Supporting Information

for

A self-assembled amphiphilic imidazolium-based ATP probe

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Experimental Section

General methods

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. *N*-(3-Imidazolyl)propyl dansylamide **3** was prepared by the literature method. Flash chromatography was carried out on silica gel (230-400 mesh). NMR spectra were recorded using Varian instruments (400 MHz). Chemical shifts were expressed in ppm and coupling constants (*J*) in Hz. Surface tension measurements were carried out by a DCA 315 tensiometer (Cahn Instruments) using a platinum plate (20 × 15 × 0.127 mm^3) at room temperature.

Calculation of the association constants

The binding constants of the inclusion complex were obtained from the fluorescence titration data. According to the Benesi-Hildebrand method, the equations for 1:1 host:guest complexes are given below:

$$\frac{1}{I - I_0} = \frac{1}{I' - I_0} + \frac{1}{K(I' - I_0)[\mathrm{M}]}$$

In the equations, I_0 is the intensity of fluorescence of **P1** without PPi, I is the intensity with a particular concentration of PPi, I' is the intensity of the fully complexed form at the highest concentration of PPi, and K is the binding constant.

General UV-vis and fluorescence spectra measurements

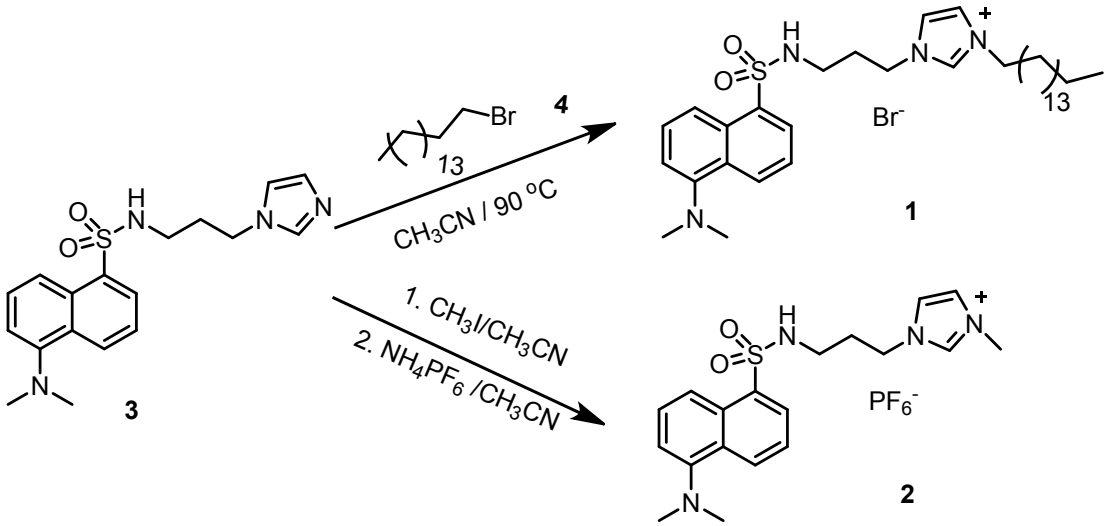
Stock solutions of anions (sodium or potassium salts, 0.01 M) were prepared in aqueous solution. Stock solutions of **1** (0.01 M) were prepared in DMSO-H_2O (1:1) solution. The concentration of **1** in the UV-vis and emission titrations was 0.02 mM in 100% aqueous solution. During the titration, anions solution ware added into a solution of **1** (2 mL) using a micro injector, and the whole volume of the final system can be considered constant because the volume of anions solution added is negligible compared to that of ligand's solution.

Cell culture and fluorescence imaging

Liver cancer HEPG-2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (Invitrogen Corp., Carlsbad, CA) and penicillin (100 units/mL)-streptomycin (100 μg/mL) liquid (Invitrogen Corp., Carlsbad, CA) at 37 °C in a humidified incubator containing 5% CO_2 in air. The cells were incubated for 2 days on an uncoated 35 mm diameter glass-bottomed dish (D110100, Matsunami, Japan). Then, the cells were incubated with DMEM containing 10% PBS and 10 μM probe **1** at 37 °C for 30 min, washed twice with PBS, and mounted on the microscope stage. Fluorescence images were captured by a Nikon A1 confocal laser-scanning microscope equipped with a live cell workstation. Then the live HeLa cells were in situ treated with 1U mL^{-1} apyrase30 minutes, and then the same set of cells was used for confocal laser-scanning microscopy measurement

