Supporting Information to Accompany "**Dual-Color Imaging of Cytosolic and Mitochondrial Zinc Ions in Live Tissue with Two-Photon Fluorescent Probes**"

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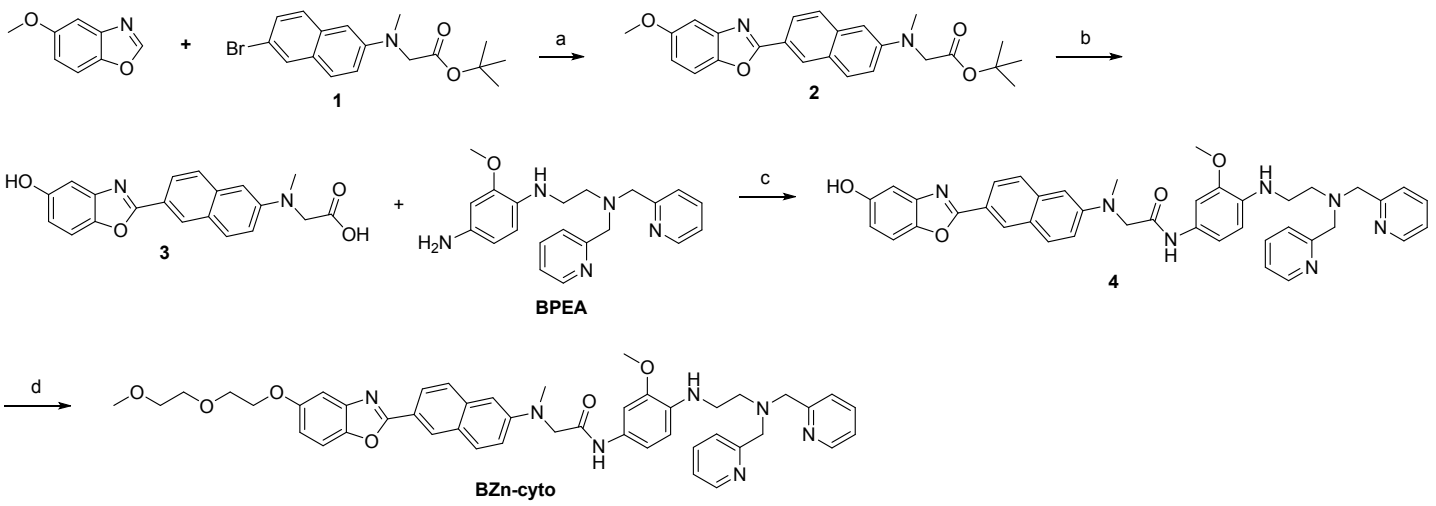
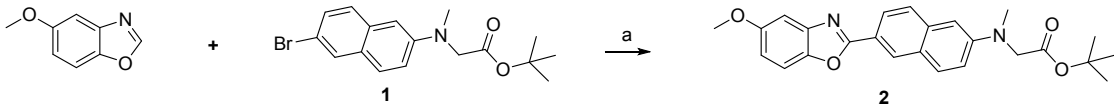
Scheme S1. Synthesis of **BZn-cyto**:

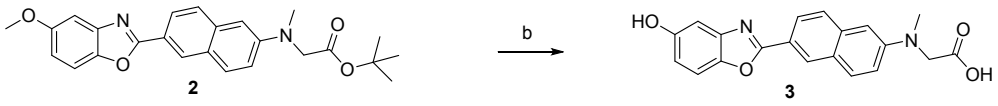


Reagents and conditions: (a) $Pd(OAc)_2$, *t*-Bu_3P, CuBr, Cs_2CO_3, Toluene, 90 °C, 16 h, 56 %; (b) BBr_3, CH_2Cl_2, -78 °C - rt, 16 h, 63 %; (c) HATU, DIPEA, BPEA, DMF, rt, 16 h, 37 %; (d) $CH_3OCH_2CH_2OCH_2CH_2OTs$, K_2CO_3, DMF, rt, 22 h, 61 %.

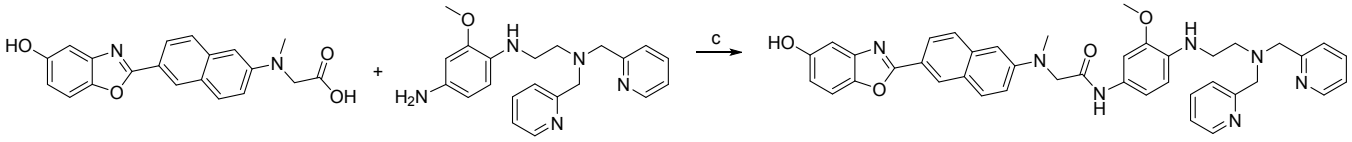
5-Methoxybenzo[*d*]oxazole[1], 2-*N*-methyl-*N*-[(*t*-butoxycarbonyl)methyl]amino-6-bromo naphthalene **1**[2] and 4-amino-2-methoxy-*N*-{2-[*N*,'*N*'-bis(2-picolyl)]amino}ethylaniline (BPEA)[3] were prepared by the literature methods. Syntheses of other compounds are described below.



Compound 2. A flame-dried round bottom flask with a magnetic stirring bar was charged with CuBr (15 mg, 0.11 mmol), $Pd(OAc)_2$ (12 mg, 0.050 mmol), *t*-Bu_3P (11 mg, 0.050 mmol), Cs_2CO_3 (160 mg, 0.50 mmol), 5-methoxybenzo[*d*]oxazole[1] (75 mg, 0.50 mmol) and compound **1**[2] (180 mg, 0.50 mmol). Toluene (3 mL) was syringed under an atmosphere of argon and the reaction mixture was heated to 90 °C for 16 h before it was cooled to room temperature and filtered through a small pad of Celite. The solid residue was washed with CH_2Cl_2 (20 mL) and the combined organic layers were evaporated under reduced pressure to deliver a crude residue which was purified by silica gel column chromatography using 9% EtOAc in *n*-hexane as the eluent to furnish compound **2** (120 mg, 0.28 mmol) in 56 % yield as a white solid. m. p. 148-150 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (s, 1H), 8.16 (d, *J* = 8.8 Hz, 1H), 7.83 (d, *J* = 8.8 Hz, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 7.46 (d, *J* = 8.8 Hz, 1H), 7.12 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.94 (d, *J* = 2.7 Hz, 1H), 6.93-6.89 (m, 2H), 4.10 (s, 2H), 3.88 (s, 3H), 3.20 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 164.6, 157.2, 148.3, 145.3, 143.2, 136.5, 130.1, 127.8, 126.8, 126.1, 124.3, 120.4, 115.9, 112.9, 110.4, 106.0, 102.6, 81.8, 55.9, 55.3, 39.8, 28.0. HRMS (FAB): calcd. for $C_{25}H_{26}N_2O_4$ $[M]^+$: 418.1893, Found 418.1893.

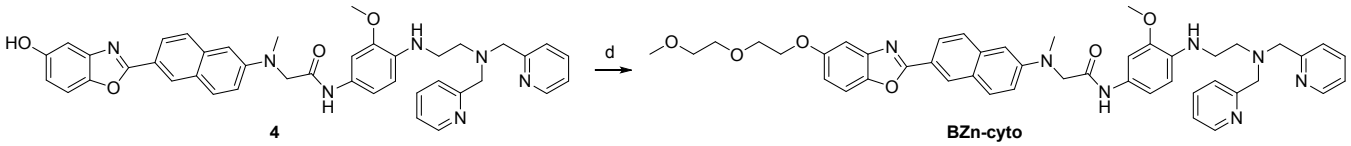


Compound 3. BBr_3 (1.0 M in CH_2Cl_2, 1.1 mL, 1.1 mmol) was added drop wise to a solution of *t*-butyl ester **2** (112 mg, 0.27 mmol) in CH_2Cl_2 (5 ml) at -78 °C for a period of 2 minutes. The reaction mixture was stirred for 2h at the same temperature before it was gradually warmed to rt and allowed to stir for 16 h. The reaction mixture was cooled to 0 °C, quenched by slow addition of ice cold water (2 mL) and diluted with CH_2Cl_2 (10 mL). The solid residue was filtered and washed successively with CH_2Cl_2 (5 mL), water (5 mL) and 5% MeOH in CH_2Cl_2 (2 x 5 mL) and dried in *vaccuo* to furnish title compound **3** (59 mg, 0.17 mmol) in 63 % yield as a dark green solid, which was taken ahead for the next step without further purification. m. p. 181-183 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.53 (brs, 1H), 8.55 (s, 1H), 8.04 (d, J = 8.8 Hz, 1H), 7.94 (d, J = 8.8 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 8.8 Hz, 1H), 7.24 (d, J = 8.8 Hz, 1H), 7.08 (d, J = 2.2 Hz, 1H), 6.99 (s, 1H), 6.82 (dd, J = 8.8, 2.2 Hz, 1H), 4.31 (s, 2H), 3.11 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.8, 163.5, 155.0, 148.7, 143.9, 142.7, 136.2, 129.9, 127.2, 126.7, 125.3, 123.8, 119.4, 116.3, 113.1, 110.5, 105.1, 104.5, 53.2. HRMS (FAB): calcd. for $C_{20}H_{17}N_2O_4$ $[M+H]^+$: 349.1188, Found 349.1188.



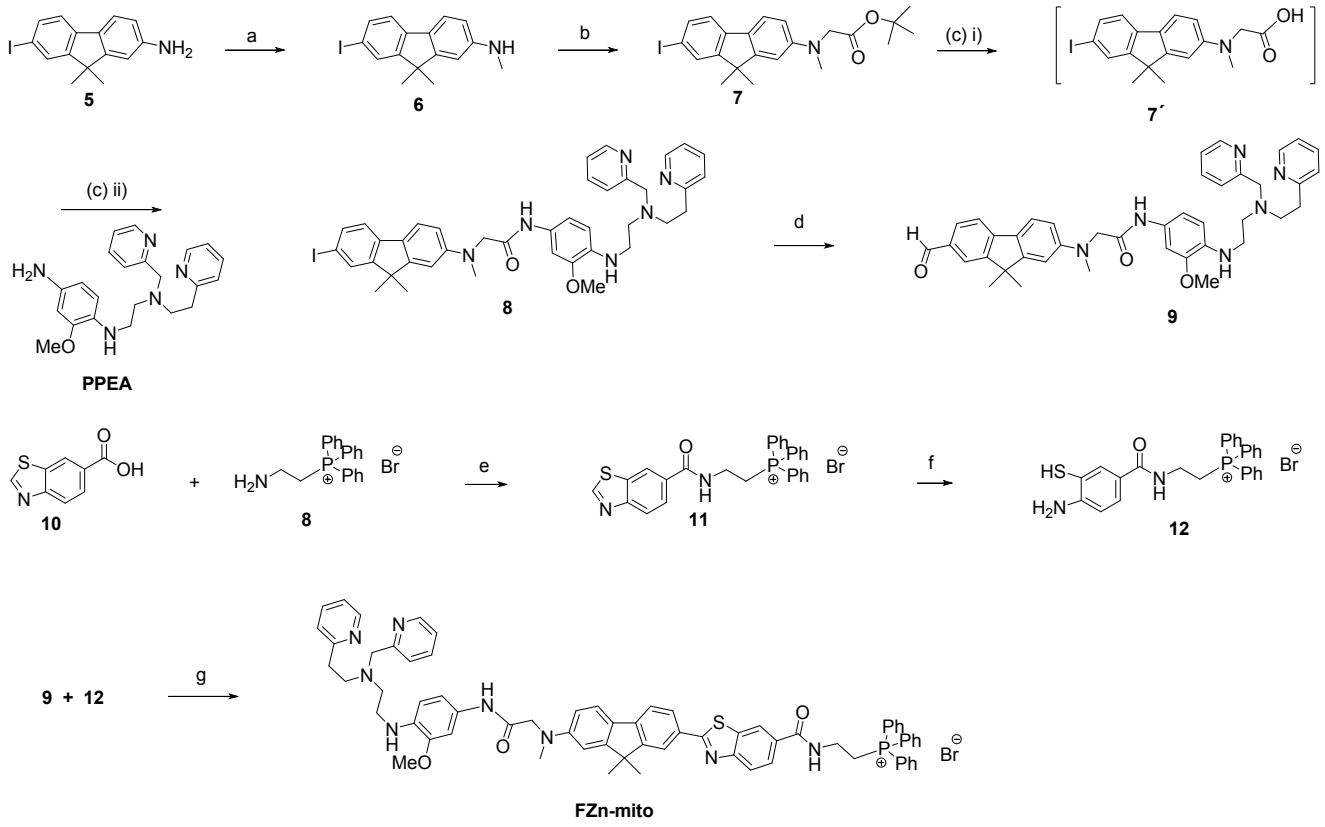
Compound 4. *O*-(7-Azabenzotriazole-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (HATU, 66 mg, 0.17 mmol) was added to the solution of compound **3** (50 mg, 0.14 mmol) in DMF (1 mL). The resulting clear solution was stirred for 10 min at room temperature, before diisopropylethylamine (DIPEA, 75 µL, 0.43 mmol)) was added drop wise followed by a solution of (BPEA)**3** (52 mg, 0.14 mmol) in DMF (0.5 mL). The reaction mixture was stirred for 16 h at rt before it was diluted with water (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic extracts were washed with brine (1 x 5 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to yield a crude residue which was purified by reverse-phase HPLC using the following conditions: YMC-Pack ODS-A, (20 x 250 mm), 5 µm, 12 nm; mobile phase, MeOH / H_2O (0.1% FA) = 40/60 to 55/45 to 100/0 to 100/0 (linear gradient, 20 then 21 then 30 min); flow rate, 10 mL/min; temperature, 40 °C; injection, 0.1 mL, detection UV (260 nm). The fraction of interest at retention time 16.5 min was collected and lyophilized to furnish compound **4** (37 mg, 0.053 mmol) in 37 % yield as a brown, gummy product. ^{1}H NMR (300 MHz, Acetone-d_6): δ 8.60-8.52 (m, 3H), 8.12 (dd, J = 8.8, 2.2 Hz, 1H), 7.99-7.87 (m, 3H), 7.83 (d, J = 8.8 Hz, 1H), 7.60-7.52 (m, 2H), 7.48 (d, J = 8.8 Hz, 1H), 7.46-7.40 (m, 2H), 7.39 (d, J = 2.2 Hz, 1H), 7.31 (dd, J = 8.8, 2.2 Hz, 1H), 7.16-7.11 (m, 2H), 6.96 (dd, J = 8.8, 2.2 Hz, 1H), 6.90 (dd, J = 8.8, 2.2 Hz, 1H), 6.46 (d, J = 8.8 Hz, 1H), 4.82 (s, 4H), 4.28 (s, 2H), 3.83 (s, 3H), 3.67 (s, 4H), 3.29 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 169.6, 165.8, 156.6, 153.2, 151.0, 150.2, 149.0, 146.5, 145.1, 140.3, 138.3, 135.1, 131.7, 131.5, 129.1, 128.6, 127.9, 125.9, 125.5, 122.2, 118.4, 114.9, 114.3, 112.1, 111.9,

107.9, 106.5, 105.6, 59.4, 58.6, 57.1, 55.3, 41.2, 40.9. HRMS (FAB): calcd. for $C_{41}H_{40}N_7O_4$ $[M+H]^+$: 694.3142, Found 694.3143.



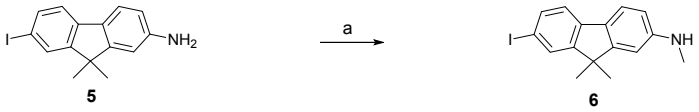
BZn-cyto. A solution of diethyleneglycol monomethyl ether tosylate (14 mg, 0.050 mmol) in DMF (1 mL) was added drop wise to a mixture of compound **4** (29 mg, 0.042 mmol) and K_2CO_3 (7.0 mg, 0.050 mmol) in DMF (2 mL) at rt under argon atmosphere. Resulting mixture was stirred for 22 h while TLC examination revealed complete consumption of the starting material. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (4 x 5 mL). Combined organic extracts were washed with brine (5 mL), dried over anhydrous $MgSO_4$ and filtered. Evaporation of the solvent under *vaccuo* afforded a crude residue which was purified by silica gel column chromatography using MeOH : $CHCl_3$: Acetone [1 : 9 : 10] as the eluent to furnish **BZn-cyto** (20 mg, 0.025 mmol) in 61 % yield as a colorless viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61 (s, 1H), 8.52-8.48 (m, 2H), 8.20 (dd, *J* = 8.8, 2.2 Hz, 1H), 8.11 (s, 1H), 7.89 (d, *J* = 8.8 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.66-7.58 (m, 2H), 7.52-7.43 (m, 4H), 7.18 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.16-7.07 (m, 3H), 6.97 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.70 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.38 (d, *J* = 8.8 Hz, 1H), 4.21 (t, *J* = 6.0 Hz, 2H), 4.10 (s, 2H), 3.94-3.81 (m, 9H), 3.78-3.72 (m, 2H), 3.63-3.57 (m, 2H), 3.40 (s, 3H), 3.23 (s, 3H), 3.16 (t, *J* = 6.0 Hz, 2H), 2.86 (t, *J* = 6.0 Hz, 2H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 169.1, 166.1, 161.5, 158.7, 151.2, 150.7, 148.5, 147.2, 145.2, 138.5, 138.0, 137.2, 131.8, 130.3, 129.3, 128.8, 128.1, 126.0, 124.7, 123.8, 122.3, 118.6, 115.4, 114.3, 112.3, 110.9, 108.0, 105.5, 105.2, 73.7, 72.2, 71.3, 70.2, 61.8, 59.8, 58.9, 56.9, 54.5, 42.8, 41.2. HRMS (FAB): calcd. for $C_{46}H_{50}N_7O_6$ $[M+H]^+$: 796.3823, Found 796.3820.

Scheme S2. Synthesis of **FZn-mito**:



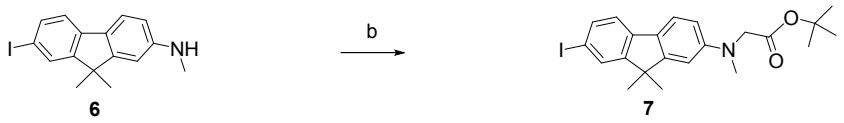
Reagents and conditions: (a) CH_3I, K_2CO_3, DMF, 0 °C, 5 h, 41 %; (b) $BrCH_2CO_2$-*t*-Bu, Proton sponge, CH_3CN, reflux, 6 h, 87 %; (c) i) TFA / CH_2Cl_2 (1/1), 0 °C - rt, 5 h; (c) ii) HATU, DIPEA, PPEA, DMF, rt, 16 h, 53 % (Over two steps); (d) *i*-PrMgCl.LiCl, DMF, THF, 0 °C - rt, 4.5 h, 43 % (e) DCC, HOBT, CH_2Cl_2, rt, 16 h, 63 %; (f) Hydrazine monohydrate, EtOH, 80 °C, 3 h, 47 %; (g) PTSA, $CHCl_3$, reflux, 16 h, 25%.

Compound **5**[4], 4-Amino-2-methoxy-*N*-{2-[*N*'-(2-picolyl)-*N*'-(2-pyridin-2-yl)ethyl]amino}ethylaniline (PPEA)[5] and (2-aminoethyl)triphenylphosphonium bromide[6] were prepared by the literature methods. Syntheses of other compounds are described below.



