Electronic Supplementary Information

NIR Fluorescence Probes With Good Water-Solubility For Detection Of Amyloid Beta Aggregates In Alzheimer's Disease

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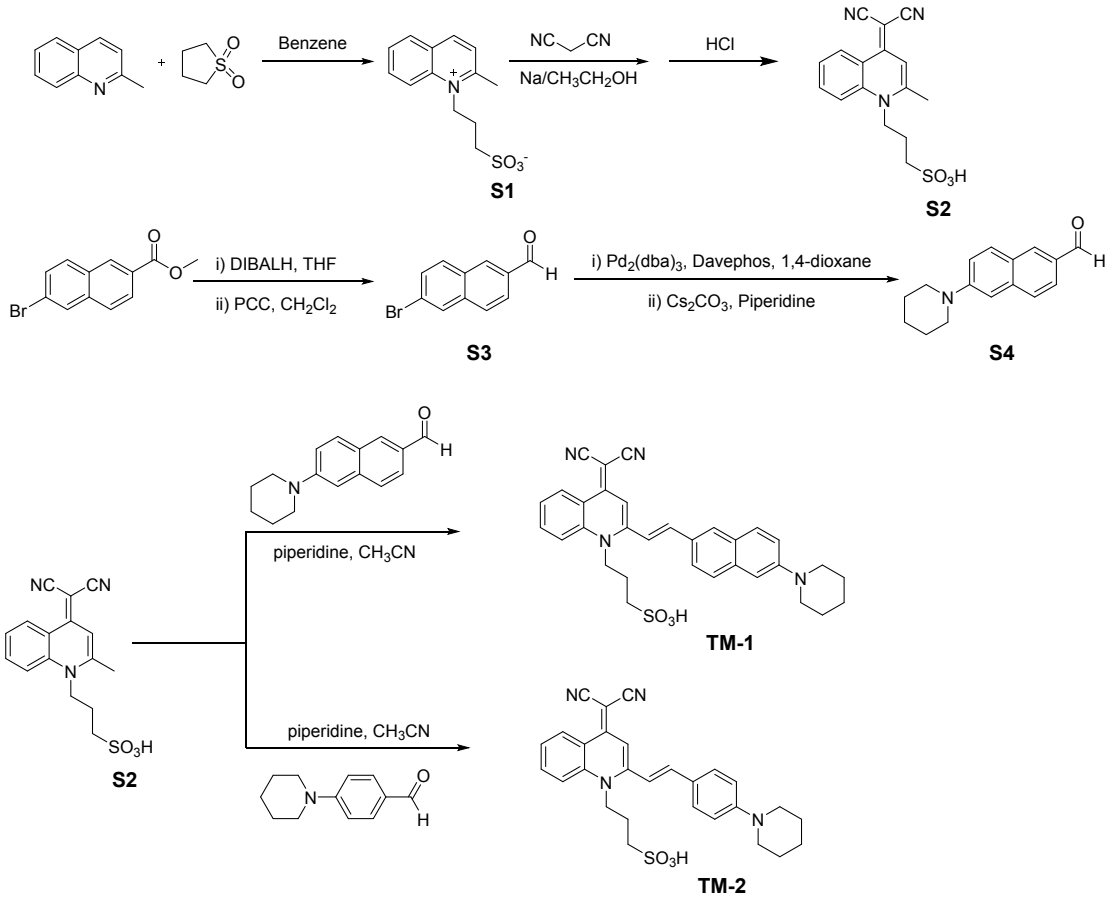
Contents

1. Materials and general methods

All of the starting materials were obtained from commercial suppliers and used as received. Moisture sensitive reactions were performed under an atmosphere of dry argon. Methyl 6-bromo-2-naphthoate and other chemicals were supplied from J&K Scientific Ltd. Aβ (1-42) monomer was purchased from GL Biochem (Shanghai) Ltd. Column chromatography was carried out on silica gel (200-300 mesh). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Mercury plus-Varian instrument. Proton chemical shifts are reported in parts per million downfield from tetramethylsilane (TMS). HR-MS was obtained on an LTQ-Orbitrap mass spectrometer (ThermoFIsher, San Jose, CA). UV-visible spectra were recorded on a Shimadzu UV-2550 spectrometer. Steady-state emission experiments at room temperature was measured on a Shimadzu instruments spectrometer (RF-9000).

All the in *vivo* experiments were performed in compliance with the relevant laws and institutional guidelines, and the institutional committees of both Fudan University and Shanghai University of Medicine & Health Sciences have approved the experiments.

2. Synthesis details of TM-1 and TM-2



Scheme S1 Synthesis routes of **TM-1** and **TM-2**

Synthesis of S1:

2-Methylquinoline (0.08 mol, 12 g) and 7-propanesultone (0.12 mol, 14.6 g) was added into 40 mL of benzene and was refluxed for 20 h. The precipitate was filtered off, washed with hot benzene, then recrystallized from ethanol to give 5.6 g (26 %) of **S1**. ^{1}H NMR (400 MHz, DMSO) δ 9.07 (d, *J* = 8.4 Hz, 1H), 8.78 (d, *J* = 9.2 Hz, 1H), 8.39 (d, *J* =8.0 Hz, 1H), 8.21 (t, *J* = 7.8 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.97 (t, *J* = 7.6 Hz, 1H), 5.14 (d, *J* = 7.6 Hz, 2H), 3.12 (s, 3H), 2.82 – 2.70 (m, 2H), 2.19 (d, J = 4.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 160.8, 145.5, 138.4, 135.2, 130.5, 128.9, 128.2, 125.5, 119.0, 50.7, 47.5, 24.6, 22.4.

Synthesis of S2:

S1 (3.8 mmol, 1 g) and malononitrile (3.8 mmol, 250 mg) was added into 5 mL of absolute ethanol in an ice-bath, stirred vigorously with adding a solution prepared from

sodium (87.4 mg) in 5 mL of absolute ethanol. Stirring was continued for overnight. Then acid was added into mixture and solid was isolated by filtration. Crude product was recrystallized from absolute ethane to give a yellow solid of **S2** (500 mg, 37.4 %). ^{1}H NMR (400 MHz, DMSO) δ 8.90 (d, *J* = 8.4 Hz, 1H), 8.23 (d, *J* = 8.8 Hz, 1H), 7.88 (t, *J* = 7.6 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 6.80 (s, 1H), 4.59 (t, *J* = 7.2 Hz, 2H), 2.78 – 2.57 (m, 6H), 2.01 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 152.4, 150.8, 138.2, 133.4, 125.0, 124.8, 120.6, 119.3, 118.3, 109.1, 47.6, 47.1, 45.8, 24.2, 21.3.

Synthesis of S3:

To a mixture of 6-bromonaphthalene-2-carboxylate (5.0 mmol, 1.3 g) and THF (15 mL) in an ice-bath, diisobutyl aluminium hydride (DIBAL-H, 1.0 M in hexane, 15 mL, 15 mmol) was dropped under argon, followed by slowly adding MeOH and a saturated sodium potassium tartrate solution at room temperature and extracted with ethyl acetate. The organic phase was washed by NaCl, dried over $NaSO_4$ and concentrated to yield the corresponding alcohol (6-bromonaphthalen-2-yl methanol) (47%).

To a suspension of pyridinium chlorochromate (1.5 g, 7.0 mmol) in anhydrous CH_2Cl_2 (60 mL) was added a solution of the above alcohol in CH_2Cl_2, and the reaction was refluxed for 5 h, cooled to room temperature and poured into diethyl ether. The solution was then filtered and concentrated under reduced pressure to yield white solid of compound **S3** (87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H), 8.30 (s, 1H), 8.07 (s, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.85 (t, *J* = 9.2 Hz, 2H), 7.66 (dd, *J* = 1.6, 1.6 Hz, 1H).^{13}C NMR (100 MHz, DMSO) δ 140.98 (s), 133.29 (s), 131.41 (s), 129.83 (s), 129.36 (s), 128.89 (s), 126.79 (s), 126.35 (s), 124.23 (s), 118.47 (s), 62.80 (s).

Synthesis of S4

$Pd_2(dba)_3$ (45.8 mg, 0.05 mmol) and davephos (19.6 mg, 0.05 mmol) was added to 1,4-dioxane. The solution was stirred for 20 min. Compound **S3**, piperidine (914 μL, 10 mmol) and Cs_2CO_3 (1.63 g, 5 mmol) were added and reflux for 12 hours. Then, the reaction was purified via silica gel flash chromatography (EtOAc: petroleum ether = 1: 10) to yield compound **S4** (54 %). ^{1}H NMR (400 MHz, DMSO) δ 9.98 (s, 1H), 8.32 (s, 1H), 7.92 (d, *J* = 9.2 Hz, 1H), 7.74 (d, *J* = 4.0 Hz, 2H), 7.44 (dd, *J* = 2.0, 2.4 Hz, 1H), 7.20 (d, *J* = 2.0 Hz, 1H), 3.37 (m, 4H), 1.61 (m, 6H). ^{13}C NMR (100 MHz, DMSO) δ

192.51 (s), 151.85 (s), 138.64 (s), 134.77 (s), 130.97 (s), 127.51 (s), 126.10 (s), 123.22 (s), 119.59 (s), 108.41 (s), 49.06 (s), 25.50 (s), 24.44 (s).