Cytotoxicity Assay by MTT Method

Liver cancer HEPG-2 cells were seeded into 96-well plates and maintained overnight in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) (v/v), and then treated using various concentrations of probe **1** (0−30 μM) at 37 °C for 24 h. After the medium was poured out, 100 μL of freshly prepared 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 1 mg/mL in phosphate buffered saline [PBS]) was added to each well and the wells were incubated for 4 h. The supernatant was removed, and the cells were lysed by adding 100 μL of DMSO per well. The plate was gently shaken for 5 min, and then the absorbance of purple formazan at 520 nm was monitored using a Spectra MAX 340PC plate reader.



Scheme S1 synthesis route of **1** and **2**

Synthesis of 1

Under a nitrogen atmosphere, *N*-(3-imidazolyl)propyl dansylamide **3** (200 mg, 0.56 mmol) and 1-bromohexadecane **4** (305 mg, 1.0 mmol) were dissolved in 30 ml acetonitrile. The solution was stirred at 90 °C for 10 h followed by evaporation of the solvent in vacuo. The crude was purified by column chromatography (CH_2Cl_2/MeOH, 98:2, v/v) to yield **1** as a yellow oil like liquid (316 mg, yield 85%). ^{1}H NMR (DMSO, 400MHz): δ = 9.60 (1 H, s), 8.39 (2 H, d, *J* = 8.4 Hz), 8.05 (1 H, d, *J*= 7.1 Hz), 7.74 (1 H, s), 7.64 (1 H, s), 7.48 (1 H, t, *J* =8.1 Hz), 7.38 (1 H, t, *J* =7.9 Hz), 7.24 (1 H, s), 7.04 (1 H, d, *J* =7.4 Hz), 4.35 (2 H, s), 4.04 (2 H, d, *J* =6.4 Hz), 3.06 (2 H, dd, J_1 =7.2 Hz, J_1 =14.3 Hz), 2.75 (6 H, s), 1.17–1.19 (33 H, m) ^{13}C NMR ($CDCl_3$, 100 MHz): 150.7, 136.1, 133.7, 129.2, 128.8, 128.2, 127.9, 127.6, 122.1, 121.7, 120.5, 118.2, 118.5, 114.4, 30.9, 29.0, 28.7, 28.6, 28.5, 28.4, 28.0, 25.3, 21.7, 13.1. ESI-MS ($M - Br^-$): 583.4021.

Synthesis of 2

Under a nitrogen atmosphere, *N*-(3-imidazolyl)propyl dansylamide **3** (200 mg, 0.56 mmol) and iodomethane (300 mg, 2.1 mmol) were dissolved in 30 ml acetonitrile. The solution was stirred at 90 °C for 10 h followed by evaporation of the solvent in vacuo to get the crude dark yellow oil-like liquid. The crude liquid was dissolved in 10 mL MeOH solution. Then NH_4PF_6 (500 mg, 3.1 mmol) was added and stirred for 2

h. The solvent was removed by evaporation under vacuum, and the crude was redissolved in DCM and filtered to remove the undissolvable aminium salts. Pure yellow solid **2** (235 mg, 0.45 mmol, 81% yield) was obtained after crystallization from hexane/DCM (1:1, v/v) solution. ^{1}H NMR (DMSO, 400MHz): δ = 9.06 (1 H, s), 8.53 (2 H, d, *J* = 8.4 Hz), 8.33 (1 H, d, *J* =8.4 Hz), 8.13 (2H, t, *J* =7.6 Hz), 7.65–7.72 (4 H, m), 7.32 (1 H, d, *J* =8.0 Hz), 4.21 (2H, t, *J* =6.8 Hz), 3.87 (s, 3H), 3.43 (6H, s), 2.83 (2H, dd, J_1 =7.2 Hz, J_2 =12.4 Hz), 1.94 (2H, t, *J* =6.8 Hz). ESI-MS (M – BF^-): 373.1810.