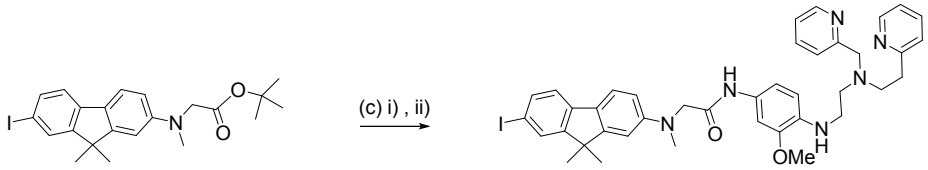
Compound 6. Methyl iodide (0.24 mL, 3.92 mmol) was added dropwise to a mixture of 2-amino-7-iodo-9,9-dimethyl-9*H*-fluorene **5**[4] (1.0 g, 3.0 mmol) and K_2CO_3 (500 mg, 3.6 mmol) in DMF (10 mL) at 0 °C. The reaction mixture was stirred at the same temperature for 5 h before it was filtered through a pad of celite and solvent was removed under reduced pressure. The crude residue was dissolved in EtOAc (30 mL), washed successively with water (2 x 5 mL), brine (5 mL), dried over anhydrous $MgSO_4$ and filtered. Evaporation of the solvent in *vaccuo* afforded the crude product which was purified

by silica gel column chromatography using 6% EtOAc in *n*-hexane as the eluent to furnish compound **6** (430 mg, 1.2 mmol) in 41 % yield as a brown solid; m. p. 119-121 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66 (d, *J* = 1.6 Hz, 1H), 7.58 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 6.63 (d, *J* = 2.2 Hz, 1H), 6.58 (dd, *J* = 7.7, 2.2 Hz, 1H), 3.90 (brs, 1H), 2.91 (s, 3H), 1.43 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.0, 154.9, 149.7, 135.7, 131.5, 127.8, 121.0, 120.2, 111.4, 106.1, 89.7, 46.8, 30.9, 27.2. HRMS (FAB) Calcd. for $C_{16}H_{16}IN$ $[M]^+$: 349.0328. Found: 349.0328.



6 7

Compound 7. A mixture of amine **6** (410 mg, 1.2 mmol), proton sponge (280 mg, 1.3 mmol) and *t*-butyl bromoacetate (0.21 mL, 1.4 mmol) in MeCN (5 mL) was refluxed for 6 h under nitrogen atmosphere. The reaction mixture was diluted with EtOAc (15 mL), filtered through a pad of Celite, washed successively with water (2 x 5 mL), brine (5 mL), dried over anhydrous $MgSO_4$ and filtered. Evaporation of the solvent under reduced pressure yielded a crude product which was purified by silica gel column chromatography using 4% EtOAc in *n*-hexane as the eluent to furnish title compound **7** (492 mg, 1.06 mmol) in 87 % yield as a colorless viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66 (d, *J* = 1.6 Hz, 1H), 7.58 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 7.7 Hz, 1H), 6.66-6.63 (m, 2H), 4.0 (s, 2H), 3.1 (s, 3H), 1.42 (s, 6H), 1.41 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 155.0, 154.5, 149.2, 139.3, 135.6, 131.4, 127.4, 120.8, 120.3, 111.1, 105.9, 89.7, 81.4, 55.5, 46.7, 40.0, 27.9, 27.1. HRMS (FAB) Calcd. for $C_{22}H_{26}INO_2$ $[M]^+$: 463.1008. Found: 463.1009.

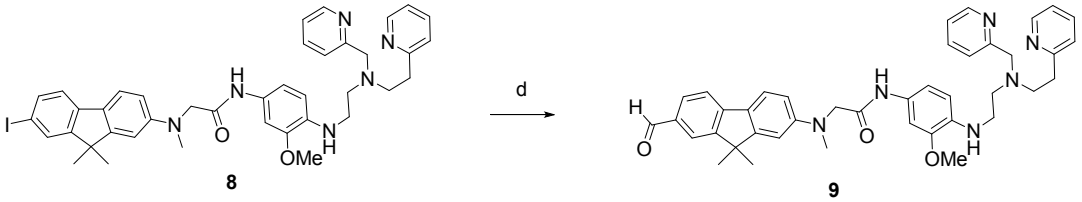


7 8

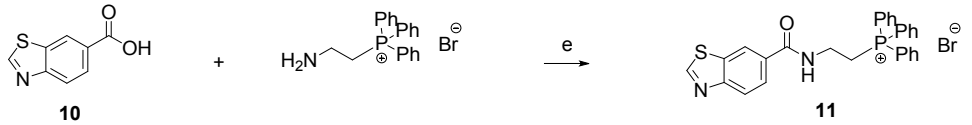
Compound 8. A solution of ester **7** (470 mg, 1.0 mmol) in 50 % TFA in CH_2Cl_2 (6 mL) was stirred at ambient temperature for 5 h. Solvent was evaporated under *vacuo* to yield a crude acid residue **7′** which was taken ahead for the next step without further purification to avoid possible decaboxyllation.

O-(7-Azabenzotriazole-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (HATU, 665 mg, 1.75 mmol) was added to the solution of crude acid **7** in DMF (2 mL). The resulting clear solution was stirred for 10 min at rt, before diisopropylethylamine (DIPEA, 0.54 mL, 3.06 mmol) was added dropwise, followed by a solution of PPEA[5] (390 mg, 1.0 mmol) in DMF (1 mL). The reaction mixture was stirred for 16 h at rt before it was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was washed with brine (2 x 5 mL), dried over anhydrous $MgSO_4$, filtered and concentrated in *vacuo* to yield a crude residue which was purified via silica gel chromatography using 2% MeOH in $CHCl_3$ as the eluent to furnish compound **8** (415 mg, 0.55 mmol) in 53 % yield as a colorless viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.49-8.42 (m, 2H), 8.20 (s, 1H), 7.69 (d, *J* = 1.6 Hz, 1H), 7.64-7.49 (m, 3H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.21 (d, *J*

= 2.2 Hz, 1H), 7.13-7.03 (m, 3H), 6.84-6.77 (m, 2H), 6.71 (dd, *J* = 8.2, 2.2, 1H), 6.39 (d, *J* = 8.2, 1H), 4.01 (s, 2H), 3.89-3.80 (m, 5H), 3.17-3.08 (m, 5H), 3.03-2.96 (m, 4H), 2.88 (t, *J* = 6.0 Hz, 2H), 1.44 (s, 6H). ^{13}CNMR (100 MHz, CDCl3): δ 168.0, 155.2, 154.9, 149.8, 149.1, 146.8, 138.7, 136.3, 136.2, 135.9, 135.7, 131.7, 129.6, 126.8, 123.3, 122.7, 121.9, 121.1, 121.0, 120.7, 113.1, 112.8, 109.5, 107.5, 103.6, 90.7, 60.1, 55.5, 54.3, 52.8, 47.0, 41.1, 40.3, 30.6, 27.2. HRMS (FAB) Calcd. for $C_{40}H_{44}IN_6O_2$ $[M+H]^+$: 767.2571. Found: 767.2571.

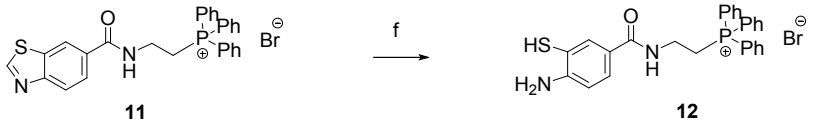


Compound 9. *i*-PrMgCl.LiCl[7] (1.0 mL, 1.3 mmol, 1.3 M in THF) was added drop wise to a solution of compound **8** (130 mg, 0.19 mmol) in THF (8 mL) at 0 °C. The reaction mixture was gradually warmed to rt and stirred for 3.5 h, before being cooled to 0 °C and subjected to a treatment with anhydrous DMF (1.5 mL, 1.9 mmol). Resulting mixture was allowed to warm to rt and stirred for 1 h while a suspension of white solid formed. The reaction was cooled to 0 °C, quenched with aq. NH_4Cl (1M, 5 mL) while suspended material got dissolved and a faint order of amine was noted. The reaction mixture was extracted with EtOAc (3 x 5 mL) and the combined organic extract was washed successively with water (2 x 5 mL), brine (5 mL), dried over anhydrous $MgSO_4$ and filtered. Evaporation of the solvent under reduced pressure provided a crude residue which was purified by silica gel column chromatography using 2% MeOH in $CHCl_3$ as the eluent to afford title compound **9** (54 mg, 0.081 mmol) in 43 % yield as yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.00 (s, 1H), 8.56-8.47 (m, 2H), 8.11 (s, 1H), 7.91 (s, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.71 (t, *J* = 8.6 Hz, 2H), 7.63 (dt, *J* = 8.6, 2.3 Hz, 1H), 7.30 (d, *J* = 8.6 Hz, 1H), 7.21 (d, *J* = 2.3 Hz, 1H), 7.18-7.13 (m, 1H), 6.92-6.78 (m, 4H), 6.75 (dd, *J* = 8.6, 2.3, 1H), 6.50 (d, *J* = 8.6, 1H), 4.05 (s, 2H), 3.95 (s, 2H), 3.86-3.80 (m, 5H), 3.26 (t, *J* = 6.2 Hz, 2H), 3.19 (s, 3H), 3.16-3.09 (m, 2H), 2.95 (t, *J* = 6.2 Hz, 2H), 1.49 (s, 6H). ^{13}CNMR (100 MHz, CDCl3): δ 192,0, 167.8, 159.2, 157.1, 153.5, 150.5, 149.3, 146.9, 145.8, 136.5, 135.8, 134.3, 131.0, 128.9, 126.8, 122.6, 122.4, 122.3, 122.0, 119.0, 113.2, 112.9, 109.4, 107.3, 103.7, 59.8, 55.5, 54.7, 48.3, 46.9, 43.4, 40.3, 29.7, 27.1. HRMS (FAB) Calcd. for $C_{41}H_{44}N_6O_3$ $[M+H]^+$: 669.3553. Found: 669.3551.



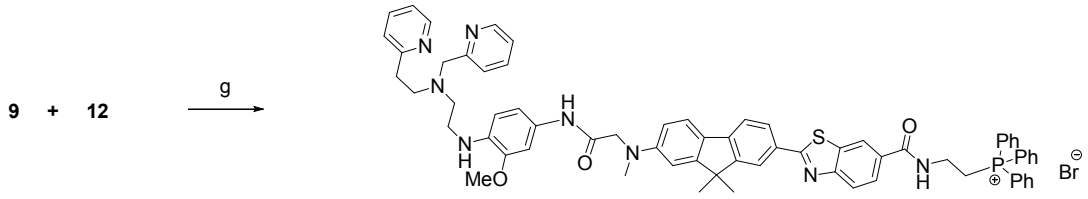
10 **11**

Compound 11. Benzothiazole-6-carboxylic acid **10** (50 mg, 0.28 mmol), 1,3-dicyclohexyl carbodiimide (DCC, 69 mg, 0.34 mmol) and 1-hydroxybenzotriazole (HOBT, 45 mg, 0.34 mmol) were dissolved in CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 1 h under a blanket of argon. (2-aminoethyl)triphenylphosphonium bromide[6] (110 mg, 0.28 mmol) was added and the reaction mixture was stirred for 16 h. Solvent was evaporated under reduced pressure and the residue was diluted with CH_3CN (10 mL) to remove urea byproduct, formed in the reaction by filtration. Filtrate on concentration in *vacuo* resulted a crude residue which was purified via silica gel column chromatography using 11% MeOH in $CHCl_3$ as the eluent to furnish title compound **11** (96 mg, 0.18 mmol) in 63 % yield as a pale yellow, gummy product. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.11 (s, 1H), 8.47 (s, 1H), 8.18-8.10 (m, 1H), 7.99-7.91 (m, 1H), 7.86-7.57 (m, 15H), 3.95-3.79 (m, 2H), 3.65-3.51 (m, 2H). ^{13}CNMR (100 MHz, $CDCl_3$): δ 166.9, 156.7, 155.1, 135.2, 133.6, 133.5, 130.6, 130.4, 125.9, 123.2, 122.3, 118.2, 117.3, 23.3, 22.8. HRMS (FAB): calcd. for $C_{28}H_{24}N_2OPS$ $[M\text{-}Br]^+$: 467.1347, Found 467.1348.



11 **12**

Compound 12. A mixture of compound **11** (81 mg, 0.15 mmol) and hydrazine monohydrate (0.10 mL, 2.2 mmol) in ethanol (2 mL) was heated at 80 °C for 3 h. The mixture was concentrated in *vacuo* to deliver a crude residue which was purified via silica gel column chromatography using 9% MeOH in $CHCl_3$ as the eluent to furnish compound **12** (37 mg, 0.070 mmol) in 47 % yield as colorless viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83-7.58 (m, 15H), 7.44 (d, J = 8.79 Hz, 1H), 7.31 (s, 1H), 7.05-6.89 (m, 1H), 6.67 (d, J = 8.79 Hz, 1H), 5.05-4.76 (brs, 2H), 3.83-3.57 (m, 2H), 3.57-3.35 (m, 2H). ^{13}CNMR (100 MHz, CDCl3): δ 166.8, 152.0, 136.1, 135.0, 133.5, 130.9, 130.4, 121.9, 118.0, 117.1, 116.4, 114.5, 23.1, 22.6. HRMS (FAB): calcd. for $C_{27}H_{25}N_2OPS$ $[M\text{-}HBr]^+$: 456.1425, Found 456.1425.



FZn-mito

FZn-mito. A solution of amino thiol **12** (19 mg, 0.035 mmol) in $CHCl_3$ (1 mL) was added drop wise to a stirred solution of aldehyde **9** (24 mg, 0.035 mmol) and *p*-toluenesulfonic acid monohydrate (PTSA, 2 mg, 0.0003 mmol) in $CHCl_3$ (4 mL) under an atmosphere argon. The reaction mixture was refluxed for 16 h while accumulation of brown solid was observed on the wall of the RB flask. Mother liquor containing the desired product was decanted carefully and the solid residue was rinsed twice with cold $CHCl_3$ (2 x 3 mL).

Evaporation of the solvent under reduced pressure yielded a crude residue which was purified by reversed-phase HPLC using the following conditions: YMC-Pack ODS-A, (20 x 250 mm), 5 μm, 12 nm; mobile phase, MeOH / H_2O (0.1% TFA) = 20/80 to 100/0 to 100/0 (linear gradient, 60 then 70 min); flow rate, 8 mL/min; temperature, 40 °C; injection, 0.1 mL, detection, UV (210 nm). The fraction of interest at retention time 49.5 min was collected and lyophilized to furnish **FZn-mito** (9 mg, 0.007 mmol) in 21 % yield as a dark brown, gummy product. ^{1}H NMR (400 MHz, Acetone-d_6): δ 8.61-8.49 (m, 2H), 8.23 (s, 1H), 8.14-7.99 (m, 10H), 7.99-7.90 (m, 4H), 7.90-7.68 (m, 11H), 7.54 (d, J = 7.8 Hz, 1H), 7.45-7.34 (m, 2H), 7.11-6.99 (m, 2H), 6.82 (d, J = 7.8, 1H), 6.65 (d, J = 7.8, 1H), 4.65 (s, 2H), 4.18 (s, 2H), 4.09-3.85 (m, 6H), 3.84-3.65 (m, 7H), 3.65-3.51 (m, 2H), 3.23 (s, 3H), 1.56 (s, 6H). ^{31}P NMR (162 MHz, DMSO-d_6): δ 22.3 ppm. HRMS (FAB$^+$): m/z calcd. for $[C_{68}H_{66}N_8O_3PS]^+$: 1105.4716, Found 1105.4711.

Water solubility. The plots of fluorescence intensity against the total amount of the dye injected to the cuvette were linear at low dye content and showed downward curvature as more dye was added (Figure S1). The maximum point in the linear region was taken as the solubility. The solubility of BZn-Cyto was 5.0 μM and that of FZn-Mito was 7.0 μM.

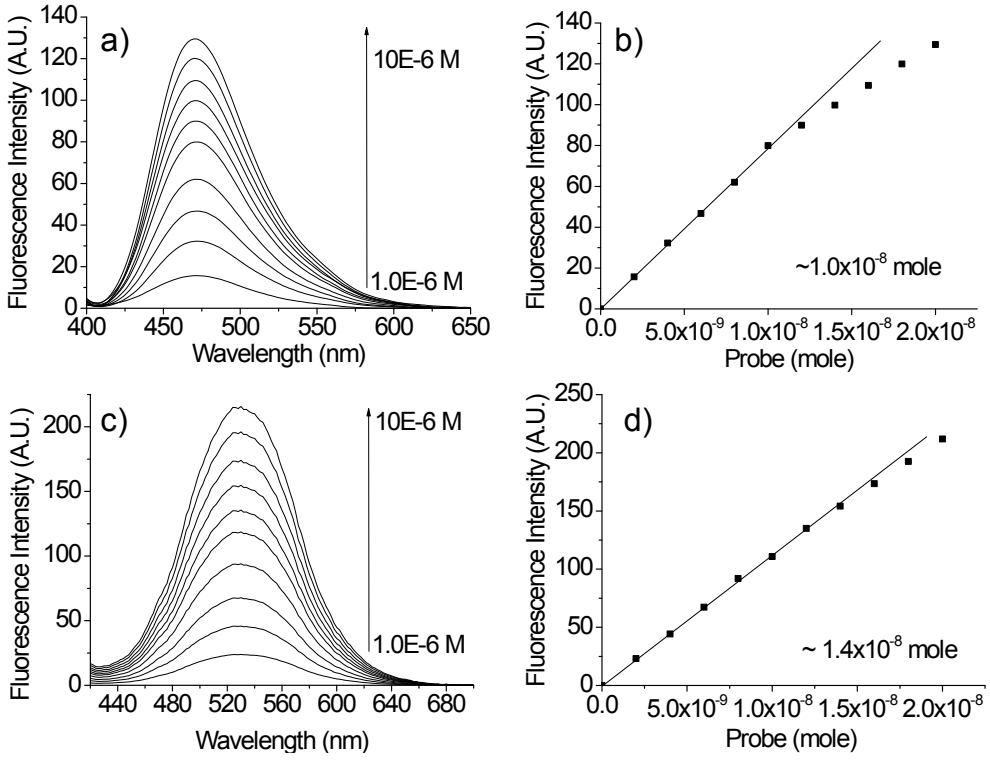


Figure S1. (a, c) One-photon fluorescence spectra and (b, d) plot of fluorescence intensity against the total amount of the probe for (a, b) BZn-Cyto in 2.0 mL MOPS buffer (BZn-Cyto: 30 mM MOPS, 100 mM KCl, pH 7.2) and (c, d) FZn-Mito in MOPS/SDS (30 mM MOPS + 1.5 mM SDS, 100 mM KCl, pH 7.2). The excitation wavelengths were (a) 367 and (c) 394 nm, respectively.