Synthesis of TM-1

S2 (0.56 mmol, 200 mg) and **S4** (0.68 mmol, 160.8 mg), piperidine (250 μL) was added into 5 mL of acetonitrile, and was refluxed for 20 h under argon. After completion, acid was added into mixture and the solid was isolated by filtration. Crude product was recrystallized from methanol to give a red solid of **TM-1 (**120 mg, 31%).^{1}H NMR (400 MHz, DMSO) δ 8.93 (d, *J* = 8.4 Hz, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 8.18 (s, 1H), 8.05 (d, *J* = 8.8 Hz, 1H), 7.91 (t, *J* = 7.6 Hz, 1H), 7.80 – 7.70 (m, 3H), 7.63 – 7.53 (m, 2H), 7.39 (d, *J* = 9.2 Hz, 1H), 7.17 (d, *J* = 12.0 Hz, 2H), 4.84 – 4.71 (m, 2H), 3.36 – 3.32 (m, 4H), 2.73 – 2.70 (m, 2H), 2.16 (m, 2H), 1.67 – 1.60 (m, 6H). ^{13}C NMR (100MHz, DMSO) δ 152.0, 150.3, 149.7, 140.8, 138.2, 135.4, 133.6, 129.6, 129.4, 126.9, 129.4, 126.9, 126.8, 125.0, 124.9, 120.7, 119.3, 118.5, 108.6, 106.2, 49.2, 47.6, 47.4, 25.2, 24.6, 24.0. HRMS (ESI, m/z): calcd for $C_{32}H_{31}N_4O_3S^+$ $[M+H]^+$, 551.2111; found: 551.2104.

Synthesis of TM-2

S2 (0.56 mmol, 200 mg), 4-(1-Piperidinyl)-benzaldehyde (0.68 mmol, 130 mg) and piperidine (250 μL) was added into 5 mL of acetonitrile, and was refluxed for 20 h under argon. Then, acid was added into to the mixture and the solid was isolated by filtration, crude product was recrystallized from methanol to give a red solid of **TM-2** (120 mg, 41%). ^{1}H NMR (400 MHz, DMSO) δ 8.92 – 8.90 (dd, *J* = 2.0 Hz, *J* = 2.0 Hz, 1H), 8.22 (d, *J* = 9.2 Hz, 1H), 7.90 – 7.86 (m, 1H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.58 (t, 1H), 7.42 (dd, *J* = 15.6, 11.6 Hz, 2H), 7.09 (s, 1H), 6.94 (d, *J* = 9.2 Hz, 2H), 4.75 – 4.71 (t, *J* = 8.0 Hz, 2H), 3.31 (m, 4H), 2.72 – 2.68 (m, 2H), 2.11 (m, 2H), 1.59 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ 152.1, 151.7, 150.0, 140.6, 138.2, 133.4, 125.0, 124.7, 124.4, 120.6, 118.3, 115.2, 114.3, 105.6, 48.3, 47.6, 47.3, 24.9, 24.5, 23.9. HRMS (ESI, m/z): calcd for $C_{28}H_{29}N_4O_3S^+$ $[M+H]^+$, 501.1955; found: 501.1942.

3. Experimental details for DLS and TEM measurements

DLS measurements: One milliliter of 250 nM of an Aβ42 PBS solution was added to a cell of DLS and balance for 2 minutes at 25°C for DLS measurement. Each sample was tested three times and then averaged.

TEM Measurement: Five microliters of 250 nM of an Aβ42 PBS solution, which was prepared from HFIP stock solution (25 μM), was dropped to a TEM grid, followed by the addition of 2 μL of a phosphotungstic acid (PTA) contrast solution to the grid. After 1 min, the liquid on the grid was carefully dried with a corner of filter paper, and the resulting grid was further dried in the air for 2−5 min.

4. Preparation of Aβ species

Aβ40/42 monomer: The preparation of Aβ40/42 monomer was performed according to the literature reported by Ran [S1]. Aβ40/42 monomers were prepared by further purification of commercially available Aβ40/42 peptide using HPLC. Purified monomers were stored as powder or in hexafluoroisopropanol (HFIP) as stock solutions. Ten microliters of Aβ40/42 monomer (25 μM) in HFIP were dried with argon gas and reconstituted in 1.0 mL of distilled water for spectral measurement.

Preparation of Aβ40/42 oligomers: The preparation of Aβ40/42 oligomers was performed according to the reported procedure by Kayed. [S2] The Aβ40/42 monomer was dissolved in 400 μL of hexafluoroisopropanol, fully depolymerized for twenty minutes. The solution was dried under nitrogen to complete evaporation, and deionized water was added and incubated at room temperature for twenty minutes at 14,000xG per minute. The mixture was centrifuged for 15 minutes, and the supernatant was taken and stirred at 500 rpm to obtain Aβ oligomers.

Preparation of Aβ40/42 aggregates: The Aβ40/42 aggregates was prepared according to the reported procedure by Jerry. [S3] The monomer of Aβ40/42 was dissolved in a phosphate buffer solution (pH =7.3) to a final concentration of 100 μM, and vigorously stirred at room temperature for three days at a rotation speed of 1200 rpm to give Aβ aggregates.

5. Confocal laser scanning microscopy (CLSM) images

Confocal fluorescence imaging was performed with an OLYMPUS ZX81 laser scanning microscopy. Excitation at 408 nm and 488 nm was carried out with a semiconductor laser, and emission was collected from 500 nm to 700 nm. In the scan mode, the lamp power of 405 nm and 488 nm for fluorescence is 0.15 mW on the focus plane of CLSM, which are applied to achieve the fluorescence image.

6. AD transgenic mouse model

AD transgenic model for amyloid plaques, 5XFAD mouse, was used to examine the possible co-localization of **TM-1** or **TM-2** staining and Aβ immunoreactivity. The 5XFAD transgenic mice were obtained from Jackson Laboratory [B6SJL-Tg (APPSwFlLon, PSEN1*M146L* L286V) 6799Vas/J; stock no. 006554, Bar Harbor, Maine, USA]. To maintain on a C57BL/6J background, the original 5XFAD mice were backcrossed to C57BL/6J mice for eight generations. These 5XFAD transgenic mice overexpress both mutant human APP(695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) Familial Alzheimer's Disease (FAD) mutations and human PS1 harboring two FAD mutations, M146L and L286V.

7. Colocalization of probe labeling and Aβ immunoreactivity in AD transgenic mouse brain

The brain tissue and immunofuorescent labeling were performed as previously described. 5 Mice were deeply anesthetized and transcardially perfused with PBS, followed by 8% formadehyde (Sigma-Aldrich) in PBS (pH = 7.3). The brains were post-fixed and frozen-sectioned (14 μm thickness) with a microtome (Leica Microsystems, GmbH, Wetzlar, Germany) for further analyses. Briefly, free-floating sections were blocked in a blocking solution containing 10% goat serum, 1% BSA and 0.4% Triton X-100. Monoclonal anti-β-Amyloid antibody was purchased from Sigma-Aldrich and used at a 1: 300 dilution. Incubation with primary antibodies took place overnight at 4 °C. After rinsing, sections were incubated in the goat anti-mouse secondary antibody (1: 300) conjugated with Alexa 488 (Molecular Probes, Carlsbad,

CA, USA) for 2 h at room temperature. APP/PS1/Tau triple transgenic mouse model was generated by crossing APP/PS1 transgenic mice with P301S mutant human Tau transgenic mice. Mice at age of 10 month old were used. In colocalization studies, images for Aβ immunofuorescent reactivity and Aβ probe labeling were taken sequentially using a microscope (BX51, Olympus, Japan) equipped with a DP72 digital camera.

8. Cytotoxicity Assay

The Cell Counting Kit-8 (CCK-8) assay was used to detect the cytotoxicity of **TM-1** and **TM-2**. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and then cultured in 5% CO_2 at 37 °C for 24 h. After the cells were incubated with **TM-1** and **TM-2** at different concentrations (0, 5, 10, 25, 50, 75, and 100 μM in cell culture medium with 10% FBS) for 12 h, CCK-8 (20 mL, 5 mg /mL) was added to each well of the 96-well assay plate for 2 h at 37 °C. The absorbance was measured at 490 nm using a microplate reader. All samples were analyzed in triplicate.

9. Additional Table

Table S1 The comparision of TM-1/TM-2 with published NIR probes for Aβ

Probes	λem	Kd	Selectivity	Enhance fold	Ref
CRANAD-58	750 nm	Aβ40: Kd = 105.8 nM, Aβ42: Kd = 45.8 nM	monomer/oligomer/ aggregates	Aβ40 (250 nM): 91.9 Aβ42 (250 nM): 113.6	29
CRANAD-102	700 nm	Soluble: Kd = 7.5 nM Insoluble: Kd = 505.9 nM	oligomer/aggregates	Oligomer (750 nM):4.34	30
CQ	654 nm	Aβ42: Kd = 86 nM	aggregates	Aβ42 (10 μM): 10	38
MC-1	685 nm	Aβ42: Kd = 59.09 nM	aggregates	/	39
DCIP-1	635 nm	Aβ42: Kd = 674.3 nM	aggregates	/	40
QM-FN-SO3	720 nm	Aβ42: Kd = 170 nM	aggregates	/	41
Fe3O4@SiO2@ SLCONHR	650 nm	/	monomer/fibrils	Aβ40 fibrils (750 μM): 11.8	42
CRANAD-3	650 nm		aggregates	/	43
Dye2	635 nm	Aβ40: Kd = 1.13 μM	aggregates	Aβ40 (20 μM): 19.5	44
TM-1/TM-2	680/650 nm	Aβ42: Kd = 35/92 nm	aggregates	Aβ42 (24 μM) : 106/96	

