Electronic Supplementary Information

Diarylethene Based Fluorescent Probes for the Detection of Amyloid-β in Alzheimer's Disease

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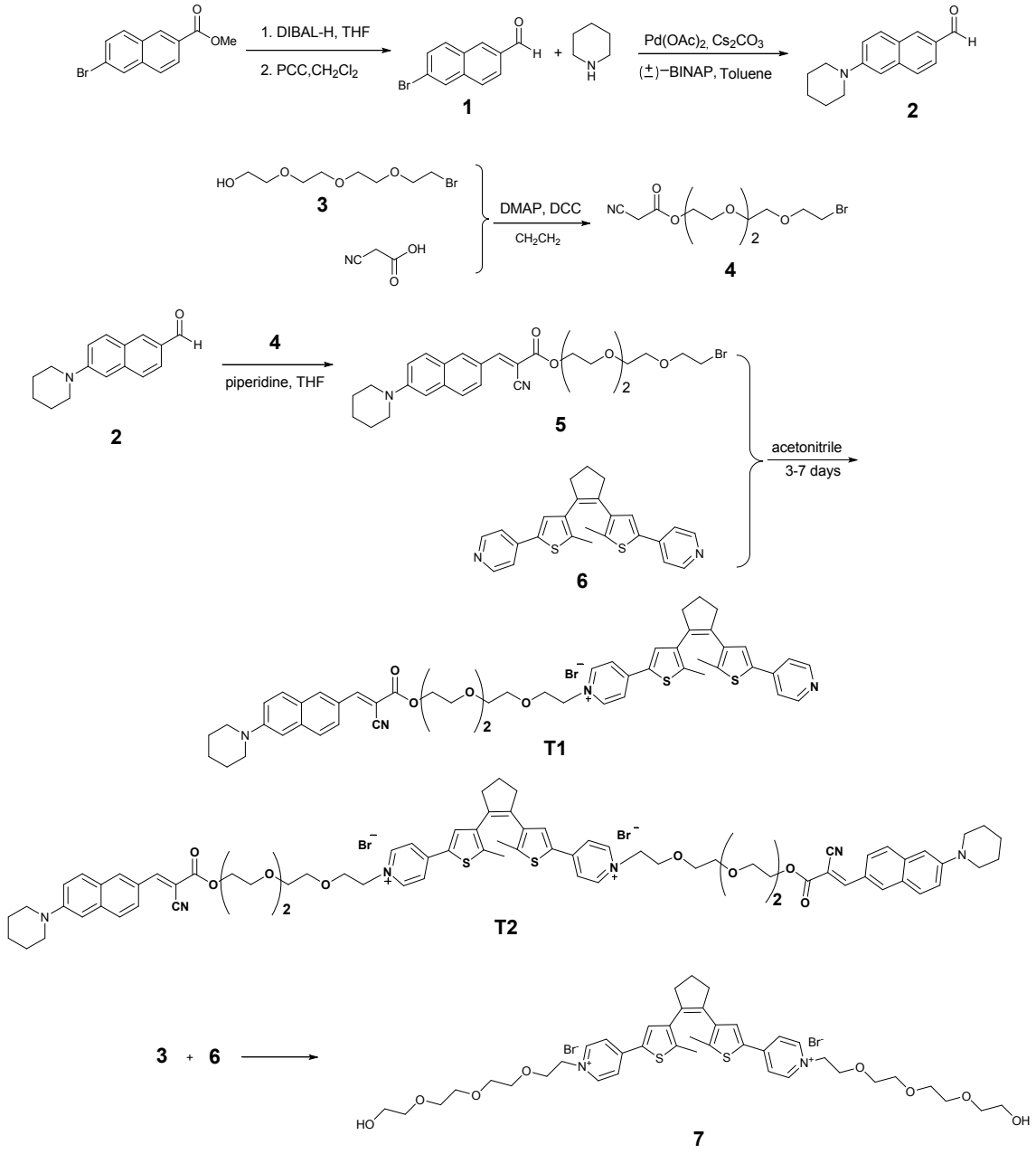
Contents

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. Palladium acetate and (±)-2,2'-bis(diphenylphosphino)-1,1'-binaphthalene were provided by Acros. 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). 1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra 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 were measured on an Edinburgh instruments spectrometer (FS-920).

2. Synthesis details of T1 and T2

The synthetic routes of **T1** and **T2** are shown in Scheme S1.



Scheme S1 Synthesis routes of **T1** and **T2** and the reference compounds

Synthesis of compound 1.[1] Diisobutyl aluminium hydride (DIBAL-H, 1.0 M in hexane, 15 mL, 15 mmol) was slowly dropped to a THF solution of 6-bromonaphthalene-2-carboxylate (1.3 g, 5.0 mmol) at 0^{o}C under argon. Then the reaction was allowed to warm to room temperature. After completion, MeOH was added, followed by a saturated sodium potassium tartrate solution and ethylacetate.