Spectroscopic measurements. Absorption spectra were recorded on a S-3100 UV-Vis spectrophotometer and fluorescence spectra were obtained with FluoroMate FS-2 fluorescence spectrometer with a 1 cm standard quartz cell. The fluorescence quantum yield was determined by using coumarin 307 (Φ = 0.95 in MeOH) as the reference by the literature method.[8]

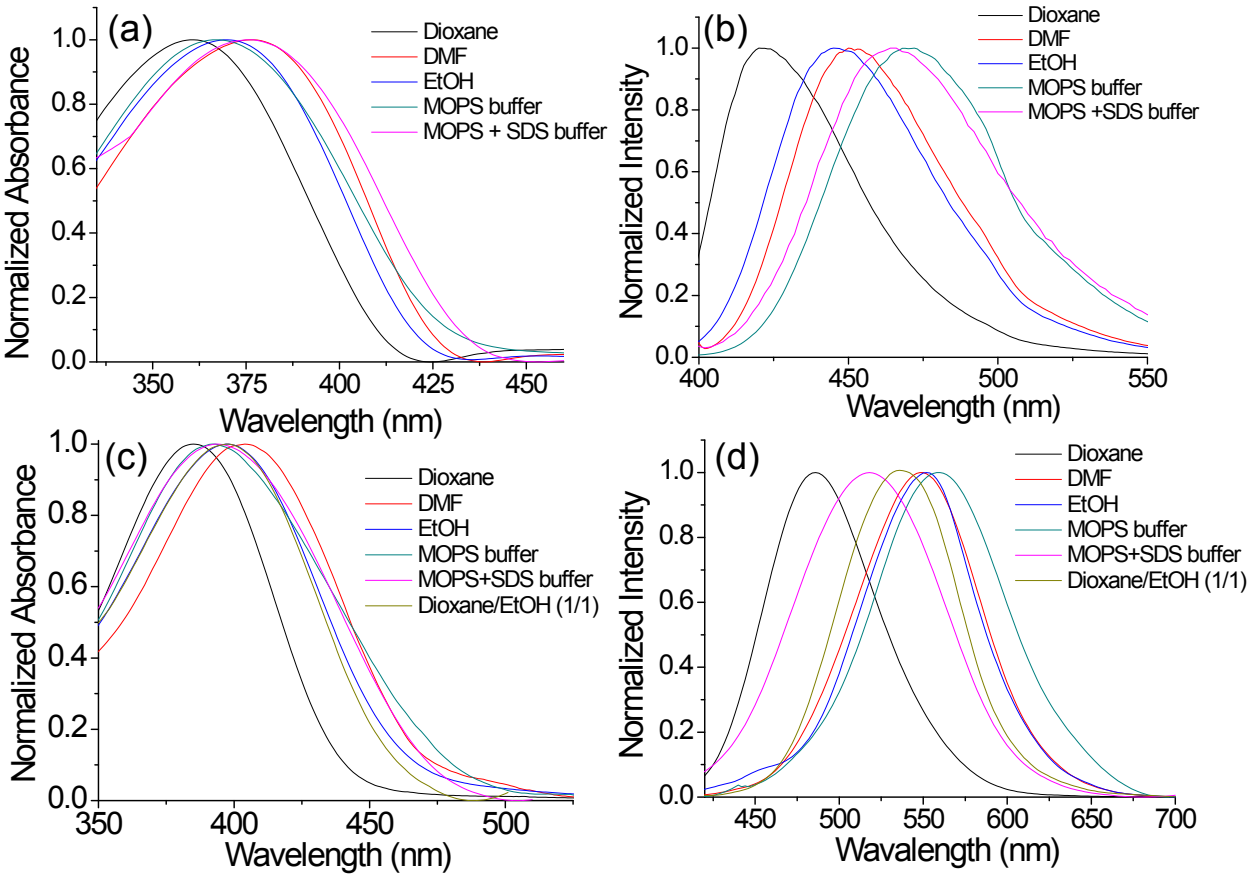


Figure S2. (a, c) Normalized absorption and (b, d) emission spectra of (a, b) BZn-Cyto and (c, d) FZn-Mito in 1,4-Dioxane, 1,4-Dioxane/EtOH (1/1), DMF, EtOH, MOPS (30 mM MOPS, 100 mM KCl, pH 7.2) and MOPS/SDS (30 mM MOPS + 1.5 mM SDS, 100 mM KCl, pH 7.2).

Table S1. Photophysical properties of BZn-Cyto and FZn-Mito in various solvents.

Solvent (E_T^N)[a]	$\lambda_{max}^{(1)}$, nm[b]		λ_{max}^{fl}, nm[b]		Φ[c]		$\lambda_{max}^{(2)}$, nm[d]		δ_{max}[e]		$\Phi\delta_{max}$[e]	
	BZn-Cyto	FZn-Mito	BZn-Cyto	FZn-Mito	BZn-Cyto	FZn-Mito	BZn-Cyto	FZn-Mito	BZn-Cyto	FZn-Mito	BZn-Cyto	FZn-Mito
1,4-Dioxane (0.164)	360	385	421	486	0.24	0.45	-	-	-	-	-	-
1,4-Dioxane/ EtOH (1/1)	-	397	-	536	-	0.31[f]	-	800	-	117	-	80
DMF (0.386)	377	404	451	548	0.25	0.13	-	-	--	-	-	-
EtOH (0.654)	369	399	445	552	0.18[f]	0.11	740		150		135	
MOPS/SDS[g]	376	394	466	520	-	-	-	-	-	-	-	-
MOPS (1.00) [h]	367	392	470	559	0.025[f]	0.023	740		194		103	
HeLa cell			450	530								

[a]The numbers in the parenthesis are normalized empirical parameter of solvent polarity.[9] [b]λ_{max} of the one-photon absorption and emission spectra in nm. [c]Fluorescence quantum yield. The uncertainty is ± 15%. [d]λ_{max} of the two-photon excitation spectra in nm. [e]Two-photon action cross section in 10^{-50} cm^4s/photon (GM), ± 15%. [f] The Φ values of FZn-Mito in 1,4-Dioxane/EtOH (1/1), BZn-Cyto in MOPS and EtOH in the presence of excess Zn^{2+} were 0.68, 0.90, and 0.53, respectively. [g] 30 mM MOPS, 100 mM KCl, 1.5 mM SDS, pH 7.2. [h] 30 mM MOPS, 100 mM KCl, pH 7.2, the E_T^N value is for water.

Determination of Apparent Dissociation Constants. A series of MOPS (4-morpholinepropanesulfonic acid) buffer solutions (30 mM, pH 7.2, 0.1 M KCl) containing various amounts of $ZnSO_4$ (0 ~ 9.5 mM) and 10 mM of EGTA (ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid) were prepared.

The $[Zn^{2+}]_{free}$ was calculated from the $K_{Zn\text{-}EGTA}^{app}$, $[EGTA]_{total}$, and $[Zn^{2+}]_{total}$ using Eq (1).[10,11]

$$[Zn^{2+}]_{free} = [Zn^{2+}]_{total} / (\alpha_{Zn} \times K_{Zn\text{-}EGTA}^{app} \times [EGTA]_{free}) \quad (1)$$

Where,

$$K_{Zn\text{-}EGTA}^{app} = K_{Zn\text{-}EGTA} / \alpha_{Zn}\alpha_{EGTA},$$

$$\alpha_{Zn} = 1 + 10^{(pH - pK_1)} + 10^{(2pH - pK_1 - pK_2)} + 10^{(3pH - pK_1 - pK_2 - pK_3)} \ldots,$$

$$\alpha_{EGTA} = 1 + 10^{(pK_1 - pH + 0.11)} + 10^{(pK_1 + pK_2 - 2pH + 0.22)} + 10^{(pK_1 + pK_2 + pK_3 - 3pH + 0.33)} \ldots,$$

and

$$[EGTA]_{free} = [EGTA]_{total} - [Zn^{2+}]_{total}$$

Thus,

$$K_{Zn\text{-}EGTA}^{app} = \frac{K_{Zn\text{-}EGTA}(1 + 10^{(pK_{Zn\text{-}EGTA} - pH)})}{(1 + 10^{(pH - pK_{Zn})})(1 + 10^{(pK_1 - pH)} + 10^{(pK_1 + pK_2 - 2pH)})}$$

The stability constant for the Zn^{2+} complex of EGTA ($K_{Zn\text{-}EGTA}$) was taken from ref. 14. Thus, for EGTA (pH 7.2, 0.1 M KCl, 25 °C),

pK_1 = 9.40, pK_2 = 8.79, pK_3 = 2.70, log $K_{Zn\text{-}EGTA}$ = 12.6.

All protonation constants must be corrected upward by 0.11 when worked out in 0.1 M ionic strength. $[EGTA]_{total}$ was set at 20 mM, and $[Zn^{2+}]_{total}$ was varied from 0-9.8 mM.

The calculated $[Zn^{2+}]_{free}$ concentration of each solution is:

$[Zn2+]_{total}$ (mM)	0.50	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.99	5.99	6.98	7.98	8.97	9.47
$[Zn2+]_{free}$ (nM)	0.14	0.29	0.46	0.66	0.87	1.1	1.4	1.8	2.6	3.9	6.1	10	22	47
$[Zn^{2+}]_{total}$ (mM)	3.50	6.00	7.00	8.00	8.30	8.80	8.90	9.00	9.20	9.40	9.50	9.60	9.70	9.80
$[Zn^{2+}]_{free}$ (nM)	1.4	3.9	6.1	11	13	19	21	24	30	41	47	63	85	130

To determine the apparent dissociation constants for the Zn^{2+} complex of probes, the fluorescence titration curves (Figures 1a and S3b) was obtained and fitted to Eq 2 (Figures 1b).[12,13]

$$F = F_0 + (F_{max} - F_0)\frac{[Zn^{2+}]_{free}}{K_d + [Zn^{2+}]_{free}} \quad (2)$$

where F is the fluorescence intensity, F_{max} is the maximum fluorescence intensity, F_0 is the fluorescence intensity in the absence of Zn^{2+}, and $[Zn^{2+}]_{free}$ is the free Zn^{2+} concentration. The K_d value that best fits the titration curve with Eq 2 was calculated by using the Excel program.

In order to determine the K_d^{TP} for the two-photon process, the TPEF spectra were obtained with a DM IRE2 Microscope (Leica) using the $xy\lambda$ mode at 800 Hz scan speed. They were excited by a mode-locked titanium-sapphire laser source (Coherent Chameleon, 90 MHz, 200 fs) set at wavelength 780 nm and output power 1180 mW, which corresponded to approximately 10 mW average power in the focal plane. The TPEF titration curves (Fig. 1b and Fig S3c) were obtained and fitted to Eq 2 (Figures 1c and Fig S3d).

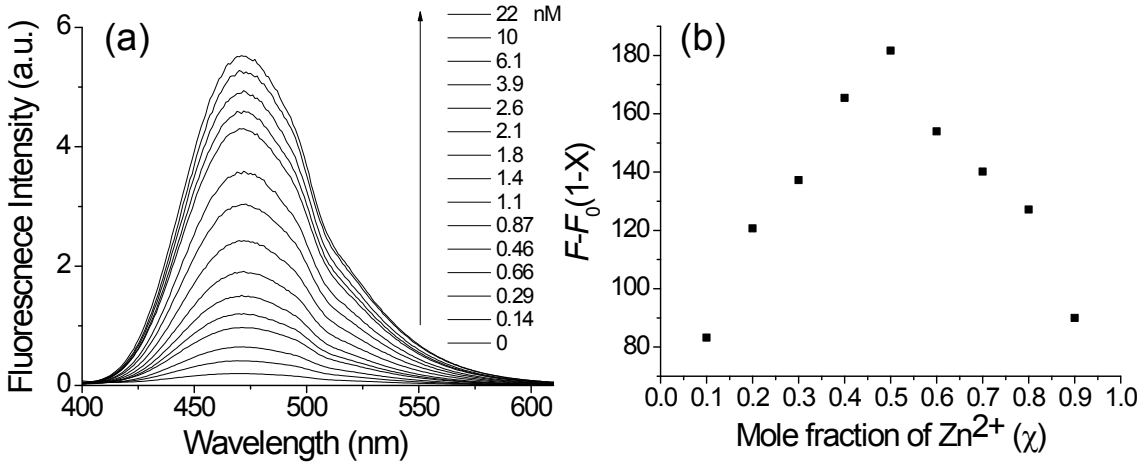


Figure S3. One-photon fluorescence spectra of 3 μM BZn-Cyto in the presence of free Zn^{2+} (0−22 nM). (b) Job's plot for the complexation of BZn-Cyto with free Zn^{2+}. Total Zn^{2+} concentration was 2 μM. All data were collected in MOPS and the excitation wavelength was 367 nm.

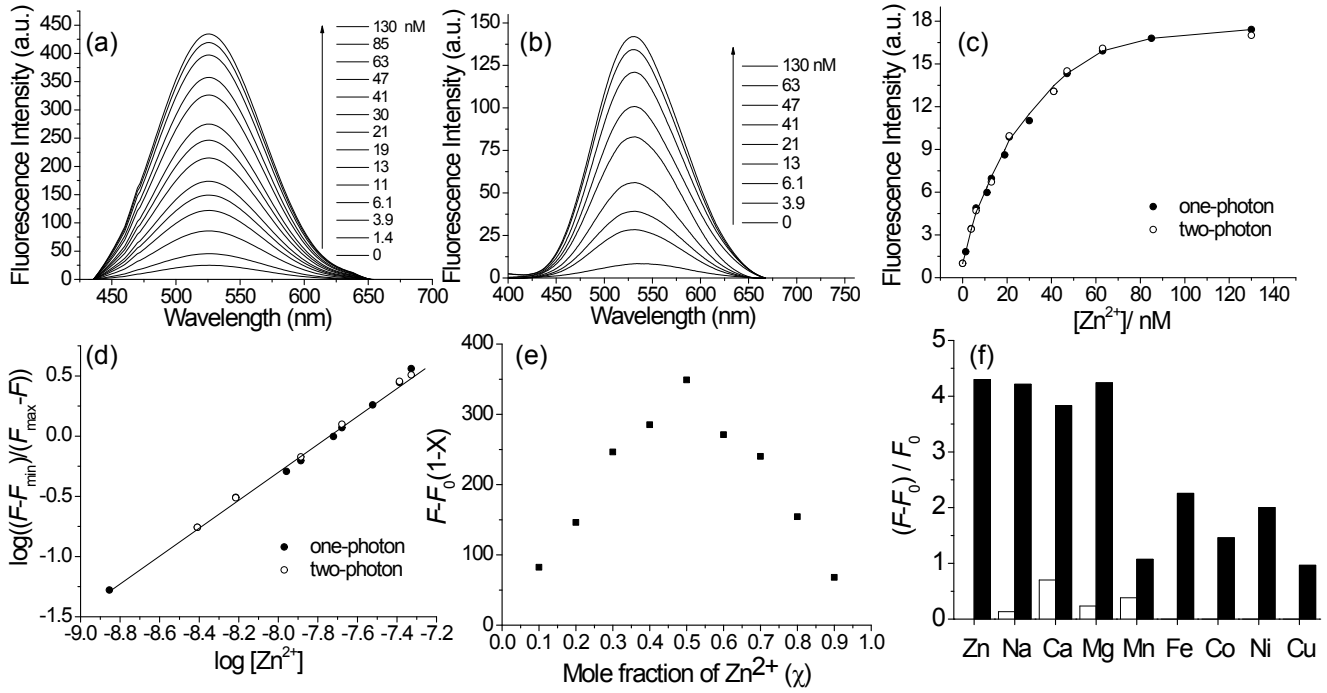


Figure S4. (a) One-photon and (b) two-photon fluorescence spectra of 3 μM FZn-Mito. (c,d) One- (●) and two-photon (○) fluorescence titration curve for the complexation and hill plots for the complexation of FZn-Mito with free Zn^{2+} (0−130 nM). (e) Job's plot for the complexation of FZn-Mito with free Zn^{2+}. Total Zn^{2+} concentration was 2 μM. (f) The relative fluorescence intensity of 3.0 μM of FZn-Mito in the presence of 5.0 mM for Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}; 300 μM for Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} (empty bars) followed by addition of 130 nM of free Zn^{2+} (filled bars). All data were collected in MOPS/SDS. The excitation wavelengths for one- and two-photon processes were 394 and 800 nm, respectively.

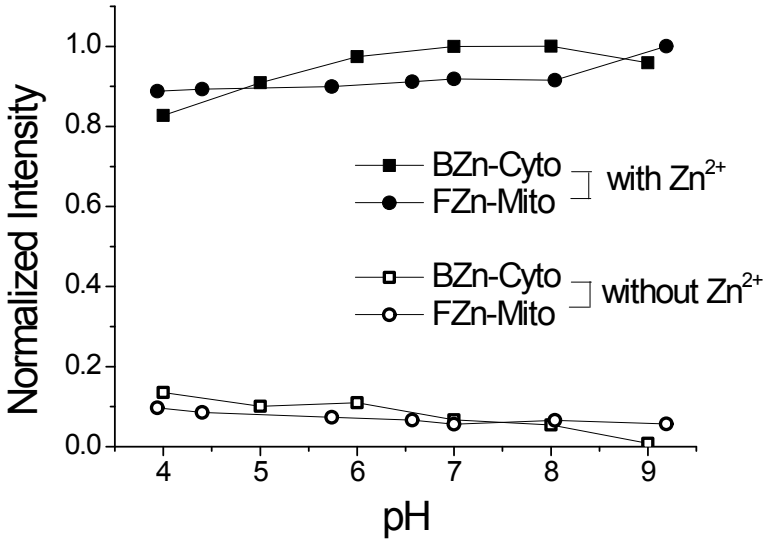


Figure S5. Effect of the pH (4.0-9.0) on the one-photon fluorescence intensity of 3 μM BZn-Cyto in MOPS and FZn-Mito in MOPS/SDS the presence of 0 (○, □) and 100 nM of Zn^{2+} ion (●, ■). The excitation wavelengths for BZn- Cyto and FZn-Mito were 367 and 394 nm, respectively.

Detection windows. For the colocalization experiments, the emission spectra of BZn-Cyto (3 μM), FZm-Cyto (3 μM), and Mitotracker Red FM (MTR) (1 μM) in the HeLa cells were compared. The detection windows were determined by considering two factors; i) the emission from the two probes should be separated as far as possible, ii) the emission intensities from the probes should be very similar. For all of the co-localization experiments conducted in this study, 400-450 (BZn-Cyto), 550-650 (FZn-Mito), and 600-700 nm (MTR) were used as the detection windows (Figure S6).

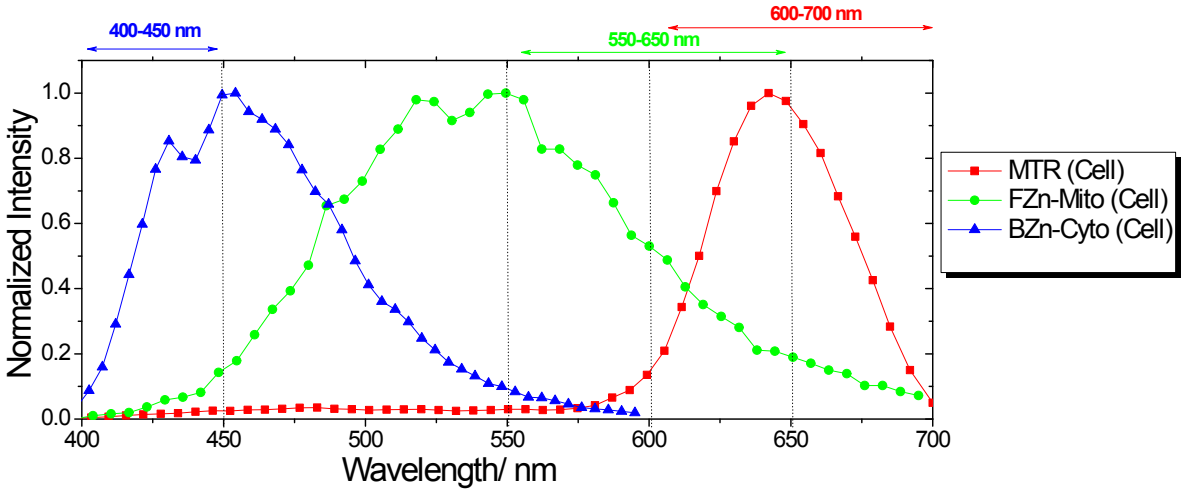


Figure S6. One-photon fluorescence spectra of Mitotracker Red FM (MTR) and two-photon excited fluorescence spectra of BZn-Cyto and FZn-Mito in HeLa cells. The excitation wavelengths were 543 (MTR) and 750 nm (BZn-Cyto and FZn-Mito), respectively.

Cell viability. To confirm that the probe couldn't affect the viability of HeLa cells in our incubation condition, we used CCK-8 kit (Cell Counting Kit-8, Dojindo, Japan) according to the manufacture's protocol. The results are shown in Figure S7.

