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

Kailash Rathore, Chang Su Lim, Young Lee, and Bong Rae Cho*

Department of Chemistry, Korea University, 1-Anamdong, Seoul 136-701, Korea.

Table of Contents

Synthesis of BZn-Cyto and FZn-Mito ................................................................. S3
Water solubility ........................................................................................................ S10
Spectroscopic measurements ................................................................................... S11
Detection windows .................................................................................................... S15
Cell Viability ............................................................................................................. S16
Photostability ............................................................................................................ S16
Reference .................................................................................................................. S17

1H, 13C NMR and HRMS Spectra of 2-12, BZn-cyto and FZn-mito.............................. S18

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....................................................................................................................................................

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)........

Figure S3. One-photon fluorescence spectra of 3 µM BZn-Cyto in the presence of free Zn2+ (0–22 nM). (b) Job’s plot for the complexation of BZn-Cyto with free Zn2+. Total Zn2+ concentration was 2 µM. All data were collected MOPS and 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 Zn2+ (0–130 nM). (e) Job’s plot for the complexation of FZn-Mito with free Zn2+. Total Zn2+ 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+, Ca2+, Mg2+; 300 µM for Mn2+, Fe2+, Co2+, Ni2+, Cu2+ (empty bars) followed by addition of 130 nM of free Zn2+ (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.

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.

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.

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 ($\lambda_{ex} = 740$ nm, ~200 fs). Cells shown are representative images from replicate experiments (n = 5).

Table S1. Photophysical properties of BZn-Cyto and FZnMito in various solvents.
Scheme S1. Synthesis of BZn-cyto:

Reagents and conditions: (a) Pd(OAc)$_2$, t-Bu$_3$P, CuBr, Cs$_2$CO$_3$, Toluene, 90 °C, 16 h, 56 %; (b) BBr$_3$, CH$_2$Cl$_2$, -78 °C - rt, 16 h, 63 %; (c) HATU, DIPEA, BPEA, DMF, rt, 16 h, 37 %; (d) CH$_3$OCH$_2$CH$_2$OCH$_2$CH$_2$OTs, K$_2$CO$_3$, 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$_3$P (11 mg, 0.050 mmol), Cs$_2$CO$_3$ (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$_2$Cl$_2$ (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$_2$O$_4$ [M]$^+$: 418.1893, Found 418.1893.
Compound 3. BB₃ (1.0 M in CH₂Cl₂, 1.1 mL, 1.1 mmol) was added drop wise to a solution of t-butyl ester 2 (112 mg, 0.27 mmol) in CH₂Cl₂ (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 CH₂Cl₂ (10 mL). The solid residue was filtered and washed successively with CH₂Cl₂ (5 mL), water (5 mL) and 5% MeOH in CH₂Cl₂ (2 x 5 mL) and dried in vacuo 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; ¹H NMR (300 MHz, DMSO-d₆): 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).

¹³C NMR (100 MHz, DMSO-d₆): 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 C₂₀H₁₇N₂O₄ [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 MgSO₄, 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 / H₂O (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. ¹H NMR (300 MHz, Acetone-d₆): 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). ¹³C NMR (100 MHz, Acetone-d₆): 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_{7}O_{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$_2$CO$_3$ (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_{7}O_{6} [M+H]^+: 796.3823, Found 796.3820.
**Scheme S2. Synthesis of FZn-mito:**

Reagents and conditions: (a) CH₃I, K₂CO₃, DMF, 0 °C, 5 h, 41 %; (b) BrCH₂CO₂-t-Bu, Proton sponge, CH₃CN, reflux, 6 h, 87 %; (c) i) TFA / CH₂Cl₂ (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, CH₂Cl₂, rt, 16 h, 63 %; (f) Hydrazine monohydrate, EtOH, 80 °C, 3 h, 47 %; (g) PTSA, CHCl₃, reflux, 16 h, 25%.

**Compound 5**

4-Amino-2-methoxy-N-{2-[N’-(2-picolyl)-N’-(2-pyridin-2-yl)ethyl]amino}ethylaniline (PPEA) and (2-aminoethyl)triphenylphosphonium bromide 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-9H-fluorene (1.0 g, 3.0 mmol) and K₂CO₃ (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 MgSO₄ and filtered. Evaporation of the solvent in vacuo 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. 1H NMR (300 MHz, CDCl3): 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). 

13C NMR (100 MHz, CDCl3): 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 C16H16NO: 295.1095. Found: 295.1098.

