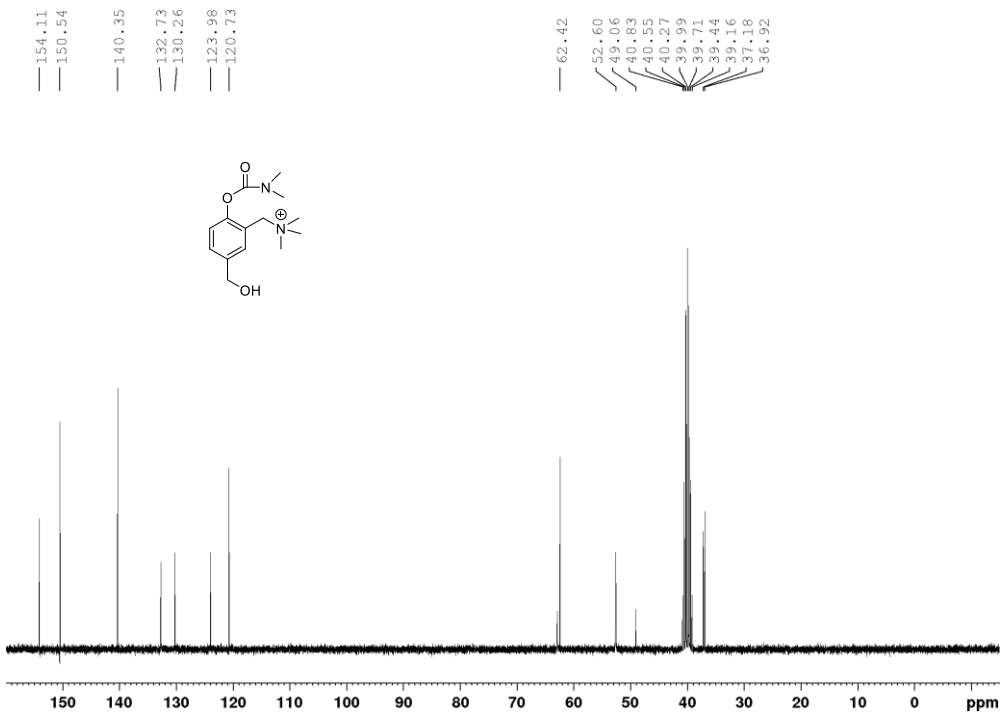


Fig. S101 ^{13}C-NMR spectrum of **P10-4** (75 MHz, 298 K, DMSO-d_6).

