Electronic Supplementary Material (ESI) for ChemComm.

Supporting information:

Self-assembly of an MRI-responsive agent under physiological conditions provides an extended time window for *in vivo* imaging

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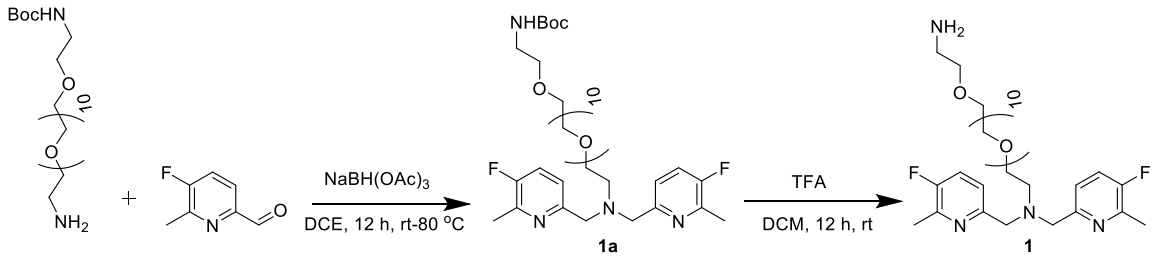
A. Materials and Methods

All the solvents and reagents were obtained from commercial suppliers. 5- fluoro-6-methylpicolinaldehyde was purchased from Pharma Block Sciences, Inc. (Nanjing, China). Boc-Amino PEG-6 Amine, Boc-Amino PEG-10 Amine were purchased from poly pure, Oslo, Norway. Benzene-1,3,5-tricarbonyl trichloride, Sodium triacetoxyborohydride and sodium sulphate were purchased from Alfa Aesar. Deuterated solvents, such as $CDCl_3$ and D_2O, were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Preparative RP-HPLC was performed on Agilent 218 purification system, equipped with an auto-sampler, a C18 column, an UV-VIS dual wavelength detector and a 440-LC fraction collector, operating with OpenLab ChemStation software. The ^{1}H NMR, ^{13}C NMR, ^{19}F NMR spectra, ^{19}F NMR Zn^{2+} binding studies, and ^{19}F-iCEST experiments were performed on a Bruker AVANCE III 9.4 T NMR spectrometer. The following abbreviations are used to describe peaks: s-singlet; d- doublet; t-triplet; m-multiplet. High-resolution mass spectrometry (HRMS) was recorded on an AB SCIEX 5800 MALDI TOF instrument at the Weizmann institute of Science mass spectrometry facility. Phantom magnetic resonance imaging (MRI) experiments were performed on a 9.4 T wide-bore MR scanner (Bruker AVANCE III system). A 25 mm, double-resonant ($^{1}H/^{19}F$) radiofrequency (RF) coil was used to acquire ^{1}H, ^{19}F, and ^{19}F-iCEST MR images. For animal surgeries, a customized stereotaxic surgery setup equipped with 2 µL Hamilton glass syringe was used. Animal MRI experiments were performed on a Bruker BioSpec 15.2 T AVANCE III HD imaging spectrometer. A dual $^{1}H/^{19}F$, 23 mm RF coil was used to acquire the ^{19}F signal, ^{1}H-MRI, and ^{19}F-MRI images.

B. Chemical Synthesis Protocols

Compound 1:

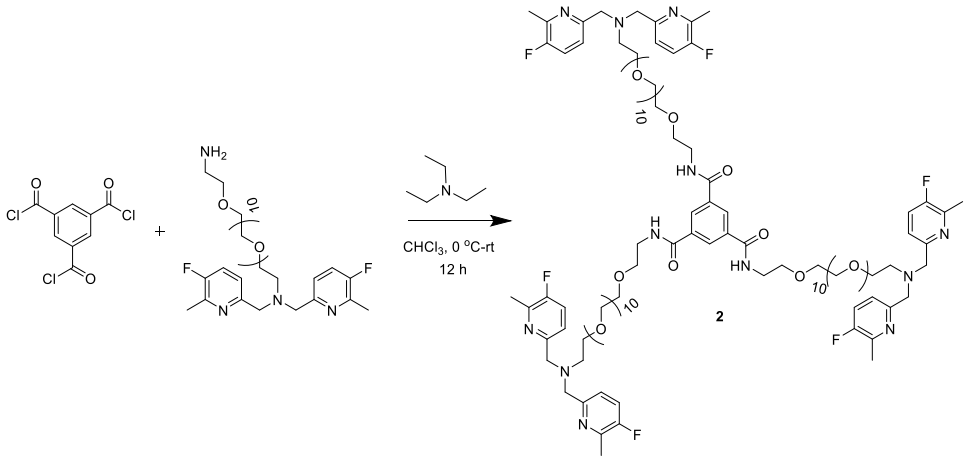


1a: 5-fluoro-6-methylpicolinaldhyde (80 mg, 581.5 µmol) was added to a solution of Boc-Amino PEG-10 Amine (150 mg, 232.1 µmol) in 1,2-dichloroethane (DCE) (6 mL) and stirred at room temperature for 30 min, followed by the addition of sodium triacetoxyborohydride (150 mg, 0.693 mmol). The reaction mixture was then stirred at room temperature for an additional 1 h before being heated to 80 ^{o}C for 12 h. Completion of the reaction was confirmed by TLC and the solvent was evaporated. The crude product was dissolved in $CHCl_3$, washed with a saturated solution of $NaHCO_3$, and extracted with $CHCl_3$ (2 X 20 mL). The combined organic phase was dried over Na_2SO_4. After filtration, the organic phase was concentrated and purified using silica gel column chromatography ($CHCl_3$/MeOH, 90:10) to obtain compound **1a** as yellow oil (180 mg, 87%). ^{1}H NMR (400.35 MHz, $CDCl_3$) δ 7.48 (m, 2H), 7.33 (m, 2H), 3.9 (s, 4H), 3.71 (m, 44H), 3.39 (m, 2H), 2.87 (s, 2H), 2.56 (s, 6H), 1.52 (s, 9H).^{19}F (376.7 MHz, $CDCl_3$) δ -129.1 (m). HRMS (ESI) calculated for $C_{43}H_{73}F_2N_4O_{13}$ [M+H] *m/z* 891.5142, found *m/z* 891.5181.

1: Compound **1a** (180 mg, 0.202 mmol) was dissolved in 6 mL of dichloromethane (DCM). Trifluoroacetic acid (TFA) (184.2 mg, 1.62 mmol) was added drop wise to the reaction mixture at room temperature and left for 12 h. Completion of the reaction was confirmed by TLC, and the resulting crude product was purified using silica gel column chromatography (EtOAc/MeOH/NH_3 Solution 90:5:5) to obtain compound **1** (143 mg, 90%) as a yellow oil. ^{1}H NMR (400.35 MHz, $CDCl_3$) δ 7.43 (m, 2H), 7.33 (m, 2H), 3.8 (s, 4H), 3.6 (m, 44H), 3.0 (t, *J*=5.0 Hz, 2H), 2.81 (t, *J*=5.8 Hz, 2H), 2.5 (s, 6H). ^{13}C{^{1}H} (100.67 MHz, $CDCl_3$) δ 158.04,155.53 (d, $J_{C\text{-}F}$ = 253

Hz), 154.72,154.67 (d, J_{C-F} = 4 Hz), 145.44,145.28 (d, J_{C-F} = 16 Hz), 122.69,122.49 (d, J_{C-F} = 19 Hz), 121.41,121.37 (d, J_{C-F} = 3 Hz), 70.69-70.12 (m), 69.56, 60.12, 53.45, 41.1, 17.89. ^{19}F (376.7 MHz, $CDCl_3$) δ -129.2 (m). HRMS (ESI) calculated for $C_{38}H_{65}F_2N_4O_{11}$ [M+H] *m/z* 791.4618, found *m/z* 791.4617.

Compound 2:



Benzene-1,3,5-tricarbonyl trichloride (10 mg, 37.67 µmol) was dissolved in dry chloroform (1 mL) and then added drop-wise over 15 min to the stirred solution of compound **1** and triethylamine (16 mg, 150.6 µmol) in dry chloroform (2 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred for an additional 12 h. Completion of the reaction was confirmed by TLC and the crude product was purified on a preparative reverse phase-high purification liquid chromatography (RP-HPLC) using mobile phase A (0.1% TFA in H_2O/CH_3CN, 9:1, v/v) and B (0.1% TFA in CH_3CN/H_2O, 9:1, v/v) to obtain compound **2** as a colorless oil (82 mg, 87%). ^{1}H NMR (400.35 MHz, $CDCl_3$) δ 8.6 (s, 3H), 7.5 (m, 6H), 7.3 (m, 6H), 4.0 (s, 12H), 3.6 (m, 138H), 2.86 (t, *J*=5.5 Hz, 6H), 2.5 (s, 18H). ^{13}C{^{1}H} (100.67 MHz, $CDCl_3$) δ 166.1, 158.09,155.57 (d, J_{C-F} = 253 Hz), 145.51,145.35 (d, J_{C-F} = 16 Hz), 135.11, 128.77, 122.73,122.53 (d, J_{C-F} = 19 Hz), 121.43, 70.53-69.56 (m), 60.14, 53.49, 40.18, 17.9. ^{19}F (376.7 MHz, $CDCl_3$) δ -129.1 (m). HRMS (ESI) calculated for $C_{123}H_{192}F_6N_{12}O_{36}$ [M+H] *m/z* 2529.3578, found *m/z* 2529.3435.

