Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Detection of Aβ plaques in mouse brain by using a disaggregation-induced fluorescence-enhancing probe

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Experimental Section

Reagents

Amyloid Protein Fragment 1-42 protein (ab120301) was purchased from abcam(330 Cambridge Science Park Cambridge CB4 0FL UK). $A\beta_{(1-42)}$ was dissolved in DMSO at a concentration 1mg/ 221.5 μl in tubes that were sealed and sonicated for 30min. and incubated 4℃ for 24h .Probe was dissolved in DMSO at a concentration of 1.0 mM and diluted 6 μM/μl with sterile saline. Mouse anti-NeuN antibody was purchased from Millipore (Merk Millipore, MA, U.S.A.). Cy3-conjugated donkey anti-mouse was purchased from Jackson Immuno Research Laboratories (West Grove, PA, U.S.A.). The other chemicals and reagents used were of high quality and obtained from various commercial sources.

Spectroscopic measurements. Absorption spectra were recorded on a diode array spectrophotometer with 2 μM solution of probe **1** in PBS buffer and photoluminescence spectra were obtained with fluorescence spectrometer with a 1 cm standard quartz cell using 3 μM solution conditions in various solvents, respectively. The fluorescence quantum yields were determined by using 9,10-diphenylanthracene as the reference by a literature (Crosby, G. A.; Demas, J. N. *Phys. Chem.* **1971**, *75*, 991).

Animals

Male ICR mice mice (26-28g, Nara Biotechnology, Korea) were used for this study. All animal protocols were approved by ethics Committee for Care and Use of Laboratory Animals at Kyung Hee University. The animals were housed in plastic cages at a constant temperature (22±2°C) and humidity (55±10%) with 12h-12h light-dark conditions. The animals were allowed free to food and water before the experiment.

Experimental animal groups

Mice were randomly divided into three groups: Aβ(+)/**1**(-), Aβ(+)/**1**(-), and Aβ(+)/**1**(-) group. The Aβ(+)/**1**(-) received a single injection of 3 μl of the aggregated $A\beta_{(1-42)}$. The Aβ(-)/**1**(+) group received a single injection of 3 μl of probe **1**. The Aβ(+)/**1**(+) group received the Aβ and **1** injection consecutively at the same region of the hippocampus.

Injection procedures

Mice were anesthetized by Zoletil (10 mg/kg, i.p.) and placed in a stereotaxic frame with rectal temperature maintained at 37°C using an electric heating pad throughout surgery. Under aseptic conditions, a 1.0 mm diameter burr hole was drilled in the skull (1.5 mm caudal and 2.0 mm lateral to bregma). Aβ and **1** were injected unilaterally (depth = 1.9 mm from the skull surface; speed = 0.6 μl/min) into the hippocampus using a micropump (model 310, K_d Scientific, USA) equipped with a 26-gauge needle Hamilton microsyringe. After completion of the injection, the needle was left in place for 5 min to prevent solution reflux. After the surgery, scalp wound was sutured and returned to the cage.

Tissue preparation and confocal observation

The mice were sacrificed under deep anesthesia 48 h after the surgery, and brains were removed. Coronal brain sections (50 μm of thickness) were made using a freezing microtome (Leica, CM3500S, Germany). Serial coronal sections were picked up on gelatin-coated glass slides and allowed to dry at dark. Without staining, glass coverslips were applied atop a fade-retardant solution and mounted with cover slips for confocal observation. The brain sections were observed at 488 nm wavelength using a Carl Zeiss LSM 510 META laser-scanning microscope. After the confocal observation (or imaging), the cover slips were taken off carefully in order to do immune-fluorescent labeling on neurons. The brain sections were fixed with 100% ethanol for 20 min and immuno-stained against NeuN which is a neuron-marker. The anti-mouse Cy3 was used for a secondary antibody. Then the brain sections were observed again at 488 nm and 543 nm wavelengths. Relative optical density in the hippocampus and average size of granules were measured using the captured images and NIH ImageJ software.

Synthesis

9,9-Diethyl-2-(3-{2-[2-(ethoxy)ethoxy]ethoxy}-1-propynyl)-fluorene (4). 9,9-diethyl-2-iodofluorene (1.45 g, 3.45 mmol) was dissolved in anhydrous toluene (50 mL) under argon. Bis(triphenyl phosphine)-palladium(II)-dichloride (24 mg, 0.036 mmol), copper(I) iodide (6.7 mg, 0.036 mmol), and triphenylphosphine (9.3 mg, 0.036 mmol) were added into the above solution. The reaction mixture was stirred at room temperature and 3-(2-(2-ethoxyethoxy)ethoxy)-1-propyne (1.20 g, 6.89 mmol) was slowly added into the reaction

mixture at 80 °C, then kept at 80 °C for 6 h under argon. After cooling to room temperature, the undissolved solid in the reaction mixture was removed by filtration. The filtrate was concentrated by rotary evaporation mixture under reduced pressure. After the mixture was stirred at 80 °C for 5 h, the product was poured into CH_2Cl_2 (200 mL) and water (200 mL). The organic layer was separated and dried over anhydrous $MgSO_4$, then the solvent was removed in vacuo. Column chromatograph using silica gel with hexane/ethyl acetate (3:1) as eluent gave a brown oil (480 mg). Yield: 35 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (m, 1H), 7.63 (dd, *J* = 8.5 Hz, 1H), 7.42 (dd, *J* = 6.8 Hz, 1H), 7.32 (d, *J* = 3.0 Hz, 1H), 7.31 (s, 2H), 3.81 (m, 2H), 3.74 (m, 2H), 3.69 (m, 2H), 3.62 (m, 2H), 3.53 (q, *J* = 7.0 Hz, 2H), 2.01 (q, *J* = 7.3 Hz, 4H), 1.21 (t, *J* = 6.9 Hz, 3H), 0.29 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.4, 150.1, 142.2, 140.9, 130.9, 127.8, 127.2, 126.5, 123.2, 121.0, 120.2, 119.7, 87.5, 85.1, 70.9, 70.8, 70.1, 69.4, 66.9, 59.5, 56.4, 32.9, 15.4, 8.7. FAB-MS m/z (M) Calcd for $C_{26}H_{32}O_3$ 396.3; Found 397.2.