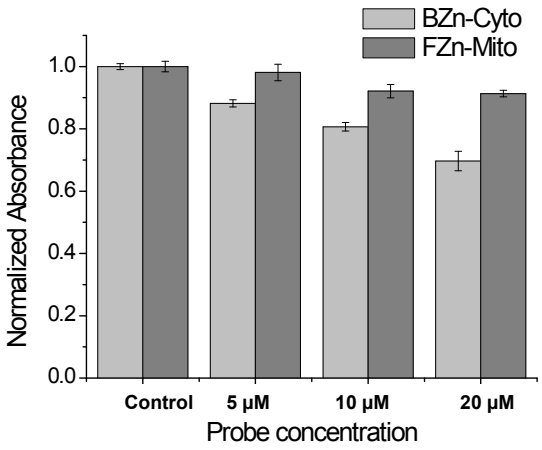


Figure S7. Viability of HeLa cells in the presence of BZn-Cyto and FZn-Mito as measured by using CCK-8 kit. The cells were incubated with BZn-Cyto and FZn-Mito for 4 hr.

Photostability. Photostability of BZn-Cyto and FZn-Mito were determined by monitoring the changes in TPEF intensity with HeLa cells chosen without bias. The TPEF intensity remained nearly the same for 1 hr, indicating high photostability.

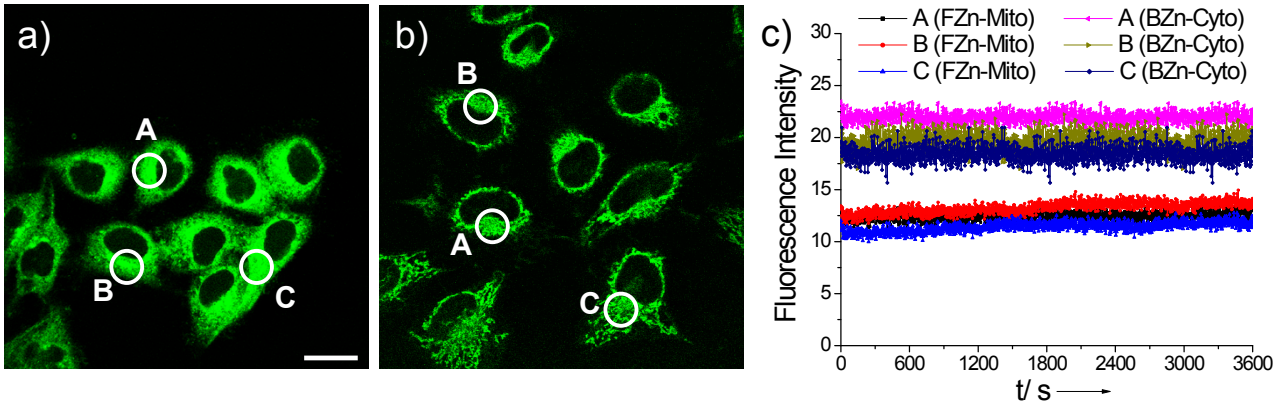


Figure S8. TPM image of HeLa cells labeled with 3 μM of BZn-Cyto (a) and FZn-Mito (b) collected at 400-650 nm. The relative TPEF intensity as a function of time (c). The digitized intensity was recorded with 2.0 sec intervals for the duration of one hour using *xyt* mode (λ_{ex} = 750 nm, ~200 fs). Cells shown are representative images from replicate experiments (n = 5). Scale bar, 30 μm.

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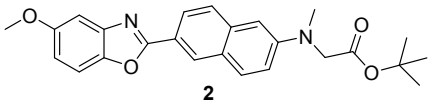
^{1}H, ^{13}C NMR and HRMS Spectra of 1-12, BZn-cyto, and FZn-mito:



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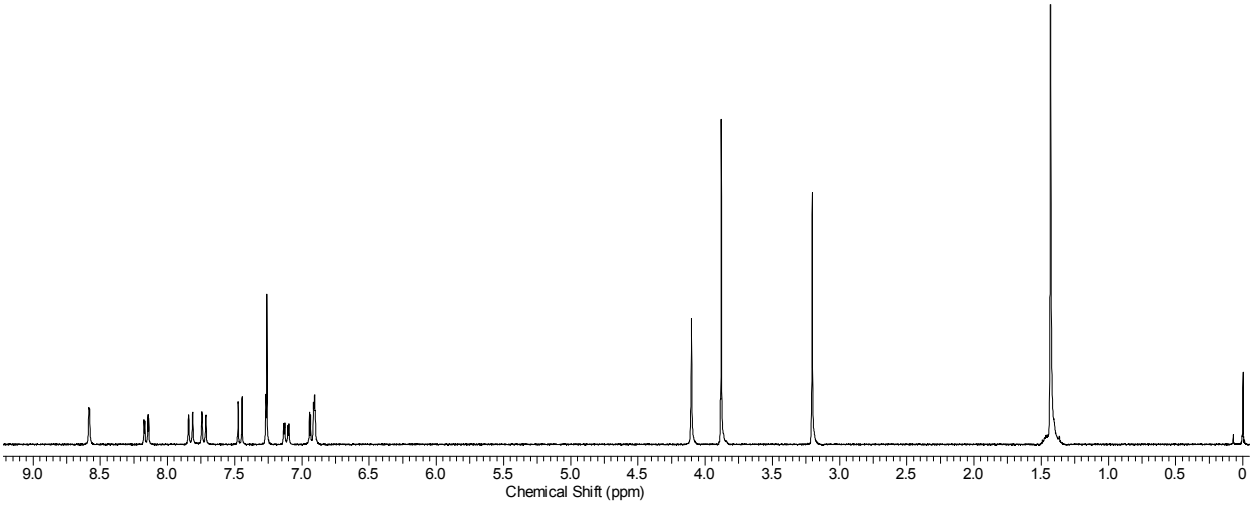
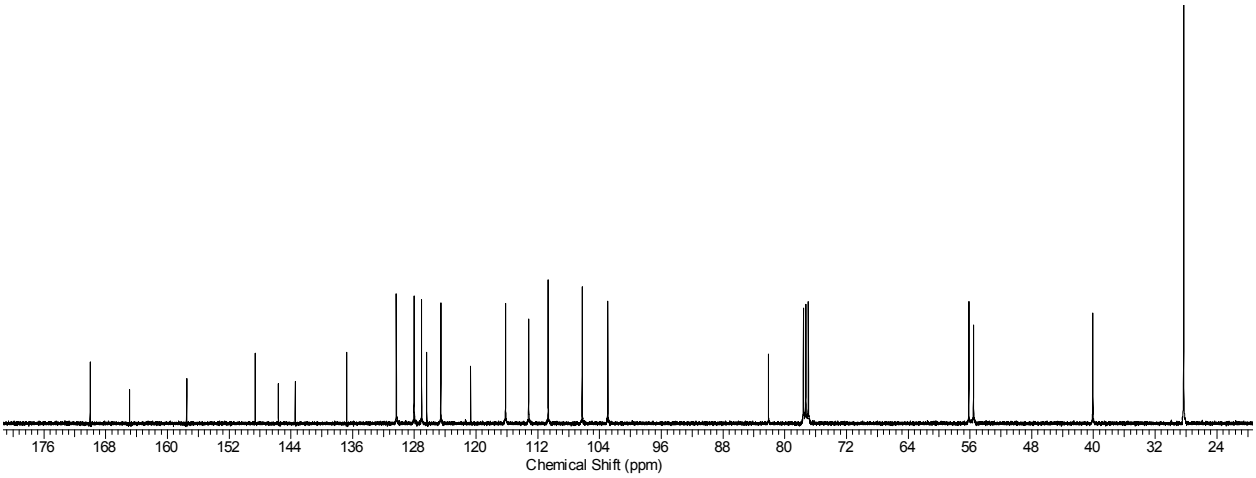
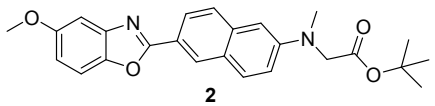


^{1}H NMR (300 MHz, $CDCl_3$) of compound **2**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **2**



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RT : 3.25 min                           Scan#: 40
Elements : C 25/0, H 26/0, O 4/0, N 2/0
Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
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Estimated m/z  Error[ppm]  U.S.     C     H     O     N
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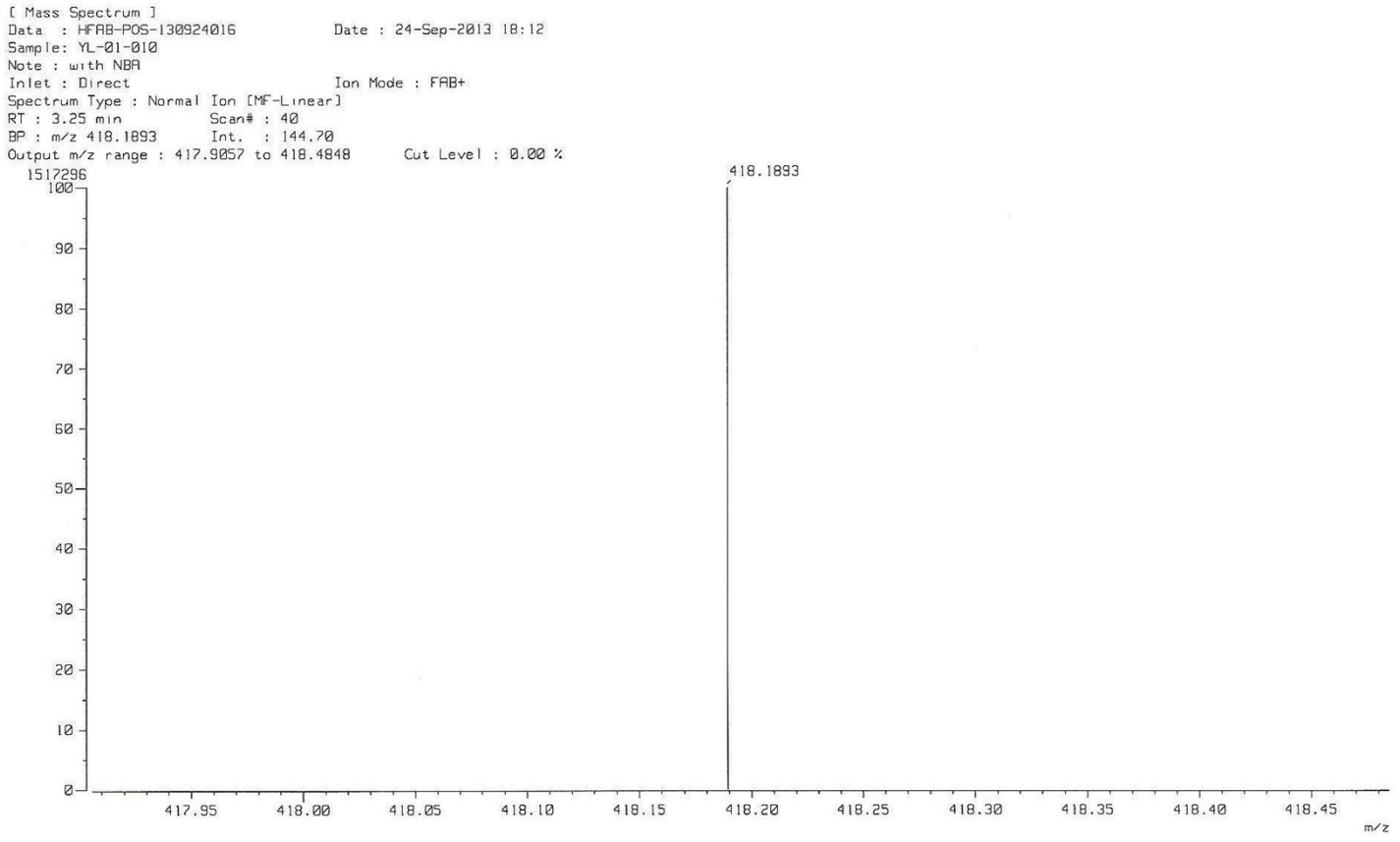
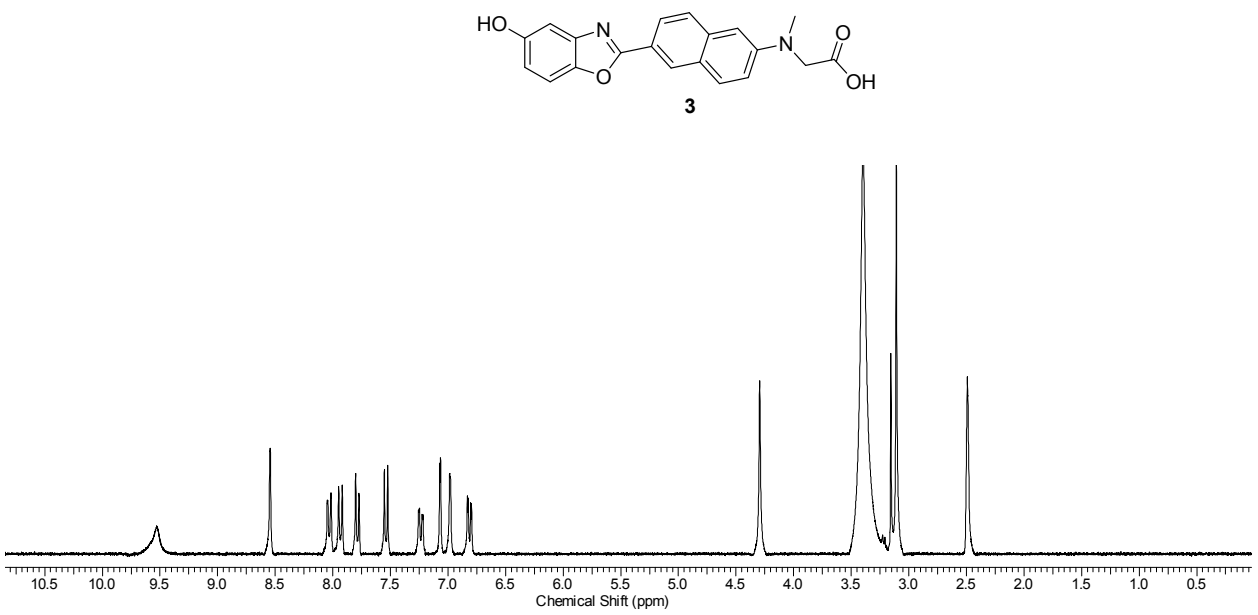
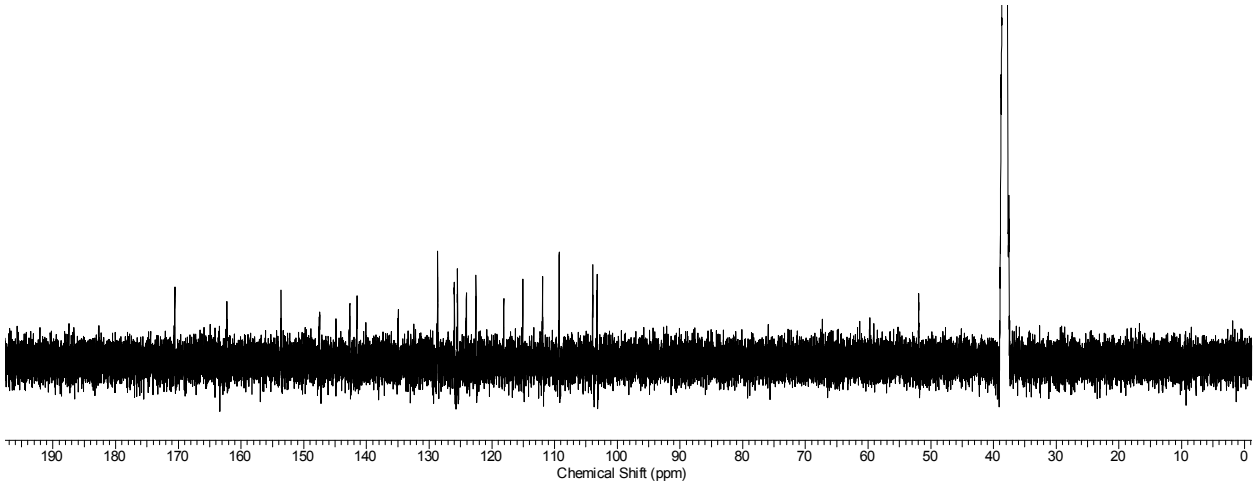