C. ^{19}F-iCEST NMR experiments

All ^{19}F-iCEST NMR experiments were performed on a 9.4 T MHz AVANCEIII NMR spectrometer (Bruker, Germany), with the sample temperature stabilized at 310 K. A pre-saturation pulse (t_{sat}) with a length of 1 sec and a pulse strength 100 Hz were applied prior to the 90° RF pulse. The frequency of the B_1 was swept from Δω= +8 ppm to Δω= -8 ppm relative to the resonance of the free ^{19}F-iCEST sensor (set to 0 ppm for convenience). Each frequency offset (Δω +/ -) was acquired with eight scans, using a repetition time of 4 sec, resulting in total iCEST experiment time of ~52 min.

D. Dynamic light scattering (DLS)

The size distribution of the nano-assembly were evaluated by DLS, measurements were performed on Malvern Nano-ZS with 10 mm quartz cuvette where water is the solvent.

E. Diffusion ^{19}F-NMR experiments

The ^{19}F diffusion NMR measurements were performed on a 9.4 T (376.7 MHz) AVANCE Ⅲ NMR spectrometer (Bruker, Germany) equipped with a 50 gauss/cm Z gradient system. For both compounds (**1** and **2**) a LED (longitudinal eddy current delay) sequence was used at 25°C and 37°C. The diffusion experiments were performed with smoothed square (SMSQ.10.100) gradients, incremented from 2% to 98% in 10 linear steps and 8 scans were acquired for each gradient. Each ^{19}F diffusion measurement was performed 3 times consecutively and an average with standard deviation were calculated and reported. For compound **1** the gradient duration was 2ms and the diffusion time was 60ms both at 25°C and 37°C. For compound **2**, at 25°C, the gradient duration was 4ms and the diffusion time was 60ms. At 37°C, two diffusion dataset were acquired one with gradient duration of 4ms and diffusion time of 60ms and another with gradient duration of 8ms and diffusion time of 100ms. To eliminate differences in temperature/viscosity/calibration of gradients the ^{1}H diffusion coefficient of the water signal was measured (^{1}H diffusion Bipolar LED δ = 2 ms Δ = 60ms) and

compared to the literature value of water self-diffusion coefficient at each temperature. The differences between the measured value and the literature value were used as a factor, which was implemented in the calculation of the reported ^{19}F diffusion coefficients.

F. MRI experiments

Phantom Studies:

Phantom experiments were performed on a 9.4 T wide-bore MR scanner (Bruker Avance system) equipped with a 25 mm, double-resonant ($^{1}H/^{19}F$) radiofrequency (RF) coil at 37 ^{o}C. Seven 5 mm NMR tubes containing 2 mM of compound **2** and 80 μM of either of the cations Mg^{2+}, Cu^{2+}, Na^{+}, Zn^{2+}, K^{+}, and Ca^{2+} in 100 mM HEPES buffer (pH ~ 7.4) were placed in a larger tube containing a 4% gelatin (w/w) solution in water. ^{1}H MRI: A RARE sequence was used to acquire ^{1}H MRI images with the following parameters: TR/TE=1000/30.7 ms; FOV=3.2 x 3.2 cm; matrix size=128 x 128; RARE factor=8; averages (NA=1). ^{19}F-iCEST MRI: A modified RAREst sequence at ^{19}F-MRI was used to perform the ^{19}F-iCESTMRI experiments with the following parameters: TR/TE=1500/8.8 ms; RARE factor=16; 8 mm slice; FOV=3.2 × 3.2 cm; matrix size=32 × 32; NA=800, and a saturation pulse of B_1= 2.5 μT for 1 s. The frequency of B_1 was swept from Δω= +8 ppm to Δω= -8 ppm in 85 Hz steps relative to the resonance of the compound **2** (set to 0 ppm).

***In vivo* MRI:**

All the animal experiments were performed on Male C57BL/6 mice, which were purchased from Envigo (Israel). In vivo experiments were performed accordance with the IACUC guidelines and regulations of the Weizmann Institute of Science. An institutional committee approved the in vivo experiments as detailed in IACUC protocol number 03480420-4, entitled “Molecular biosensors for in vivo mapping of labile zinc in brain”. Note that all studied animals survived the in vivo experiments with no observable undesired effects.

The following coordinates were used for intracranial injection of the probe delivery: For delivering compound **1** or **2** to the CA3 region of the hippocampus, following

coordinates were used (based on the mouse brain atlas): 2.42 mm (ML), 2.15 mm (AP), and 2.27 mm (DV), relative to bregma. After delivery of probe at the location of interest in the animal brain, skin was sutured then animal was transferred to the Bruker BioSpec 15.2 Tesla AVANCE III HD imaging spectrometer equipped with a dual $^{1}H/^{19}F$, 23 mm RF coil. ^{1}H MRI: A RARE sequence was used to acquire the ^{1}H MRI images with the following parameters: TR/TE=5000/34.7 ms; FOV=2.2 x 2.2 cm; matrix size=128 x 128; RARE factor=16, NA=2. ^{19}F-NMR experiment was performed to observe the probe washout at different time points with TR=1500, NA=400 and obtained frequency offset was used to acquire ^{19}F-MRI with the following parameters: A modified RAREst sequence, TR/TE=1500/10.9 ms; RARE factor=16; 8 mm slice; FOV=2.2 × 2.2 cm; matrix size=32 × 32; NA=400.

	T_1	T_2
Compound 1	1.5 s	121 ms
Compound 2 (non-assembled)	1.0 s	104 ms
Compound 2 (large assembly)	515 ms	94 ms

Table S1. T1 and T2 values of both 1 and 2 at 37 ^{o}C, as determined from ^{19}F-NMR experiments (inversion recovery and CPMG) performed on a 9.4 T NMR spectrometer.

* For compound **2**, two values were evaluated from the two peaks at the ^{19}F-NMR spectrum, one for the non-assembled structure and one for the larger assembly of the tripod.

G. Supporting Figures

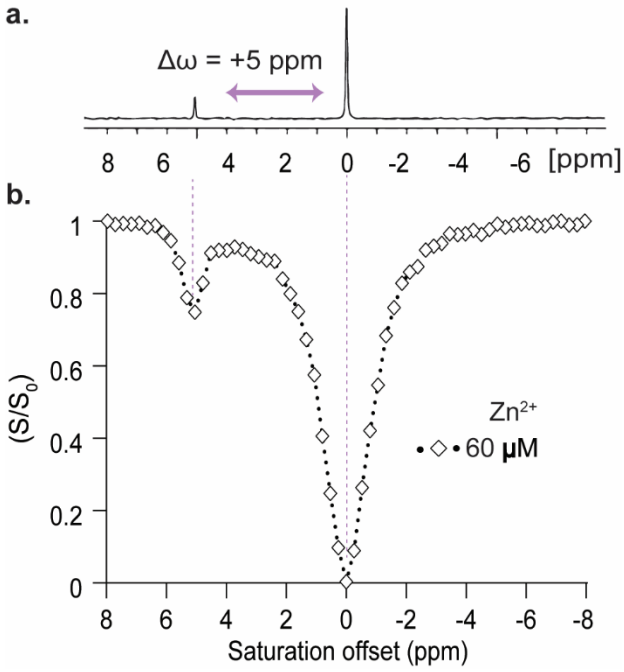


Fig. S1 ^{19}F-NMR and ^{19}F-iCEST spectra of compound **1** : a) ^{19}F-NMR spectra of 3 mM compound **1** and 0.6 mM of Zn^{2+} was dissolved in 100 mM HEPES buffer pH=7.4) and the obtained Δω between the peak of the free ligand (set at 0.0 ppm) and the Zn^{2+}-bound ligand. b) ^{19}F-iCEST spectra of 3 mM compound **1** in the presence of 60 μM of Zn^{2+} in 100 mM HEPES buffer pH=7.4 at 37 °C.

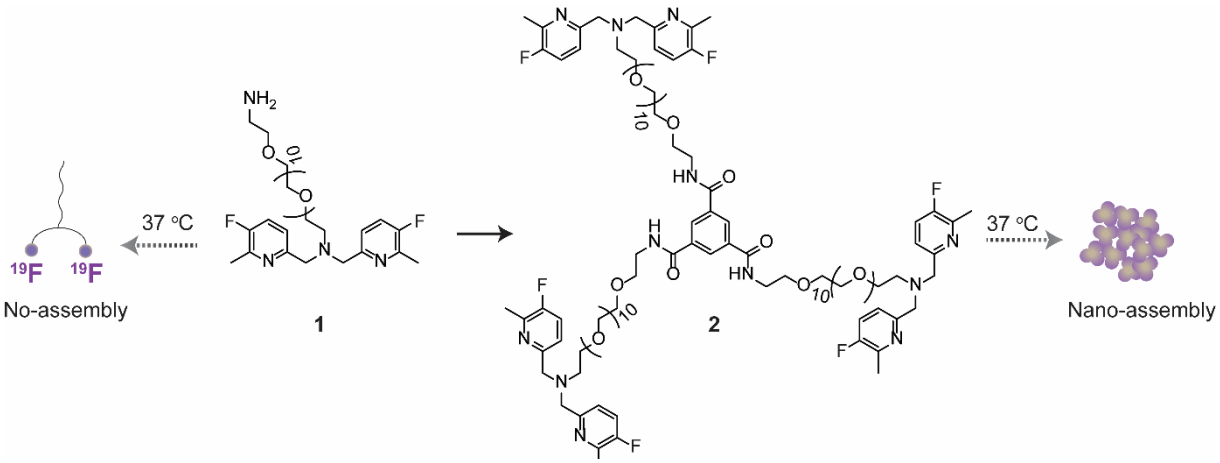


Fig. S2 The molecular structures of **1** and **2** and schematic illustration of their appearances at 37 °C, as dispersed (**1**, left) or nano-assembly (**2**, right) structure. Reaction conditions: Benzene-1,3,5-tricarbonyl trichloride, **1** and Et_3N in dry $CHCl_3$ for 12 hr.