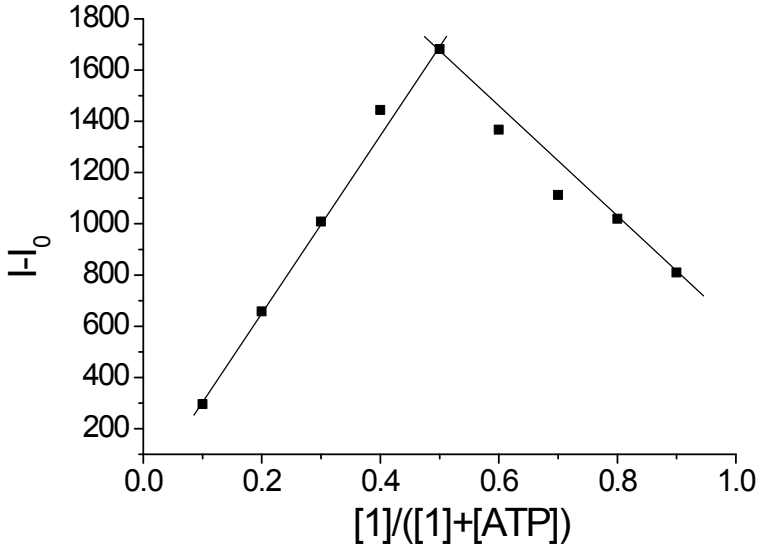


Fig. S1 Job plot of **1**-ATP according to the emission data

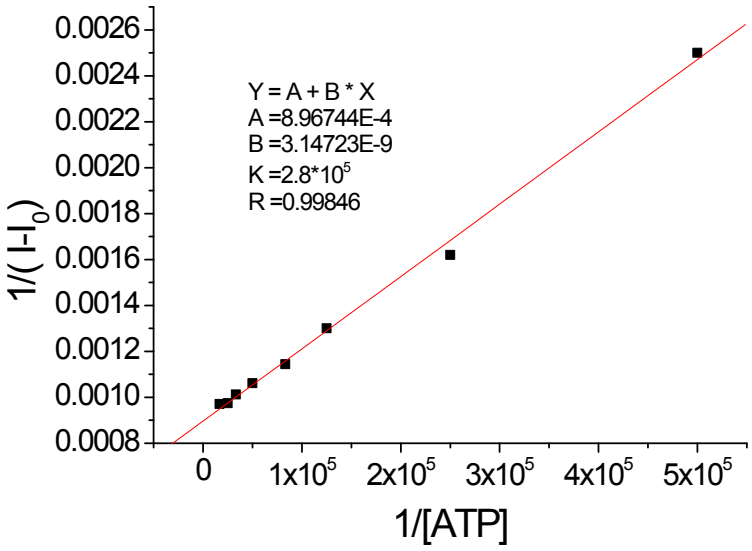


Fig. S2 Benesi-Hildebrand plot of **1**-ATP according to the emission data

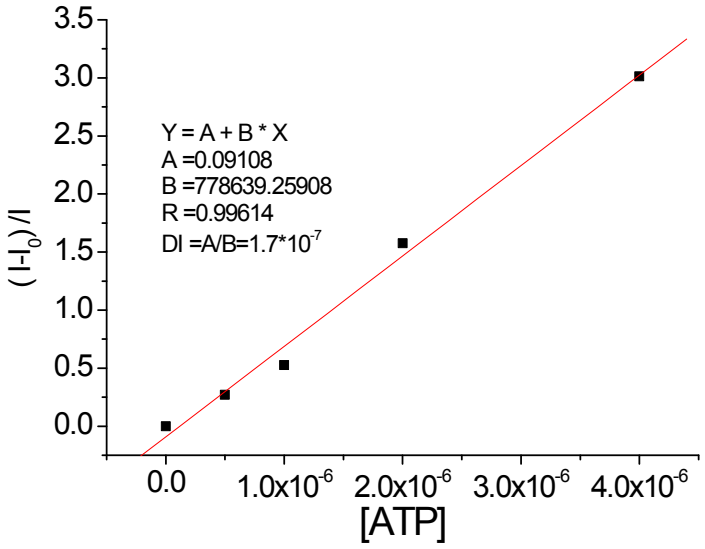


Fig. S3 Emission intensity of **1** at 508 nm upon addition of different concentrations of ATP (0–5 μM) normalized between the minimum emission and the maximum emission intensity