10. Additional absorption and fluorescent spectra

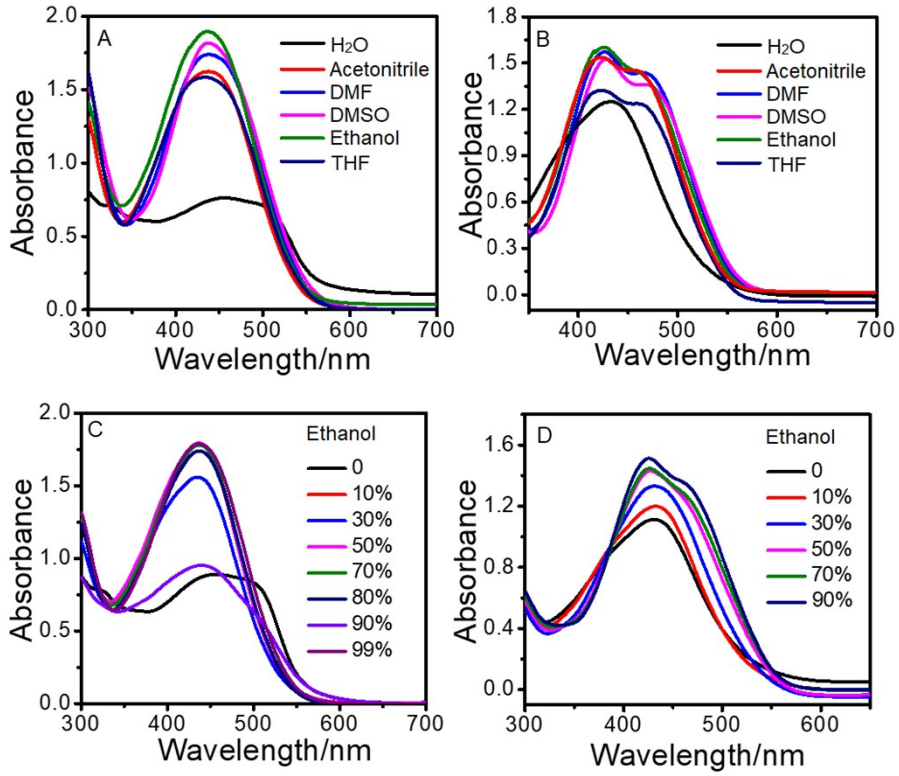


Fig. S1 Solvent dependent absorption spectra of **TM-1** and **TM-2**. (A, B) absorption spectra of **TM-1** and **TM-2** in different solvents; (C, D) absorption spectra of **TM-1** and **TM-2** with different ethanol fractions (f_e) in a mixture of ethanol /water.

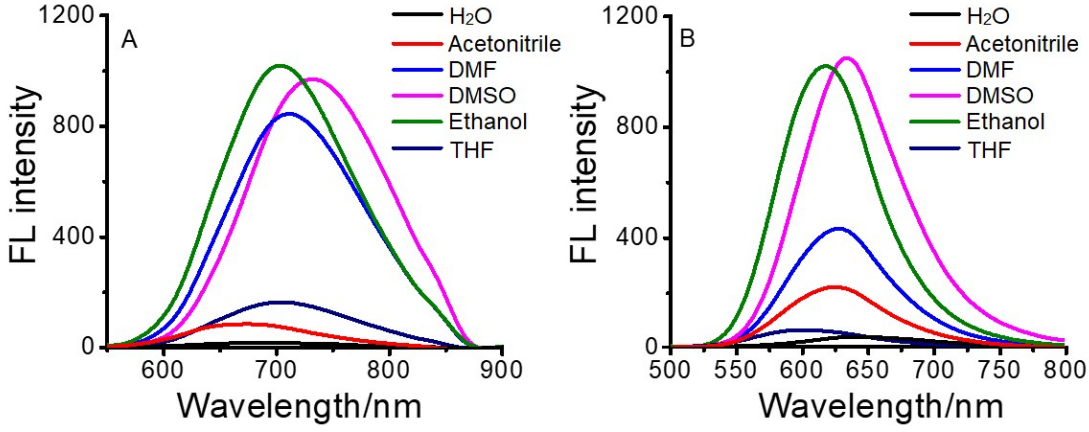


Fig. S2. Emission spectra of **TM-1** (A) and **TM-2** (B) in different solvents.

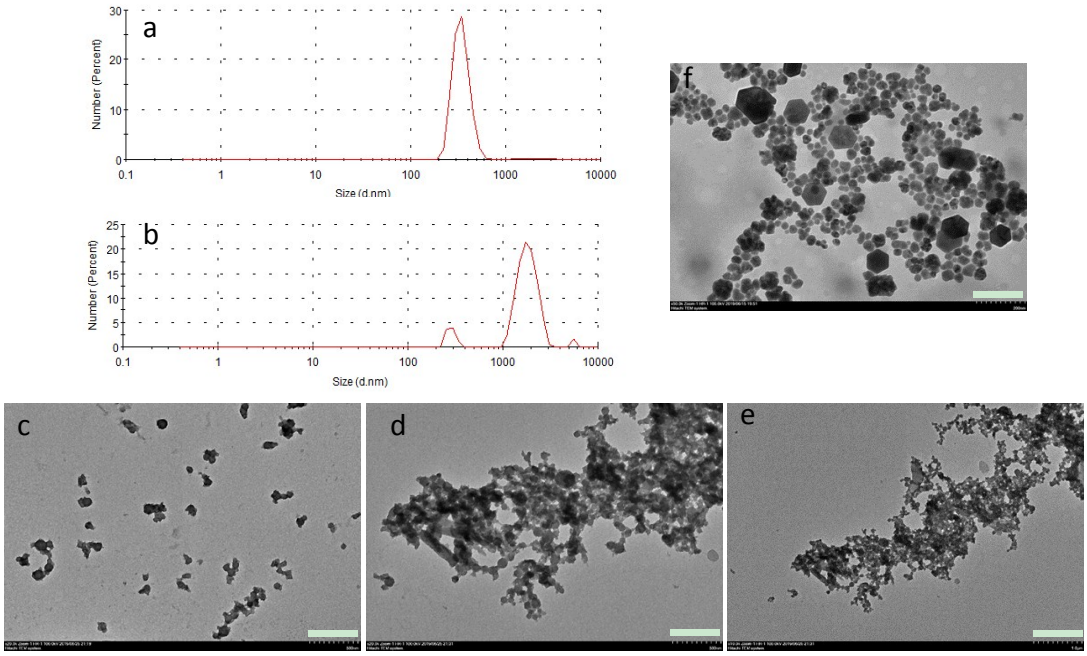


Fig. S3 Dynamic light scattering of Aβ oligomer (a), aggregates (b) in PBS (pH = 7.3); TEM images of Aβ oligomer (c), Aβ aggregates (d and e) and prion (f). Scale bar: c and d, 500 nm; e, 1000 nm; f, 500 nm

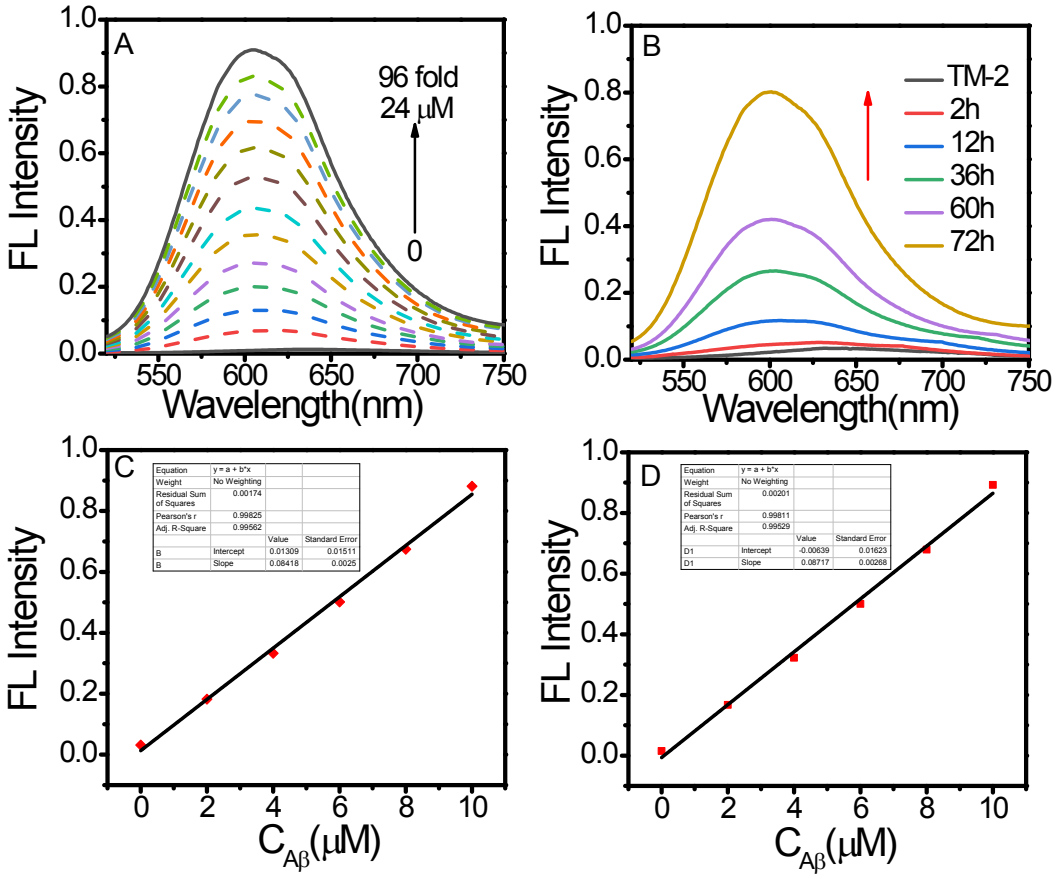


Fig. S4 Normalized fluorescence spectral change of **TM-2** at (A) different concentrations of Aβ aggregates ranging from 0 to 24 μM. (B) Different incubation time of the Aβ monomer over 72 h. Standard curve: **TM-1** (C) and **TM-2** (D) with different concentrations of Aβ aggregates ranging from 0 to 10 μM.

