SUPPORTING INFORMATION

Near-Infrared Fluorescence Activation Probes based on Disassembly-Induced Emission

Cyanine Dye

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**Materials and instruments**

Chemicals and reagents were purchased from Sigma-Aldrich and TCI and were used without further purification. All solvents were used after appropriate distillation or purification. Besides the hCAII and SNAP-tag protein which were expressed and purified in the laboratory, all other proteins used in the selectivity test were purchased from Sigma-Aldrich. PBS buffer (0.9 mM KCl, 2.67 mM KH₂PO₄, 138 mM NaCl, 8.1 mM Na₂HPO₄) was diluted 10-times from commercially available concentrates supplied by Amersco. Thin layer chromatography (TLC) was performed on TLC-aluminum sheets (Silica gel 60 F254, Merck). Flash column chromatography was performed with silica gel (230-400 mesh, Merck). HPLC analysis was performed with analytical column (EC 150/4.6 Nucleosil 300-5 C18, Macherey-Nagel). Products were purified by semi-preparative column (VP 150/21 Nucleosil 300-5 C18, Macherey-Nagel).

¹H, and ¹³C nuclear magnetic resonance (NMR) spectra were recorded either on Varian MR-400, Varian Unityinova-500, Mercury-400 with ¹H chemical shifts (δ) reported in ppm relative to the solvent residual signals of d-methanol (3.30 ppm), d-DMSO (2.49 ppm). ¹³C chemical shifts (δ) were reported in ppm relative to the solvent residual signals of d-methanol (49.0 ppm) and d-DMSO (39.5 ppm). Coupling constants were reported in Hz. Absorption spectra were recorded on Ocean Optics USB4000 spectrophotometer and using long wavelength pass filter (LAMBDA GG-400-25.4). Fluorescence spectra were recorded using Hitachi F-4500 fluorescence spectrophotometer and TECAN Infinite M200Pro. High resolution mass spectra (HRMS) were recorded on Varian 901-FTMS.
SNAP-tag and hCAII protein expression and purification

Plasmid pET51b-SNAP and pET51b-HCA both with C-terminal His-tag were transformed to *E. coli* strain BL21. The bacteria were cultured at 37 °C in LB broth medium to OD$_{600\text{nm}}$ of 1.2. Protein expression was induced by adding 1 mM IPTG. The bacteria were grown for an additional 16 hours at 18 °C and harvested by centrifugation. The cells were lysed by sonication and insoluble protein and cell debris were removed by centrifugation. The SNAP-tag and hCAII protein were then purified by Ni-NTA. The purified protein was concentrated and transferred in PBS buffer using Amicon® Ultra centrifugal filters and snap frozen in liquid nitrogen before being stored at -78 °C. The concentration of protein was determined using BCA assay. The purity of the protein was checked by SDS-PAGE and stained by Instant Blue.
Fluorescence imaging of transfected HeLa Cells

The cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. 1.5 × 10^4 cells were seeded in 8-well chamber slides and cultured overnight at 37 °C in air with 5% CO₂. Cells were then transfected by using X-treme GENE HP DNA transfection reagent (Roche Applied Science) according to the manufacturer’s protocol. Thirty hours after transfection, the cells were washed with DMEM supplemented with 10% FBS twice and cultured overnight. The cells were washed twice with Opti-MEM and stained with 0.5 μM Hoechst 34580. After thirty minutes incubation, excess Hoechst 34580 was removed. The cells were treated with 0.5 μM of probe prepared in Opti-MEM (1.0 % DMSO (v/v)). The fluorescence imaging was carried out by using Laser Scanning Confocal Microscope (LSM 700, Zeiss, Germany). For Cy5 channel, the images were taken by using 639 nm laser and LP640 emission filter. For Hoechst, we used 405 nm laser and SP490 emission filter. For CFP, we used 405 nm laser and SP555 emission filter.