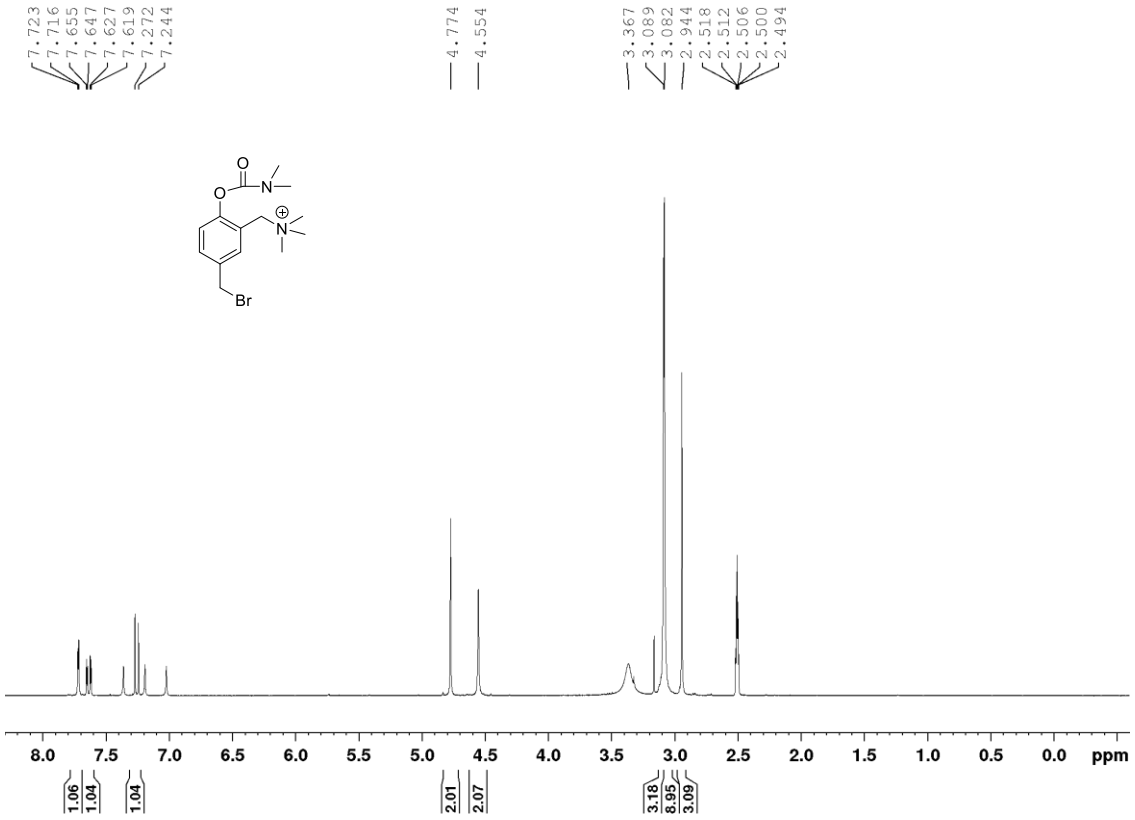


Fig. S102 ^{1}H-NMR spectrum of **P10-5** (300 MHz, 298 K, DMSO-d_6).

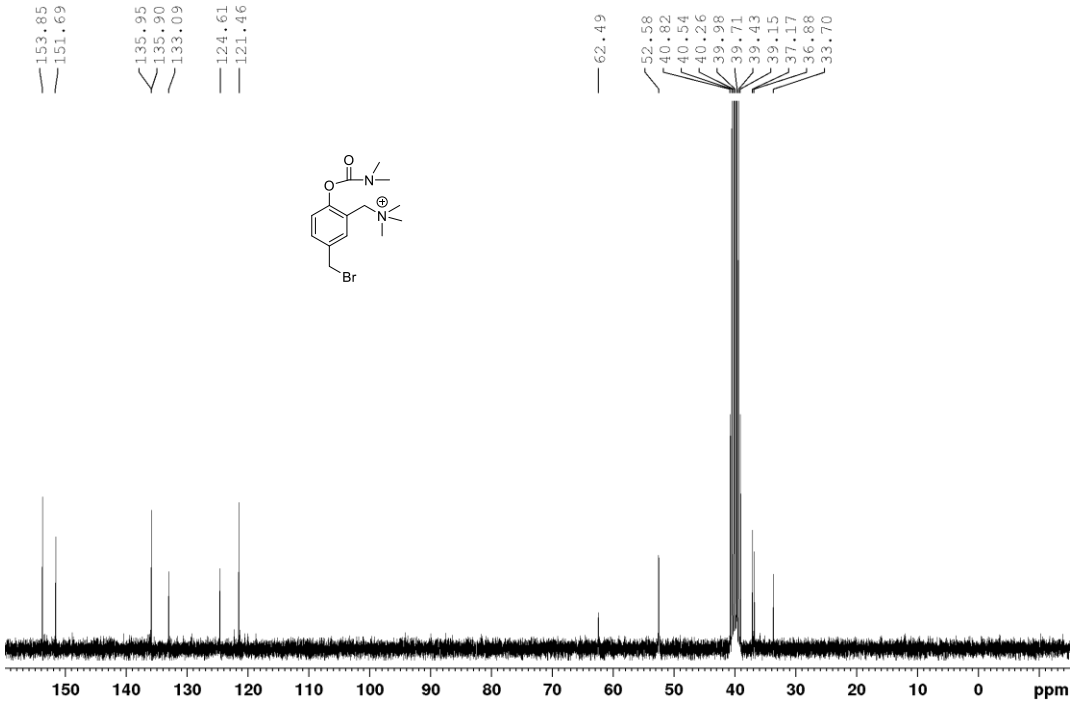


Fig. S103 ^{13}C-NMR spectrum of **P10-5** (75 MHz, 298 K, DMSO-d_6).