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 MgSO4 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. 1H NMR (300 MHz, CDCl3): δ 7.66 (d, J = 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). 13C NMR (100 MHz, CDCl3): 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 C22H26INO2 [M]+: 463.1008. Found: 463.1009.

Compound 8. A solution of ester 7 (470 mg, 1.0 mmol) in 50% TFA in CH2Cl2 (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 PPEA5 (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 MgSO4, filtered and concentrated in vacuo to yield a crude residue which was purified via silica gel chromatography using 2% MeOH in CHCl3 as the eluent to furnish compound 8 (415 mg, 0.55 mmol) in 53% yield as a colorless viscous oil. 1H NMR (300 MHz, CDCl3): 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). 13CNMR (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, 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 C40H44N6O2 [M+H]+: 767.2571. Found: 767.2571.

**Compound 9.** i-PrMgCl.LiCl7 (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. NH4Cl (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 MgSO4 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 CHCl3 as the eluent to afford title compound 9 (54 mg, 0.081 mmol) in 43 % yield as yellow viscous oil. 1H NMR (300 MHz, CDCl3): 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). 13CNMR (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 C41H44N6O3 [M+H]+: 669.3553. Found: 669.3551.
**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$_2$Cl$_2$ (3 mL). The reaction mixture was stirred at room temperature for 1 h under a blanket of argon. (2-amoethyI)tripheny1phosphonium 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$_3$CN (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$_2$OPS [M-Br]$^+$: 467.1347, found 467.1348.

**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, CDCl$_3$): 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$_2$OPS [M-HBr]$^+$: 456.1425, found 456.1425.

**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 / H₂O (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. ¹H NMR (400 MHz, Acetone-d₆): 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). ³¹P NMR (162 MHz, DMSO-d₆): δ 22.3 ppm. HRMS (FAB⁺): m/z calcd. for [C₇₆H₆₀N₅O₅PS⁺]: 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.

<table>
<thead>
<tr>
<th>Solvent (E_T)</th>
<th>BZn- Cyto</th>
<th>BZn- Mito</th>
<th>BZn- Cyto</th>
<th>BZn- Mito</th>
<th>BZn- Cyto</th>
<th>BZn- Mito</th>
<th>BZn- Cyto</th>
<th>BZn- Mito</th>
<th>BZn- Cyto</th>
<th>BZn- Mito</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-Dioxane (0.164)</td>
<td>360</td>
<td>385</td>
<td>421</td>
<td>486</td>
<td>0.24</td>
<td>0.45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,4-Dioxane/ EtOH (1/1)</td>
<td>-</td>
<td>397</td>
<td>-</td>
<td>536</td>
<td>-</td>
<td>0.31</td>
<td>-</td>
<td>800</td>
<td>-</td>
<td>117</td>
</tr>
<tr>
<td>DMF (0.386)</td>
<td>377</td>
<td>404</td>
<td>451</td>
<td>548</td>
<td>0.25</td>
<td>0.13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EtOH (0.654)</td>
<td>369</td>
<td>399</td>
<td>445</td>
<td>552</td>
<td>0.18</td>
<td>0.11</td>
<td>740</td>
<td>150</td>
<td>135</td>
<td>-</td>
</tr>
<tr>
<td>MOPS/SDS</td>
<td>376</td>
<td>394</td>
<td>466</td>
<td>520</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MOPS (1.00)</td>
<td>367</td>
<td>392</td>
<td>470</td>
<td>559</td>
<td>0.025</td>
<td>0.023</td>
<td>740</td>
<td>194</td>
<td>103</td>
<td>-</td>
</tr>
<tr>
<td>HeLa cell</td>
<td>450</td>
<td>530</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The numbers in the parenthesis are normalized empirical parameter of solvent polarity. The values of absorption and emission spectra in nm. The uncertainty is ± 15%. The 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. \[ \text{Zn}^{2+} \] values of FZn-Mito in 1,4-Dioxane/EtOH (1/1), BZn-Cyto in MOPS and EtOH in the presence of excess Zn^{2+}.