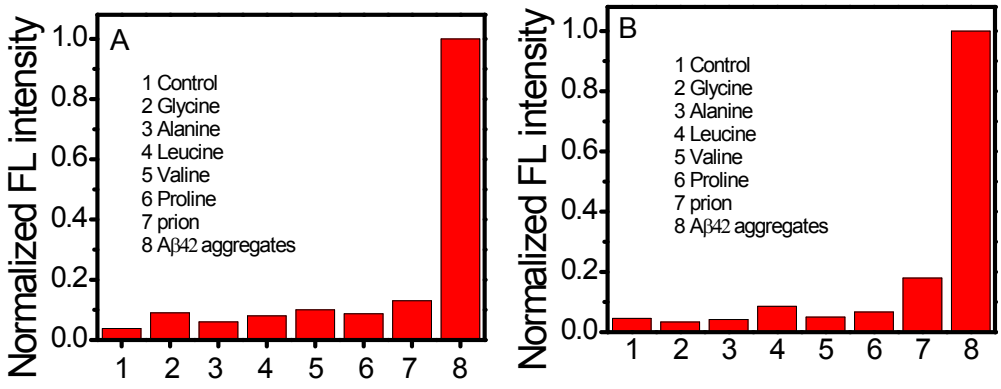


Fig. S5 Normalized fluorescence selectivity spectrum of **TM-1** (A) and **TM-2** (B) toward amino acids, prion and Aβ aggregates.

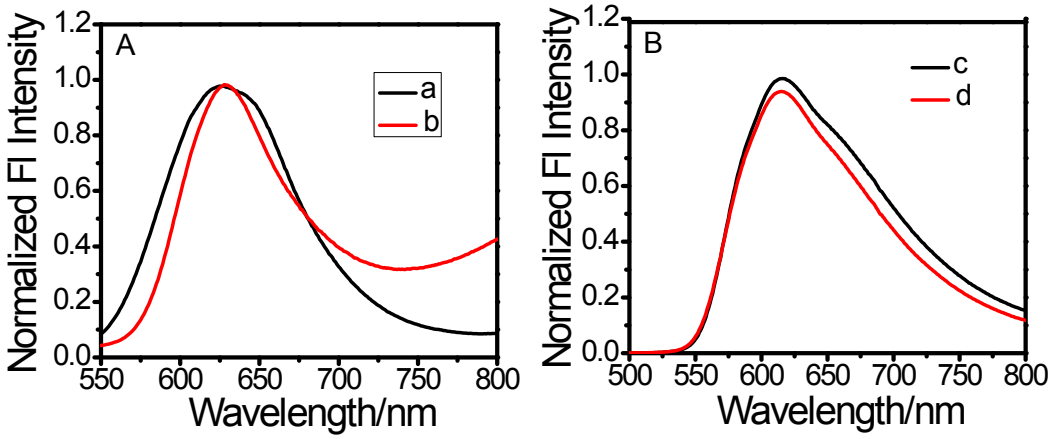


Fig. S6 The affect of **TM-1** and **TM-2** in the aggregation process of Aβ. Fluorescence spectral change of **TM-1** (A) and **TM-2** (B) when (a, c) **TM-1** or **TM-2** was added to Aβ aggregates and (b, d) **TM-1** or **TM-2** was added to Aβ monomer followed by stirring for 72 h.

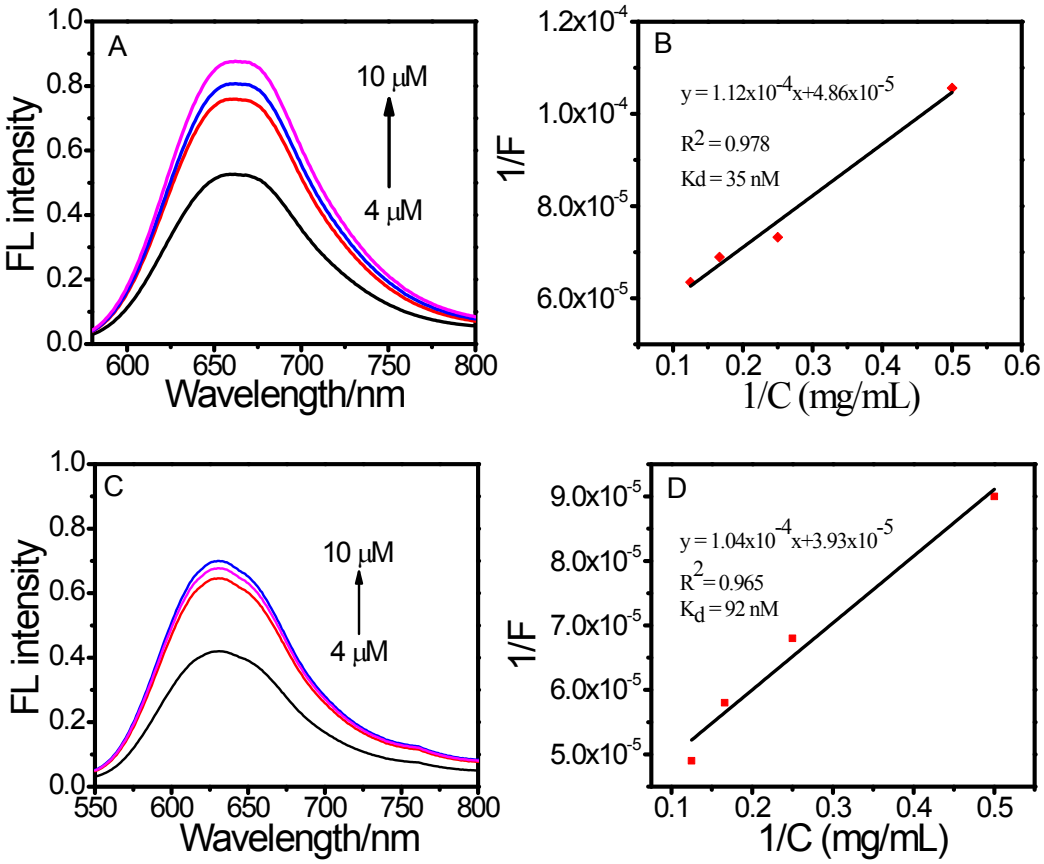


Fig. S7 Determination of the apparent binding constant (K_d) of **TM-1**(4, 6, 8, 10 μM.) (A,B) and **TM-2** (4, 6, 8, 10 μM.) (C,D) to Aβ aggregates (5 μM).

11. Toxicity of TM-1 and TM-2

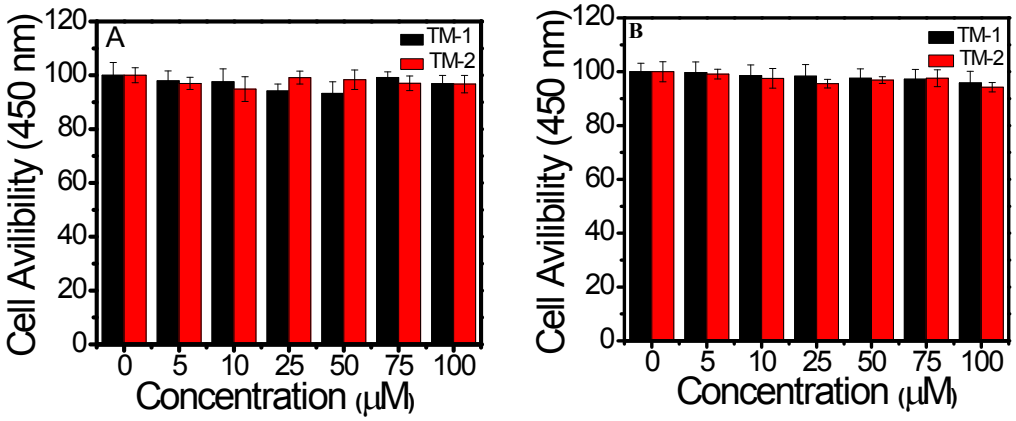


Fig. S8 Cell viability values (%) estimated by CCK-8 assay (A) in Hela cells and (B) in Raw cells, which were cultured in the presence of 0-100 μM **TM-1** and **TM-2** for 12 h at 37 °C.

12. Additional brain section images

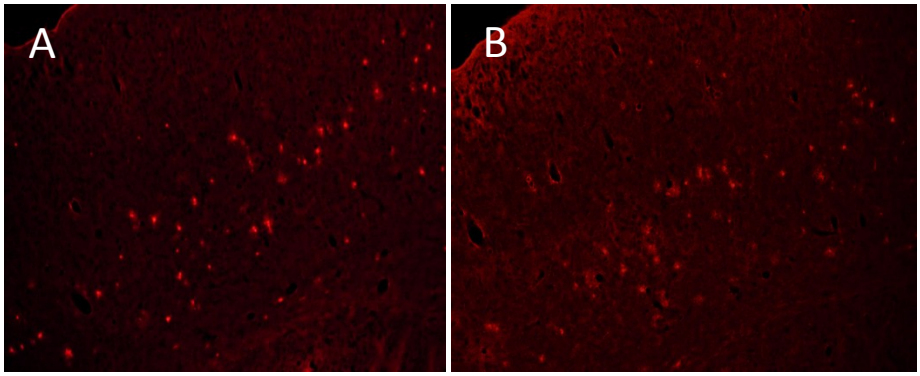


Fig. S9 CLSM images of brain sections: (A) **TM-1** (excitation = 500 nm), (B) **TM-2** (excitation = 430 nm)**.**