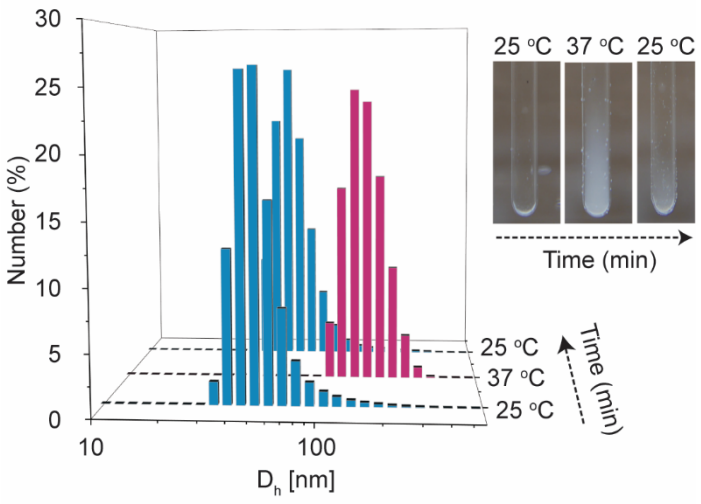


Fig. S3. DLS plot of 100 µM compound **2**, with photograph of tubes containing 3 mM compound **2** at 25 °C, 37°C and cooling to 25 °C.

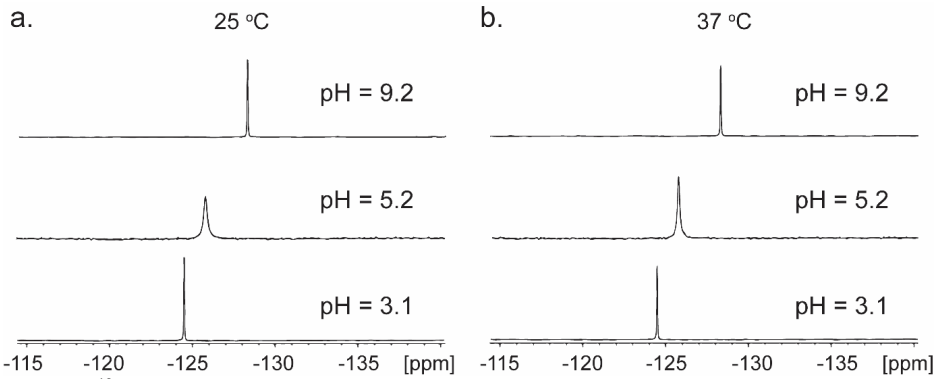


Fig. S4. ^{19}F-NMR of aqueous solutions of **1** at 25 °C (a) and 37 °C (b). The studied solutions were prepared in 100 mM HEPES buffer with 3 mM of **1** and the pH was adjusted to3.1, 5.2, and 9.2, as noted in the Figure. of aqueous solutions of **1** at 25 °C (a) and 37 °C (b). The studied solutions were prepared in 100 mM HEPES buffer with 3 mM of **1** and the pH was adjusted to3.1, 5.2, 9.2 as noted in the Figure.

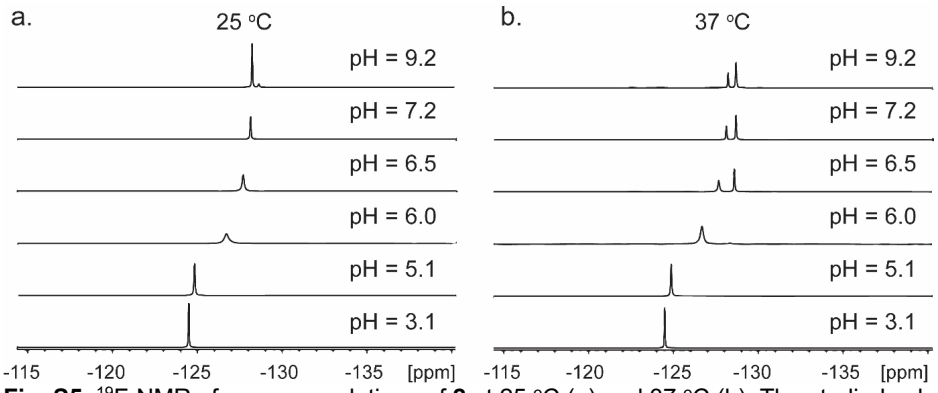


Fig. S5. ^{19}F-NMR of aqueous solutions of **2** at 25 °C (a) and 37 °C (b). The studied solutions were prepared in 100 mM HEPES buffer with 3 mM of **1** and the pH was adjusted to3.1, 5.1, 6.0, 6.5, 7.2, and 9.2, as noted in the Figure.

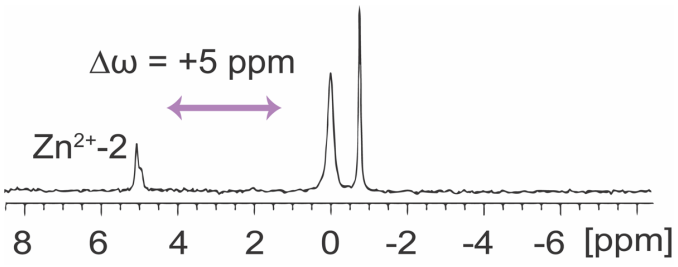


Fig. S6 ^{19}F-NMR spectra of 2 mM **2** in the presence of 1.2 mM of Zn^{2+} and the obtained Δω between the peak of the free **2** (set at 0.0 ppm) and the Zn^{2+}-bound **2**.

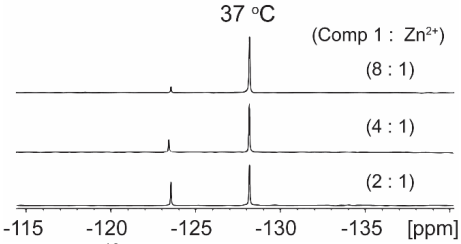


Fig. S7. ^{19}F-NMR spectra of **1** at 37 °C in the presence of different Zn^{2+} concentrations. Samples were prepared in 100 mM HEPES buffer, pH=7.2, and contained 3 mM of **1** and (i) 1.5 mM of Zn^{2+} (2:1 ratio), (ii) 0.75 mM of Zn^{2+} (4:1 ratio), or (iii) 0.375 mM of Zn^{2+} (8:1 ratio).

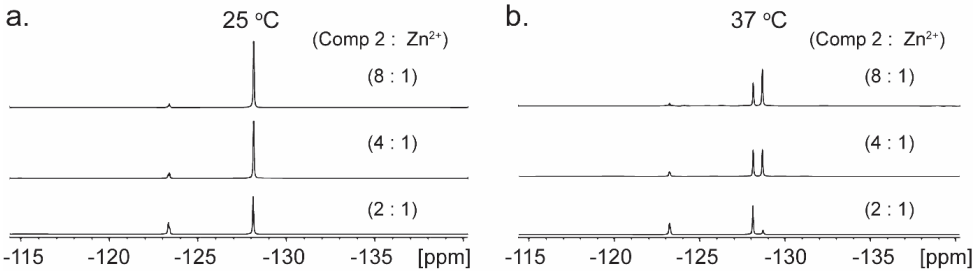


Fig. S8. ^{19}F-NMR spectra of **2** at 25 °C (a) and 37 °C (b) in the presence of different Zn^{2+} concentrations. Samples were prepared in 100 mM HEPES buffer, pH=7.2, and contained 3 mM of **1** and (i) 1.5 mM of Zn^{2+} (2:1 ratio), (ii) 0.75 mM of Zn^{2+} (4:1 ratio), or (iii) 0.375 mM of Zn^{2+} (8:1 ratio).