9,9-Diethyl-2-(3-{2-[2-(ethoxy)ethoxy]ethoxy}-1-propyl)-fluorene (5). Pd/C (10%) (300 mg) was added to a dried THF solution (50 mL) of **4** (480 mg, 1.05 mmol) under argon. The reaction mixture was hydrogenated under normal pressure (H_2 balloon) at room temperature for 24 h. Pd/C was removed by filtration through a pad of celite. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography with ethyl acetate/hexane (3:1) as eluent to give a brown oil (420 mg). Yield: 86.5 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67-7.64 (m, 2H), 7.60 (d, *J* = 8.27, 1H), 7.34-7.28 (m, 3H), 7.15 (d, *J* = 4.8, 1H), 7.13 (s, 1H), 3.70-3.67 (m, 4H), 3.63-3.59 (m, 4H), 3.54 (q, *J*= 7.0 Hz, 2H), 3.49 (t, *J* = 6.5 Hz, 2H), 2.76 (t, *J* = 7.6 Hz, 2H) 1.99 (q, *J* = 7.3 Hz, 4H), 1.9 (m, 2H), 1.22 (t, *J* = 7.0 Hz, 3H), 0.30 (t, *J* = 7.3 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.3, 150.0, 141.8, 141.2, 139.5, 127.3, 126.9, 126.7, 123.2, 123.0, 119.6, 119.5, 70.9, 70.8, 70.7, 70.4, 70.1, 66.9, 56.1, 32.9, 32.7, 31.7, 15.4, 8.7. FAB-MS m/z (M^+) calcd $C_{26}H_{32}O_3$ 396.3; Found 396.2.

9,9-Diethyl-2-(3-{2-[2-(ethoxy)ethoxy]ethoxy}-1-propyl)-9-iodo-fluorene (6). 9,9-Diethyl-2-(3-{2-[2-(ethoxy)ethoxy]ethoxy}-1-propyl)-fluorene (420 mg, 1.05 mmol) was dissolved in acetic acid at 40 °C in a 250 mL flask. To this solution, I_2 (179 mg, 0.70 mmol) and a solution of iodic acid (80 mg, 0.35 mmol) in a water were added. The resulting solution was heated to 90 °C for 4 h. At the end of this period, the solution was allowed to cool to room temperature and poured into CH_2Cl_2 (100 mL) and water (100 mL). The organic layer was

separated and dried over anhydrous $MgSO_4$, then the solvent was removed in vacuo. The crude product was then subjected to column chromatography (silica gel, ethyl acetate/hexane (3:1)) to yield **6** (330 mg, 59 %) as a brown oil. ^{1}H NMR (300 MHz, CD_2Cl_2): δ 7.64–7.60 (m, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.14 (d, *J* = 7.7 Hz, 1H), 7.11 (s, 1H), 3.69-3.64 (m, 4H), 3.62-3.58 (m, 4H), 3.53 (q, *J* = 7.0 Hz, 2H), 3.48 (t, *J* = 6.5 Hz, 2H), 2.75 (t, *J* = 7.9 Hz, 2H) 2.00-1.91 (m, 6H), 1.21 (t, *J* = 6.9 Hz, 4H), 0.29 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.4, 149.6, 141.9, 141.4, 138.4, 135.8, 132.1, 127.4, 123.0, 121.2, 119.7, 91.9, 70.8, 70.7, 70.5, 70.3, 69.9, 66.8, 56.3, 32.8, 32.6, 31.6, 15.3, 8.6. FAB-MS m/z (M^+) calcd $C_{26}H_{35}IO_3$ 522.2; Found 522.1.

9,9-Diethyl-2-(3-{2-[2-(ethoxy)ethoxy]ethoxy}-1-propyl)-9-{(4-dimethylaminophenyl)ethenyl}-fluorene(1). 9,9-Diethyl-2-(3-{2-[2-(ethoxy)ethoxy]ethoxy}-1-propyl)-9-iodo-fluorene (330 mg, 0.631 mmol) was dissolved in anhydrous acetonitrile (20 mL). Bis(triphenylphosphine)-palladium(II)-dichloride (44 mg, 0.063 mmol), and lithium chloride (2.7 mg, 0.063 mmol) were added into the above solution. The reaction mixture was stirred at room temperature and *N*,*N*-dimethyl-4-aminostyrene (185 mg, 1.26 mmol) was slowly added into the reaction mixture at 90 °C, then kept at 90 °C for 20 h. After cooling to room temperature, the solution was concentrated by rotary evaporation mixture under reduced pressure. The crude product was poured into CH_2Cl_2 (200 mL) and water (200 mL). The organic layer was separated and dried over anhydrous $MgSO_4$, then the solvent was removed *in vacuo*. Column chromatograph using silica gel with hexane/ethyl acetate (4:1) as eluent gave a brown oil (98 mg). Yield: 28 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, *J*= 8.3 Hz, 1H), 7.58 (d, *J*= 8.0 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.43 (s, 1H), 7.31-7.27 (m, 1H), 7.15 (d, *J* = 5.4 Hz, 1H), 7.13 (s, 1H), 7.10 (d, *J* = 15.6 Hz, 1H), 7.04 (d, *J* = 7.35 Hz, 1H) 6.74 (d, *J* = 8.8 Hz, 2H), 3.70-3.66 (m, 4H), 3.63-3.59 (m, 4H), 3.54 (q, *J* = 7.0 Hz, 2H), 3.49 (t, *J* = 6.7 Hz, 2H), 2.76 (t, *J* = 7.6 Hz, 2H) 2.04-1.93 (m, 6H), 1.22 (t, *J* = 7.0 Hz, 4H), 0.33 (t, *J* = 7.3 Hz, 6H). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 150.7, 150.6, 150.5, 141.5, 140.9, 139.5, 137.2, 128.2, 127.7, 127.5, 126.1, 125.6, 124.5, 120.4, 119.8, 119.6, 112.7, 71.0, 70.9, 70.6, 70.3, 66.8, 56.2, 40.6, 33.1, 32.9, 32.0, 30.1, 15.4, 8.7. FAB-MS m/z (M^+) $C_{36}H_{47}NO_3$ 541.3; Found 541.3.

Solvent	λ_{max}^{em} [nm]	Φ
Toluene	430	0.36
1,4-Dioxane	436	0.43
EA	449	0.31
MC	455	0.27
IPA	455	0.25
Acetonitrile.	472	0.23
DMSO	482	0.35
Water	460	0.07

Table S1. Fluorescence quantum yields of **1** in various solvents using 9,10-diphenylanthracene. Φ_f = (9,10-diphenylanthracene) 0.95 in EtOH.