Characteristic of the compounds

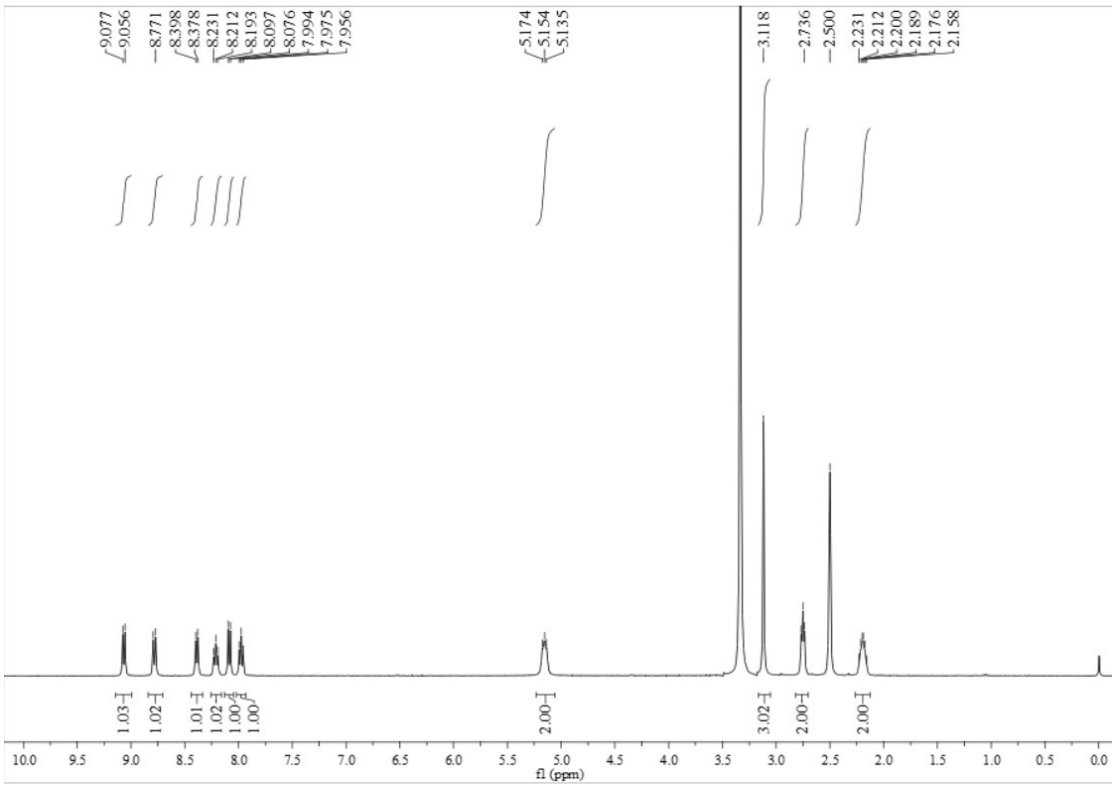


Fig. S10 The ^{1}H NMR spectrum of **S1**

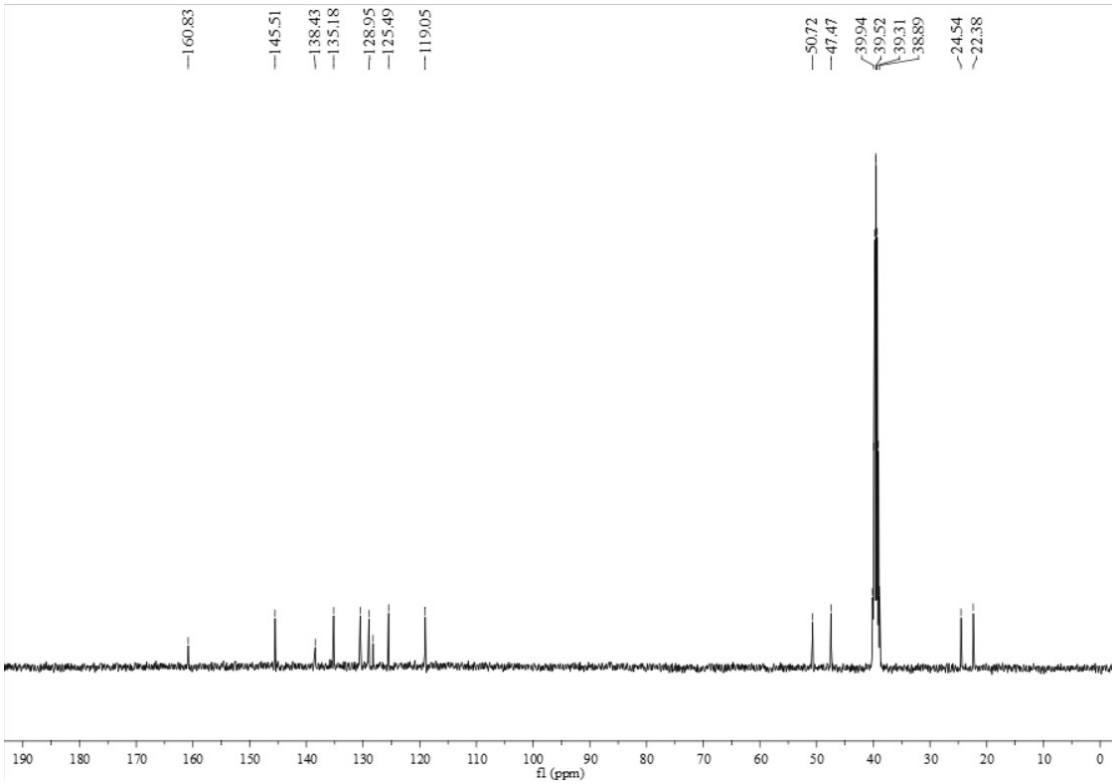


Fig. S11 The ^{13}C NMR spectrum of **S1**

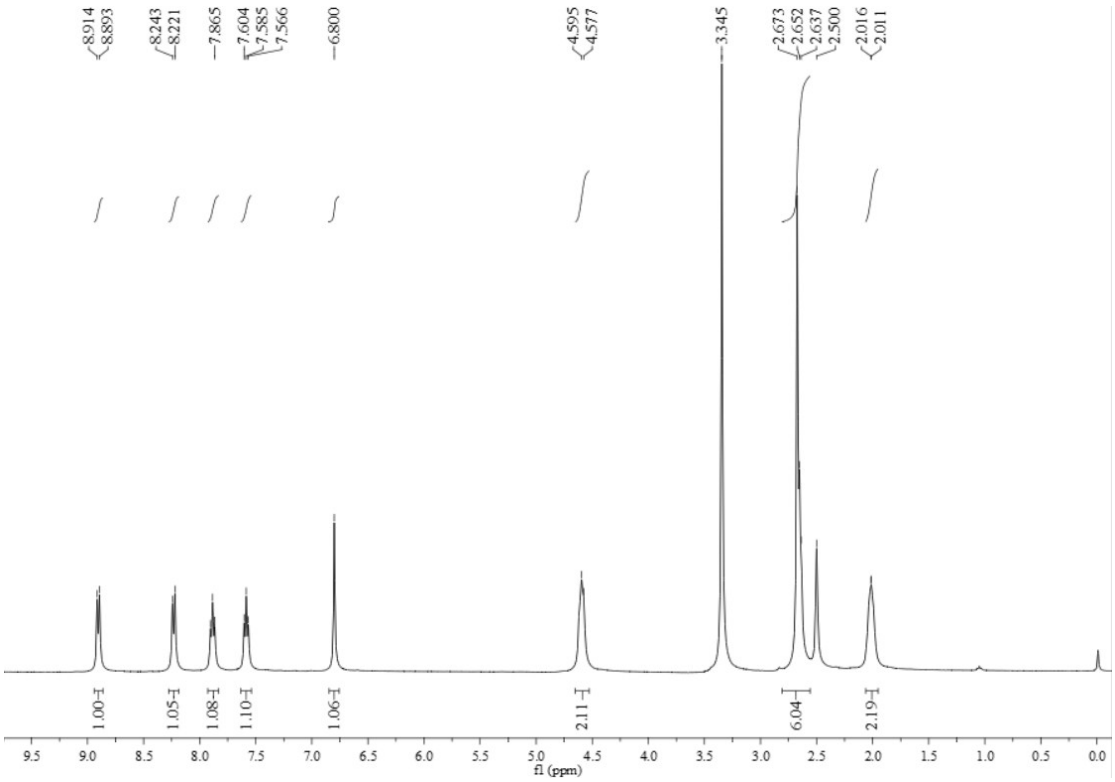


Fig. S12 The ^{1}H NMR spectrum of **S2**

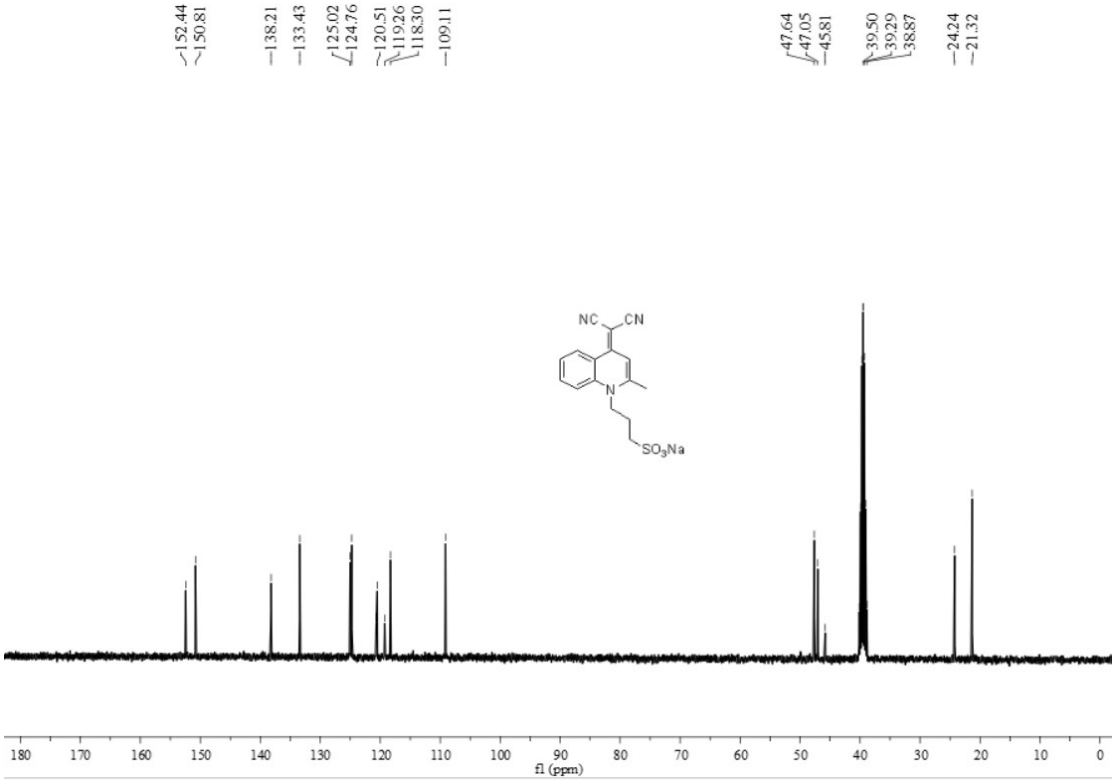