Fluorescence imaging of HeLa cells under hypoxia condition

The cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. 2 × 10^4 cells were seeded in 8-well chamber slides and cultured 24 hours at 37 °C in air with 5% CO₂. The cells were cultured for 24 hours at 37°C in Opti-MEM under hypoxic conditions (<0.1% O₂) generated with an Anaero Pack (Mitsubishi Gas Chemical Company Inc.). For hypoxia-mimetic conditions, the cells were cultured in Opti-MEM with 200 μM DFO for 24 hours at 37 °C in air with 5% CO₂. The cells were imaged in Opti-MEM with the addition of 0.5 μM probe1 (1% DMSO (v/v)) in the absence or presence of 100 μM EZA (1% DMSO (v/v)). The images were taken after staining without any washing operations.
Transmission electron microscopy (TEM)

Sample (5 µM 1 or with 10 µM hCAII) were prepared by drop casting onto 200 mesh carbon-coated copper grids and air-dried overnight. The samples were washed with water before measurement. TEM imaging was carried out with JEOL-2100 microscope with an accelerating voltage of 160 kV.

Dynamic light scattering (DLS)

DLS analyses were performed on Brookhaven 90Plus at 25°C in PBS buffer using a plastic cuvette (3 mL volume). At least six measurements were taken. All samples were incubated for 15 minutes before measurement.

Western blot

Cell extracts were prepared by washing cells with PBS buffer and solubilizing 5 × 10^7 cells in 100 µL of RIPA buffer (150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0). After incubation for 15 minutes on ice, the lysates were centrifuged at 12,000 rpm for 20 min at 4 °C and the supernatant was collected. 16 µL/lane of cell lysate was loaded to 10% SDS-PAGE gel. Following electrophoretic transfer of proteins onto poly(vinyl difluoride) membranes, the membranes were blocked with 5% nonfat dry milk in PBST buffer (138 mM NaCl, 2.68 mM KCl, 1.76 mM KH₂PO₄, 10.14 mM Na₂HPO₄, 0.5% tween 20, pH 7.4). The membranes were washed and then incubated with anti-hCAIX antibody (0.2 µg/mL, from Santa Cruz, CA, USA) at 4 °C overnight. After three 10-min wash with PBST buffer, the membranes were incubated with a secondary antibody (anti-mouse IgG, 0.2 µg/mL) for 1 hr. The membranes were washed before being visualized by 4CN PLUS chromogenic substrate (Perkin Elmer).
**Determination of probe 1 quantum yields**

The quantum yields of 1 in the absence or presence of hCAII were determined by comparing the integrated area of the corrected emission spectrum of Probe 1 with a Cy5 reference (φ =0.28 in PBS buffer). The quantum yield can be calculated using the following equation:

$$\Phi = \Phi_R \left( \frac{m}{m_R} \right) \left( \frac{n^2}{n^2_R} \right)$$

Where

- $m$ = the slope of the line obtained from the plot of the integrated fluorescence intensity versus absorbance at 600 nm.
- $m_R$ = the slope of the line obtained from the plot of the integrated fluorescence intensity versus absorbance of reference at 600 nm.
- $n$ = the solvent refractive index.
- $n_R$ = the solvent refractive index of reference Cy5.
- $\phi$ = quantum yield of 1.
- $\phi_R$ = quantum yield of Cy5 reference.