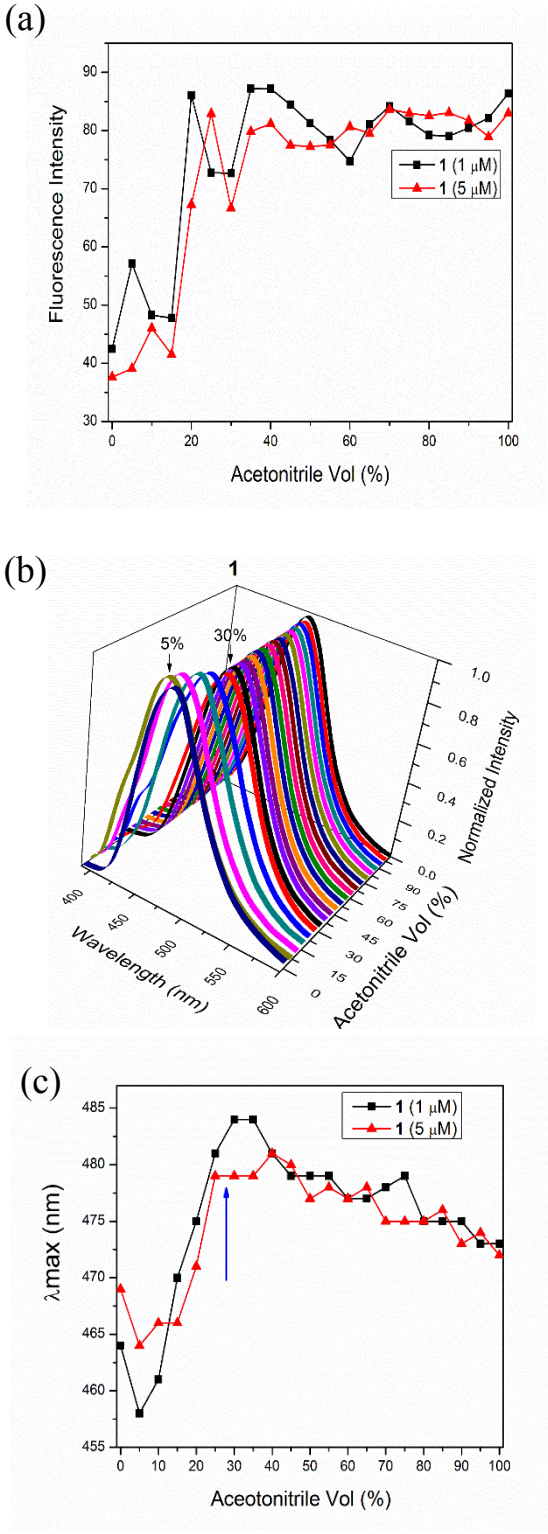

Figure S1. (a) Plot of the fluorescence intensity of **1** (1 and 5 μM) at λ_{em} = 481 nm. (b) Fluorescence intensity of **1** (1 μM), 3D view. (c) λ_{max} of **1** (1 and 5 μM) in Acetonitrile - Water mixtures, excitation at 367 nm.

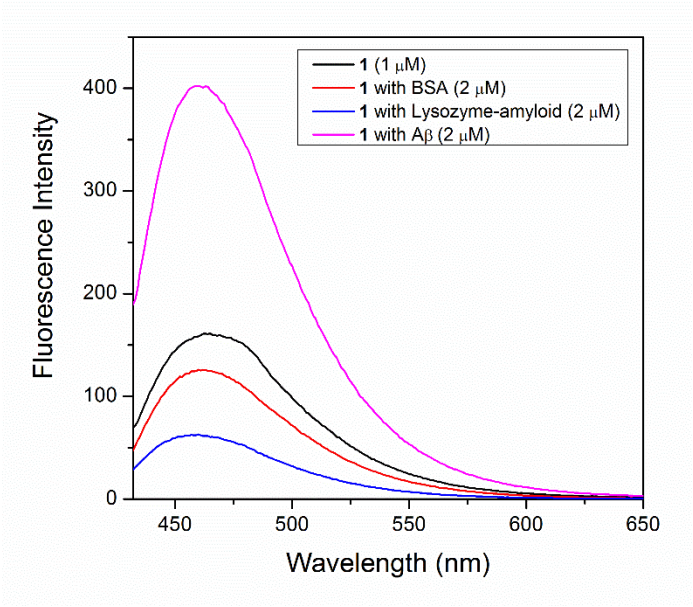

Figure S2. Fluorescence spectra **1** (1 μM) upon interaction with BSA (2 μM). Lysozyme-amyloid (2 μM) and Ab_{42} oligomer in PBS buffer (pH 7.4)/DMSO = 99.8/0.2 (v/v), excitation at 412 nm, slit width 3/5.

H-NMR, ^{13}C-NMR and MS Analyses:

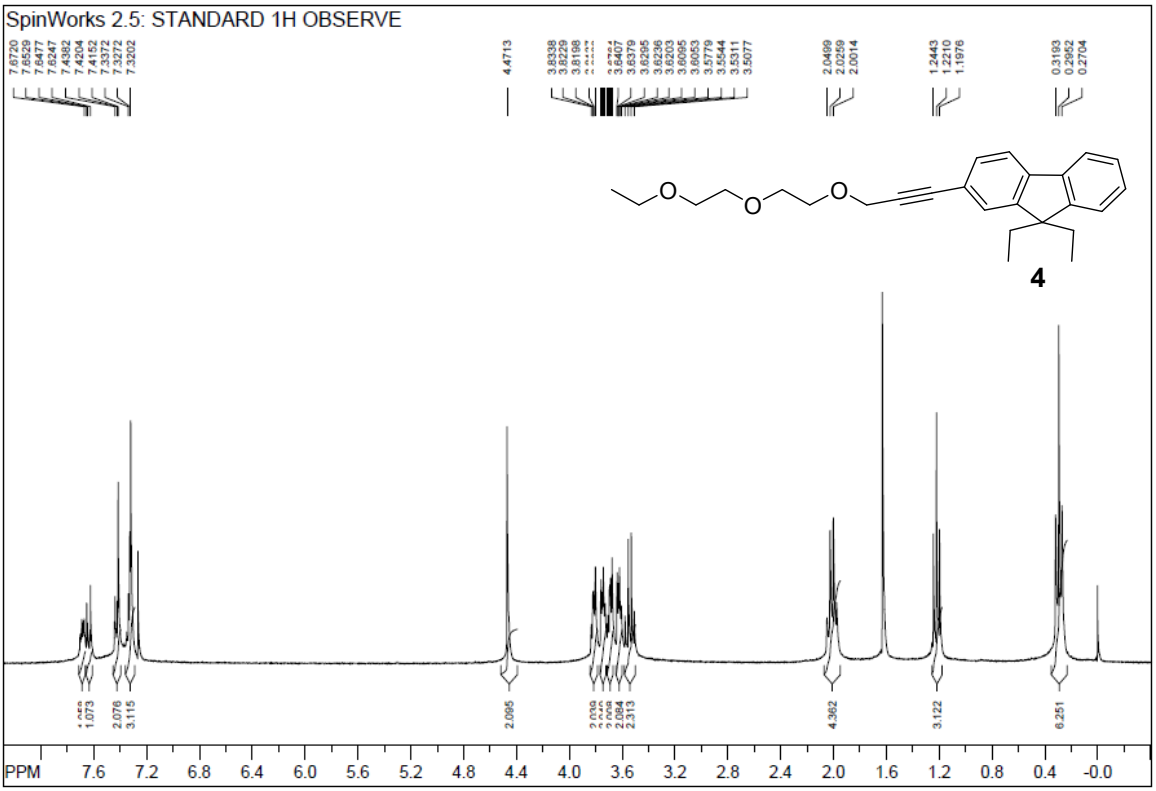

Figure S3. ^{1}H NMR spectrum (300 MHz) of **4** in $CDCl_3$.