Fig. S13 The ^{13}C NMR spectrum of **S2**

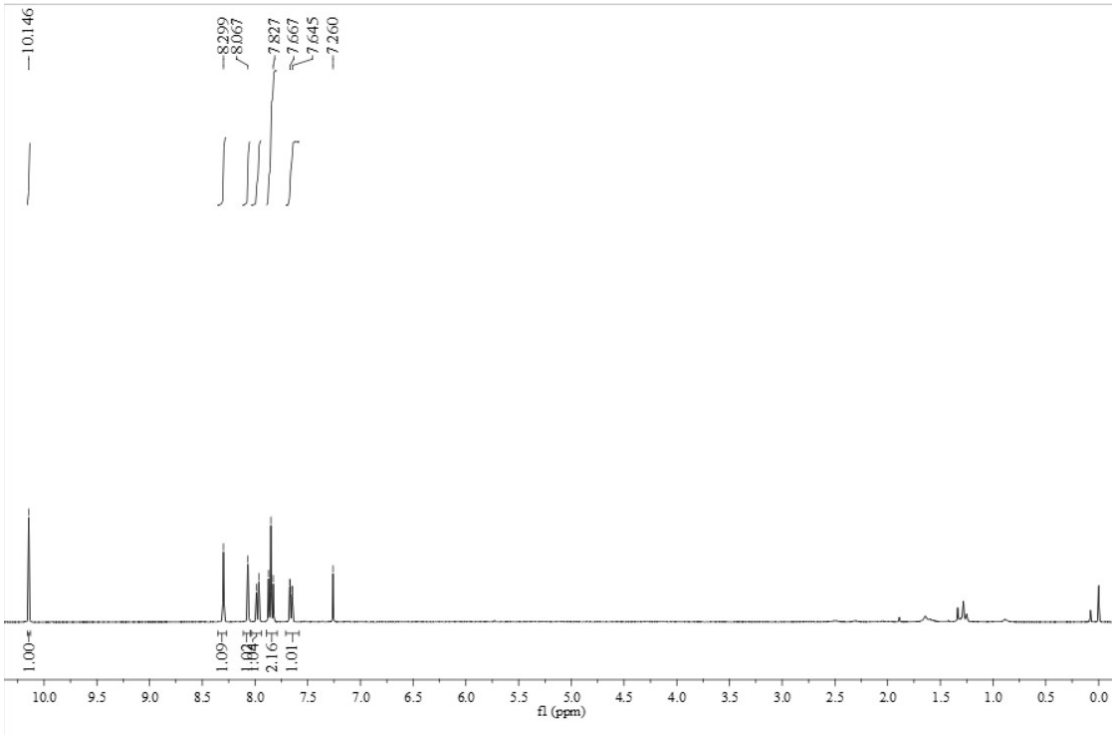


Fig. S14 The ^{1}H NMR spectrum of **S3**

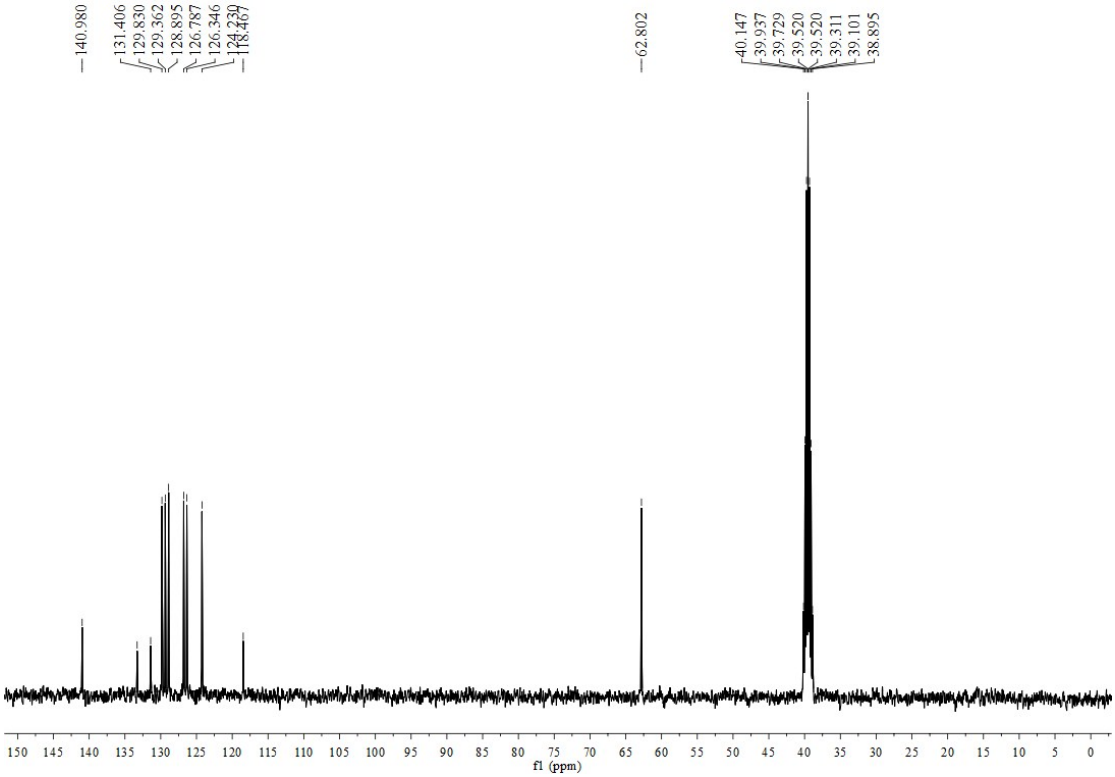


Fig. S15 The ^{13}C NMR spectrum of **S3**

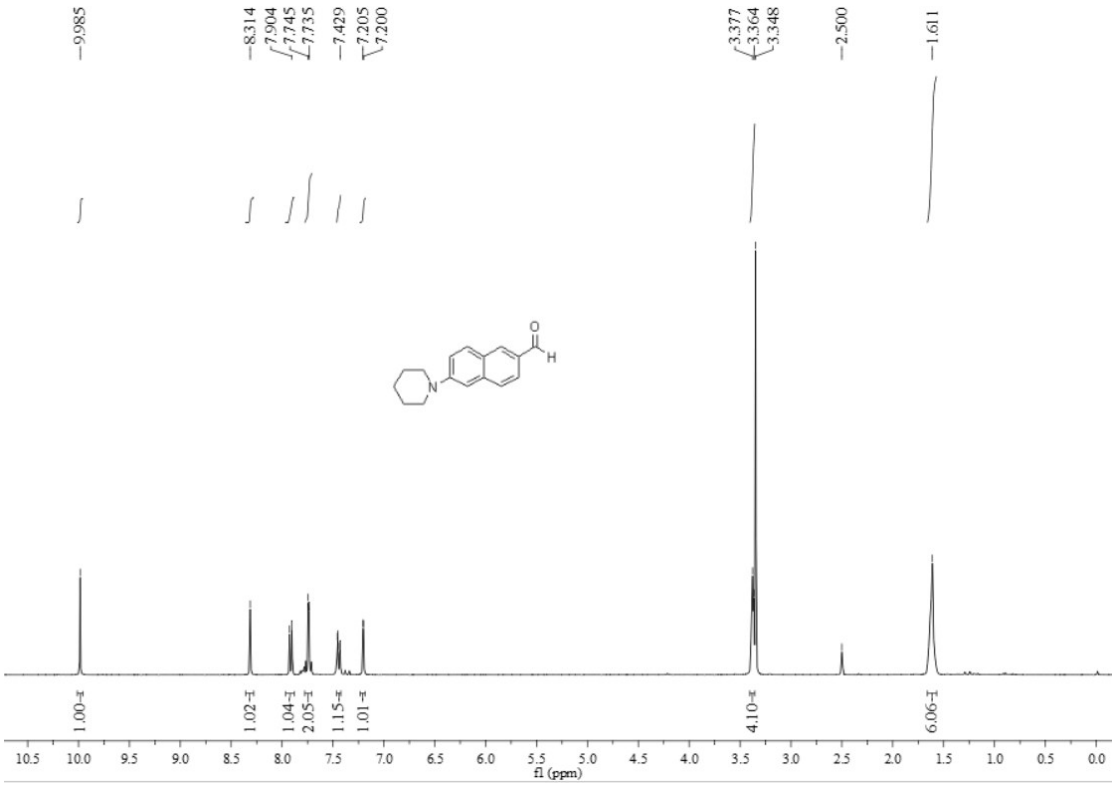


Fig. S16 The ^{1}H NMR spectrum of **S4**