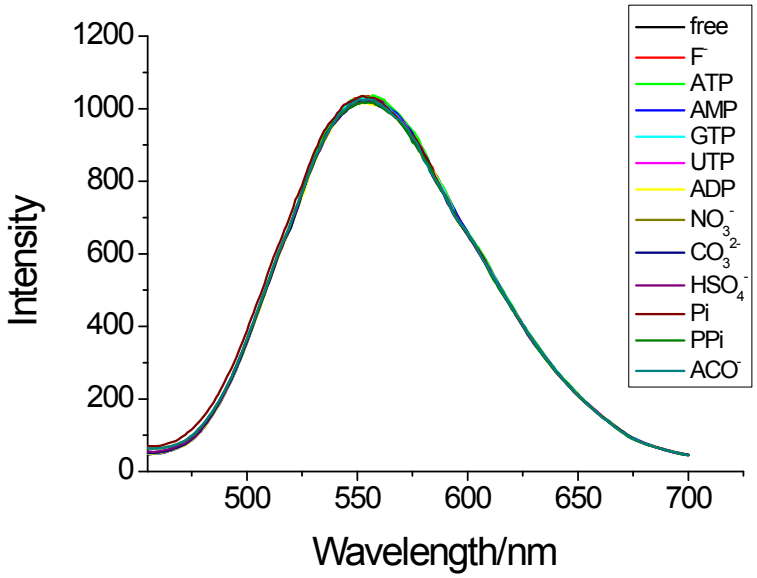


Fig. S4 The emission spectrum of **2** (10 μM) upon addition of various anions in aqueous solution

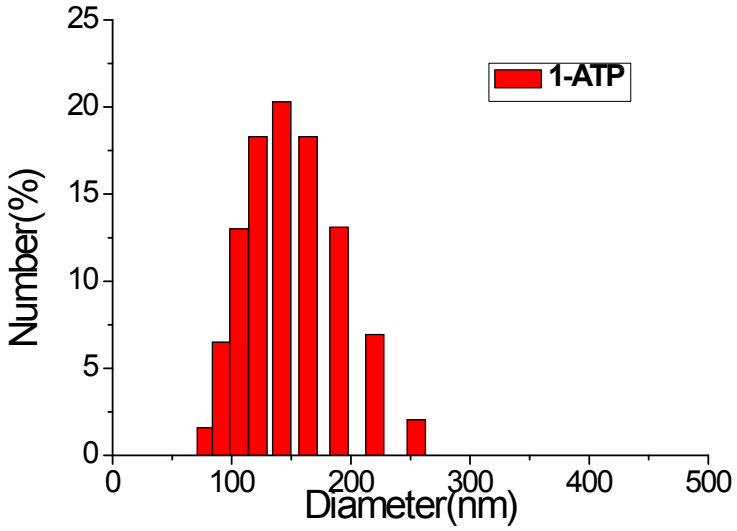


Fig. S5 Size distribution of **1**-ATP (10 μM) in aqueous solution measured by DLS.

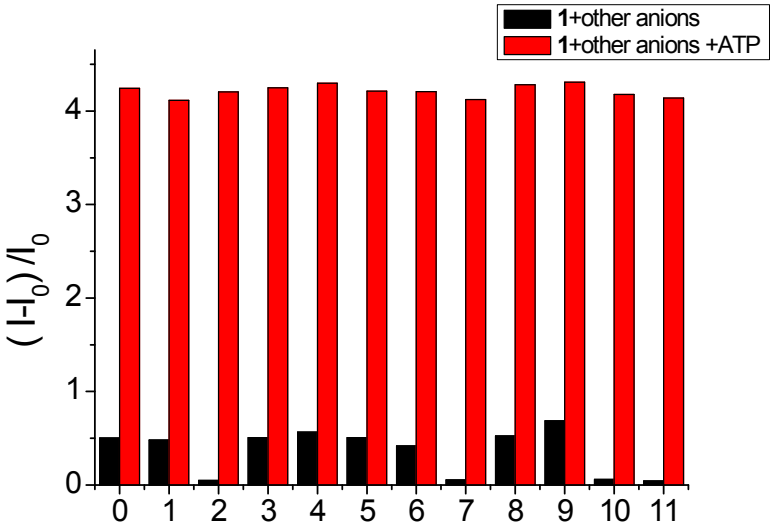


Fig. S6 Competition experiment of **1** (0) $H_2PO_4^-$ (1) HSO_4^- (2) ACO^- (3) UTP (4)ADP (5) AMP (6) NO_3^- (7) CO_3^{2-} (8) F^- (9) PPi (10) Cl^- (11) Br^-