Cy5 reference (K. Umezawa, A. Matsui, Y. Nakamura, D. Citterio and K. Suzuki, *Chem. Eur. J.* 2009, **15**, 1096-1106).
Fig. S1. Job’s plot analysis of probe 1 with hCAII.
**Fig. S2.** Limit of detection (LOD) for probe 1 to detect hCAII concentrations. The LOD was calculated from three times the standard deviation of background. The LOD is about 17 nM. For the calculation of LOD, we used the following equation, LOD = mean fluorescent signal of blank (without target protein) + three times the standard deviation corresponding to the blank controls (N = 10).
**Fig. S3.** Fluorescence intensity of 1 (5 µM) in the absence or presence of 0.5 µM hCAII in the indicated mediums. $\lambda_{ex} = 600$ nm, $\lambda_{em} = 668$ nm. FBS used is fetal bovine serum.
Fig. S4. Absorption spectra of 5 µM 1 with increasing hCAII concentrations.
**Fig. S5.** Absorption spectra of compound 2 with different concentrations in PBS and CAN.
Fig. S6. (a) Plots of the fluorescence intensity ($\lambda_{em} = 668$ nm) with increasing concentrations of compound 2 in PBS buffer. (b) Fluorescence spectra of 10 µM compound 2 in the absence or presence of 20 µM hCAII. The fluorescence enhancement ratio was around 4.5-fold.
Fig. S7. DLS analyses of the particle size distribution of 5 µM 1 in the presence of 10 and 20 µM hCAII. Probe 1 was completely disassembled (<1.5 nm) in the presence of 20 µM hCAII.
**Fig. S8.** DLS analyses of the particle size distribution of different probe 1 concentrations in PBS buffer.
Fig. S9. Reaction mechanism of SNAP-tag protein with O6-BG compound.
Fig. S10. Selectivity test of 3 (5 µM) with SNAP-tag and thirteen non-target proteins (1 µM).
Fig. S11. (a) Job’s plot analysis of probe 3 with SNAP-tag. (b) Absorption spectra of 5 µM probe 3 in ACN, PBS or with the addition of 10 µM SNAP-tag. (c) DLS analyses of the particle size distribution of 5 µM probe 3 and in the presence of 10 µM SNAP-tag. The mean diameter of 3 is about 130 nm. Negligible particle size (<1.5 nm) was observed in the presence of SNAP-tag.
Fig. S12. Kinetic study of SNAP-tag labeling with Probe 3. (a) Time course of fluorescence increase for the reaction of 5 μM Probe 3 with 5 μM SNAP-tag. The fluorescence intensity was recorded at 668 nm with excitation at 600 nm. (b) Fluorescence increase of Probe 3 (1–5 μM) in labeling of SNAP-tag (200 nM). The inset shows the linear relationship plot of probe concentration versus calculated $k_{obs}$. The second-order rate constant ($k_2$) for the reaction between probe 3 and SNAP-tag was determined to be about 5930 M$^{-1}$s$^{-1}$. 
Fig. S13. (a) Fluorescence spectra of 10 µM probe 4 in the absence or presence of 5 mM fructose. (b) Linear calibration curve for the determination of fructose detection limit, which is around 10 µM fructose.
Fig. S14. (a) Absorption spectra of 10 \( \mu \)M probe 4 in ACN, PBS or with the addition of 5 mM fructose. (b) DLS analyses of the particle size distribution of 10 \( \mu \)M probe 4 and in the presence of 5 mM fructose. The mean diameter of 10 \( \mu \)M 4 in PBS is about 1081 nm. In the presence of 5 mM fructose, particle size was reduced to around 197 nm.
**Fig. S15.** No-wash live-cell imaging of HeLa cells expressing SNAP-tag on cell surface. (a) HeLa cells expressing SNAP-PDGFR proteins treated with 0.5 µM probe 3, and (b) upon addition of 100 µM BG inhibitor. (c) Non-transfected HeLa cells treated with 0.5 µM probe 3. All cellular images were taken on the same day with identical microscope setup. Scale bar: 20 µm.
**Fig. S16.** HeLa cells cultured under hypoxia-mimetic condition with 200 μM DFO for 24 hours. (a) 0.5 μM probe 1 and (b) upon addition of 100 μM EZA. All cellular images were taken on the same day with identical microscope setup. Scale bar: 20 μm.
**Fig. S17.** No-wash imaging of hypoxia-induced (a) HeLa cells and (b) HEK293 cells. All cellular images were taken on the same day with identical microscope setup. Scale bar: 20 µm.
**Fig. S18.** Western-blot analysis of hCAIX expression levels in HeLa and HEK293 cells. (a) full view of Western-blot PVDF membrane. hCAIX was detected at around 55KDA. (b) Expanded view of PVDF membrane. α-tubulin in the cells was detected with anti-α-tubulin antibody and used as the positive control.
Scheme S1. Synthesis of Cy5 probe 1-4. Reagents and conditions: (i) DMF, POCl₃, NaClO₄, 24hrs, 70%. (ii) NaOH, quantitative. (iii) HCl, aniline, 4hrs, quantitative. (iv) Bromohexanoic acid, ACN, 65%. (v) DMF, NaOAc, 7 and 11 reflux 20h, 70%; AcOH, acetic anhydride, 9 and 11 reflux 16hrs, 50%. (vi) EDC, HOBt, TEA, DMF, r.t., 8-16hrs, 50-70%.