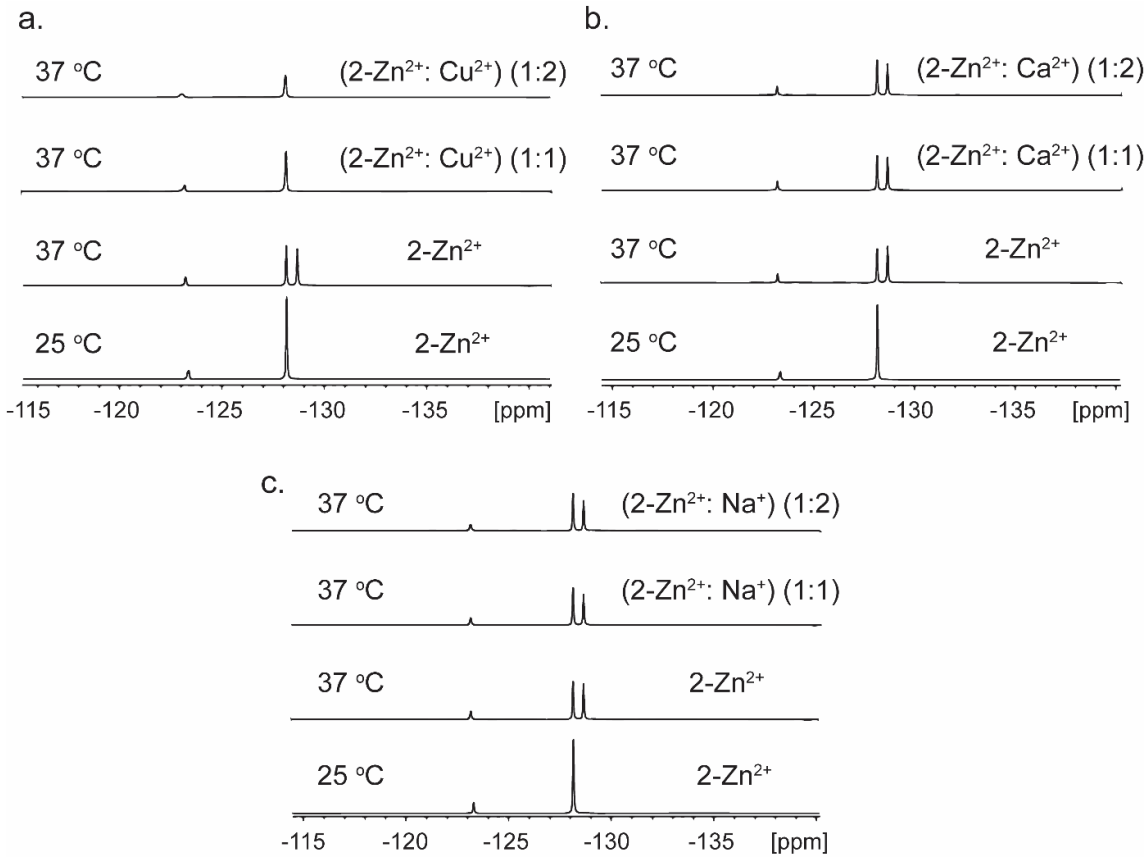


Fig. S9. Competition assays between Cu^{2+}, Ca^{2+}, or Na^{+} in the presence of **2** bound to Zn^{2+}. a) ^{19}F-NMR of sample containing 2 mM of **2** and 1.2 mM of Zn^{2+} without or with the addition of 1.2 mM and 2.4 mM of Cu^{2+}. b) ^{19}F-NMR of sample containing 2 mM of **2** and 1.2 mM of Zn^{2+} without or with the addition of 1.2 mM and 2.4 mM of Ca^{2+}. c) ^{19}F-NMR of sample containing 2 mM of **2** and 1.2 mM of Zn^{2+} without or with the addition of 1.2 mM and 2.4 mM of Na^{+}.

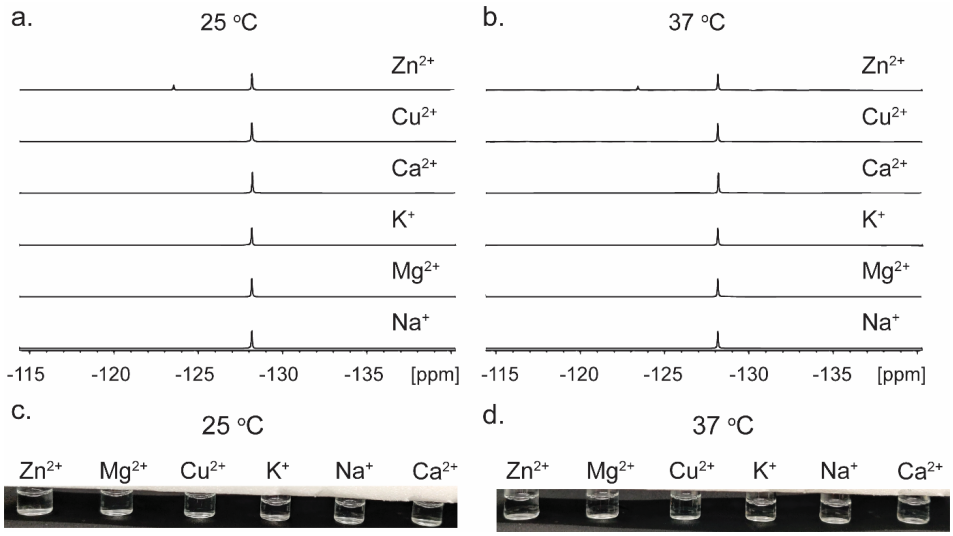


Fig. S10. The effect of metal ions on the assembly of **1** at 25 °C (a and c) or 37 °C (b and d). Samples of 3 mM of **1** and 0.6 mM of each of the studied cations, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cu^{2+}, or Zn^{2+} were prepared in 100 mM HEPES buffer, pH=7.2. ^{19}F-NMR spectra were acquired with 9.4 T NMR spectrometers at 25 °C (a) or 37 °C (b). Photographs of the studied solutions 25 °C (c) or 37 °C (d) show transparent solutions with no evidence of the formation of large assemblies of **1** at the two studied temperatures and in the presence of all studied cations.

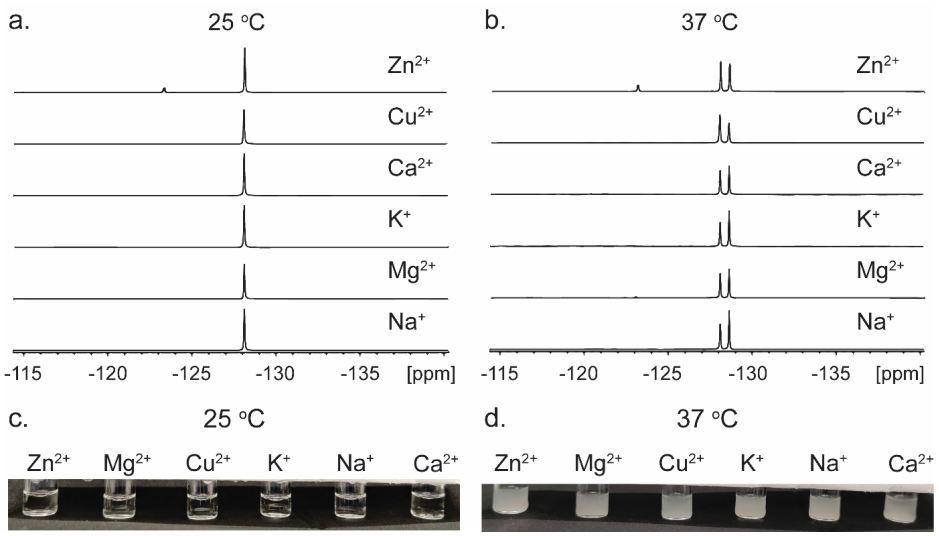


Fig. S11. The effect of metal ions on the assembly of **2** at 25 °C (a and c) or 37 °C (b and d). Samples of 3 mM of **2** and 0.6 mM of each of the studied cations, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cu^{2+}, or Zn^{2+} were prepared in 100 mM HEPES buffer, pH=7.2. ^{19}F-NMR spectra were acquired with 9.4 T NMR spectrometers at 25 °C (a) or 37 °C (b). Photographs of the studied solutions 25 °C (c) or 37 °C (d) show transparent solutions with no evidence to the formation of large assemblies of **2** at 25 °C. At 37 °C, all studied solutions showed the formation of large assemblies, as evident from the ^{19}F-NMR spectrum, and the pictures of the solutions showed the cloudiness of the large assemblies formed.

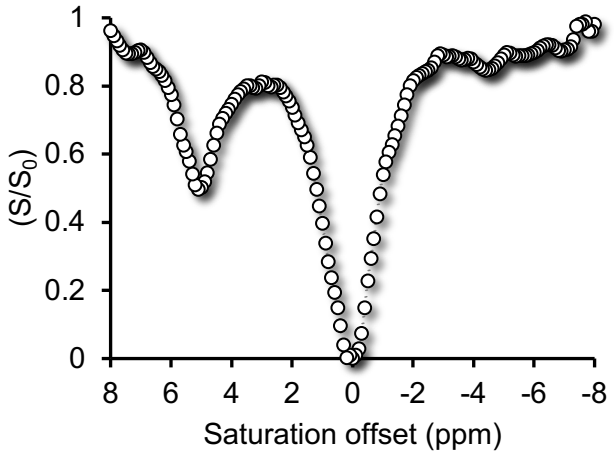


Fig. S12 ^{19}F-iCEST MRI Spectra of Phantom tube containing Zn^{2+}: 2 mM of compound **2** and 80 μM of Zn^{2+} was dissolved in 100 mM HEPES buffer (pH=7.4) and ^{19}F-iCEST MRI experiment were performed on 9.4 T wide-bore MR scanner at 37 ºC.