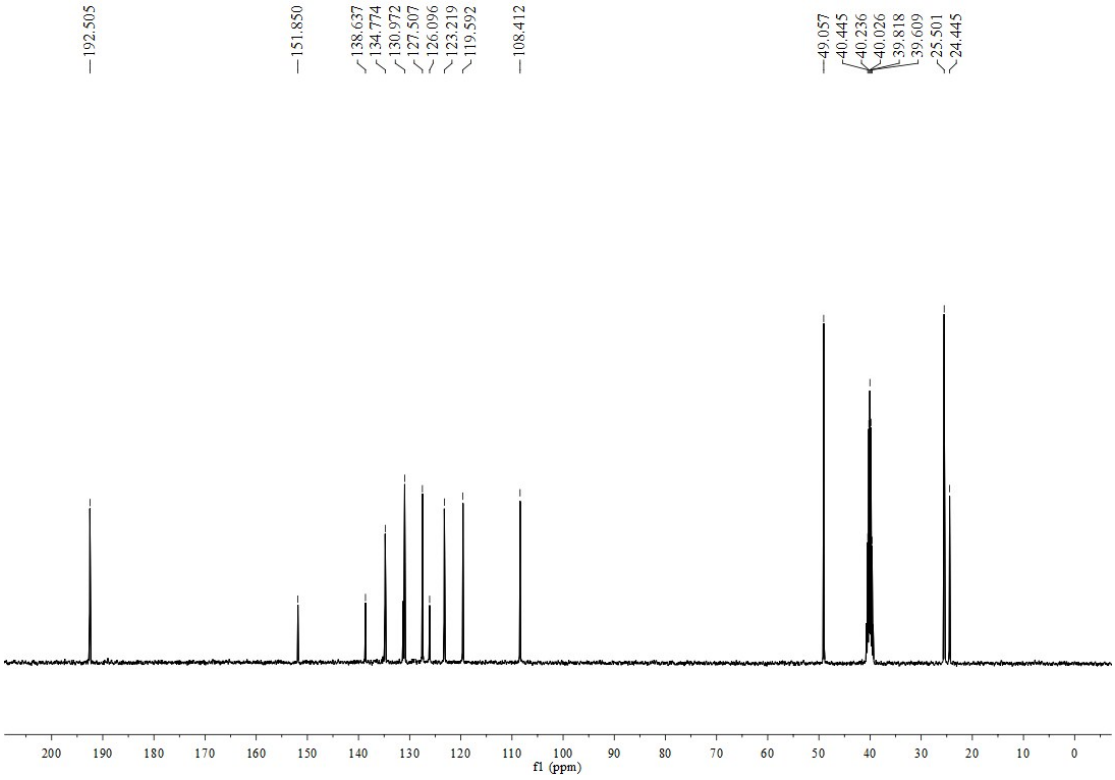


Fig. S17 The ^{13}C NMR spectrum of **S4**

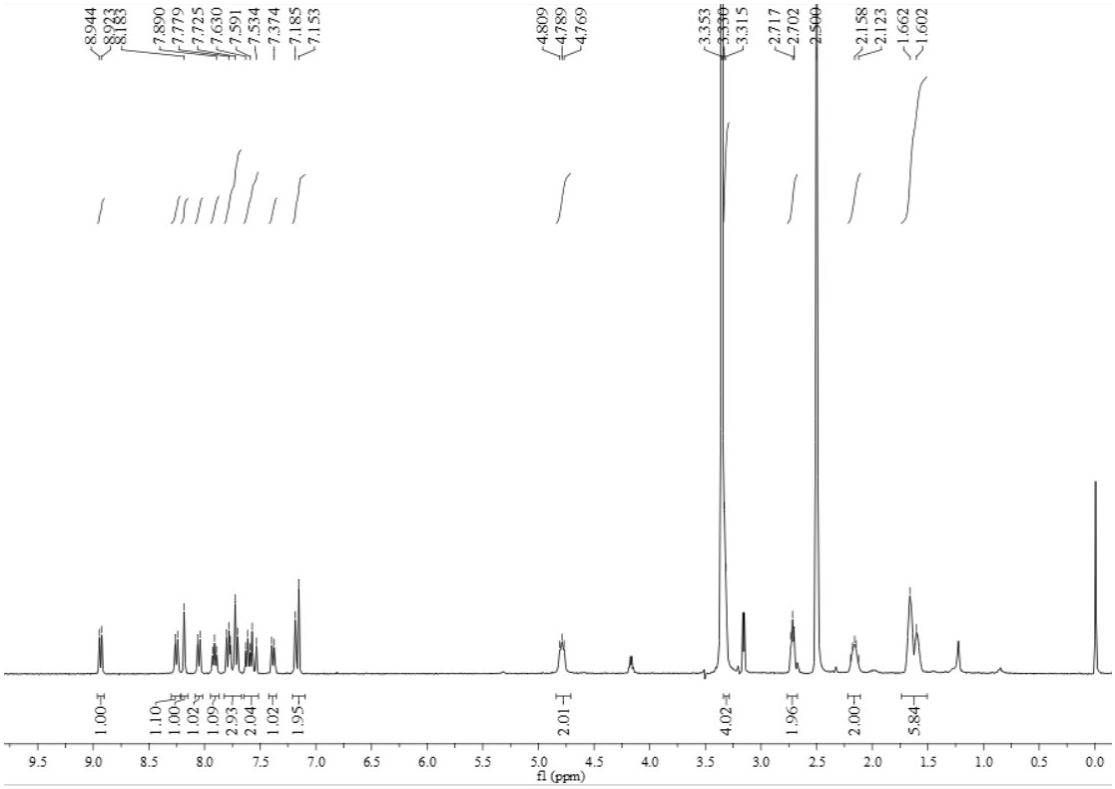


Fig. S18 The ^{1}H NMR spectrum of **TM-1**

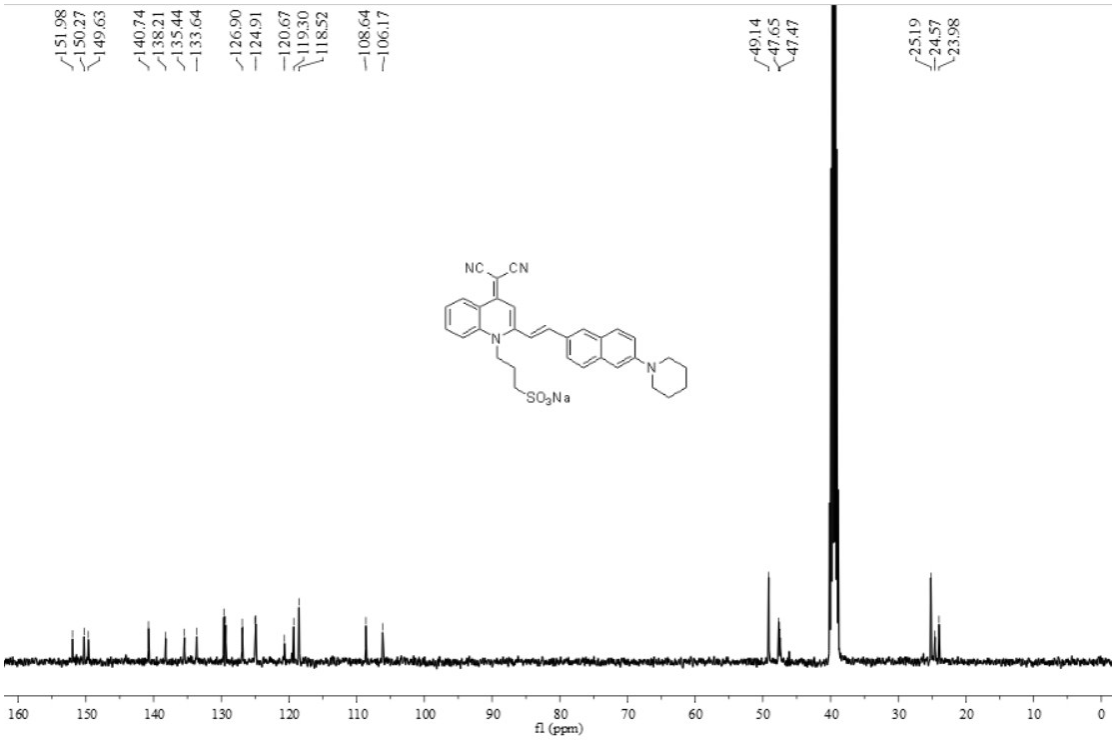


Fig. S19 The ^{13}C NMR spectrum of **TM-1**

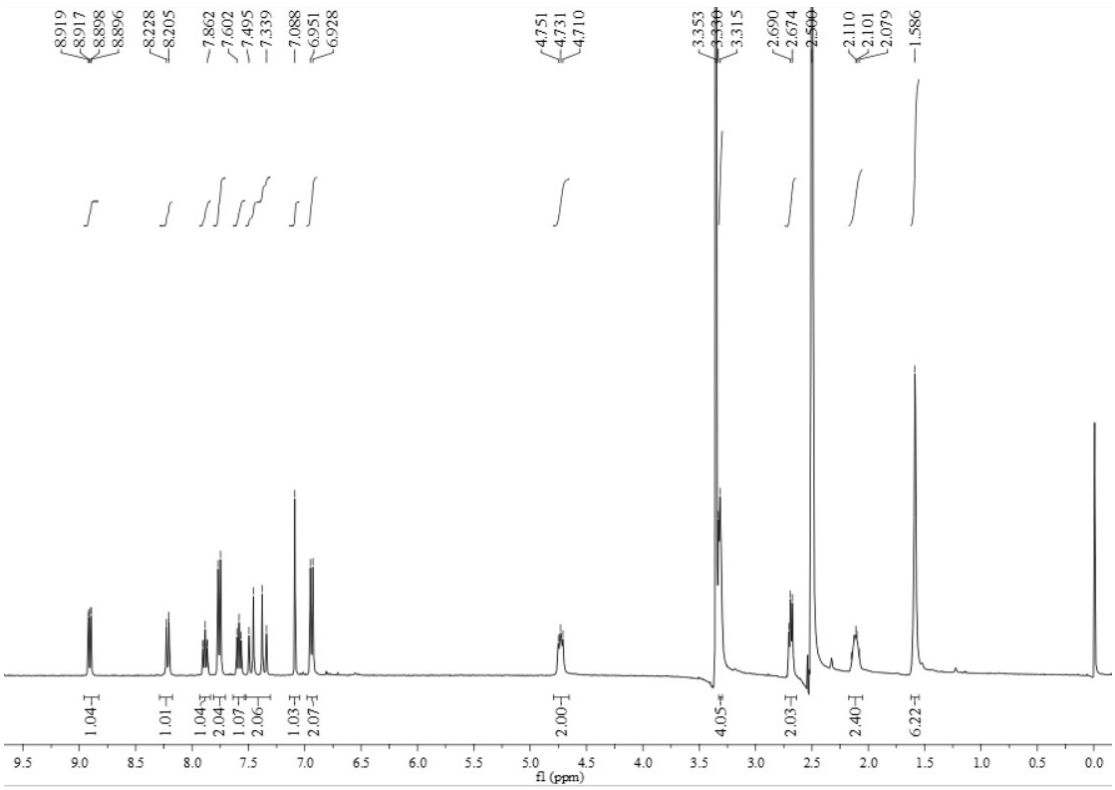


Fig. S20 The ^{1}H NMR spectrum of **TM-2**