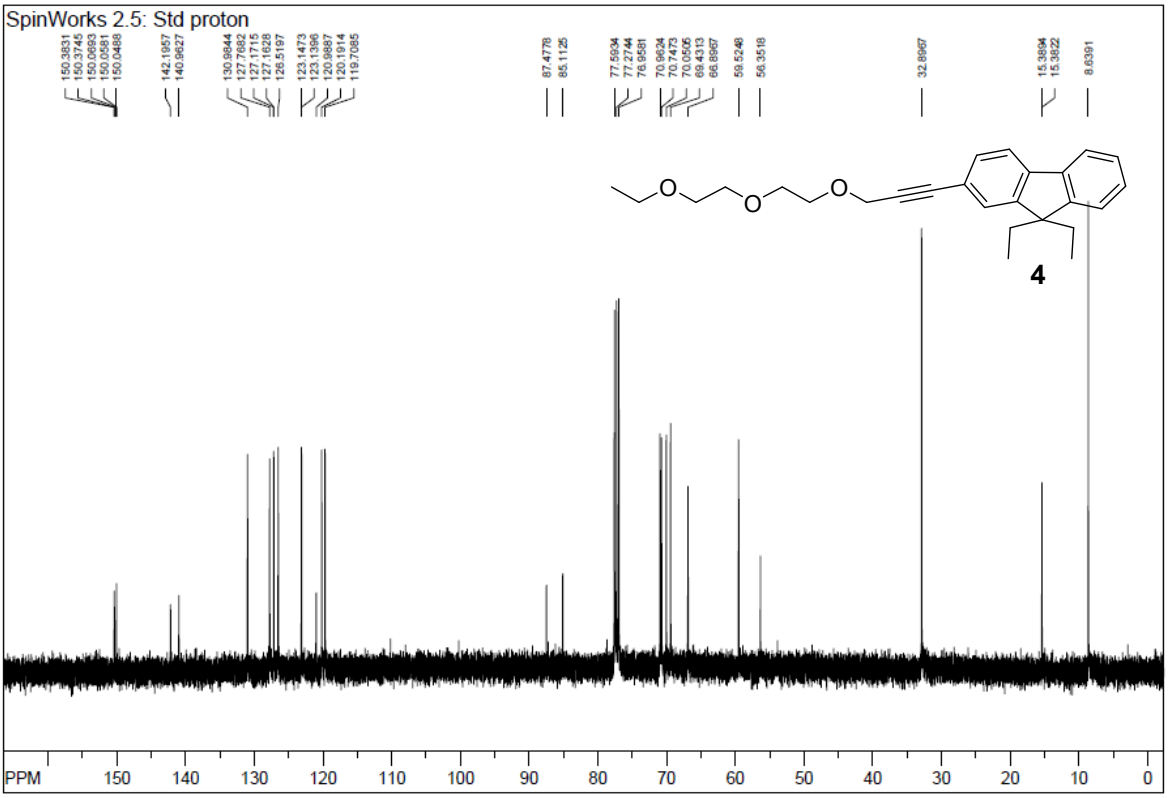

Figure S4. ^{13}C NMR spectrum (100 MHz) of **4** in $CDCl_3$.

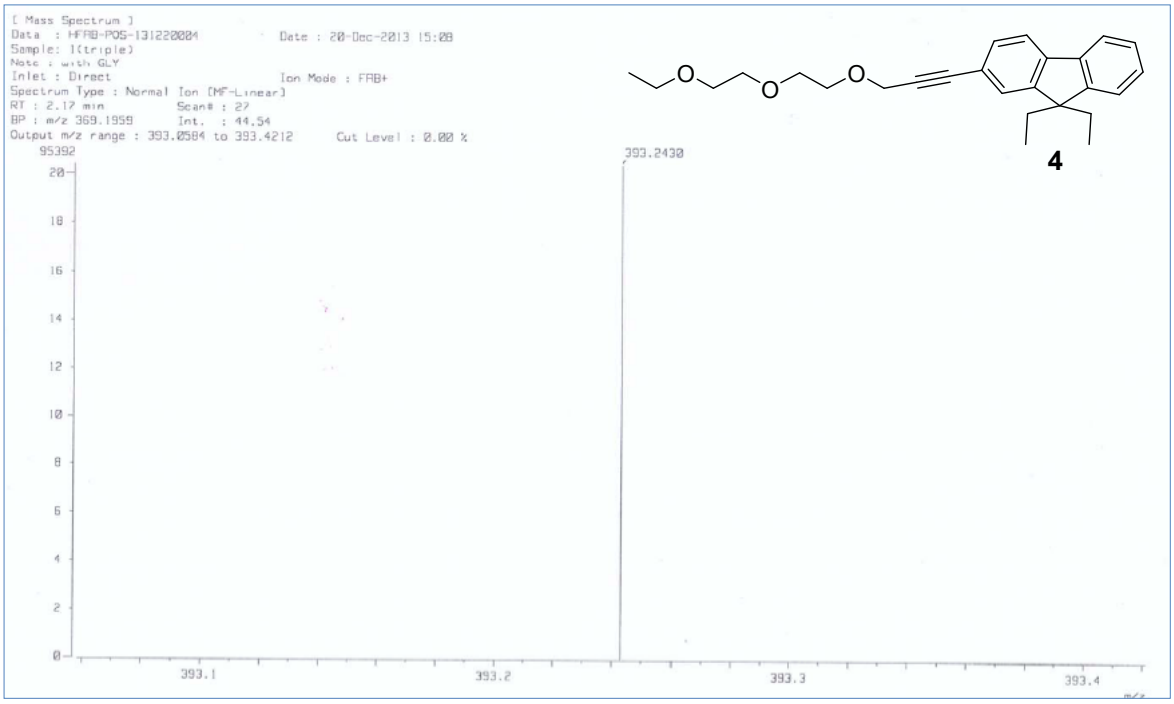

Figure S5. FAB-MS of **4**.