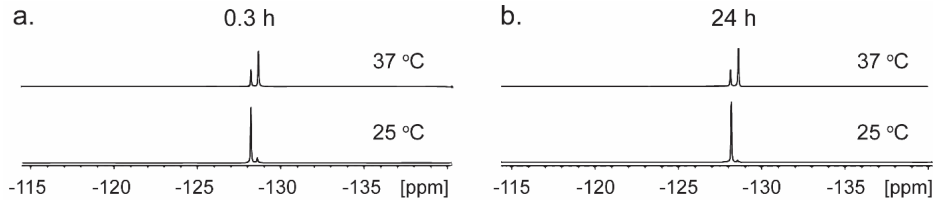


Fig. S13. ^{19}F-NMR of **2** in DMEM+10%FBS. Two mM of **2** were dissolved in a cell culture medium (DMEM+10%FBS) and the ^{19}F-NMR spectra were recorded at 20 min (a) and 24 hours (b) after its preparation at both 25 ºC (bottom spectra) and 37 ºC (top spectra).

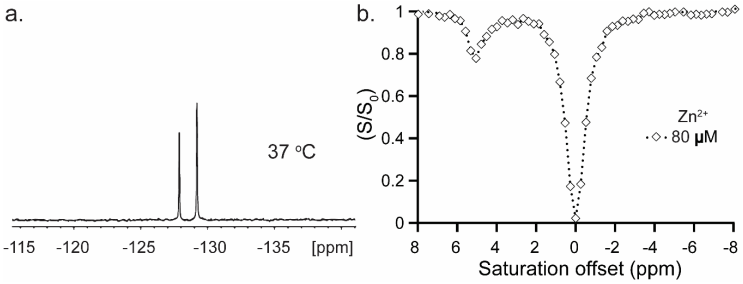


Fig. S14. Studying **2** in the presence of 50% DMSO in the examined solutions. a) ^{19}F-NMR of sample containing 2 mM of **2** and 1.2 mM of Zn^{2+} dissolved in 1:1 DMSO:water solution (100 mM HEPES buffer). b) Sample containing 2 mM of **2** and 80 μ of Zn^{2+} dissolved in 1:1 DMSO:water solution (100 mM HEPES buffer). Measurements were performed on a 9.4 T NMR spectrometer at 37 ºC.

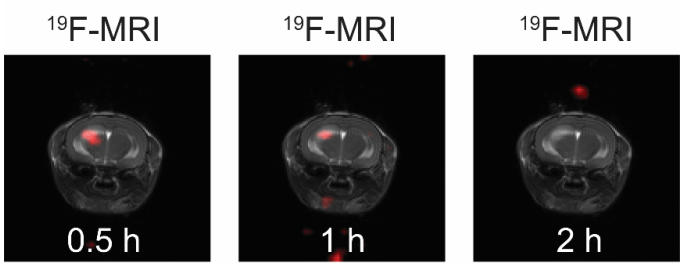


Fig. S15. *In vivo* ^{19}F-MRI: ^{19}F-MRI of live mouse obtained at 0.5 h, 1 h and 2 h after intracranial injection of **1** (1 µL of 225 mM in DMSO, 0.1 µL/min rate).

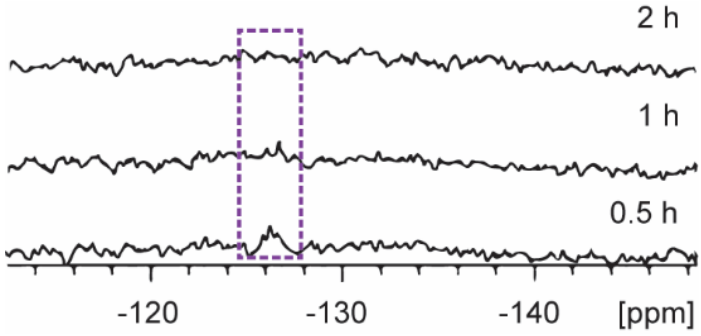


Fig. S16 Washout studies of compound **1** *in vivo*: ^{19}F-NMR spectra obtained from animal brain following intracranial injection of (1 µL of 75 mM compound **1** in DMSO, 0.1µL/min rate). The set of ^{19}F-NMR spectra obtained at different time points representing the probe washout from the tissue.

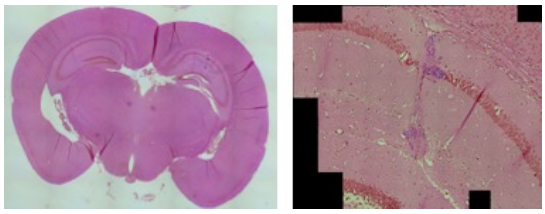


Figure S17. H&E staining of excised brain 24 hours after the injection of 2. Microscope images of excised brain 24 hours after the delivery of **2**. Shown is histological H&E (hematoxylin and eosin) stain showing the hydrogel formation within the tissue (hippocampus of the right hemisphere) with no significant damage to the tissue.

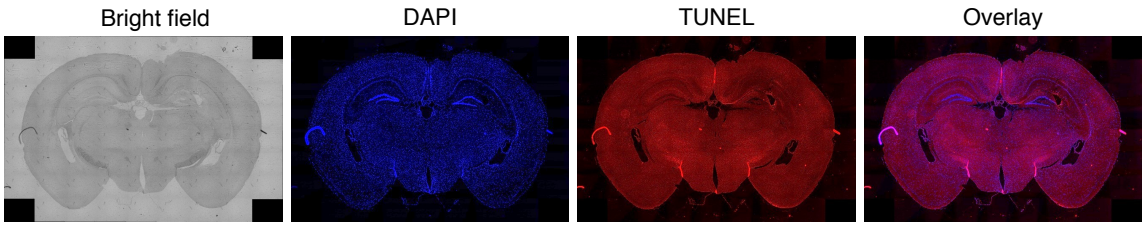
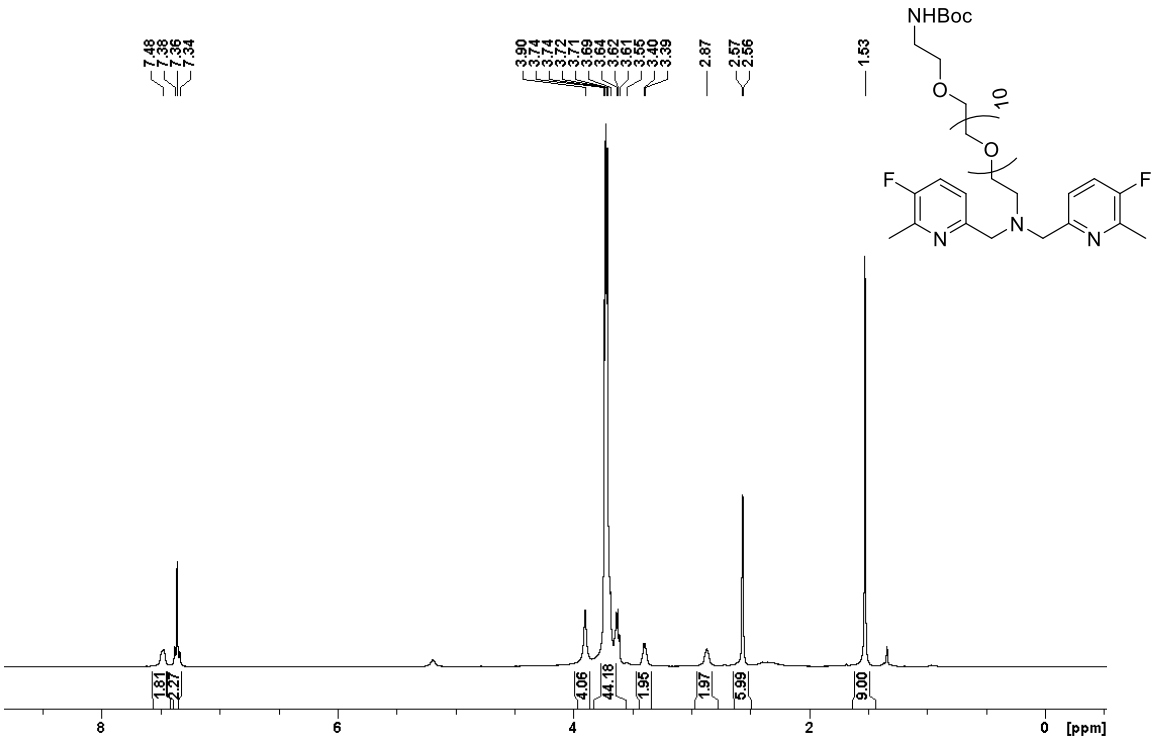
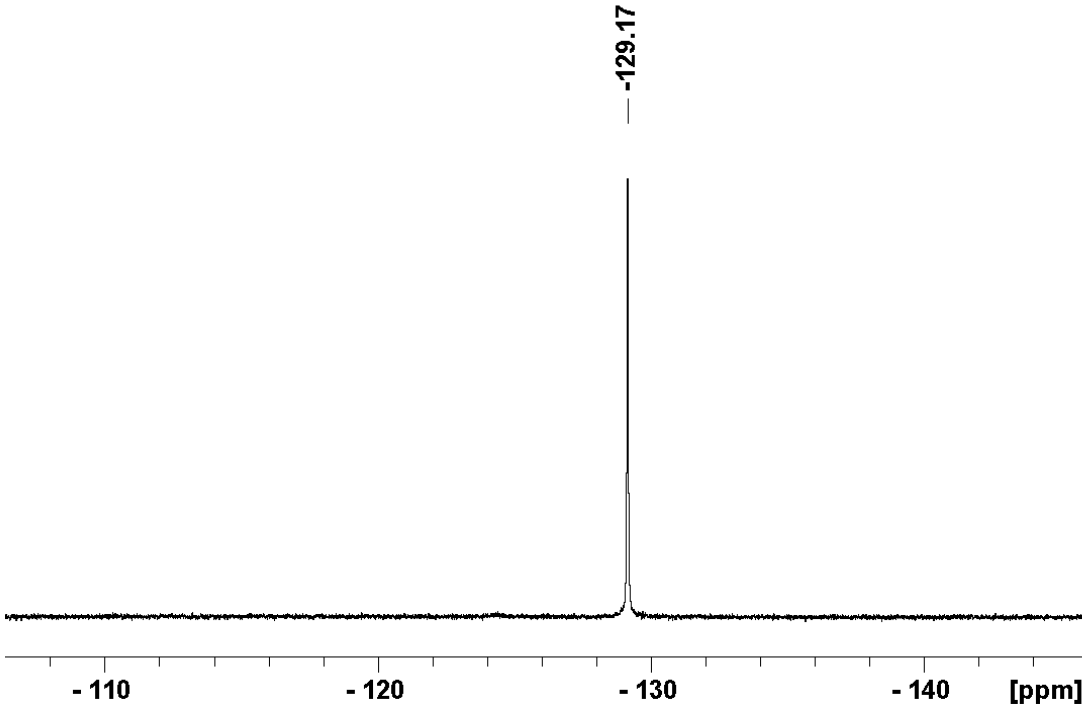