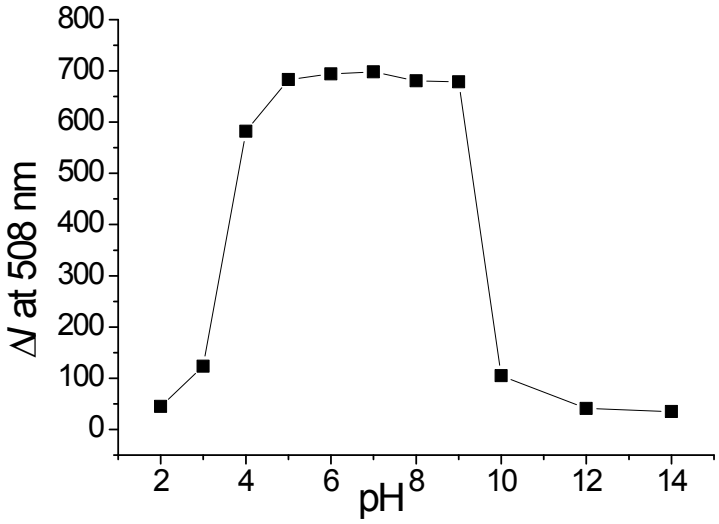


Fig. S7 Fluorescence intensities of **1** (10 μm) at 508 nm before and after the additional of ATP (1.0 equiv) at various pH values in H_2O solution

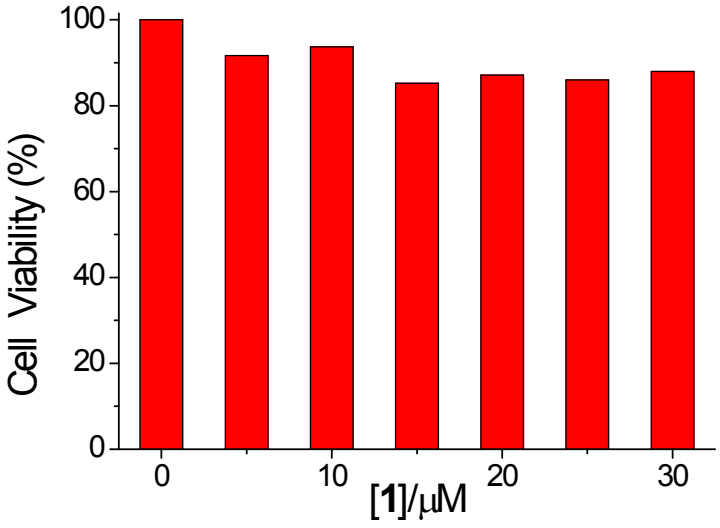


Fig. S8 Cell viability results after incubation of HEPG-2 liver cancer cells with various concentrations of **1** in aqueous solution.