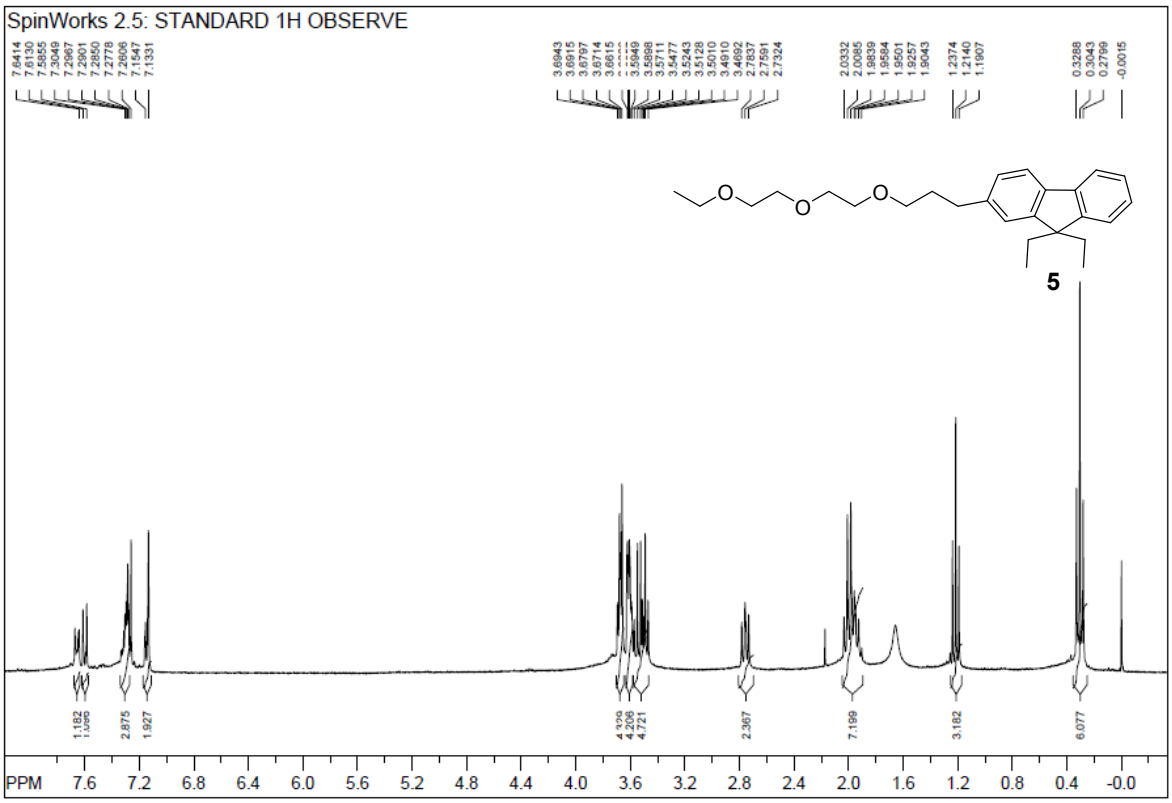

Figure S6. ^{1}H NMR spectrum (300 MHz) of **5** in $CDCl_3$.

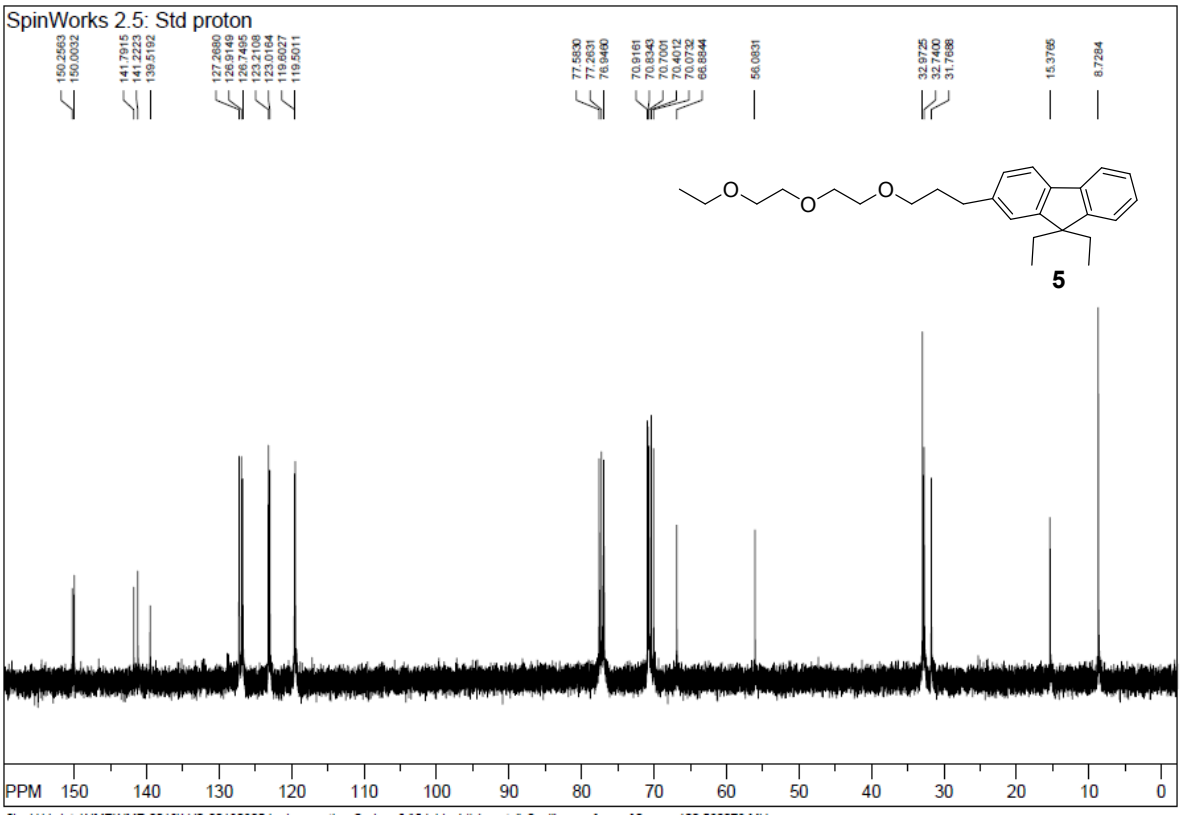

Figure S7. ^{13}C NMR spectrum (100 MHz) of **5** in $CDCl_3$.