Figure S18. TUNEL staining of excised brain 24 hours after the injection of 2. Microscope images of excised brain 24 hours after the delivery of **2**. Shown from left to right: bright field image, DAPI staining (showing the nuclei of the cells), TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining (as a marker for cellular apoptosis) and an overlay of the TUNEL image over a DAPI image. No significant difference in the staining intensity is shown when compared the injected site (hippocampus of the right hemisphere) to the contralateral region.

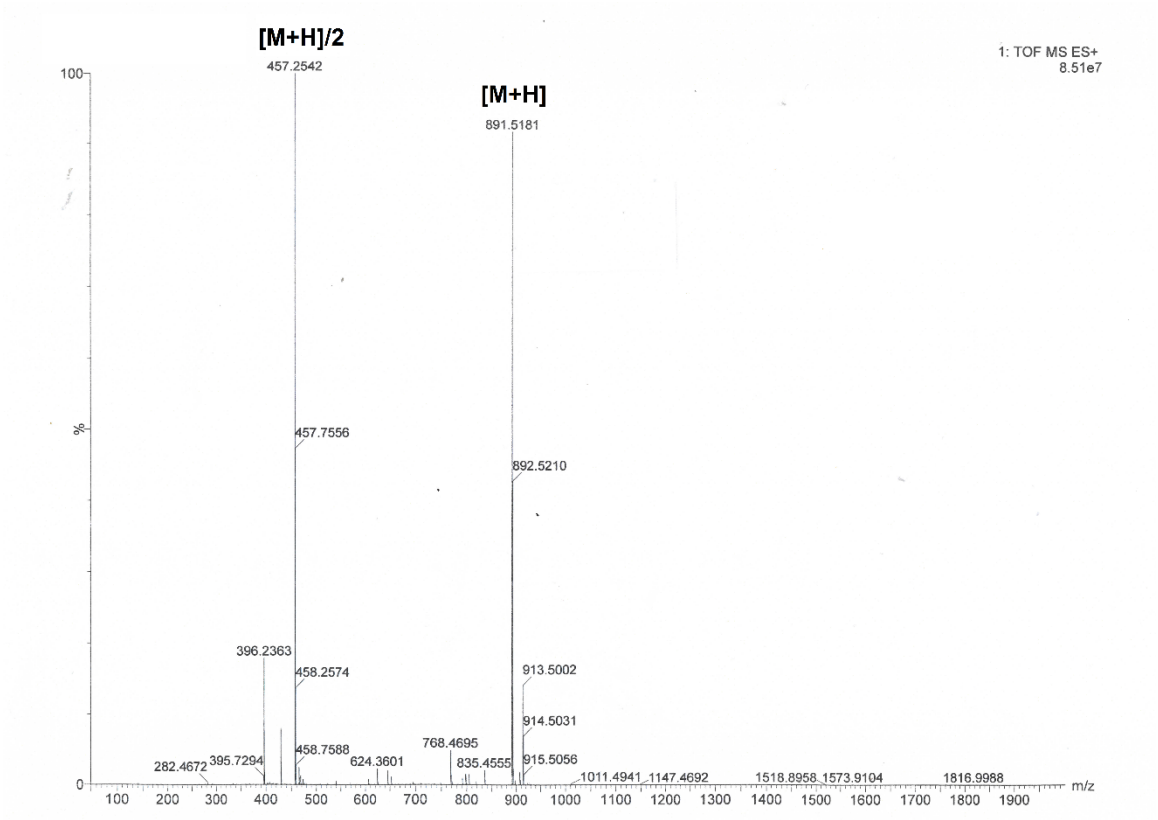
I. NMR (^{1}H, ^{13}C, ^{19}F) & HRMS Spectra



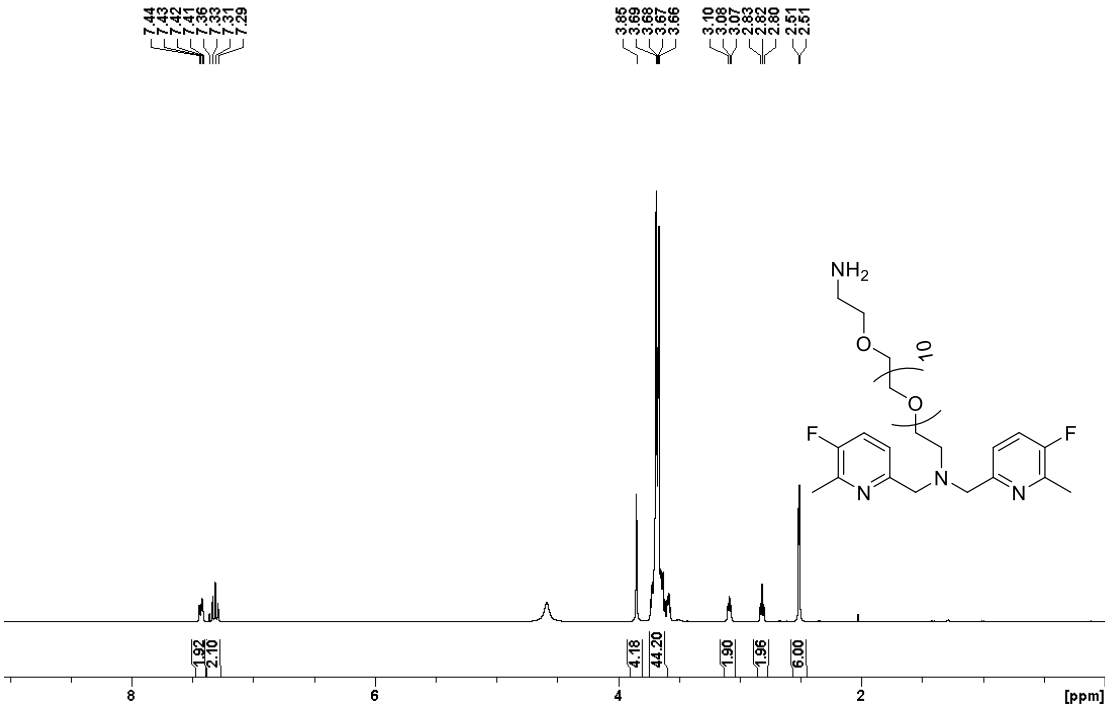
^{1}H-NMR (400.35 MHz, $CDCl_3$, 25^{o}C) spectrum of compound **1a**.