Synthesis of compound 7

POCl₃ (2.1 mL, 22.5 mmol) was added dropwise to dry DMF (10 mL) at 0 °C. The mixture was stirred for one hour at room temperature. Subsequently, phenylacetic acid (1.02 g 7.5 mmol) was
added to the solution mixture. The clear solution formed was heated at 95 °C for 4 hours and then at room temperature overnight. The dark mixture was poured onto 20 g crushed ice followed by saturated NaClO₄ solution. The resulting white crystalline was filtered and washed two times with 5 mL water to afford compound 6 in 70 % yield (1.07 g). Compound 6 was used in the next step without further purification. Compound 6 (1.07 g, 5.9 mmol) was added to 6 mL warm NaOH (1.15 g, 28.6 mmol) solution, and the mixture was heated with stirring at 90 °C until compound 6 was dissolved. The reaction mixture was cooled to room temperature and diluted with 10 mL water. The solution was acidified to pH 5 with 10% HCl solution resulting in the precipitation of the compound 7 (quantitative yield). ¹H NMR (400 MHz, d₆-DMSO) δ 8.52 (s, 2H), 7.48 (d, J = 7.5 Hz, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.22 (t, J = 7.2 Hz, 1H) ppm; ¹³C NMR (101 MHz, d₆-DMSO) δ 180.05, 131.57, 129.24, 127.67, 126.64, 120.99 ppm. HRMS (EI): m/z calc. for [C₉H₈O₂]⁺ 148.0524 found 148.0523 [M]⁺.

Synthesis of compound 9

To a solution of 1,1,3,3-tetramethyl propylene oxide (1.05 ml 6 mmol) in 0.5 M HCl was added aniline (1.11 mL, 12 mmol) at room temperature. The reaction mixture was heated at 50 °C for four hours. The reaction mixture was filtered, washed with ether (3 X 30 mL), and the orange solid was dried under reduced pressure to give compound 11 in quantitative yield (1.5 g). Rf:0.7 (DCM: MeOH = 9: 1); ¹H NMR (400 MHz, MeOD): δ 8.64 (d, J = 9.8 Hz, 2H), 7.47 (t, J = 7.9 Hz, 4H), 7.36 (d, J = 7.8 Hz, 4H), 7.29 (t, J = 7.3 Hz, 2H), 6.23 (t, J = 11.6 Hz, 1H) ppm; ¹³C NMR (126 MHz, MeOD) δ 159.79, 139.81, 131.14, 127.70, 118.76, 99.37 ppm; HRMS (ESI): m/z calc. for [C₁₅H₁₅N₂]⁺ 223.1230 found 223.1230 [M]⁺.
Synthesis of compound 11

To a stirred solution of 2,3,3-trimethyl-3H-indole (1 g, 6.28 mmol) in acetonitrile (5 mL) was added 6-bromohexanoic acid (1.2 g, 6.28 mmol) at room temperature. The reaction flask was fitted with a reflux condenser and heated at reflux for 24 hours. Subsequently, the heating bath was removed and the reaction mixture was allowed to cool to -20°C. The precipitates were filtered and dried under vacuo to provide compound 11 as a purple solid in 65% yield (1.3 g). Rf: 0.1 (DCM: MeOH = 9:1); \(^1\)H NMR (400 MHz, d\(_6\)-DMSO): \(\delta\) 8.00 (dd, \(J = 6.0, 2.9\) Hz, 1H), 7.85 (dd, \(J = 6.0, 2.7\) Hz, 1H), 7.65 – 7.49 (m, 2H), 4.47 (t, \(J = 7.7\) Hz, 2H), 2.87 (s, 3H), 2.21 (t, \(J = 7.2\) Hz, 2H), 1.95 – 1.74 (m, 2H), 1.53 (s, 8H), 1.47 – 1.37 (m, 2H) ppm; \(^{13}\)C NMR (101 MHz, d\(_6\)-DMSO): \(\delta\) 196.50, 174.28, 141.85, 141.02, 129.34, 128.90, 123.52, 115.52, 54.15, 47.49, 33.37, 26.94, 25.38, 24.01, 22.00, 14.22 ppm; HRMS (ESI): m/z calc. for [C\(_{17}\)H\(_{24}\)NO\(_2\)]\(^+\) 274.1802 found 274.1800 [M]\(^+\).