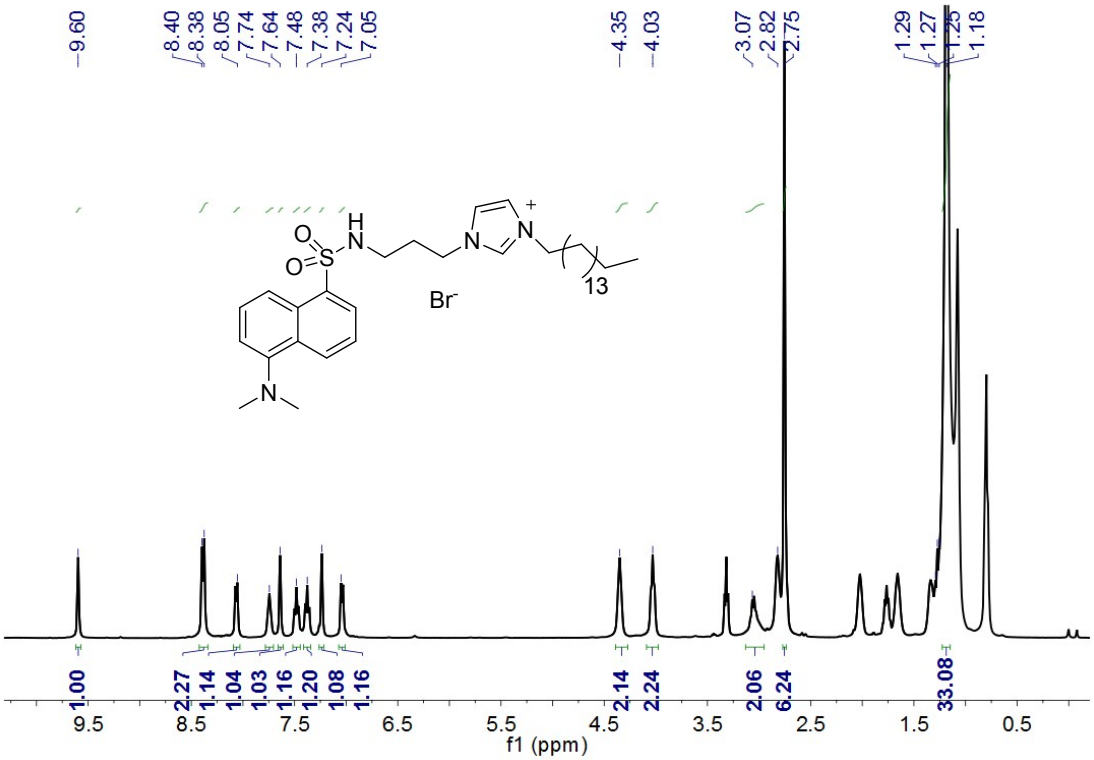


Fig. S9 ^{1}H NMR spectra of **1** in DMSO-d6 solution

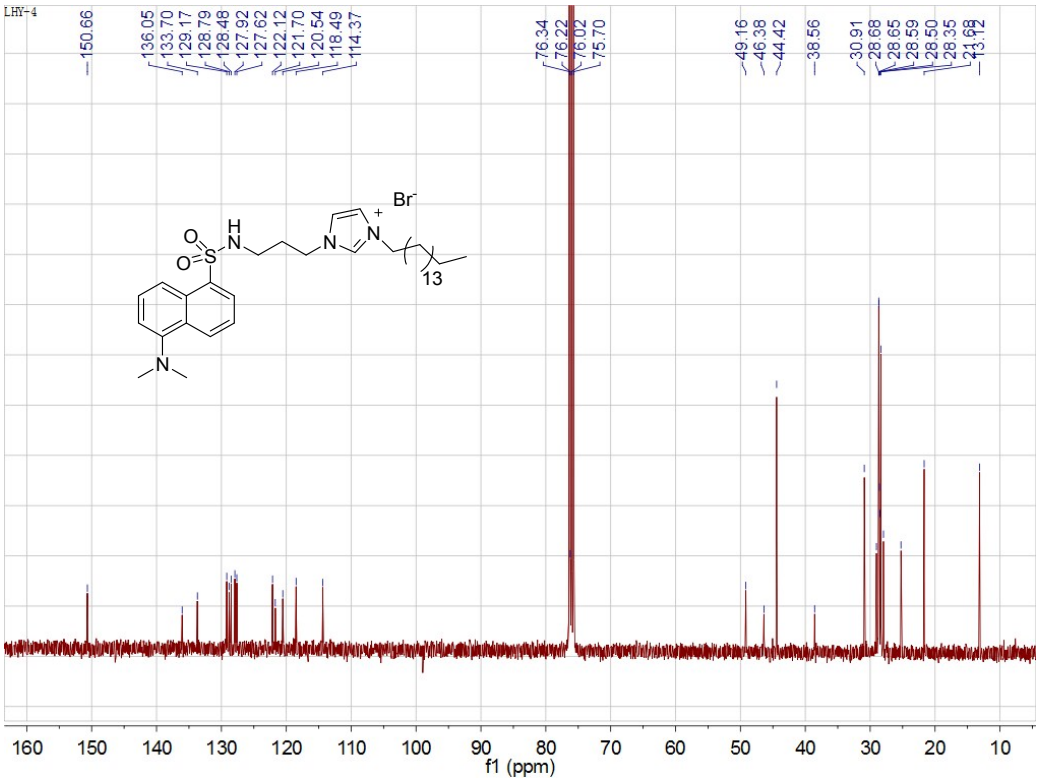