**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''-tetraacetic acid) were prepared.

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

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

Where,

\[
K_{Zn-EGTA}^{app} = K_{Zn-EGTA} / \alpha_{Zn} \alpha_{EGTA},
\]

\[
\alpha_{Zn} = 1 + 10^{(pH - pK_1)} + 10^{(2pH - pK_1 - pK_2)} + 10^{(3pH - pK_1 - pK_2 - pK_3)} + ...
\]

\[
\alpha_{EGTA} = 1 + 10^{(pK_1 - pH + 0.11)} + 10^{(pK_1 + pK_2 - 2pH + 0.22)} + 10^{(pK_1 + pK_2 + pK_3 - 3pH + 0.33)} + ...
\]

and

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

Thus,\[
K_{Zn-EGTA}^{app} = \frac{K_{Zn-EGTA} (1 + 10^{(pK_{Zn-EGTA} - pH)})}{(1 + 10^{pH - pK_{Zn}})(1 + 10^{pK_1 - pH}) + 10^{(pK_1 + pK_2 - 2pH)}})
\]
The stability constant for the Zn$^{2+}$ complex of EGTA ($K_{\text{Zn-EGTA}}$) was taken from ref. 14. Thus, for EGTA (pH 7.2, 0.1 M KCl, 25 °C), $pK_1 = 9.40$, $pK_2 = 8.79$, $pK_3 = 2.70$, $\log K_{\text{Zn-EGTA}} = 12.6$.

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

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

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

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).$^{12,13}$

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

where $F$ is the fluorescence intensity, $F_{\text{max}}$ is the maximum fluorescence intensity, $F_0$ is the fluorescence intensity in the absence of Zn$^{2+}$, and [Zn$^{2+}$]$_{\text{free}}$ 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 $K_{d\text{TP}}$ 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.

![Cell viability graph](image)

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.

![Photostability images](image)

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.
References


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

$^1$H NMR (300 MHz, CDCl$_3$) of compound 2

$^{13}$C NMR (100 MHz, CDCl$_3$) of compound 2
**Elemental Composition**

Data: HFAB-POS-130924016  
Sample: YL-01-010  
Note: with NEA  
Inlet: Direct  
Ion Mode: FAB+  
RT: 3.25 min  
Scan#: 40  
Elements: C 26/0, H 26/0, O 4/0, N 2/0  
Mass Tolerance: 10mmu  
Unsaturation (U.S.): 0.0 - 100.0

<table>
<thead>
<tr>
<th>Observed m/z</th>
<th>Int%</th>
<th>Estimated m/z</th>
<th>Error[ppm]</th>
<th>U.S.</th>
<th>C</th>
<th>H</th>
<th>O</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>418.1893</td>
<td>100.0</td>
<td>418.1893</td>
<td>+0.1</td>
<td>14.0</td>
<td>25</td>
<td>26</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

**Mass Spectrum**

Data: HFAB-POS-130924016  
Sample: YL-01-010  
Note: with NEA  
Inlet: Direct  
Ion Mode: FAB+  
Spectrum Type: Normal Ion [MF-Linear]  
RT: 3.25 min  
Scan#: 40  
BP: m/z 418.1893  
Int.: 144.70  
Output m/z range: 417.3867 to 418.4840  
Cut Level: 0.00 %

**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
[ Elemental Composition ]
Data : HPAB-POS-130924015       Date : 24-Sep-2013 17:44
Sample: KR-01-028
Note : with NBA
Inlet : Direct          Ion Mode : FAB+
RT : 3.17 min               Scan#: 39
Elements : C 20/0, H 17/0, O 4/0, N 2/0
Mass Tolerance : 10 ppm
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z    Int%
349.1188    65.3

Estimated m/z    Error[ppm]    U.S.    C    H    O    N
349.1188    -0.2    13.5    20    17    4    2

[ Mass Spectrum ]
Data : HPAB-POS-130924015       Date : 24-Sep-2013 17:44
Sample: KR-01-028
Note : with NBA
Inlet : Direct          Ion Mode : FAB+
Spectrum Type : Normal Ion (M-Linear)
RT : 3.17 min       Scan#: 39
BP : m/z 299.1845    Int. : 83.66
Output m/z range : 348.0531 to 349.4834        Cut Level : 0.00 %

HRMS Spectrum of compound 3
\( ^1H \text{ NMR} \) (300 MHz, Acetone-\( d_6 \)) of compound 4

\( ^{13}C \text{ NMR} \) (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 : 10ppm
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

[ Mass Spectrum ]
Data : HFAB-POS-130923001 Date : 23-Sep-2013 17:28
Sample: KR-02-014
Note : with GLY
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 2.42 min Scan# : (25,35)
BP : m/z 549.3383 Int. : 5.83
Output m/z range : 694.0000 to 694.6047 Cut Level : 8.20 %