Synthesis of compound 12

Sodium acetate (31 mg, 0.39 mmol) and 7 (52 mg, 0.35 mmol) were added to a solution of 11 (300 mg, 0.88 mmol) in dry ethanol (15 mL) at room temperature. The reaction flask was fitted with a reflux condenser and heated at reflux for 20 hours. The solvent was removed and the residue was purified by flash column chromatography (MeOH/DCM) to afford compound 12 as a blue solid in 70% yield (160 mg). \(^1\)H NMR (400 MHz, MeOD) \(\delta\) 8.33 (d, \(J = 14.0\) Hz, 2H), 7.62 (t, \(J = 7.6\) Hz, 2H), 7.57 – 7.43 (m, 3H), 7.37 (t, \(J = 7.7\) Hz, 2H), 7.32 – 7.18 (m, 6H), 5.73 (d, \(J = 14.0\) Hz, 2H), 3.73 (t, \(J = 7.3\) Hz, 4H), 2.17 (t, \(J = 7.3\) Hz, 4H), 1.76 (s, 12H), 1.66 – 1.53 (m, 4H), 1.53 – 1.40 (m, 4H), 1.31 – 1.11 (m, 4H) ppm; \(^{13}\)C NMR (101 MHz, MeOD) \(\delta\) 181.88, 174.54, 154.08, 143.43, 142.62, 137.13, 136.86, 131.28, 130.58, 129.73, 129.40, 126.37, 123.39, 112.05, 102.89, 50.50, 44.97, 38.54, 28.07, 27.9, 27.82, 27.05 ppm; HRMS (ESI): m/z calc. for
Synthesis of compound 13
To compound 9 (254 mg 0.717 mmol) in a mixture of 10 mL (Ac)₂O/AcOH (1:1) was added compound 11 (195 mg 0.79 mmol) at room temperature. The reaction was heated at reflux for 16 hours. The solvent was removed and the residue was purified by flash-column chromatography (MeOH/DCM) to afford compound 13 as a blue solid in 50% yield (209 mg). ¹H NMR (400 MHz, d₆-DMSO) δ 8.51 (d, J = 12.7 Hz, 1H), 8.40 – 8.25 (m, 2H), 7.58 (d, J = 7.4 Hz, 2H), 7.46 – 7.28 (m, 2H), 7.26 – 7.12 (m, 2H), 7.07 – 6.88 (m, 1H), 6.67 – 6.52 (m, 1H), 6.23 (d, J = 13.7 Hz, 2H), 4.03 (s, 4H), 2.05 (s, 4H), 1.62 (s, 12H), 1.51 – 1.06 (m, 8H) ppm; ¹³C NMR (126 MHz, d₆-DMSO) δ 172.55, 153.93, 141.88, 141.14, 128.49, 126.08, 125.98, 124.76, 122.54, 111.07, 103.28, 48.93, 43.29, 36.11, 27.17, 26.64, 25.84, 24.93 ppm; HRMS (ESI): m/z calc. for [C₃₇H₄₇N₂O₄]⁺ 583.3530 found 583.3532 [M]⁺.

Synthesis of compound 1
To a reaction flask containing compound 6 (15 mg, 0.02 mmol), HOBt.H₂O (8 mg, 0.06 mmol), and EDC.HCl (12 mg, 0.06 mmol, 3 equiv) was added 2 mL DMF at room temperature. After 10 minutes, triethylamine (28 uL) and 4-aminomethylbenzenesulfonamide hydrochloride (13 mg, 0.06 mmol) were added respectively and stirred overnight at room temperature. The solvent was removed and the crude mixture was purified by reversed-phase preparative HPLC to give product 1 as a blue solid in 65% yield (13 mg). ¹H NMR (400 MHz, MeOD) δ 8.34 (d, J = 14.0 Hz, 2H), 7.83 (d, J = 8.3 Hz, 4H), 7.56 (t, J = 7.7 Hz, 2H), 7.49 (d, J = 7.4 Hz, 2H), 7.42 (d, J = 8.2 Hz, 5H), 7.37 (d, J = 7.8 Hz, 2H), 7.31 – 7.18 (m, 6H), 5.73 (d, J = 13.9 Hz, 2H), 4.41 (s, 4H), 3.74 (t, J = 7.4 Hz, 4H), 2.20 (t, J = 7.3 Hz, 4H), 1.76 (s, 12H), 1.62 – 1.48 (m, 8H), 1.25 –
1.16 (m, 4H) ppm; $^{13}$C NMR (101 MHz, MeOD) $\delta$ 175.66, 174.64, 154.09, 144.85, 143.81, 143.37, 142.63, 137.27, 136.79, 131.34, 130.54, 129.78, 129.34, 128.93, 127.40, 126.48, 123.47, 112.02, 102.90, 50.54, 44.82, 43.59, 36.62, 27.91, 27.83, 27.43, 26.32 ppm; HRMS (ESI): m/z calc. for [C$_{57}$H$_{67}$N$_6$O$_6$S$_2$]$^+$ 995.4558 found 995.4559 [M]$^+$.