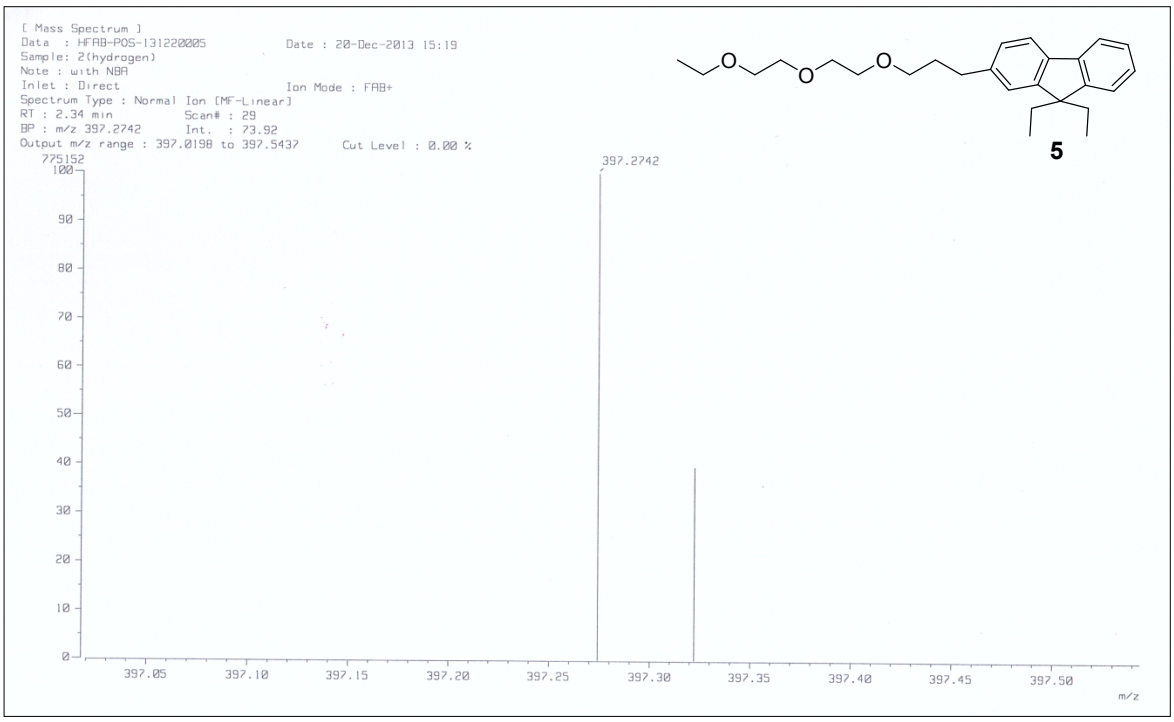

Figure S8. FAB-MS of **5**.

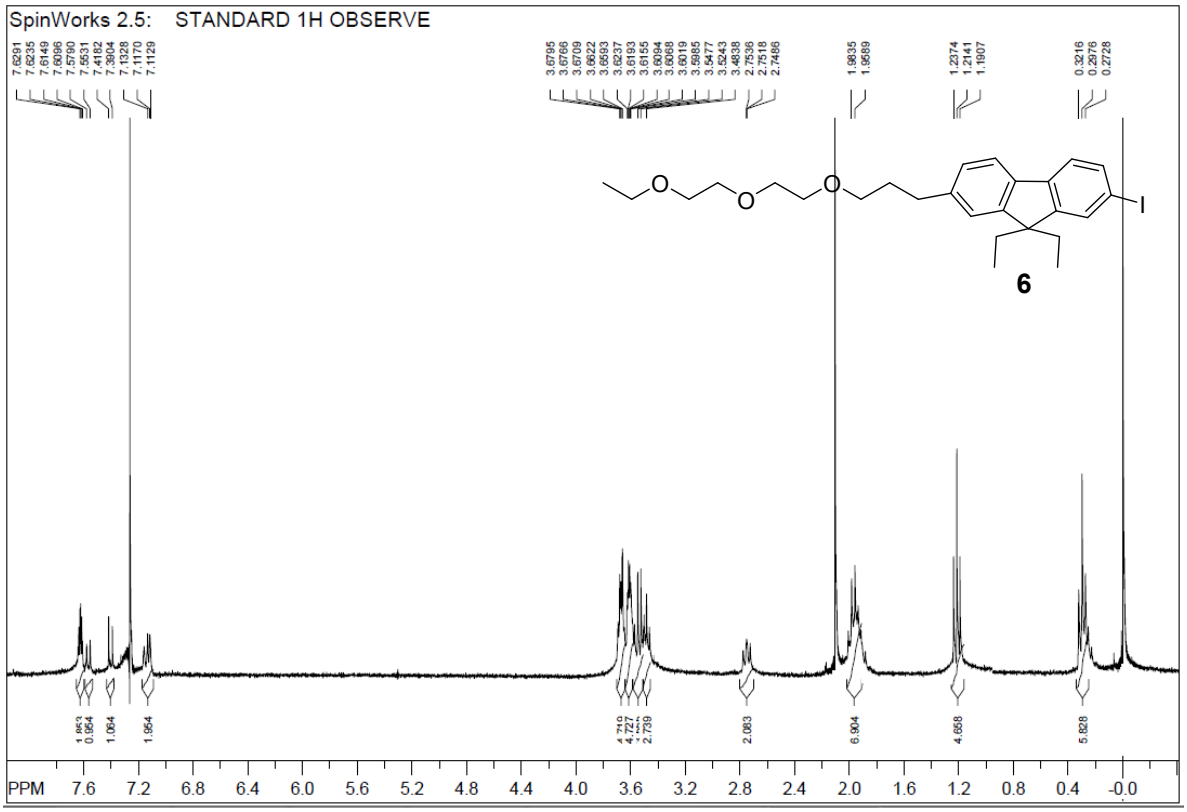

Figure S9. ^{1}H NMR spectrum (300 MHz) of **6** in $CDCl_3$.