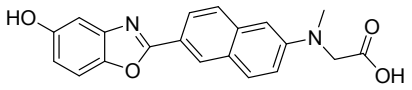
HRMS Spectrum of compound **2**



^{1}H NMR (300 MHz, DMSO-d_6) of compound **3**



^{13}C NMR (100 MHz, DMSO-d_6) of compound **3**



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RT : 3.17 min                           Scan#: 39
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Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
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Estimated m/z  Error[ppm]  U.S.     C     H     O     N
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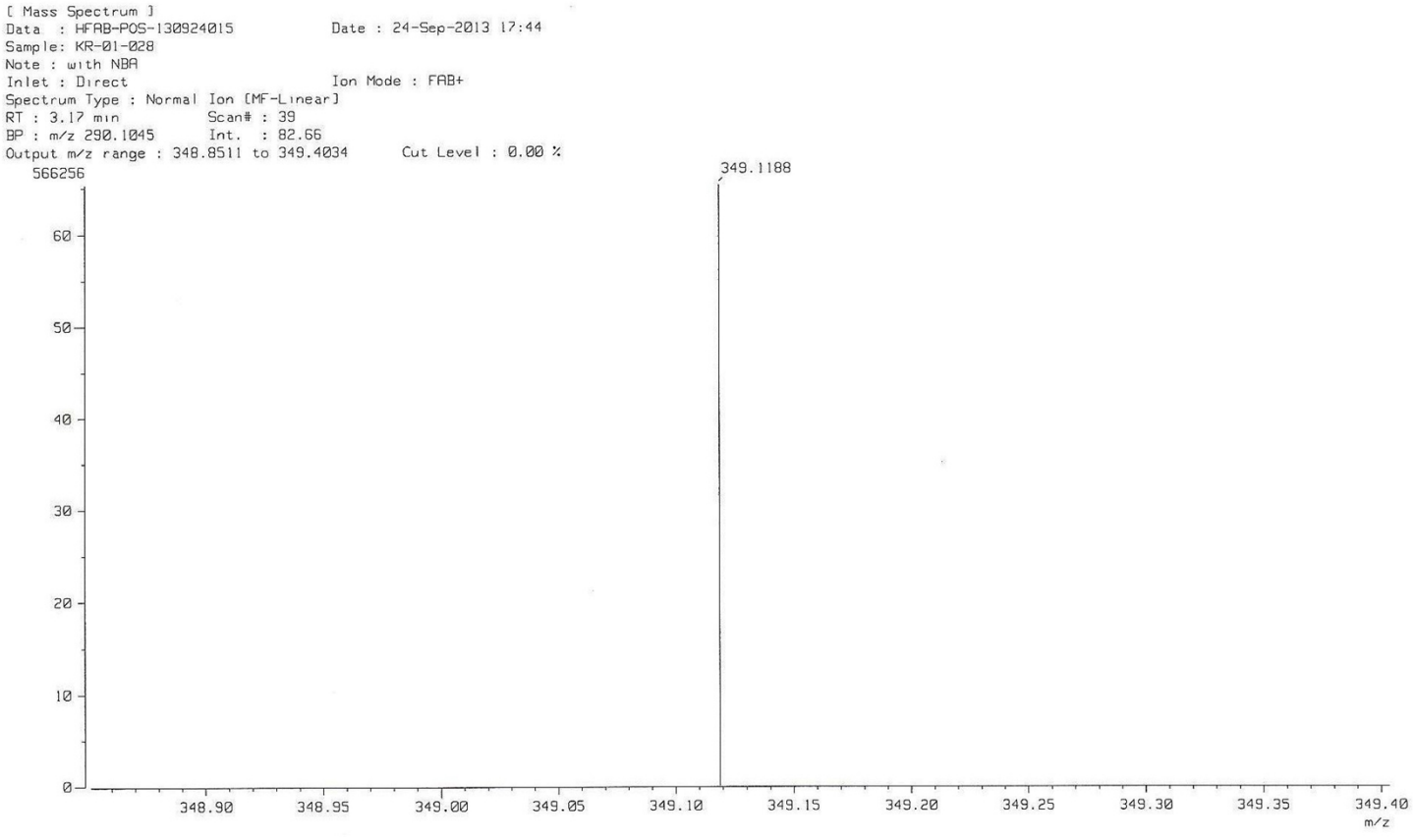
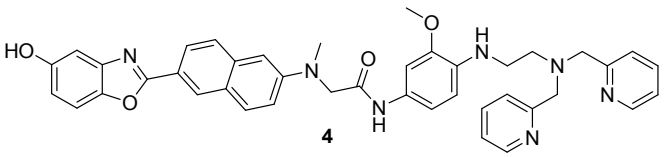
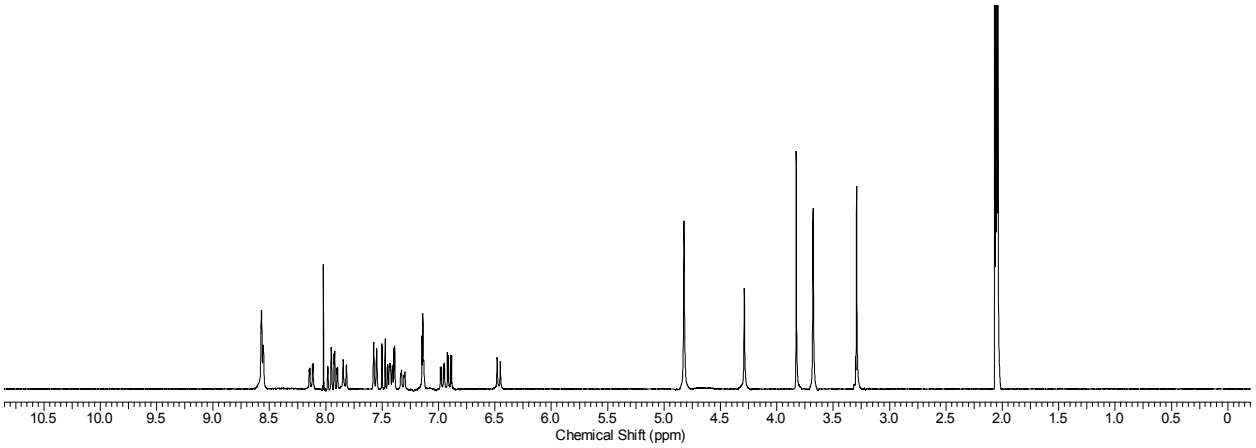
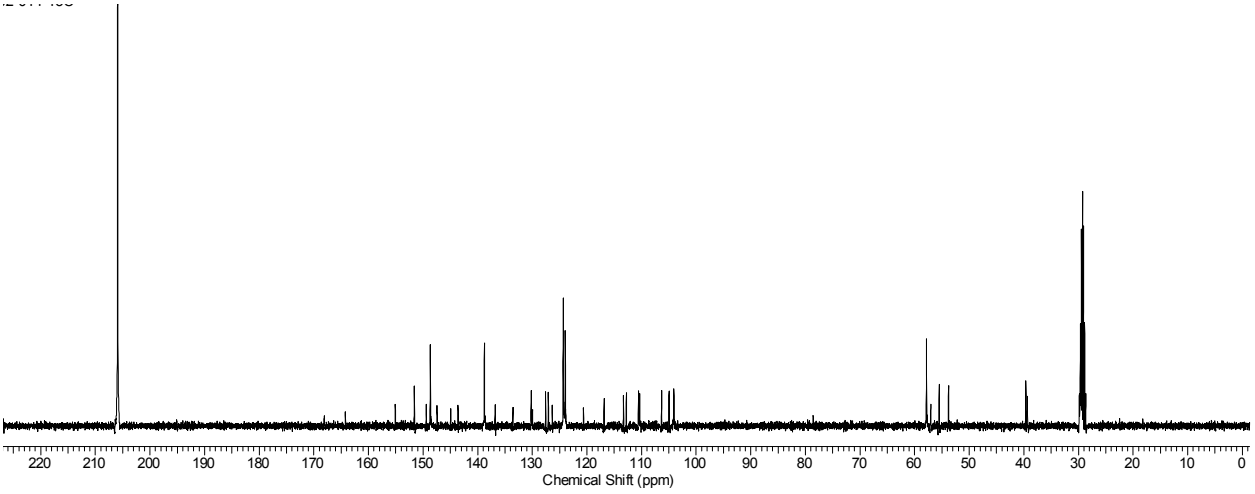


HRMS Spectrum of compound **3**

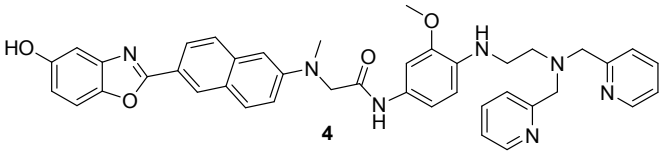




^{1}H NMR (300 MHz, Acetone-d_6) of compound **4**

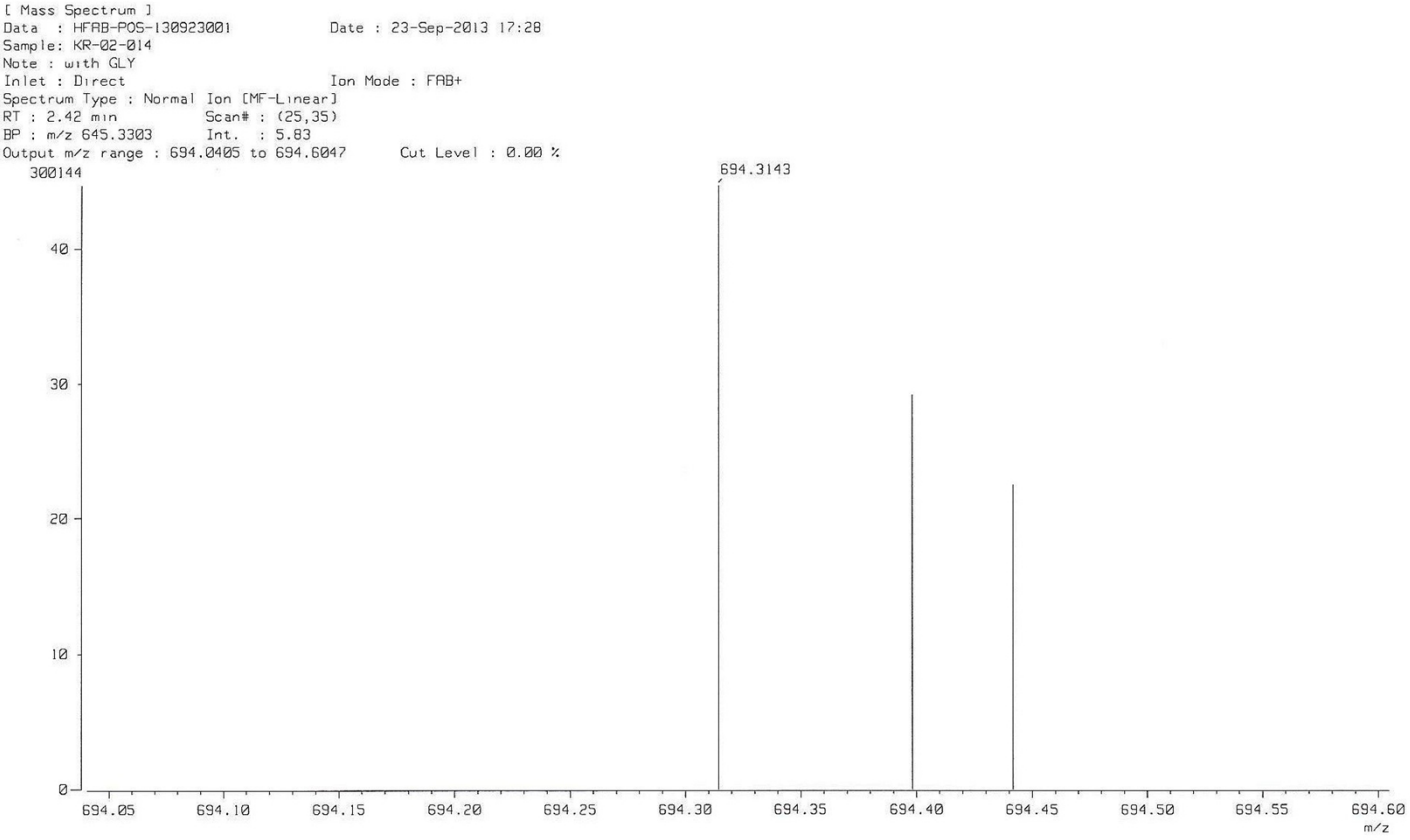


^{13}C NMR (100 MHz, Acetone-d_6) of compound **4**



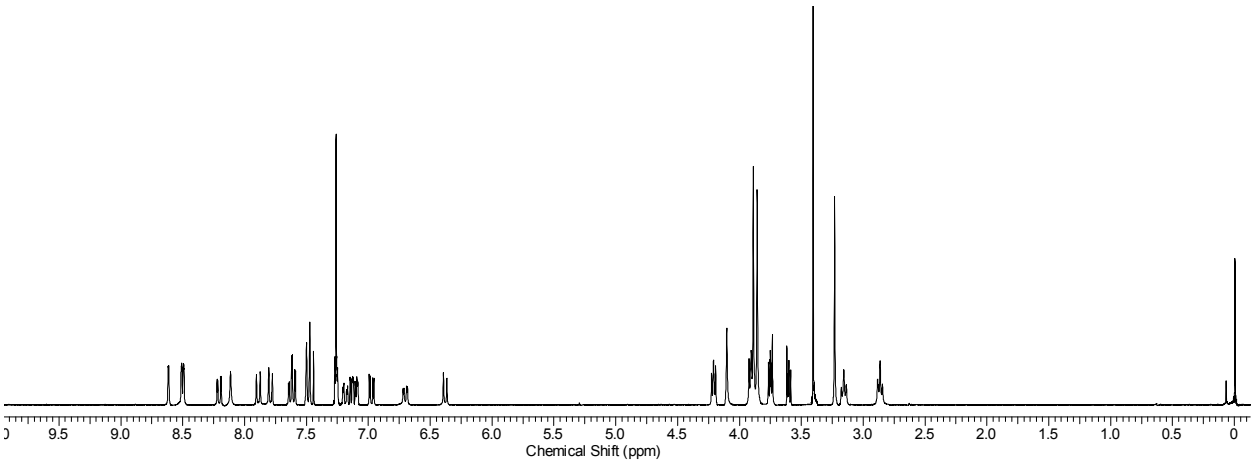
[Elemental Composition]
Data : HFAB-POS-130923001 Date : 23-Sep-2013 17:28
Sample: KR-02-014
Note : with GLY
Inlet : Direct Ion Mode : FAB+
RT : 2.42 min Scan#: (25,35)
Elements : C 41/0, H 40/0, O 4/0, N 7/0
Mass Tolerance : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z	Int%					
694.3143	44.6					
Estimated m/z	Error[ppm]	U.S.	C	H	O	N
694.3142	+0.2	25.5	41	40	4	7

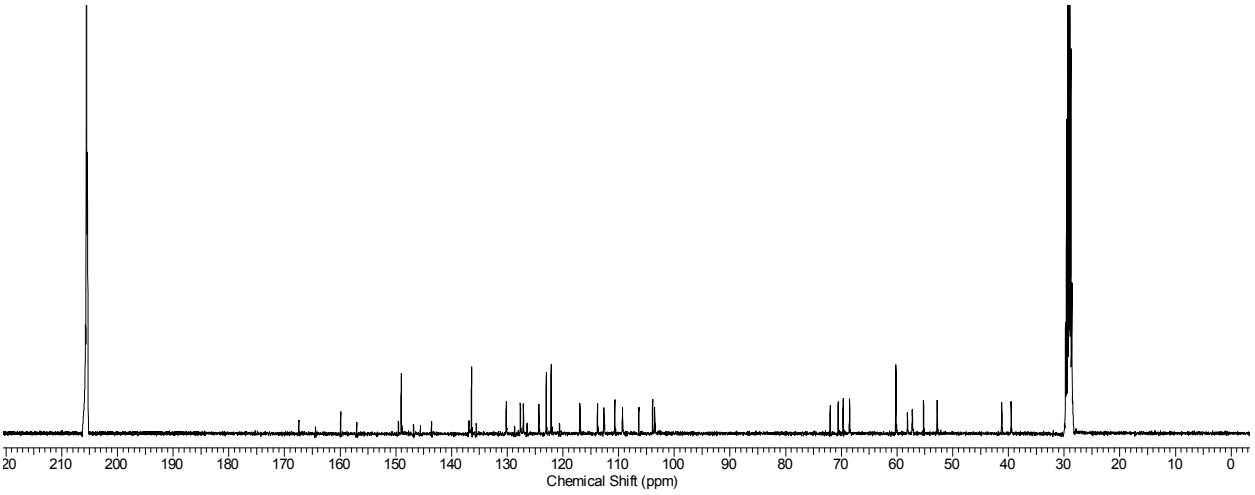


HRMS Spectrum of compound **4**

BZn-cyto



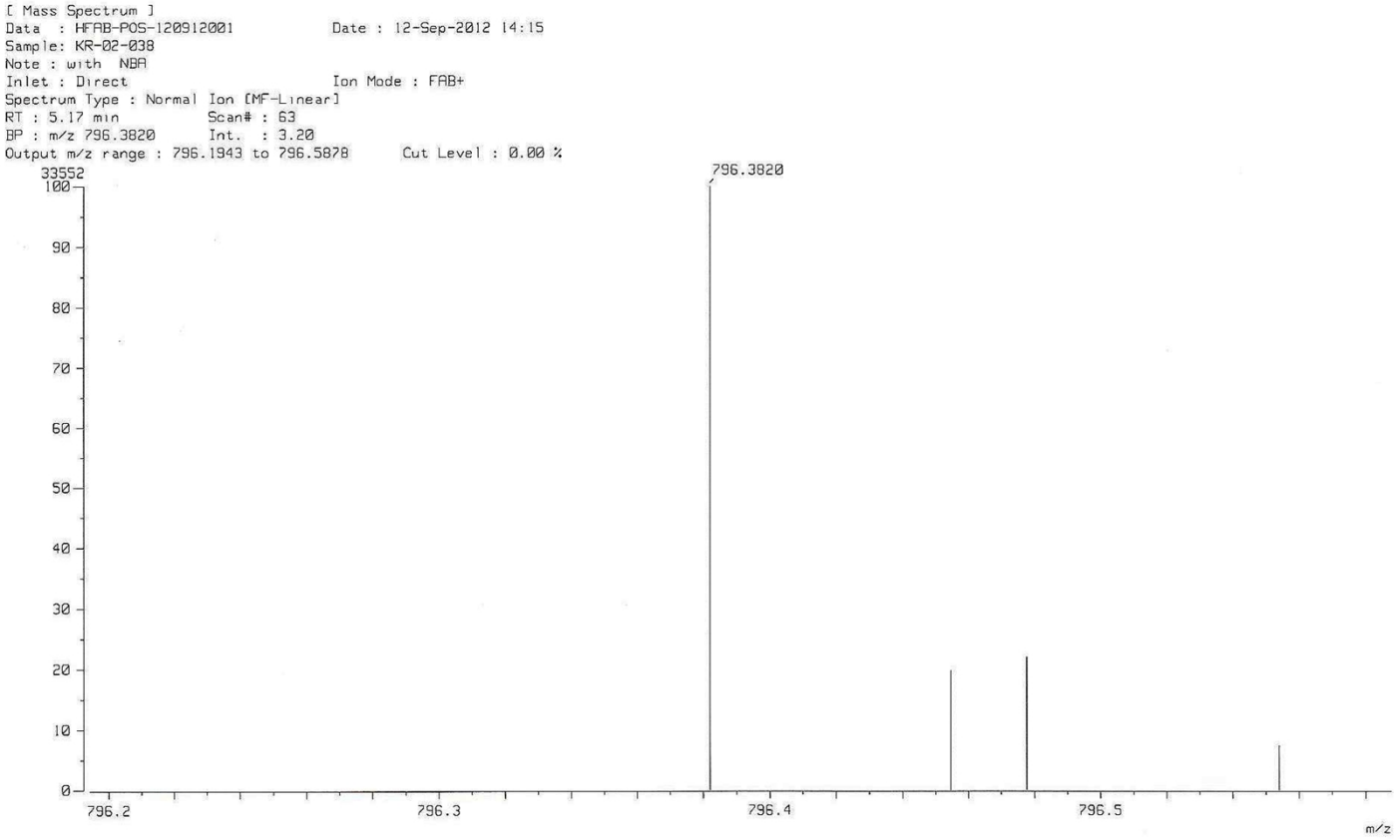
^{1}H NMR (300 MHz, $CDCl_3$) of **BZn-cyto**



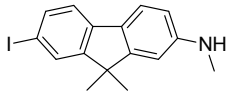
^{13}C NMR (100 MHz, Acetone-d_6) of **BZn-cyto**

BZn-cyto

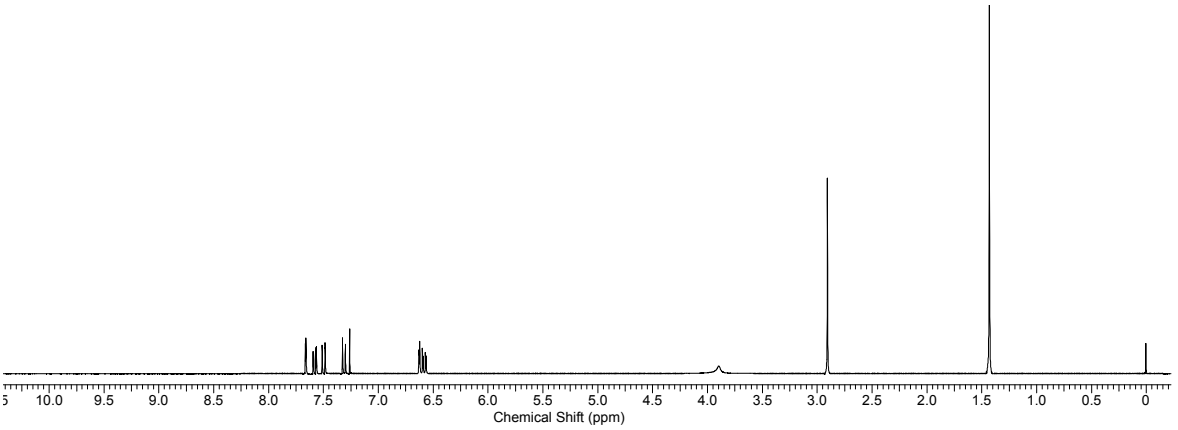
```
[ Elemental Composition ]
Data  : HFAB-POS-120912001              Date : 12-Sep-2012 14:15
Sample: KR-02-038
Note : with  NBA
Inlet : Direct                          Ion Mode : FAB+
RT : 5.17 min                           Scan#: 63
Elements : C 46/0, H 50/0, O 6/0, N 7/0
Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
  796.3820     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     N
  796.3823       -0.3      25.5    46    50     6     7
```