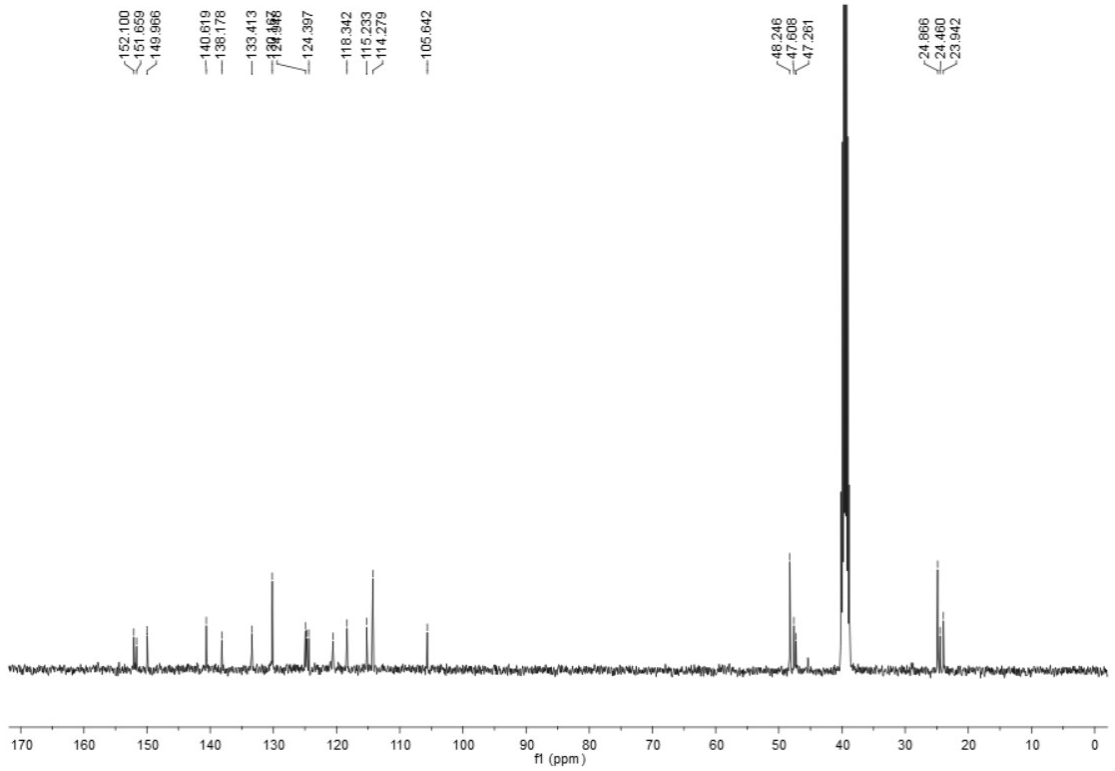


Fig. S21 The ^{13}C NMR spectrum of **TM-2**

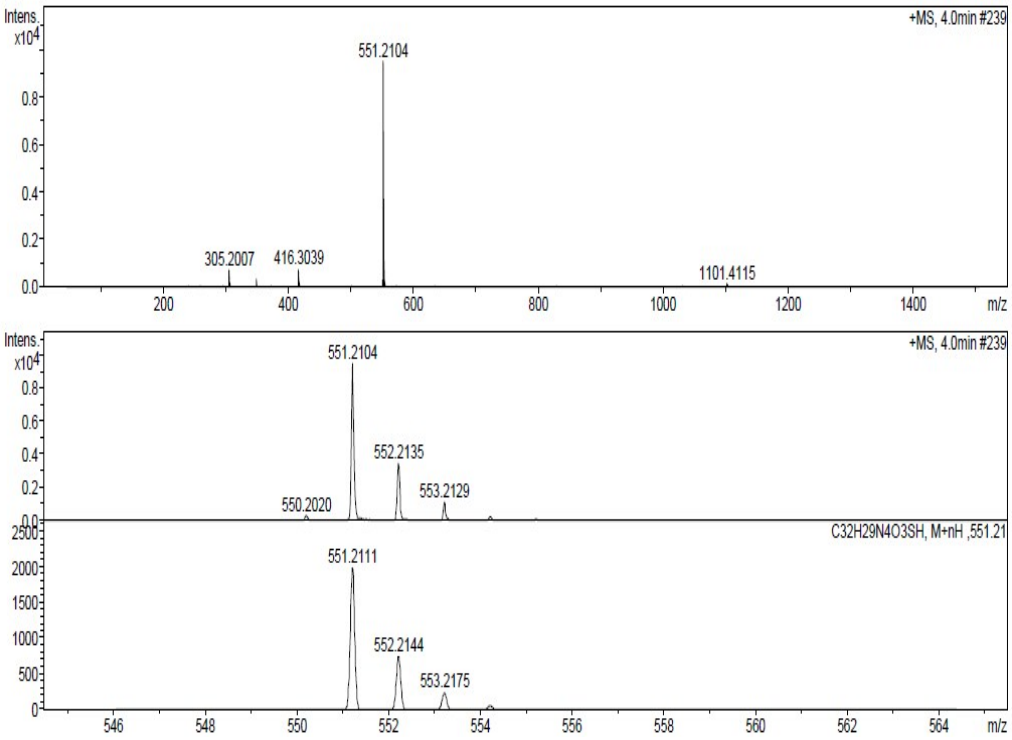


Fig. S22 The HR-MS spectrum of **TM-1**

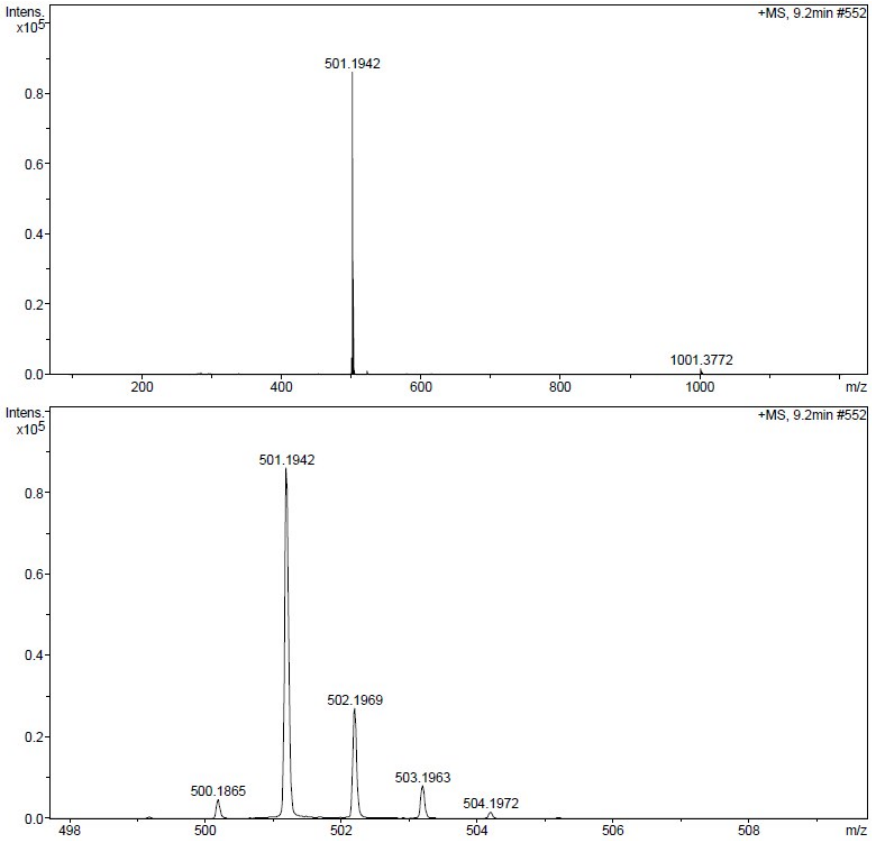


Fig. S23 The HR-MS spectrum of **TM-2**

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