Fig. S10 ^{13}C NMR spectra of **1** in $CDCl_3$ solution

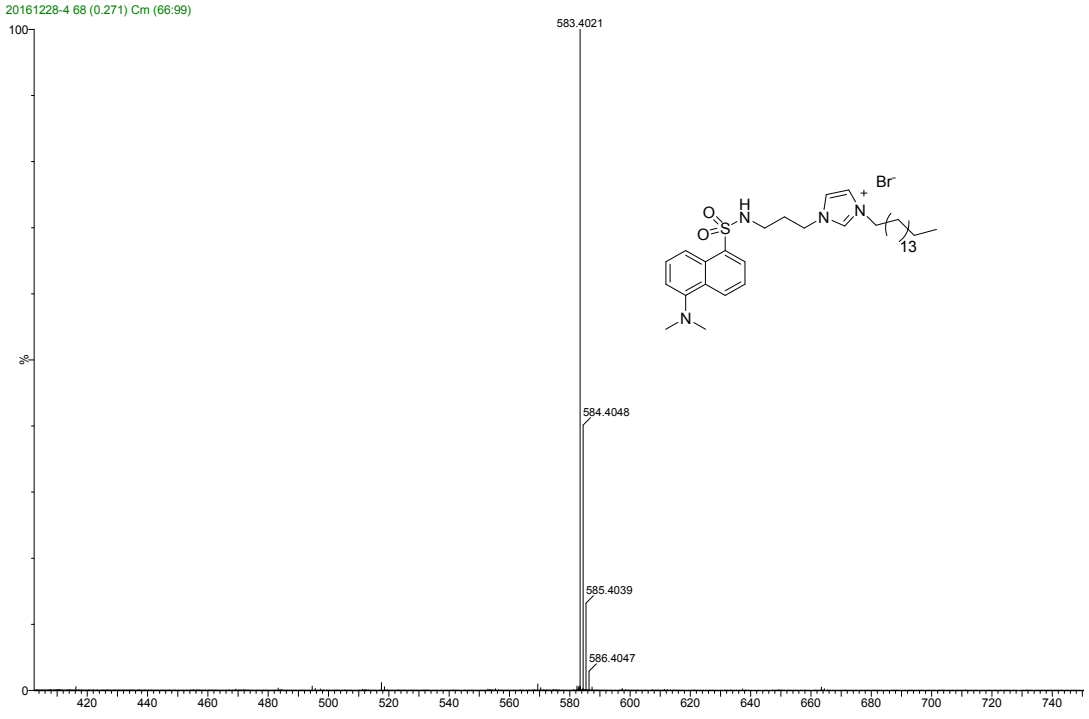


Fig. S11 ESI-TOF-MS spectra of **1**

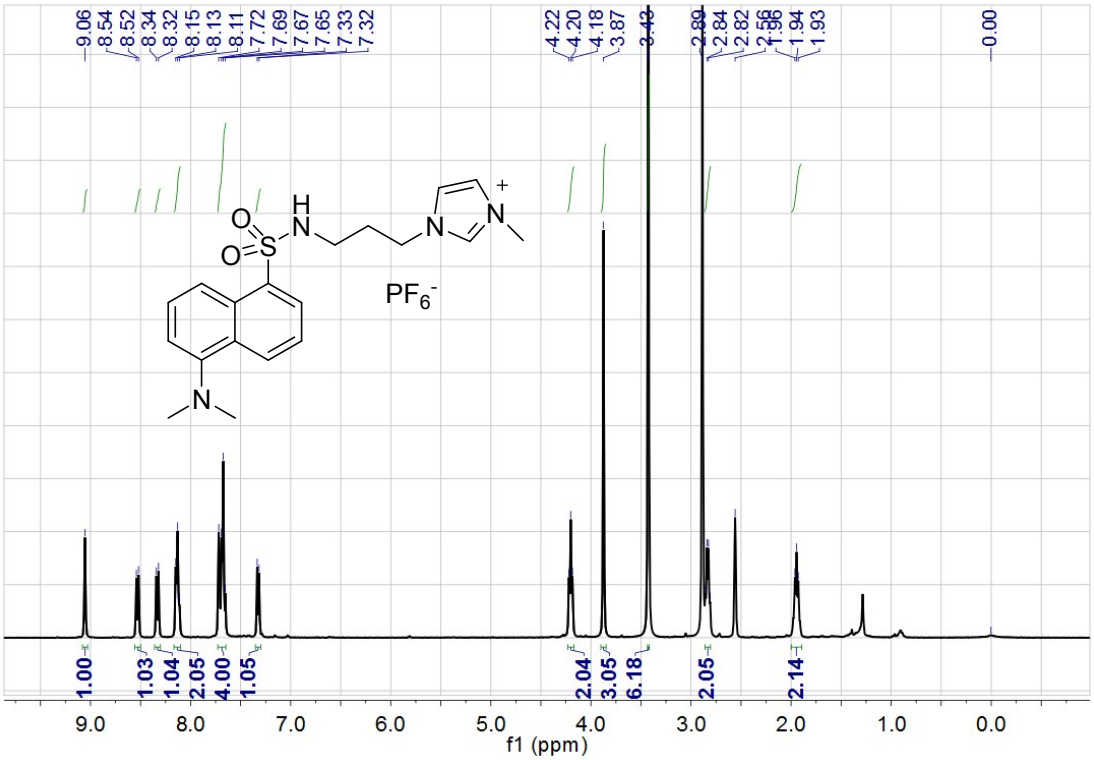