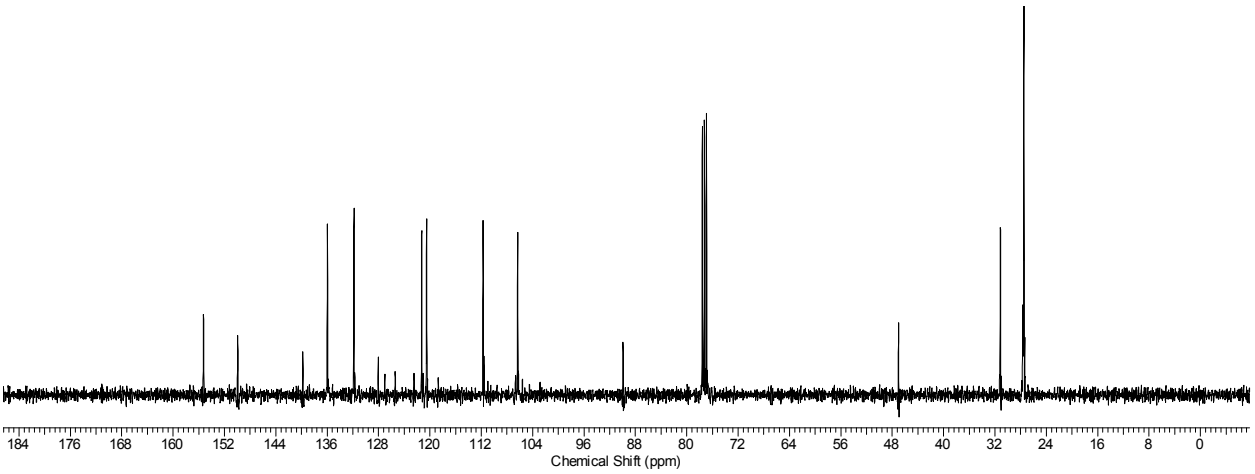
HRMS Spectrum of **BZn-cyto**



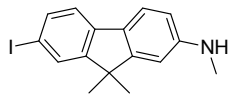
6



^{1}H NMR (300 MHz, $CDCl_3$) of compound **6**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **6**

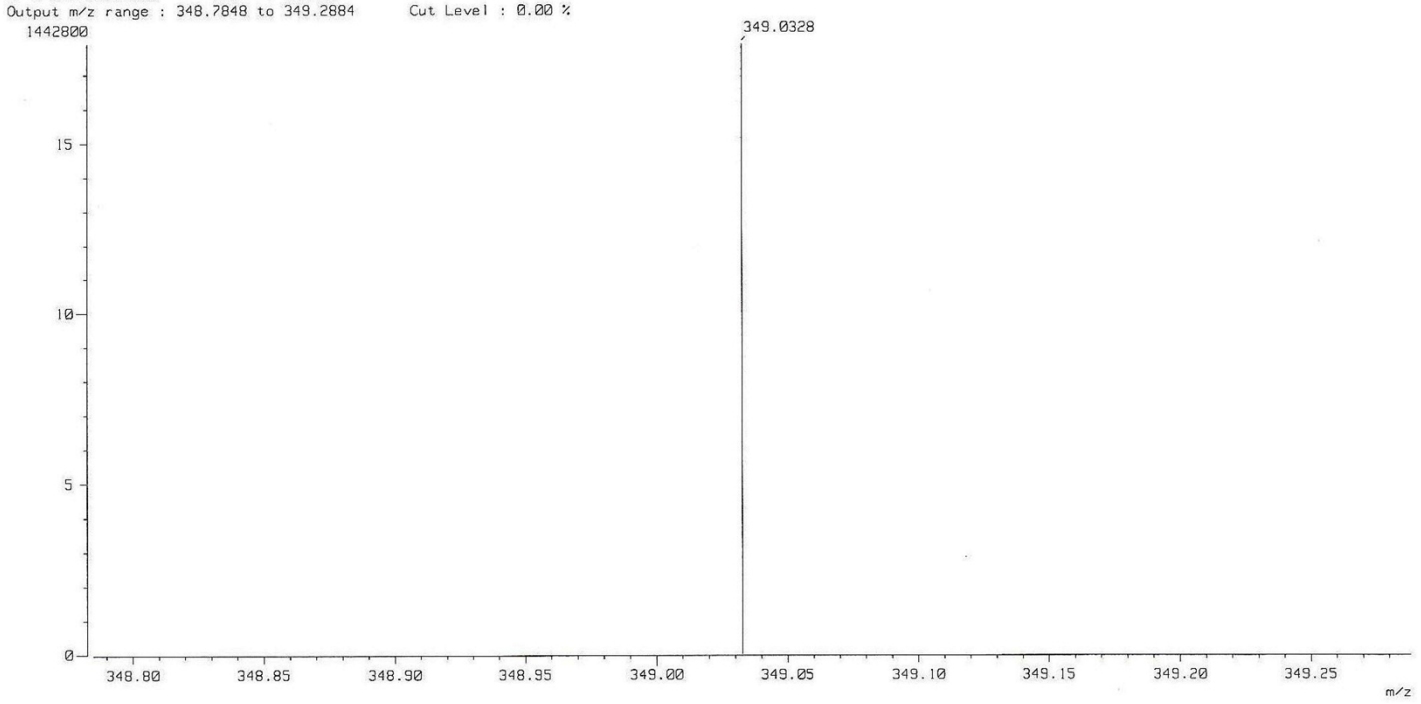


6

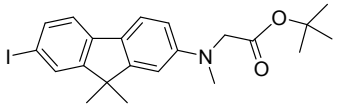
[Elemental Composition]
Data : HFAB-POS-130924013 Date : 24-Sep-2013 17:03
Sample: KR-01-022
Note : with NBA
Inlet : Direct Ion Mode : FAB+
RT : 4.09 min Scan#: 50
Elements : C 16/0, H 16/0, N 1/0, I 1/0
Mass Tolerance : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z	Int%					
349.0328	17.9					
Estimated m/z	**Error[ppm]**	**U.S.**	**C**	**H**	**N**	**I**
349.0328	+0.1	9.0	16	16	1	1

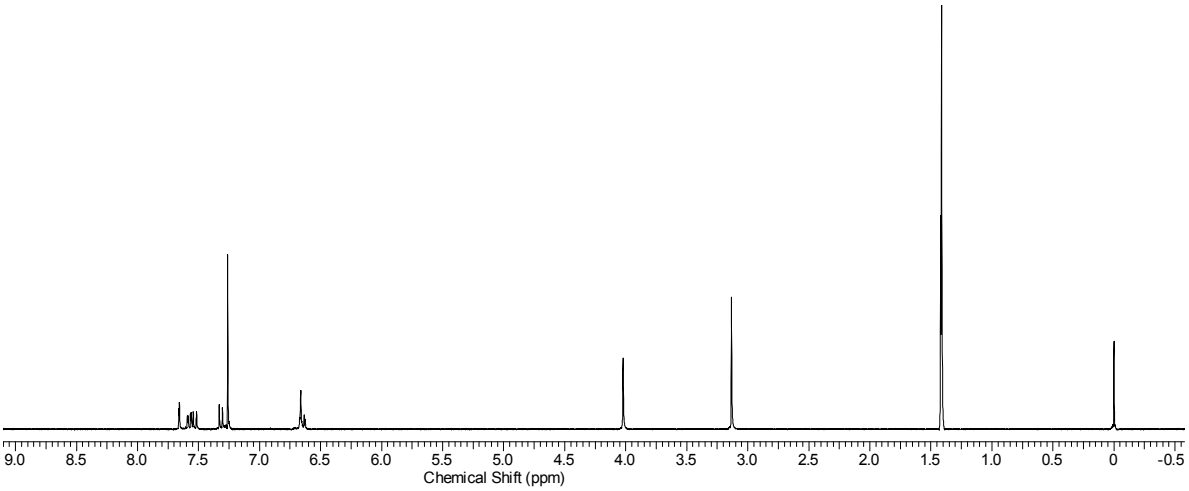
[Mass Spectrum]
Data : HFAB-POS-130924013 Date : 24-Sep-2013 17:03
Sample: KR-01-022
Note : with NBA
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 4.09 min Scan# : 50
BP : m/z 335.0118 Int. : 768.94
Output m/z range : 348.7848 to 349.2884 Cut Level : 0.00 %



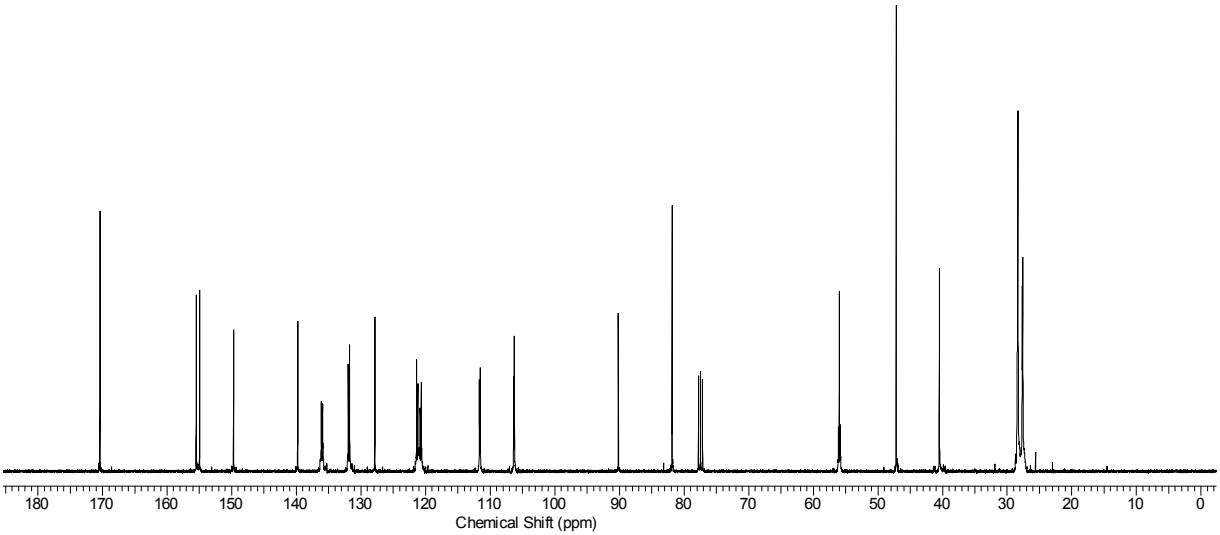
HRMS Spectrum of compound **6**



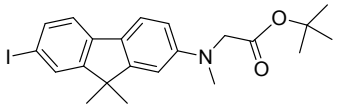
7



^{1}H NMR (300 MHz, $CDCl_3$) of compound **7**

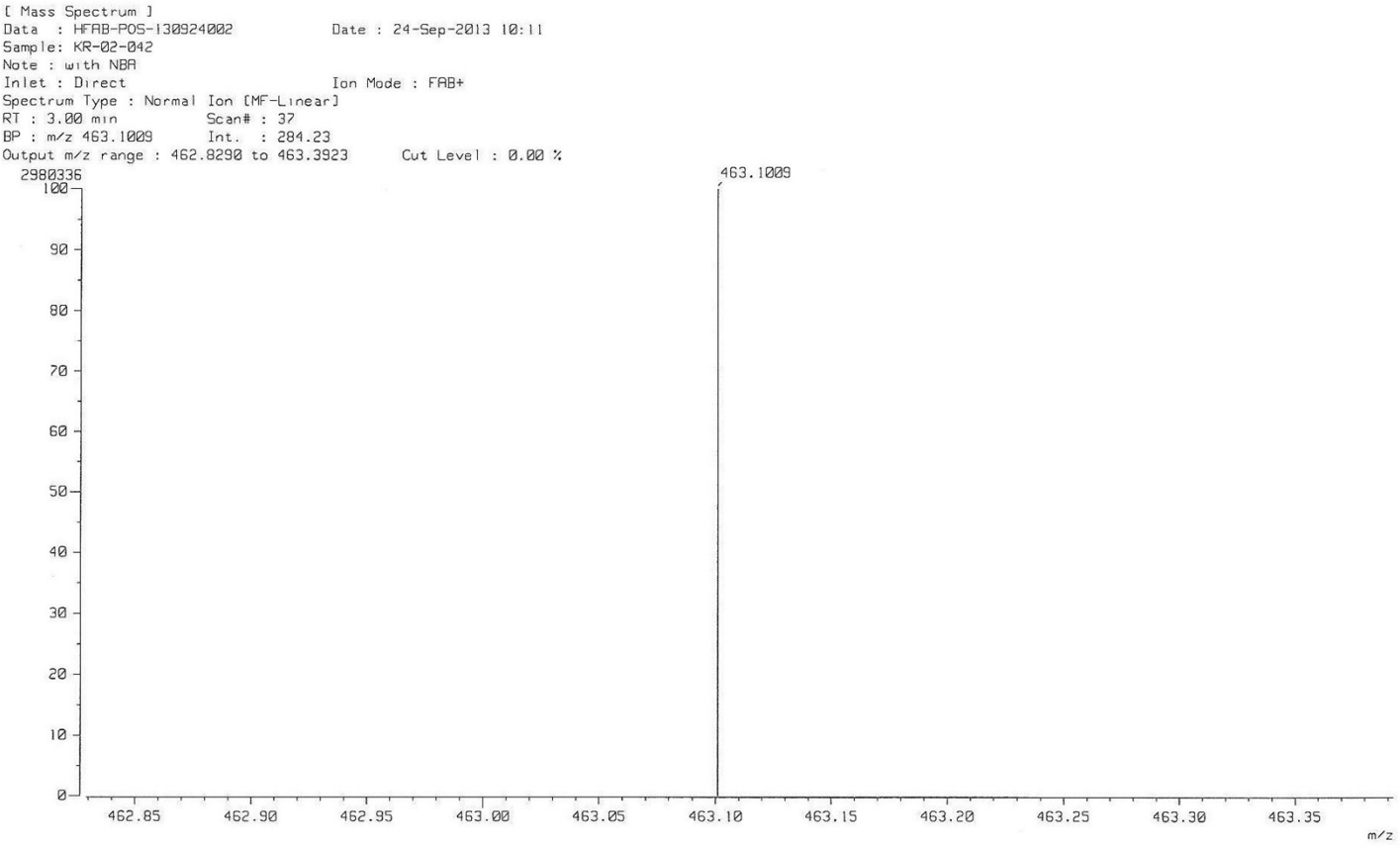


^{13}C NMR (100 MHz, $CDCl_3$) of compound **7**

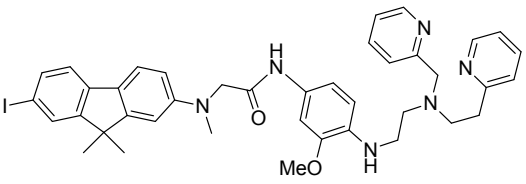


7

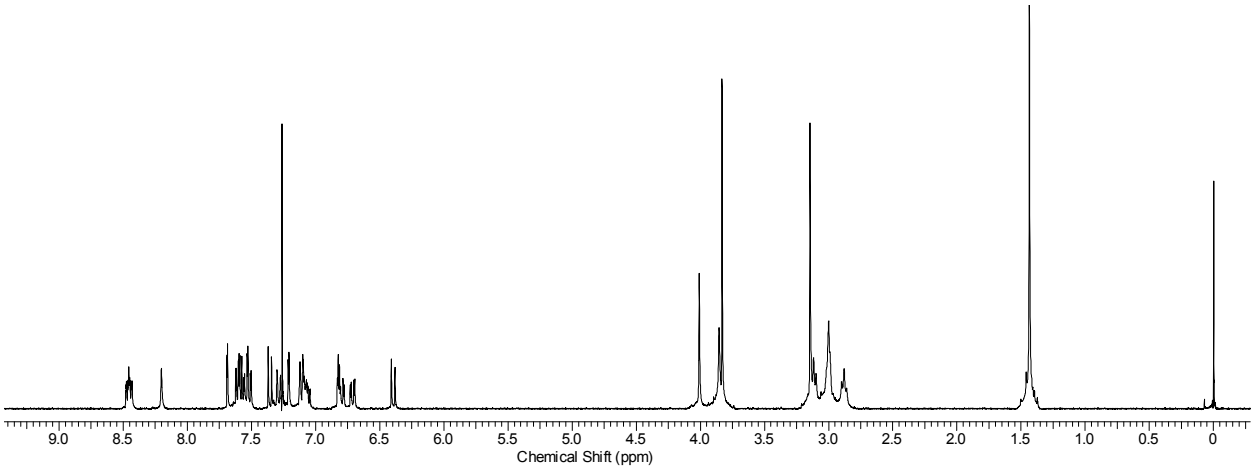
```
[ Elemental Composition ]
Data  : HFAB-POS-130924002              Date : 24-Sep-2013 10:11
Sample: KR-02-042
Note : with NBA
Inlet : Direct                          Ion Mode : FAB+
RT : 3.00 min                           Scan#: 37
Elements : C 22/0, H 26/0, O 2/0, N 1/0, I 1/0
Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
  463.1009     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     N     I
  463.1008       +0.1      10.0    22    26     2     1     1
```