**Synthesis of compound 2**

Based on the synthetic procedure of compound 1, compound 2 was obtained as a blue solid in 50% yield (9 mg). $^1$H NMR (500 MHz, d$_6$-DMSO) $\delta$ 8.40 (t, $J = 6.0$ Hz, 2H), 8.37 – 8.28 (m, 2H), 7.74 (d, $J = 8.3$ Hz, 3H), 7.61 (d, $J = 7.4$ Hz, 2H), 7.38 (t, $J = 7.1$ Hz, 5H), 7.31 (s, 2H), 7.27 – 7.21 (m, 2H), 6.58 (t, $J = 12.3$ Hz, 1H), 6.29 (d, $J = 13.9$ Hz, 2H), 4.28 (d, $J = 5.9$ Hz, 4H), 4.07 (d, $J = 7.2$ Hz, 4H), 2.14 (t, $J = 7.3$ Hz, 4H), 1.67 (s, 12H), 1.60 – 1.51 (m, 4H), 1.36 (d, $J = 7.0$ Hz, 4H), 1.23 (d, $J = 9.9$ Hz, 4H) ppm; $^{13}$C NMR (126 MHz, d$_6$-DMSO) $\delta$ 172.59, 172.09, 154.03, 143.78, 142.55, 142.00, 141.09, 128.45, 127.42, 125.66, 124.70, 122.46, 111.10, 103.16, 48.90, 43.30, 41.64, 35.04, 27.16, 26.73, 25.80, 24.90 ppm; HRMS (ESI): m/z calc. for [C$_{51}$H$_{63}$N$_6$O$_6$S$_2$]$^+$ 919.4245 found 919.4199 [M]$^+$.

**Synthesis of compound 3**

Based on the synthetic procedure of compound 1, compound 3 was obtained as a blue solid in 70% yield (16 mg). $^1$H NMR (400 MHz, MeOD) $\delta$ 8.35 (d, $J = 14.0$ Hz, 2H), 8.27 (s, 2H), 7.58 (t, $J = 7.5$ Hz, 2H), 7.47 (t, $J = 6.3$ Hz, 7H), 7.38 – 7.27 (m, 8H), 7.24 (t, $J = 7.2$ Hz, 2H), 7.17 (d, $J = 8.0$ Hz, 2H), 5.73 (d, $J = 13.9$ Hz, 2H), 5.60 (s, 4H), 4.35 (s, 4H), 3.72 (t, $J = 7.3$ Hz, 4H), 2.18 (t, $J = 7.3$ Hz, 4H), 1.76 (s, 12H), 1.61 – 1.46 (m, 8H), 1.21 (dd, $J = 14.9$, 8.1 Hz, 4H) ppm; $^{13}$C NMR (101 MHz, MeOD) $\delta$ 175.52, 174.64, 161.10, 158.44, 154.14, 153.75, 143.34, 142.63, 140.95, 137.27, 136.83, 135.47, 131.36, 130.53, 130.21, 129.74, 129.33, 128.84, 126.44, 123.45, 123.95.
111.97, 102.88, 70.69, 50.54, 44.82, 43.78, 36.64, 27.90, 27.80, 27.43, 26.36 ppm; \textbf{HRMS (ESI)}: m/z calc. for [C_{69}H_{75}N_{14}O_{4}]^{+} 1163.6090 found 1163.6099 [M].