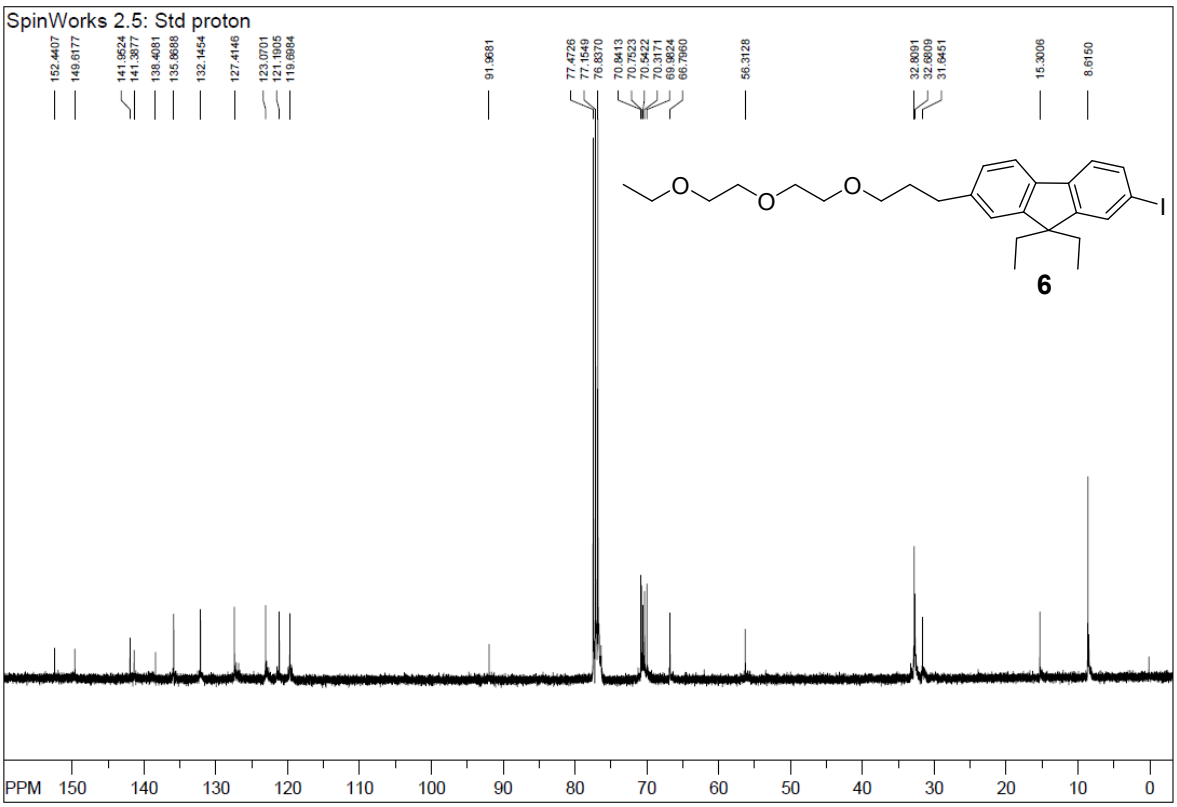

Figure S10. ^{13}C NMR spectrum (100 MHz) of **6** in $CDCl_3$.

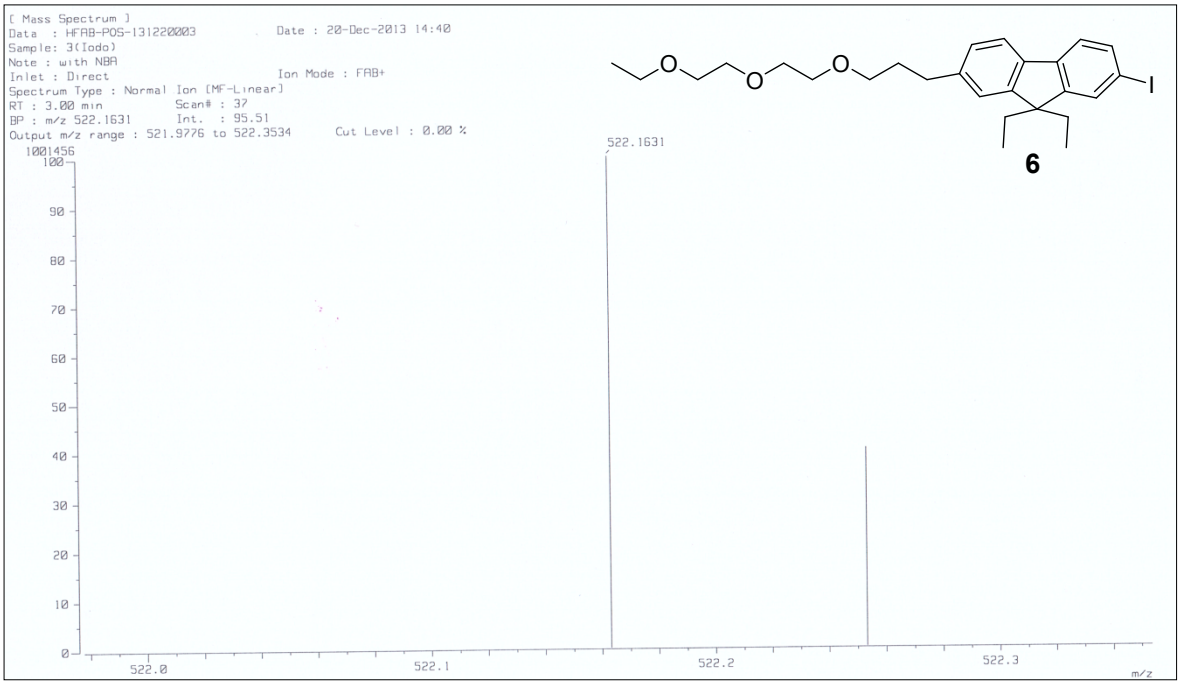

Figure S11. FAB-MS of **6**.

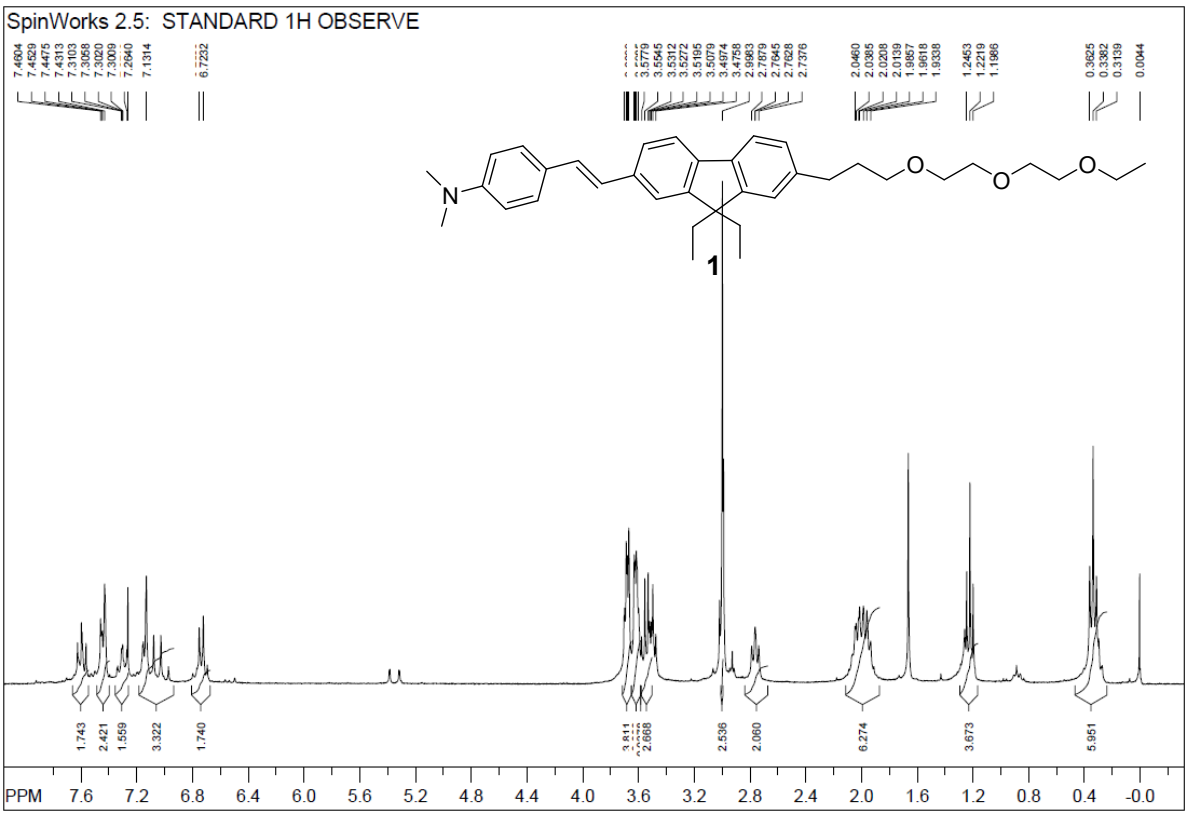

Figure S12. ^{1}H NMR spectrum (300 MHz) of **1** in $CDCl_3$.