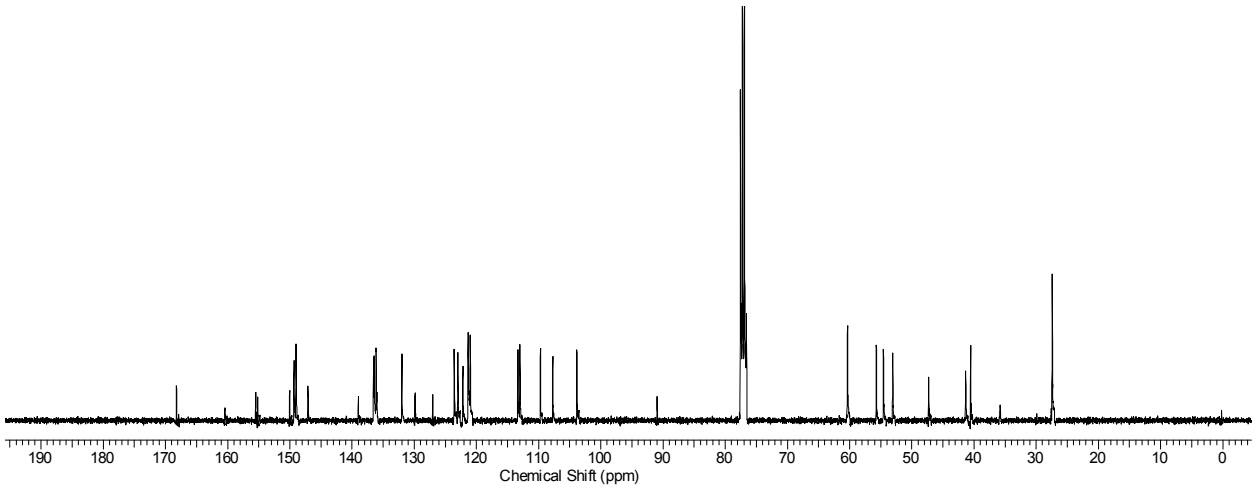
HRMS Spectrum of compound **7**



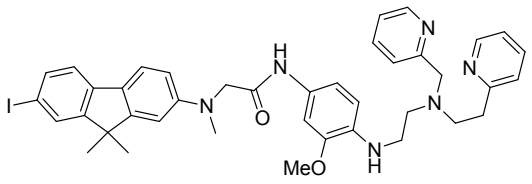
8



^{1}H NMR (300 MHz, $CDCl_3$) of compound **8**

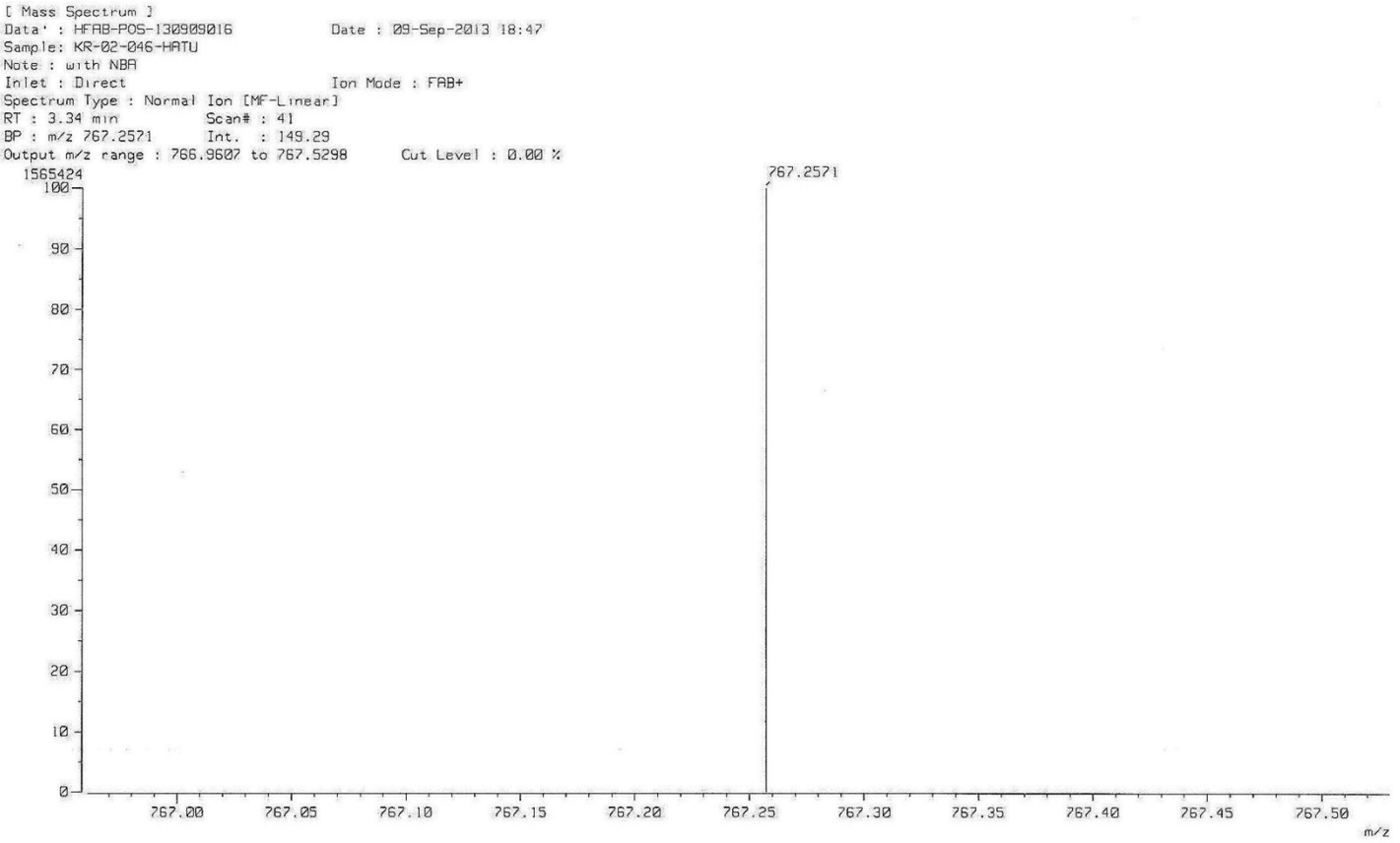


^{13}C NMR (100 MHz, $CDCl_3$) of compound **8**

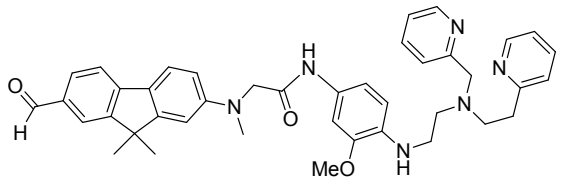


8

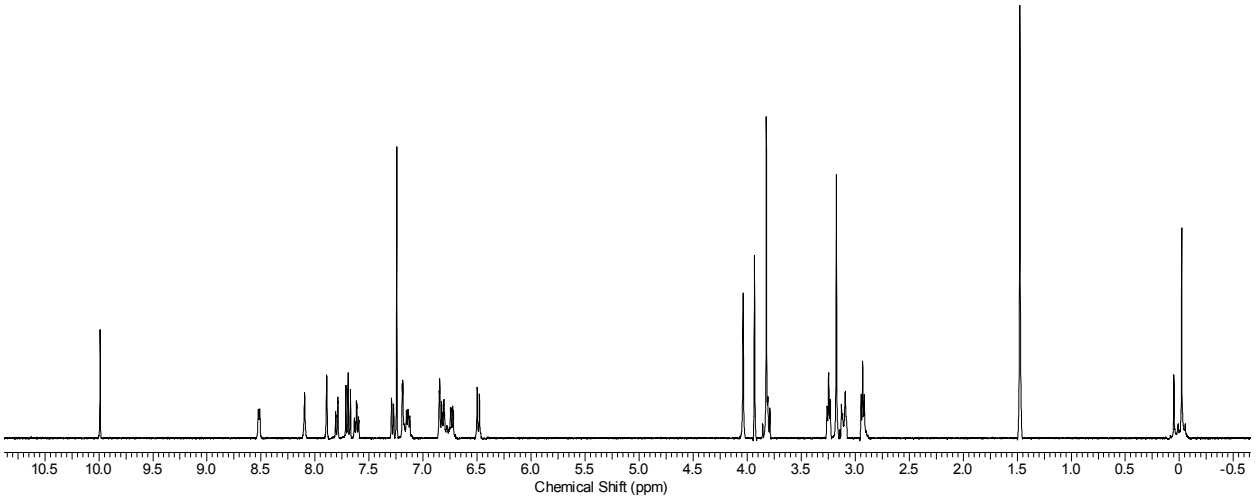
```
[ Elemental Composition ]
Data  : HFAB-POS-130909016              Date : 09-Sep-2013 18:47
Sample: KR-02-046-HATU
Note : with NBA
Inlet : Direct                          Ion Mode : FAB+
RT : 3.34 min                           Scan#: 41
Elements : C 40/0, H 44/0, O 2/0, N 6/0, I 1/0
Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
  767.2571     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     N     I
  767.2571       +0.1      21.5    40    44     2     6     1
```



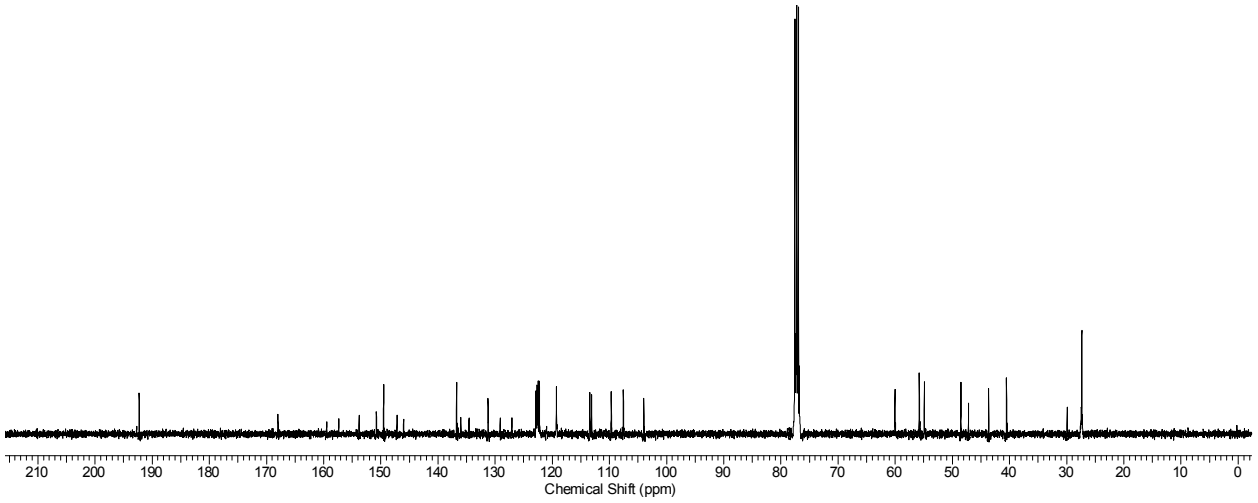
HRMS Spectrum of compound **8**



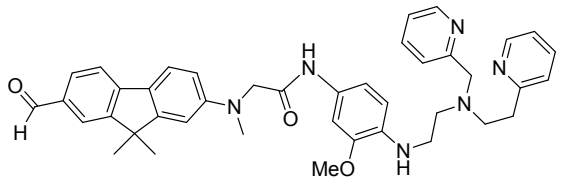
9



^{1}H NMR (300 MHz, $CDCl_3$) of compound **9**



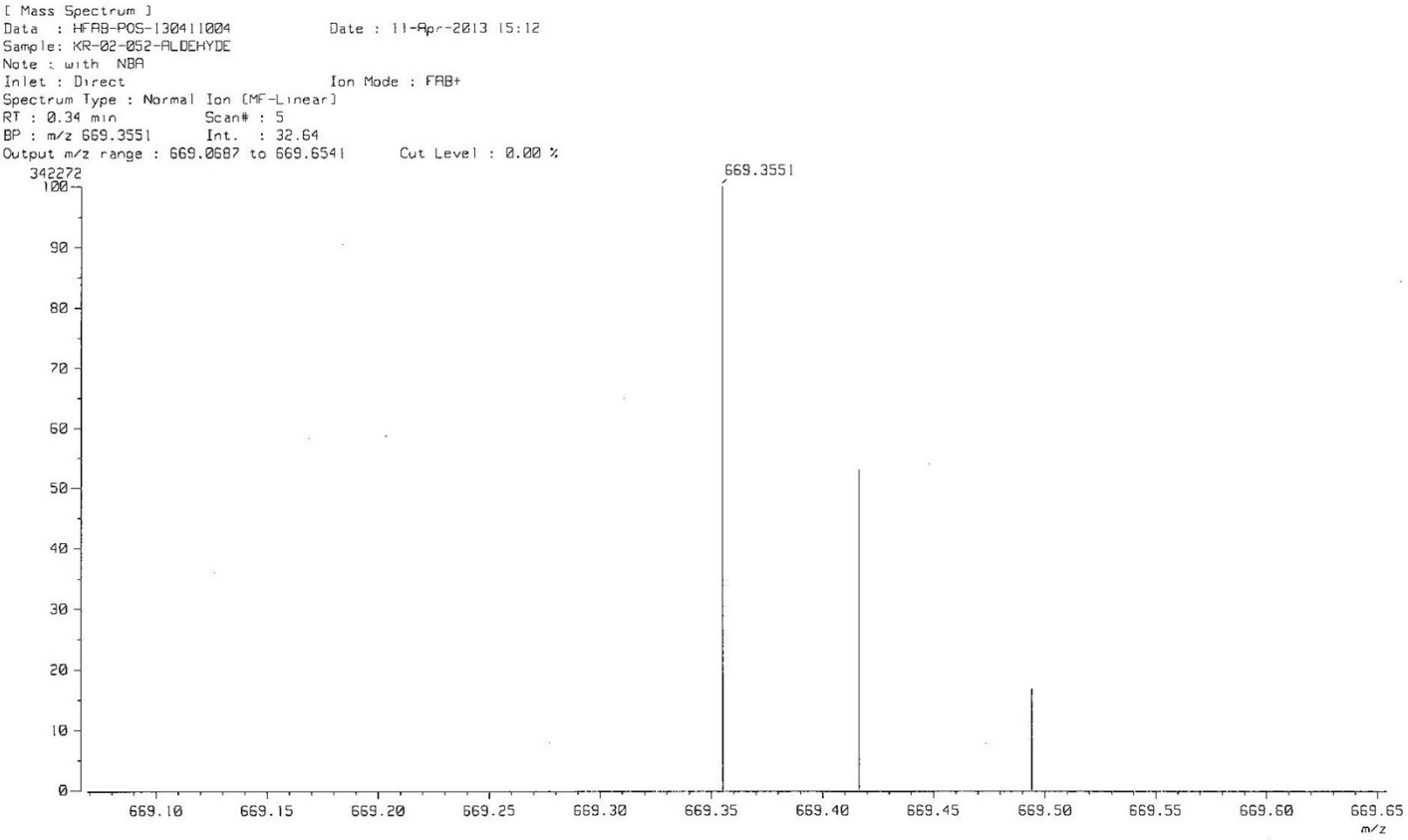
^{13}C NMR (100 MHz, $CDCl_3$) of compound **9**



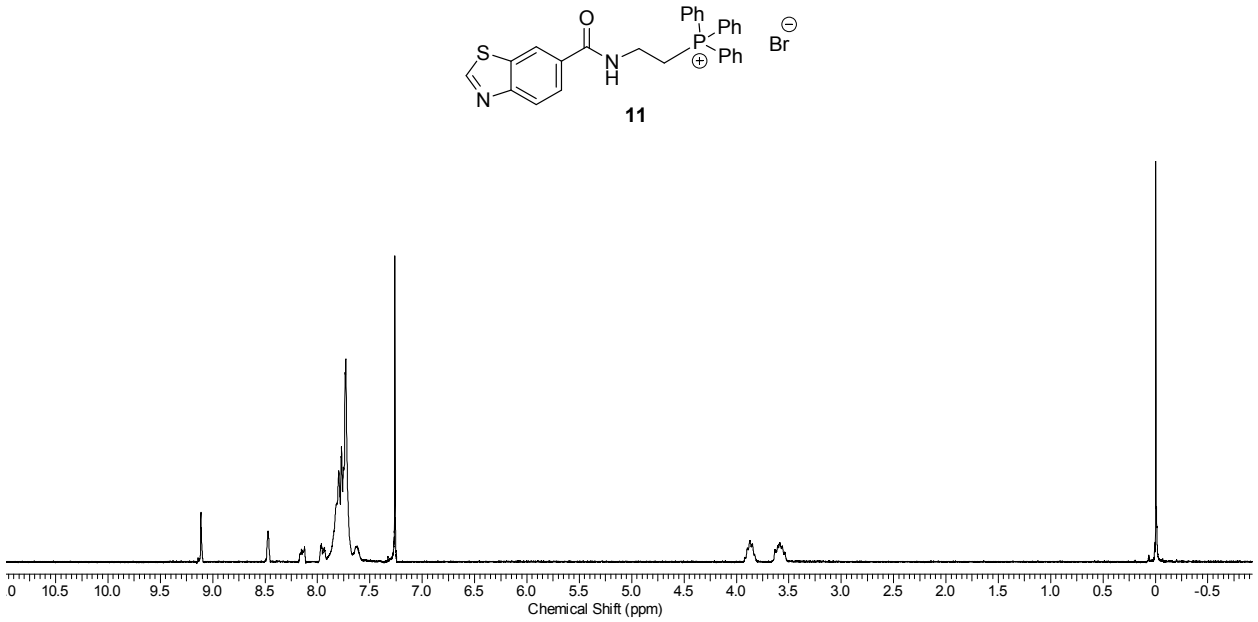
9

[Elemental Composition]
Data : HFAB-POS-130411004 Date : 11-Apr-2013 15:12
Sample: KR-02-052-ALDEHYDE
Note : with NBA
Inlet : Direct Ion Mode : FAB+
RT : 0.34 min Scan#: 5
Elements : C 41/0, H 45/0, O 3/0, N 6/0
Mass Tolerance : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

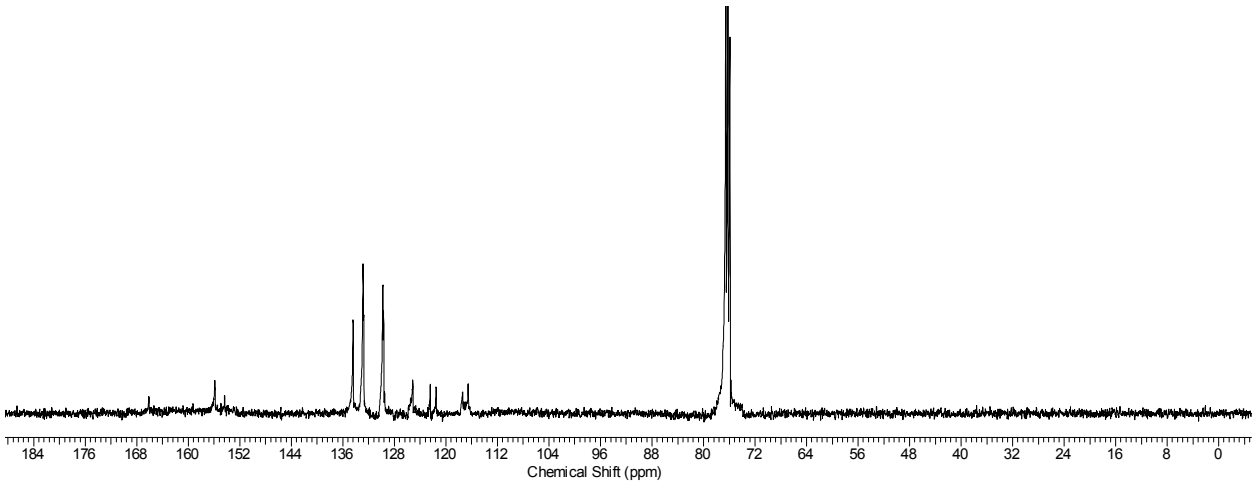
Observed m/z	Int%					
669.3551	100.0					
Estimated m/z	Error[ppm]	U.S.	C	H	O	N
669.3553	-0.3	22.5	41	45	3	6



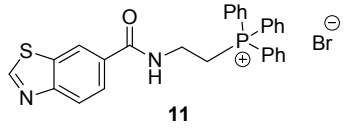
HRMS Spectrum of compound **9**



^{1}H NMR (400 MHz, $CDCl_3$) of compound **11**

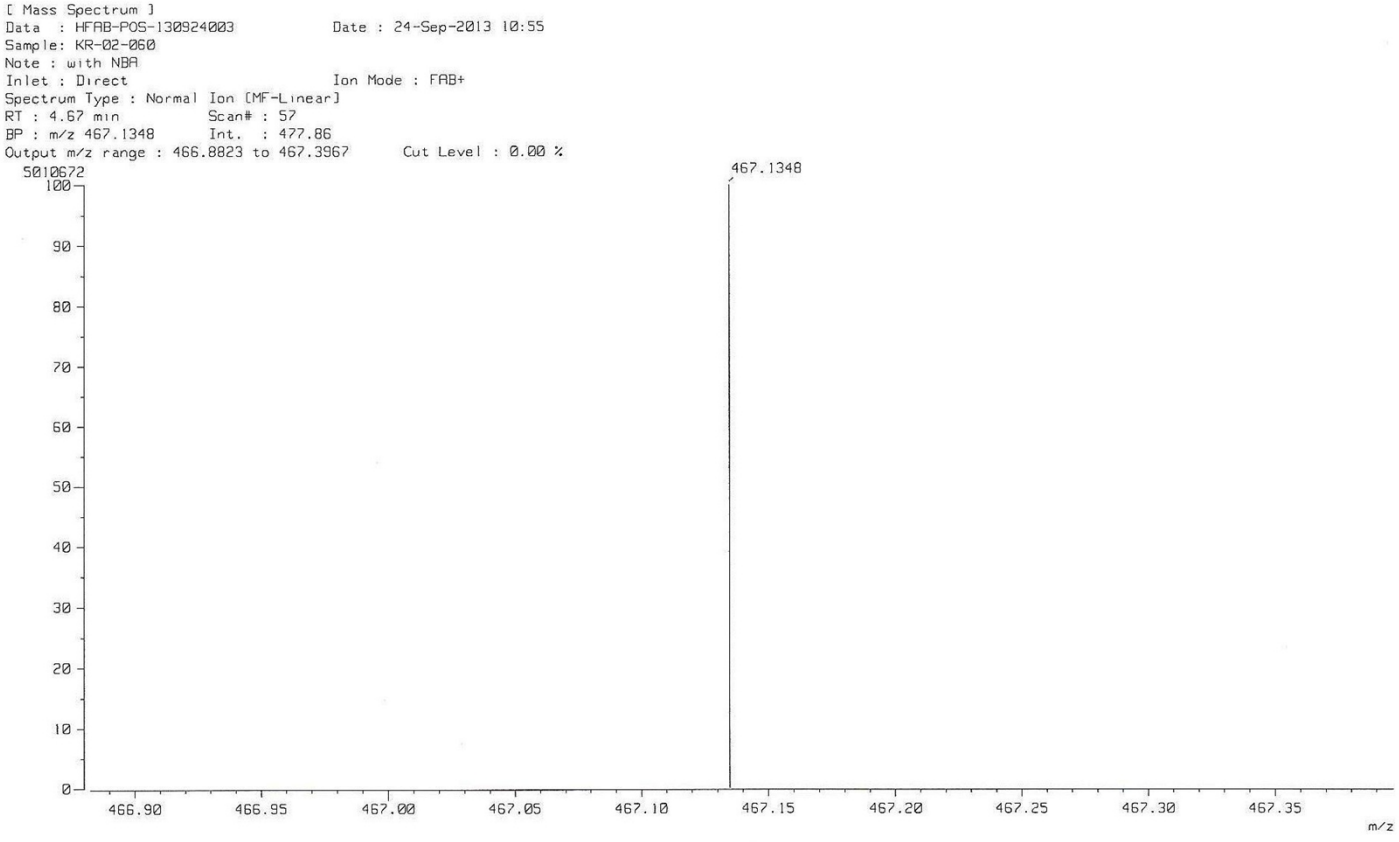


^{13}C NMR (100 MHz, $CDCl_3$) of compound **11**

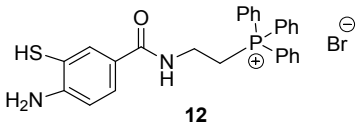


[Elemental Composition]
Data : HFAB-POS-130924003 Date : 24-Sep-2013 10:55
Sample: KR-02-060
Note : with NBA
Inlet : Direct Ion Mode : FAB+
RT : 4.67 min Scan#: 57
Elements : C 28/0, H 24/0, O 1/0, N 2/0, P 1/0, S 1/0, I 1/0
Mass Tolerance : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