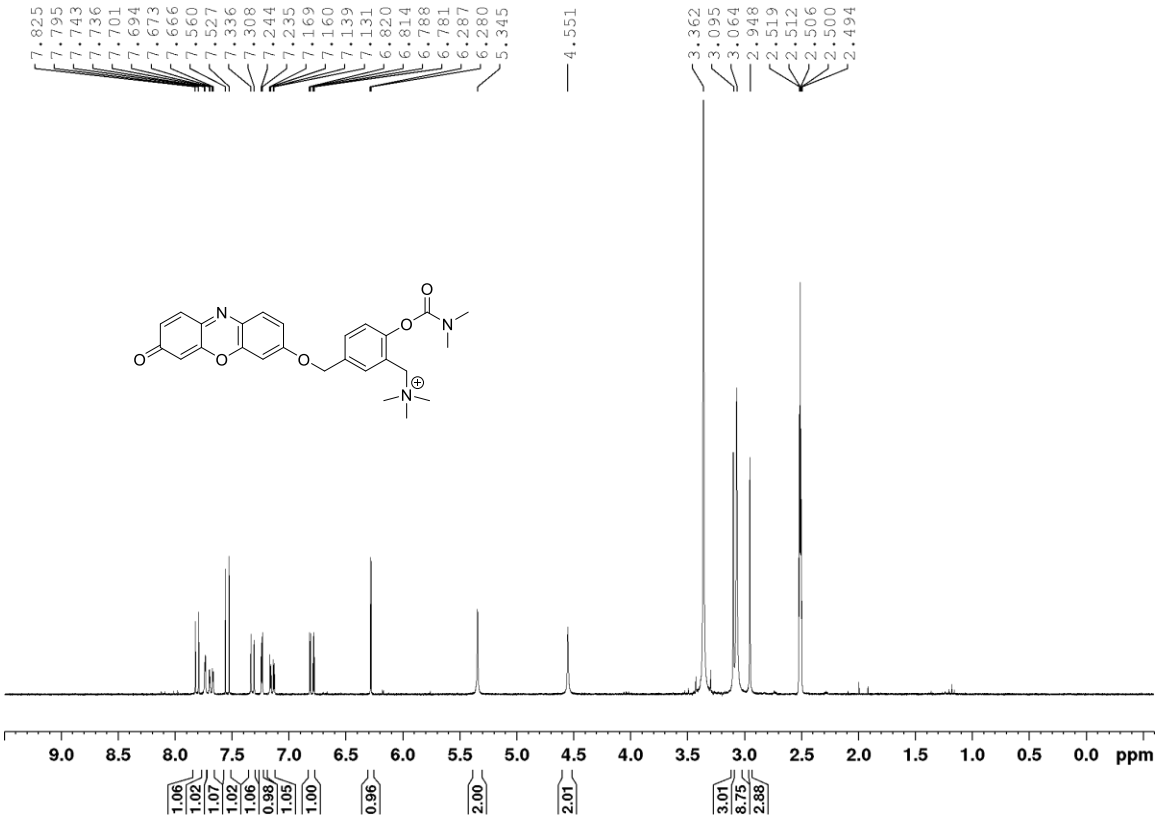


Fig. S104 ^{1}H-NMR spectrum of **P10** (300 MHz, 298 K, DMSO-d_6).

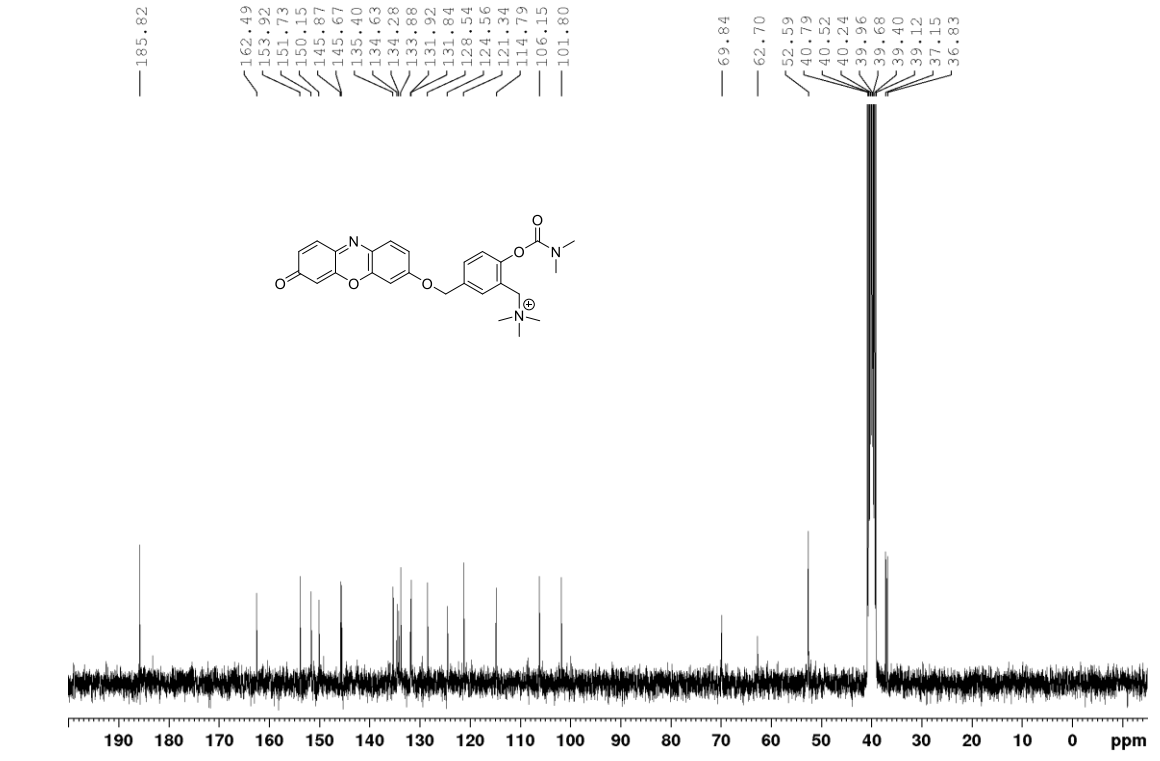


Fig. S105 ^{13}C-NMR spectrum of **P10** (75 MHz, 298 K, DMSO-d_6).

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