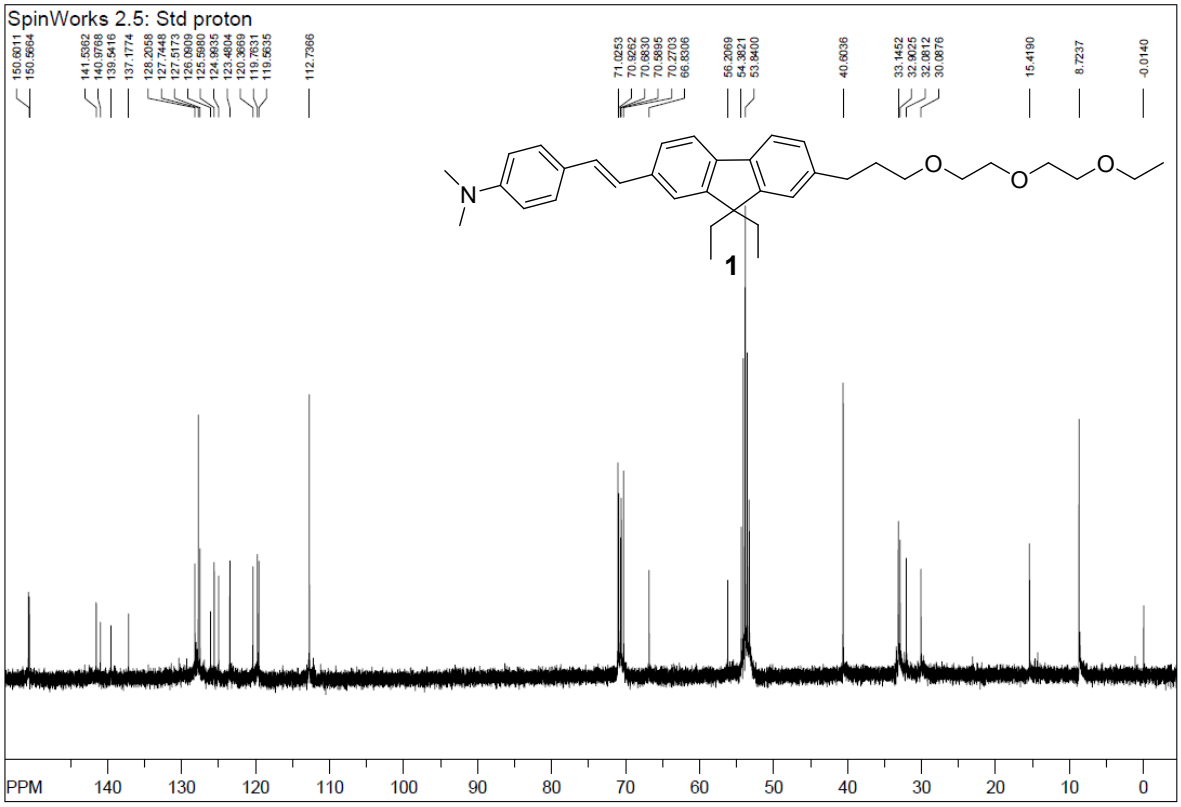

Figure S13. ^{13}C NMR spectrum (100 MHz) of **1** in CD_2Cl_2.

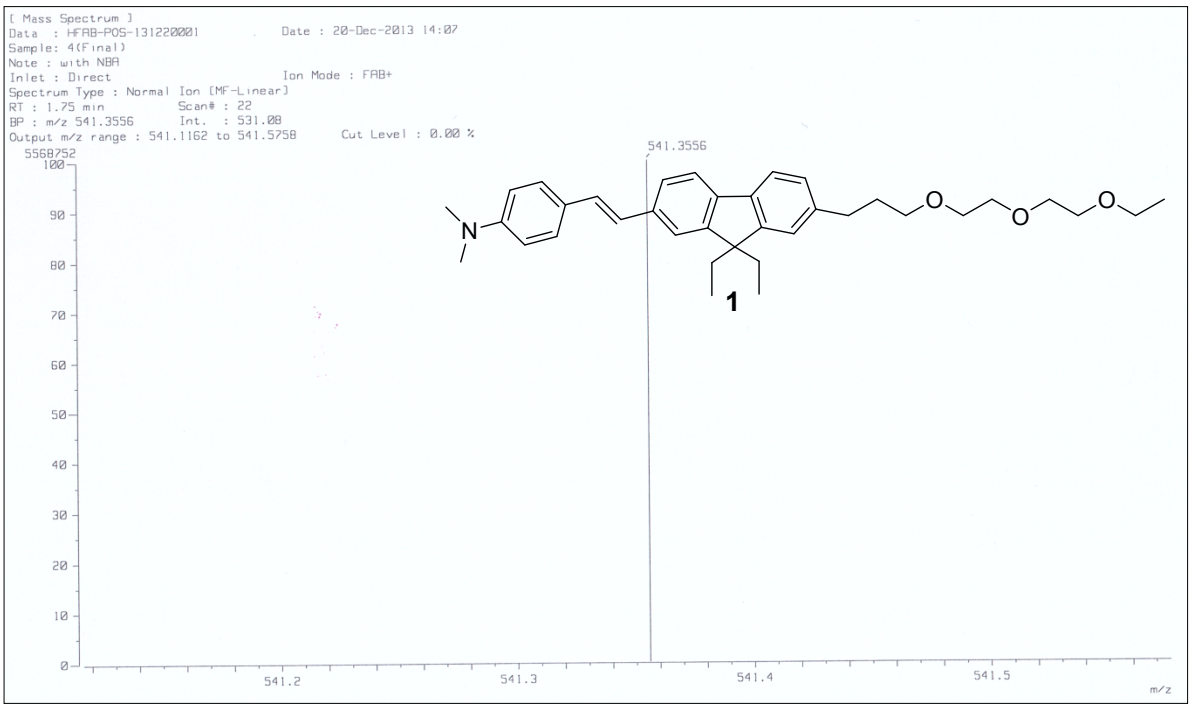

Figure S14. FAB-MS of **1**.