Fig. S12 ^{1}H NMR spectra of **2** in DMSO-d_6 solution

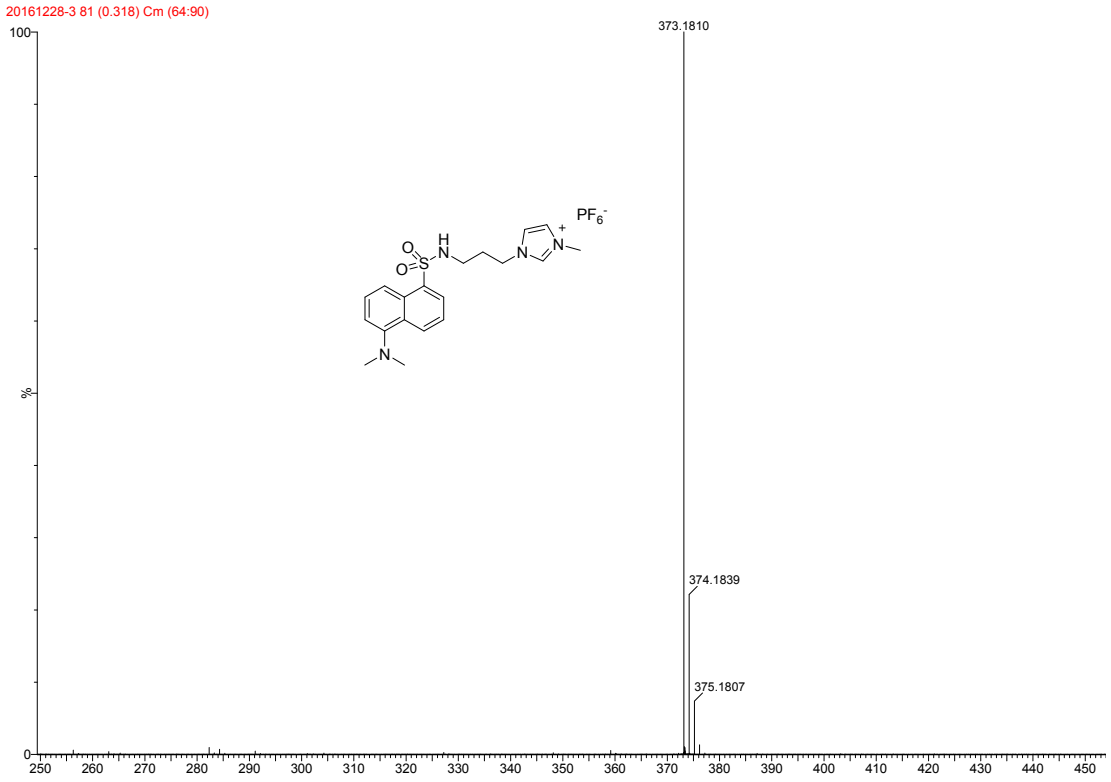


Fig. S13 TOF-MS spectra of **2**