HRMS Spectrum of compound 4
$^1$H NMR (300 MHz, CDCl$_3$) of BZn-cyto

$^{13}$C NMR (100 MHz, Acetone-$d_6$) of 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

[ Mass Spectrum ]
Data : HFAB-POS-120912001 Date : 12-Sep-2012 14:15
Sample: KR-02-038
Note : with NBA
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion (VF-Linear)
RT : 5.17 min Scan#: 63
BP : m/z 796.3820 Int. : 3.28
Output m/z range : 795.1943 to 796.5879 Cut Level : 8.88 %
HRMS Spectrum of BZn-cyto

\[ \text{Chemical Shift (ppm)} \]

\[ \text{Chemical Shift (ppm)} \]

\[^{1}\text{H NMR (300 MHz, CDCl}_3\text{)}\text{ of compound 6}\]

\[^{13}\text{C NMR (100 MHz, CDCl}_3\text{)}\text{ of compound 6}\]
[ Elemental Composition ]
Data : HFAB-POS-130924013 Date : 24-Sep-2013 17:03
Sample: KR-01-022
Note : with NBA
Inlet : Direct
RT : 4.09 min
Ion Mode : FAB+
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
Spectrum Type : Normal Ion (MF-Linear)
RT : 4.03 min
Scan# : 50
BP : m/z 349.0328 Int. : 768.34
Output m/z range : 348.7848 to 349.2684 Cut Level : 0.03 %

S27
HRMS Spectrum of compound 6

\[ \text{Chemical Shift (ppm)} \]

\( ^1H \) NMR (300 MHz, CDCl\(_3\)) of compound 7

\[ \text{Chemical Shift (ppm)} \]

\( ^{13}C \) NMR (100 MHz, CDCl\(_3\)) of compound 7

S28
[ 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

[ Mass Spectrum ]
Data: HFAB-POS-130924002  Date: 24-Sep-2013 10:11
Sample: KR-02-042
Note: with NBA
Inlet: Direct  Ion Mode: FAB+
Spectrum Type: Normal Ion (RF-Linear)
RT: 3.00 min  Scan#: 37
DP: m/z 453.1009  Int.: 264.23
Output m/z range: 452.0298 to 463.3523  Cut Level: 0.00 %

HRMS Spectrum of compound 7
$^1$H NMR (300 MHz, CDCl$_3$) of compound 8

$^{13}$C NMR (100 MHz, CDCl$_3$) of compound 8
[Elemental Composition]
Data: HPAB-POS-130909016
Sample: KR-02-046-HATU
Note: with NBA
Inlet: Direct
RT: 3.34 min
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

[Mass Spectrum]
Data: HPAB-POS-130909016
Sample: KR-02-046-HATU
Note: with NBA
Inlet: Direct
 Ion Mode: FAB+
Spectrum Type: Normal Ion (DF-Linear)
RT: 3.34 min
Scan#: 41
BP: m/z 767.2571 Int.: 145.29
Output m/z range: 765.9997 to 767.5298 Cut Level: 0.00 %

HRMS Spectrum of compound 8
$^1$H NMR (300 MHz, CDCl$_3$) of compound 9

$^{13}$C NMR (100 MHz, CDCl$_3$) of compound 9
HRMS Spectrum of compound 9
$\text{H NMR (400 MHz, CDCl}_3\text{) of compound 11}$

$\text{C NMR (100 MHz, CDCl}_3\text{) of compound 11}$
[ 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
$^{1}H$ NMR (400 MHz, CDCl$_3$) of compound 12

$^{13}C$ NMR (100 MHz, CDCl$_3$) of compound 12
HRMS Spectrum of compound 12
$^1$H NMR (400 MHz, Acetone-$d_6$) of FZn-mito

$^{31}$P NMR (162 MHz, DMSO-$d_6$) of FZn-mito
[Elemental Composition]
Data: HPAB-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: 10 ppm
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

[Mass Spectrum]
Data: HPAB-POS-130716006  Date: 16-Jul-2013 14:48
Sample: KR-02-098-Miro-Ri-49.5
Note: with GLY
Inlet: Direct  Ion Mode: FAB+
Spectrum Type: Normal Ion (MF-Linear)
RT: 2.00 min  Scan#: 25
P*: ratio 1000.2997  Int.: 21.30
Output m/z range: 1105.2418 to 1105.7243  Cut Level: 0.00%
HRMS Spectrum of \textbf{FZn-mito}