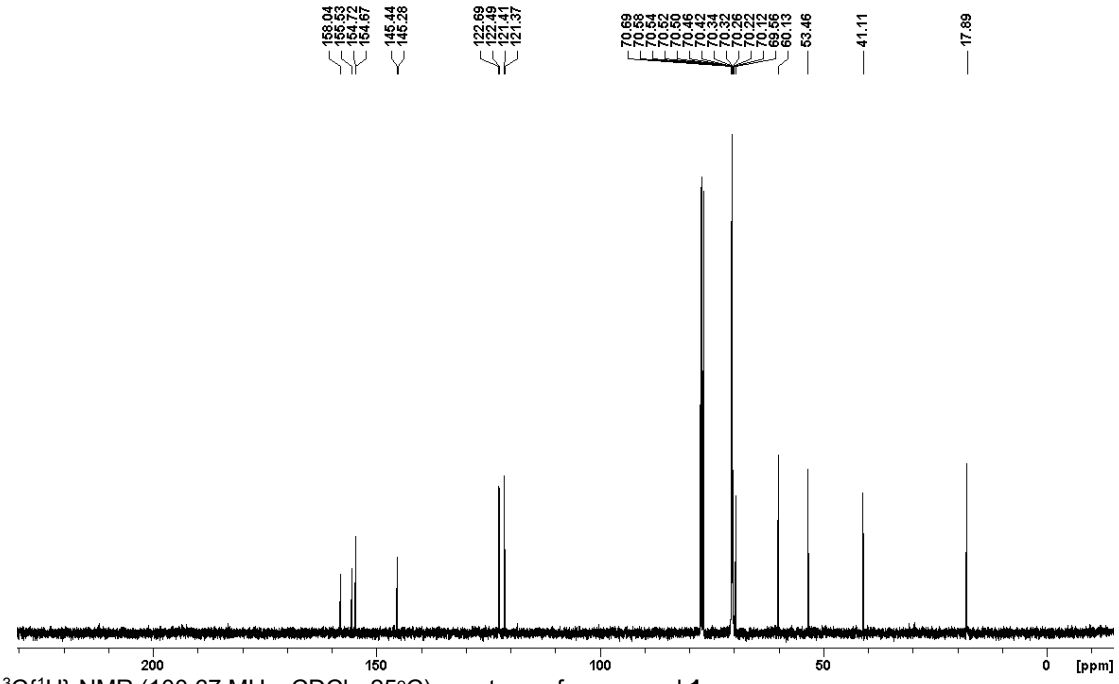
^{19}F-NMR (376.7 MHz, $CDCl_3$, 25^{o}C) spectrum of compound **1a**.



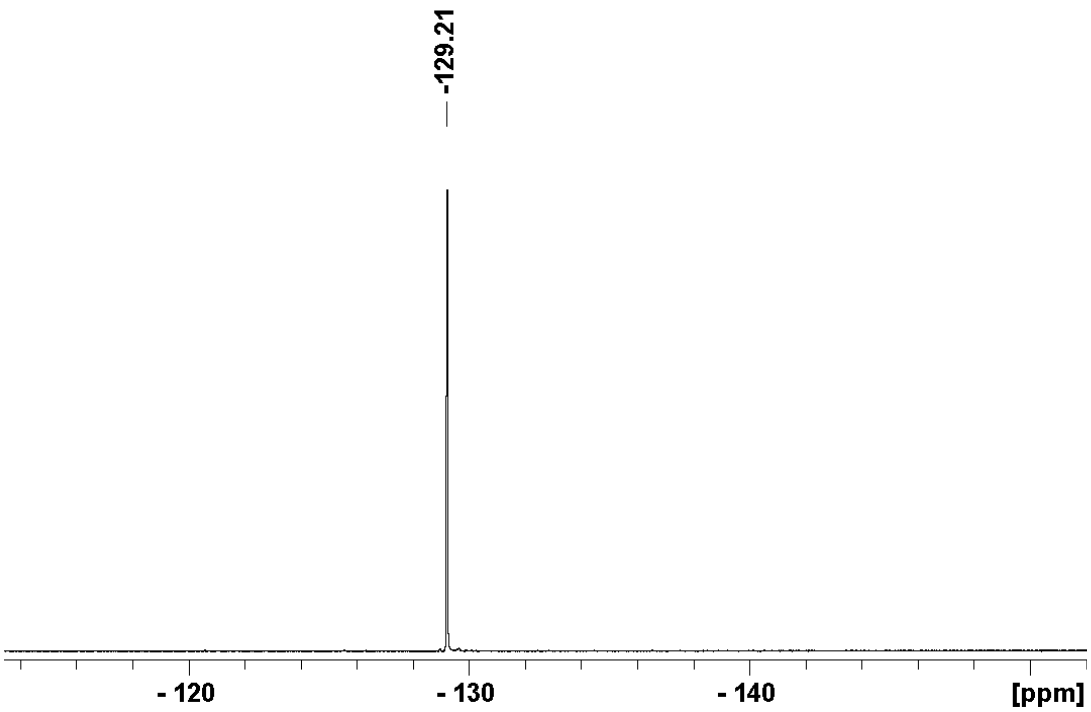
HRMS (ESI) spectrum of compound **1a**.



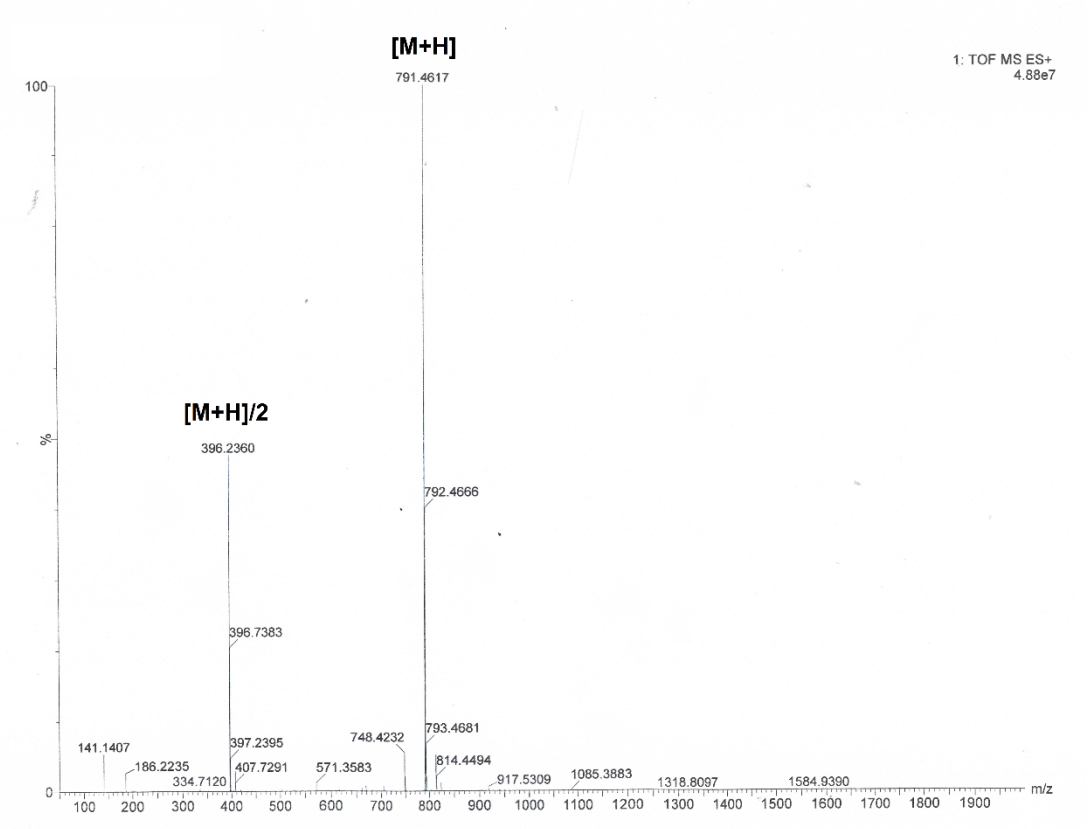
^{1}H-NMR (400.35 MHz, $CDCl_3$, 25ºC) spectrum of compound **1**.



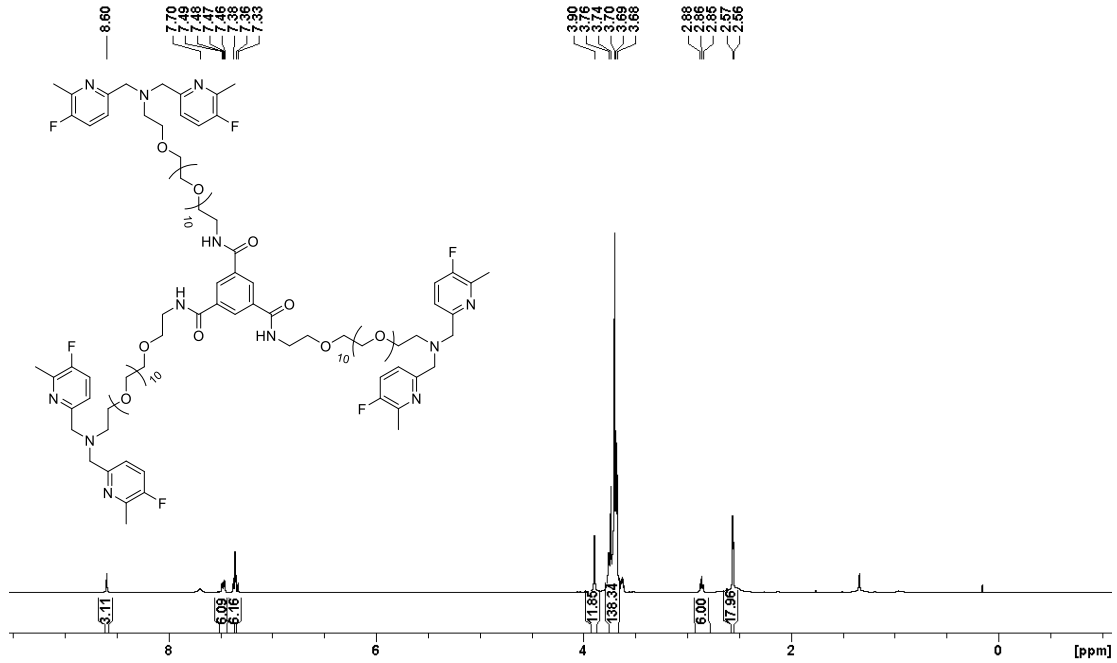
^{13}C{^{1}H}-NMR (100.67 MHz, $CDCl_3$, 25ºC) spectrum of compound **1**.