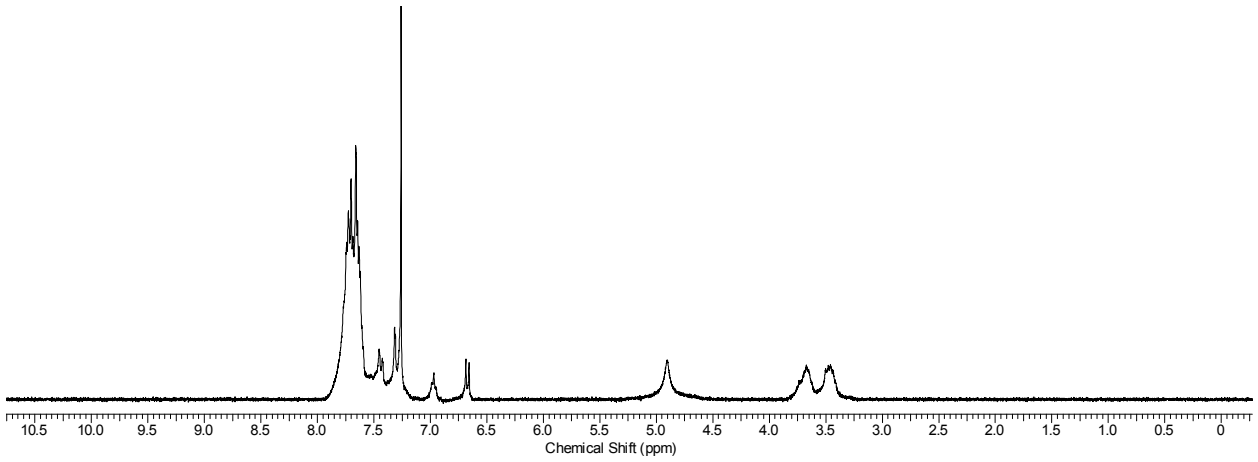
Observed m/z	Int%								
467.1348	100.0								
Estimated m/z	Error[ppm]	U.S.	C	H	O	N	P	S	I
467.1347	+0.3	20.5	28	24	1	2	1	1	-



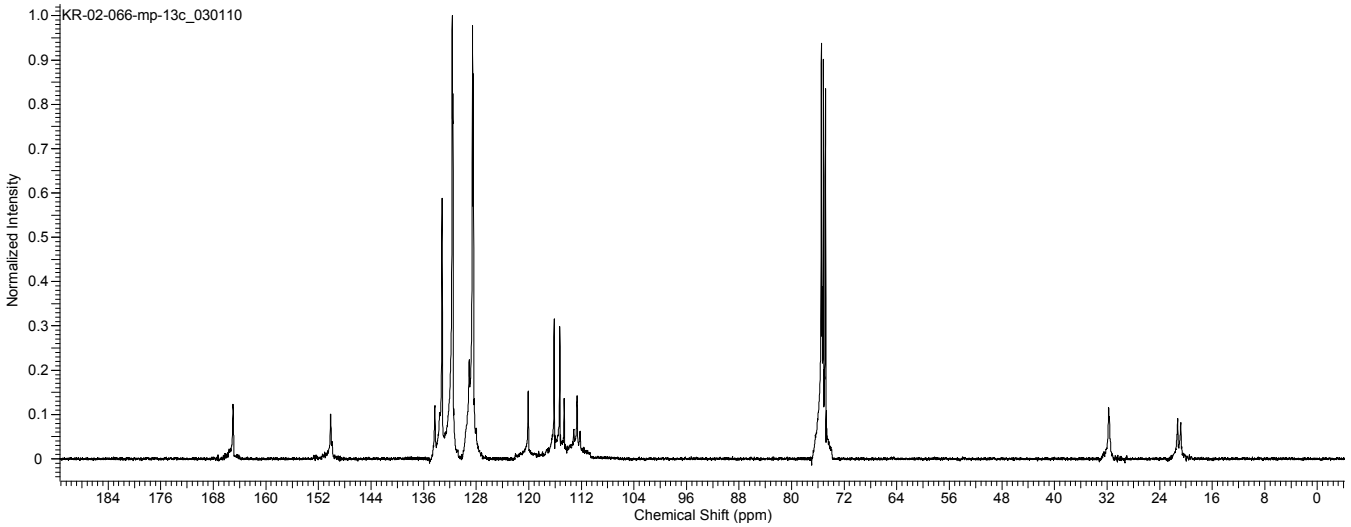
HRMS Spectrum of compound **11**



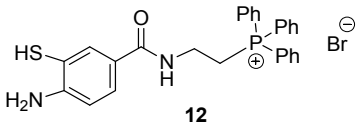
12



^{1}H NMR (400 MHz, $CDCl_3$) of compound **12**

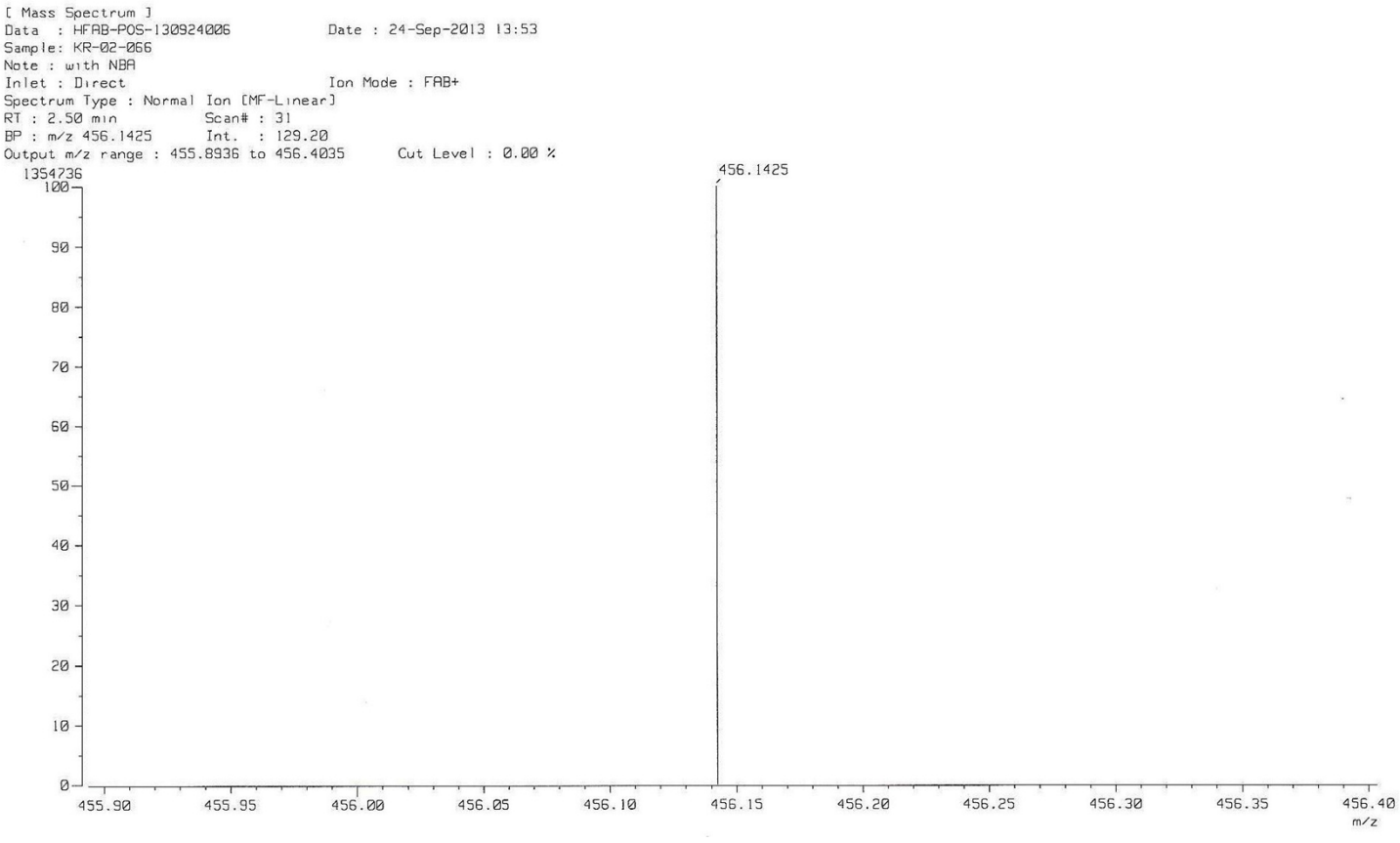


^{13}C NMR (100 MHz, $CDCl_3$) of compound **12**

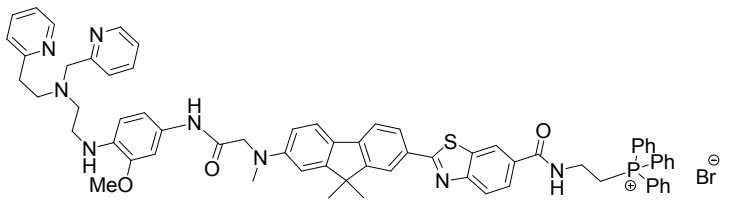


12

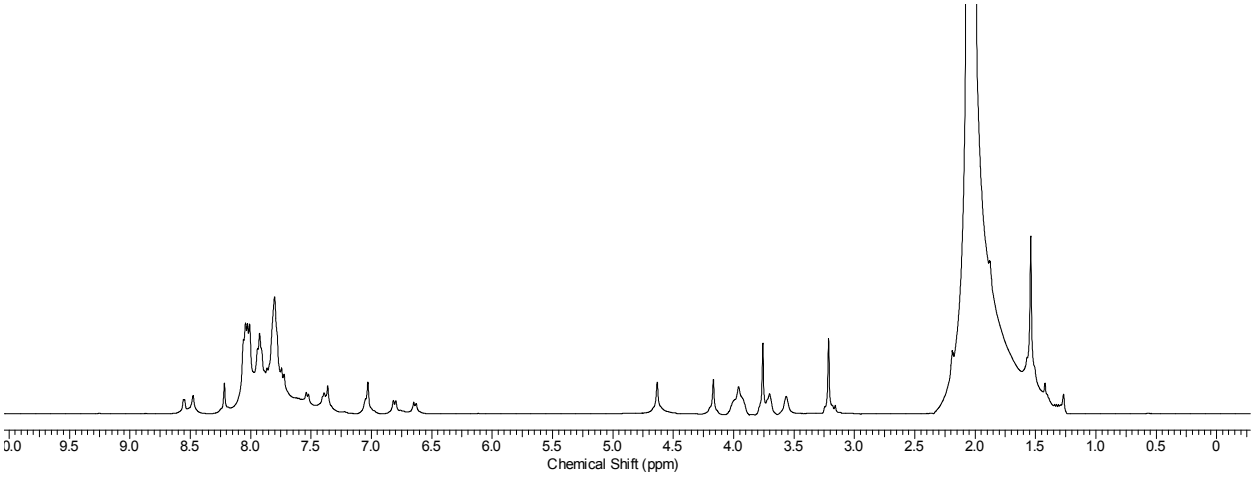
```
[ Elemental Composition ]
Data  : HFAB-POS-130924006              Date : 24-Sep-2013 13:53
Sample: KR-02-066
Note : with NBA
Inlet : Direct                          Ion Mode : FAB+
RT : 2.50 min                           Scan#: 31
Elements : C 27/0, H 25/0, O 1/0, N 2/0, P 1/0, S 1/0, I 1/0
Mass Tolerance      : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
  456.1425     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     N     P     S     I
  456.1425       -0.1      19.0    27    25     1     2     1     1     -
```



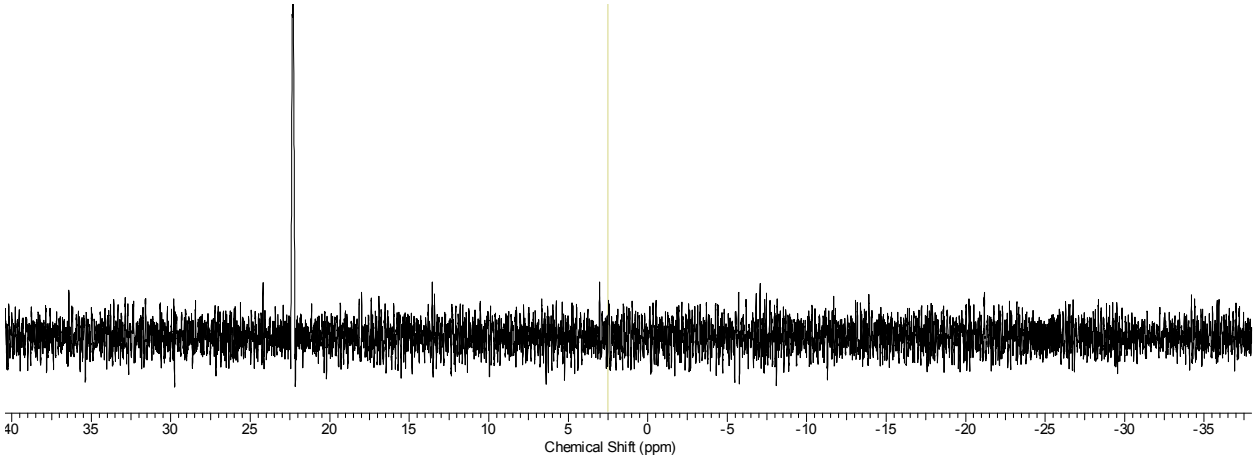
HRMS Spectrum of compound **12**



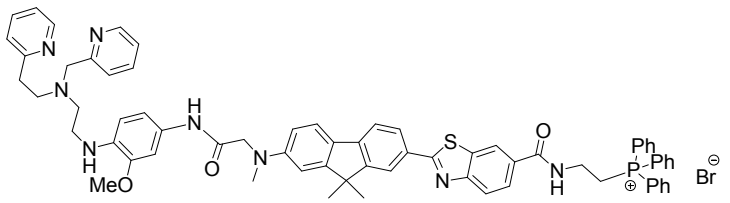
FZn-mito



^{1}H NMR (400 MHz, Acetone-d_6) of **FZn-mito**

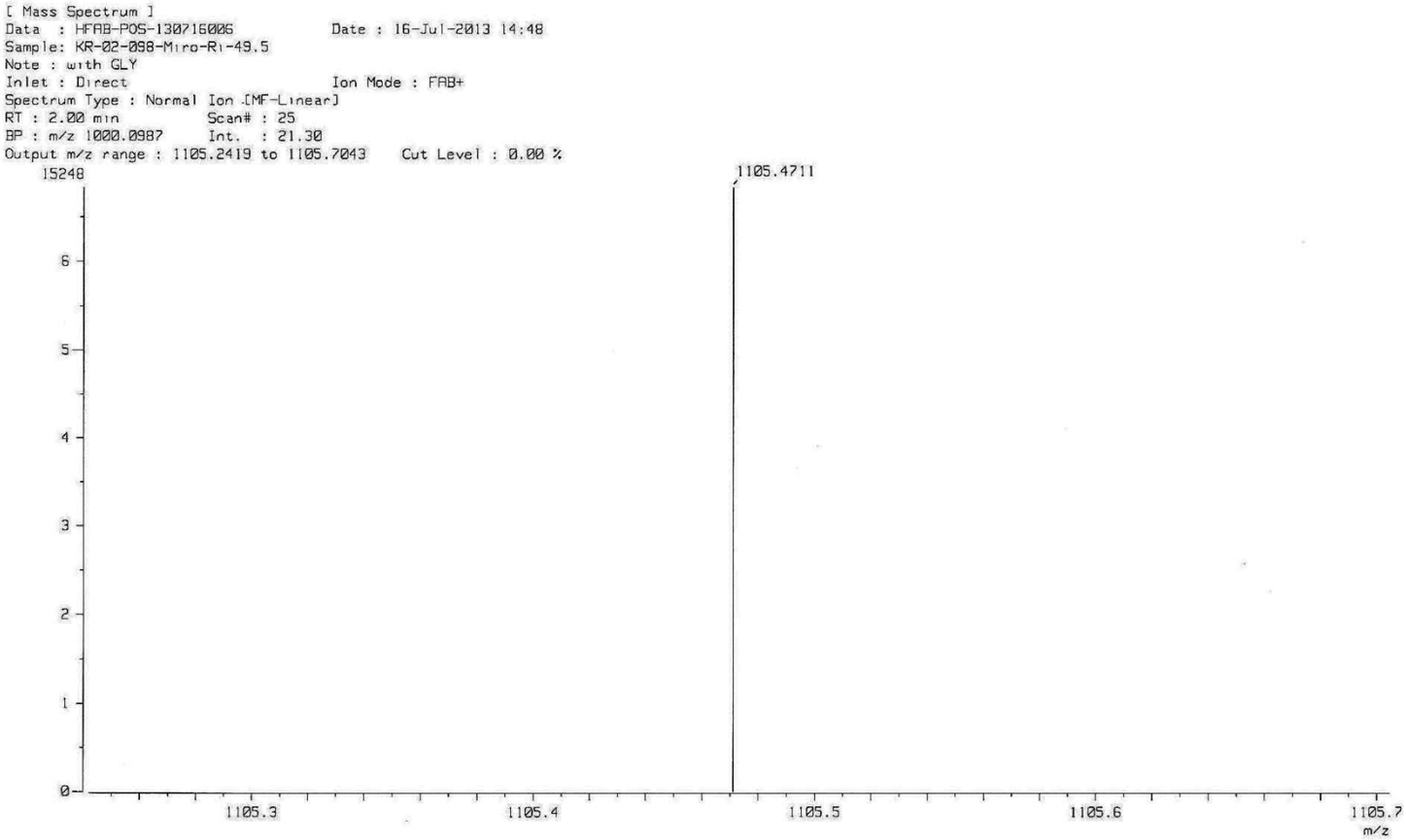


^{31}P NMR (162 MHz, DMSO-d_6) of **FZn-mito**



FZn-mito

```
[ Elemental Composition ]
Data  : HFAB-POS-130716006              Date : 16-Jul-2013 14:48
Sample: KR-02-098-Miro-Ri-49.5
Note : with GLY
Inlet : Direct                          Ion Mode : FAB+
RT : 2.00 min                           Scan#: 25
Elements : C 68/0, H 67/0, O 3/0, N 8/0, P 1/0, S 1/0
Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
 1105.4711       6.8
Estimated m/z  Error[ppm]  U.S.     C     H     O     N     P     S
 1105.4716       -0.5      42.5    68    66     3     8     1     1
```



HRMS Spectrum of **FZn-mito**