\textbf{Synthesis of compound 4}

Based on the synthetic procedure of compound 1, compound 4 was obtained as a blue solid in 70 \% yield (13 mg). $^1$H NMR (500 MHz, MeOD) $\delta$ 8.42 – 8.29 (m, 3H), 7.56 (t, $J = 7.6$ Hz, 3H), 7.49 (d, $J = 7.4$ Hz, 3H), 7.44 (s, 2H), 7.37 (t, $J = 7.7$ Hz, 2H), 7.27 (dt, $J = 15.5$, 7.6 Hz, 8H), 7.20 (d, $J = 8.0$ Hz, 2H), 5.72 (d, $J = 14.0$ Hz, 2H), 4.34 (s, 4H), 3.72 (t, $J = 7.3$ Hz, 4H), 2.17 (t, $J = 7.3$ Hz, 4H), 1.76 (s, 12H), 1.62 – 1.46 (m, 8H), 1.25 – 1.15 (m, 4H) ppm; $^{13}$C NMR (126 MHz, MeOD) $\delta$ 175.45, 154.12, 143.36, 142.63, 134.27, 133.81, 131.32, 130.56, 129.78, 129.37, 128.81, 126.45, 123.46, 112.02, 102.89, 49.85, 44.79 , 44.26 , 36.65 , 27.87, 27.79, 27.35 , 26.36 ppm; \textbf{HRMS (ESI)}: m/z calc. for [C_{57}H_{67}B_{2}N_{4}O_{6}+H]^{+} 926.5319 found 926.5327 [M+H].
| Parameter          | Value                           |
|--------------------|---------------------------------|
| Data File Name     | T2O4                            |
| Title              | T2O4                            |
| Comment            | VT143Apure                      |
| Origin             | Varian                          |
| Owner              |                                 |
| Site               | vnmrs                           |
| Solvent            | dmo                            |
| Temperature        | 24.0                           |
| Pulse Sequence     | s2pu1                          |
| Experiment         | 1D                             |
| Number of Scans    | 8                              |
| Receiver Gain      | 10000                          |
| Relaxation Delay   | 0.0000                         |
| Pulse Width        | 0.0000                         |
| Acquisition Time   | 2:5559                         |
| Acquisition Date   | 2014-11-27T00:15:41            |
| Modification Date  | 2014-11-27T00:17:58            |
| Spectrometer Period| 399.77                         |
| Spectral Width     | 641.03                         |
| Lowest Frequency   | -795.7                         |
| Nucleus            | 1H                             |
| Acquired Size      | 10384                          |
| Spectral Size      | 192/78                         |

![Chemical Structure](image)

![NMR Spectrum](image)
$^{13}$C NMR (101 MHz, DMSO) δ 196.50, 174.28, 141.35, 141.02, 129.34, 123.90, 123.52, 115.52, 54.15, 47.49, 33.37, 26.94, 25.38, 24.01, 22.00, 14.22.
| Parameter          | Value                       |
|-------------------|-----------------------------|
| 1 Data File Name  | TC26-C                      |
| 2 Title           | TC26-C                      |
| 3 Comment         | TC26                        |
| 4 Origin          | Varian                      |
| 5 Owner           | vnmr                        |
| 6 Site            | c85od                       |
| 7 Temperature     | 25.0                        |
| 8 Pulse Sequence  | 37pu1                       |
| 9 Number of Scans | 776                         |
| 10 Receiver Gain  | 30                          |
| 11 Relaxation Delay | 1.0000                    |
| 12 Pulse Width    | 0.0000                      |
| 13 Acquisition Time | 1.2845                    |
| 14 Acquisition Date | 2014-08-29T16:23:45       |
| 15 Modification Date | 2014-08-29T16:53:44       |
| 16 Spectrometer Frequency | 100.53                |
| 17 Spectral Width | 235.04                      |
| 18 Lowest Frequency | -1697.8                   |
| 19 Nucleus        | 13C                         |
| 20 Acquired Size  | 33768                       |
| 21 Spectral Size  | 65536                       |
$^1$H NMR (400 MHz, MeOD): 8.64 (d, $J$ = 9.8 Hz, 1H), 7.47 (t, $J$ = 7.9 Hz, 4H), 7.36 (d, $J$ = 7.8 Hz, 4H), 7.29 (t, $J$ = 7.3 Hz, 2H), 6.23 (t, $J$ = 11.6 Hz, 1H), 4.19, 4.06, 2.8, 1.09.