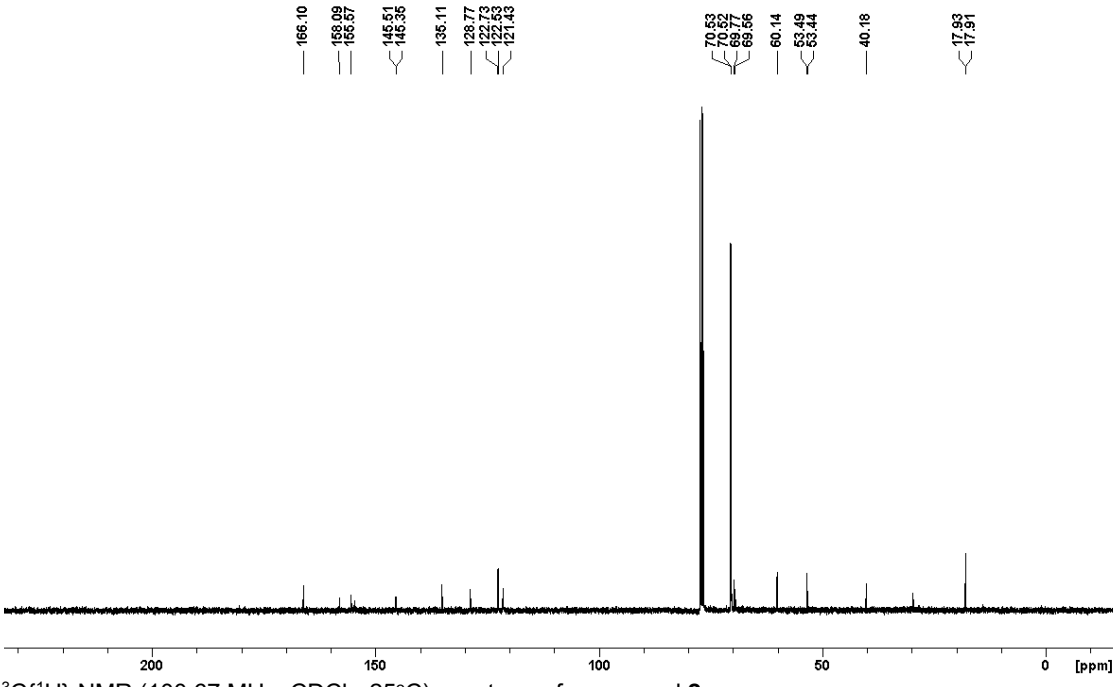
^{19}F-NMR (376.7 MHz, $CDCl_3$, 25^{o}C) spectrum of compound **1**.



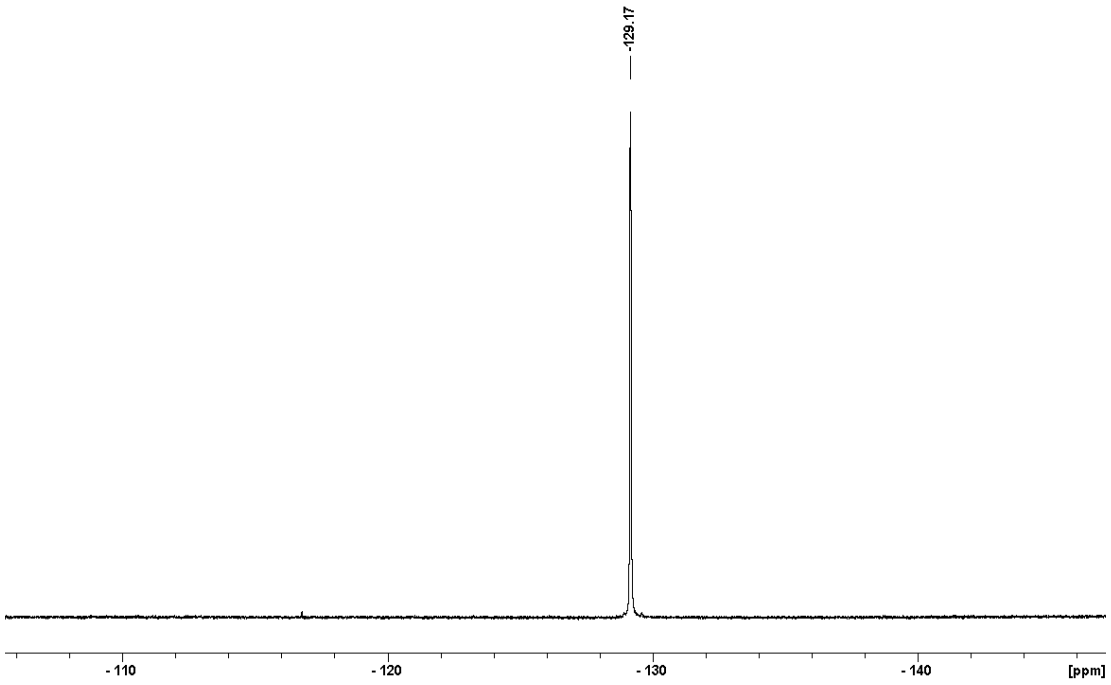
HRMS (ESI) spectrum of compound **1**.



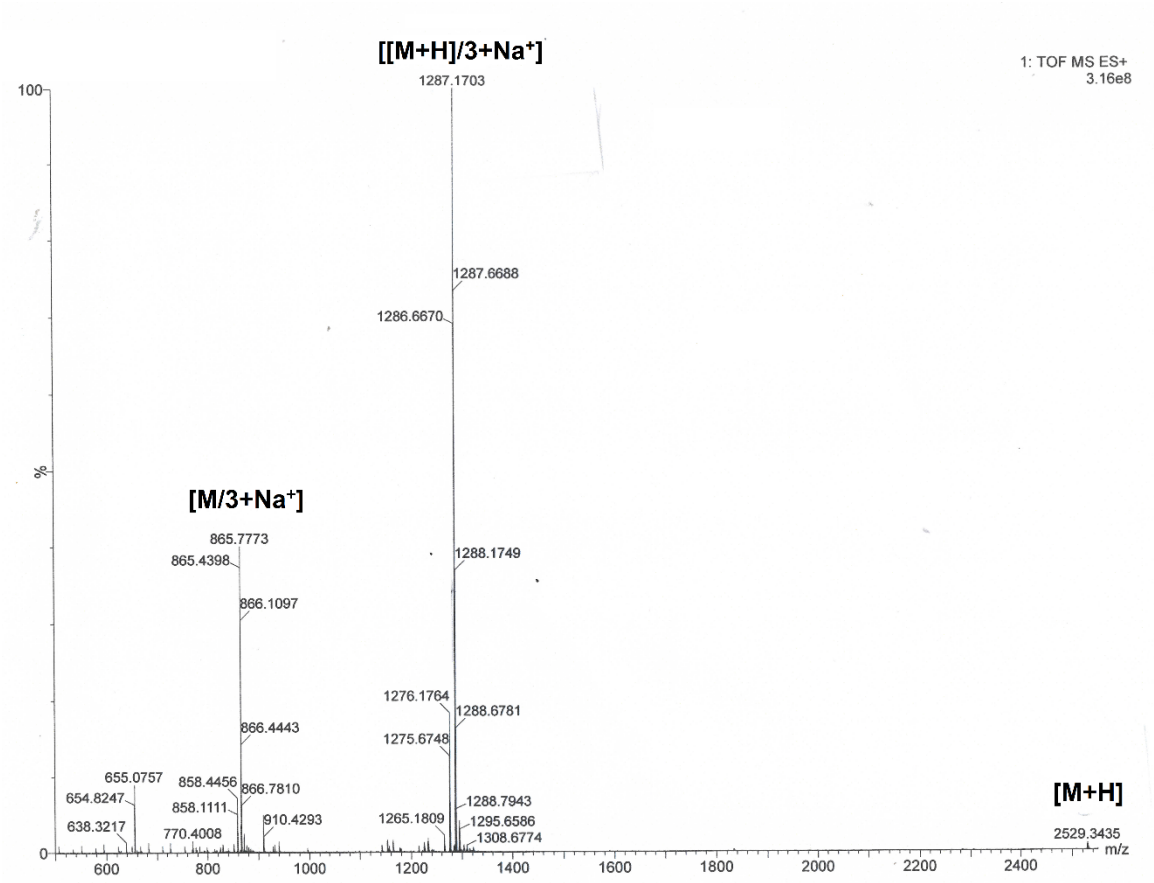
^{1}H-NMR (400.35 MHz, $CDCl_3$, 25^{o}C) spectrum of compound **2**.



^{13}C{^{1}H}-NMR (100.67 MHz, $CDCl_3$, 25^{o}C) spectrum of compound **2**.



^{19}F-NMR (376.7 MHz, $CDCl_3$, 25°C) spectrum of compound **2**.



HRMS (ESI) spectrum of compound **2**.