The organic phase was washed by NH_4Cl_4 and brine, dried over $MgSO_4$ and concentrated to yield the corresponding alcohol (6-bromonaphthalen-2-yl)methanol, which was resolved in anhydrous CH_2Cl_2, ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 7.99 (s, 1H); 7.77 (s, 1H); 7.74 (d, J = 8.4 Hz, 1H); 7.69 (d, J = 8.8 Hz, 1H)); 7.55 (d, J = 8.8 Hz, 2H); 7.49 (d, J = 8.4 Hz, 1H), 4.84 (s, 2H).

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 hours, cooled to room temperature and poured into diethyl ether. The solution was then filtered and concentrated under reduced pressure to yield compound **1** (white solid, 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); 7.83-7.88 (m, 2H); 7.66 (d, J = 8.8 Hz, 1H).

Synthesis of compound 2. $Pd(OAc)_2$ (9.0 mg, 0.04 mmol) and (±)-BINAP ((±)-2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene) (25.0 mg, 0.04 mmol) were added to dry toluene. The solution was stirred for 20 minutes. Compound **1**, piperidine (129 μL, 1.3 mmol) and Cs_2CO_3 (325 mg, 1.3 mmol) were added and the reaction left stirring under reflux for 12 hours. Upon completion, the reaction was cooled to room temperature and was purified via silica gel flash chromatography (EtOAc: petroleum ether = 1: 10) to yield compound **2** (75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.03 (s, 1H)); 8.16 (s, 1H); 7.81-7.85 (m, 2H); 7.68 (d, J = 8.4 Hz, 1H); 7.32 (d, J = 8.8 Hz, 1H); 7.10 (s, 1H); 3.37-3.39 (m, 4H); 1.66-1.75 (m, 6H).

Synthesis of compound 4. To a mixture of 2-cyanoacetic acid (382.6 mg, 4.0 mmol) and compound **3**[2] (1.024 g, 4.0 mmol) in CH_2Cl_2 (2.5 mL), DMAP (4.9 mg, 0.04 mmol) was added dropwise at 0 °C. Finally, DCC (206.3 mg, 4.5 mmol) was added and the reaction mixture was stirred at 0 °C for 6 hours. The reaction slurry was diluted with CH_2Cl_2 and was filtered. The filtrate was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (dichloromethane: acetone = 20: 1) to yield **4** (colorless liquid, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.37 (t, J = 4.8 Hz, 2H); 3.81 (t, J = 6.4 Hz, 2H); 3.74 (t, J = 4.8 Hz, 2H); 3.66 (m, 8H); 3.52 (s, 2H); 3.48 (t, J = 6.4 Hz, 2H).

Synthesis of compound 5. To the mixture of compound **2** (50.2 mg, 0.21 mmol) and compound **4** (74.3 mg, 0.23 mmol) in THF, piperidine (2.0 μL, 0.02 mmol) was added and the mixture was stirred at 50 ^{o}C for 6 hours. After completion, the crude mixture was concentrated under reduced pressure and the product was obtained via flash column chromatography (EtOAc: petroleum ether = 1: 5) as a red liquid (83% yields). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.21 (s, 1H), 8.11 (s, 1H); 8.01 (d, J = 8.4 Hz, 1H); 7.66 (d, J = 8.8 Hz, 1H); 7.56 (d, J = 8.8 Hz, 1H); 7.22-7.19 (m, 1H); 6.96 (s, 1H); 4.39 (t, J = 4.8 Hz, 2H); 3.77-3.70 (m, 4H); 3.66-3.60 (m, 8H); 3.38 (t, J = 6.4 Hz, 2H); 3.31 (t, J = 5.6 Hz, 4H); 1.65-1.58 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.3, 155.4, 151.8, 137.7, 134.6, 130.6, 127.2, 126.3, 124.9, 125.5, 119.2, 116.3, 108.3, 98.5, 71.1, 70.7, 70.6, 70.5, 70.4, 68.7, 65.3, 49.3, 42.6, 30.3, 29.6.

Synthesis of T1

Compound **5** (272 mg, 0.3 mmol) and compound **6**[3] (248 mg, 0.6 mmol) were dissolved in acetonitrile (3.0 mL). The mixture was stirred at 75^{o}C for three days. Then the reaction mixture was concentrated under reduced pressure and purified through flash column chromatography (Dichloromethane : MeOH = 10:1) to give **T1** as a yellow solid with 43% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.29 (s, 2H); 8.53 (m, 2H); 8.26 (s, 1H); 8.16 (s, 1H); 8.05 (d, J = 8.0 Hz, 1H);7.86 (d, J = 4.4 Hz, 2H); 7.74 (d, J = 9.2 Hz, 1H); 7.64-7.61 (m, 2H); 7.37 (d, J = 4.4 Hz, 2H); 7.19 (s, 1H); 7.03 (s, 1H); 5.03 (m, 2H); 4.46 (d, J =4.4 Hz, 2H); 4.03 (s, 2H); 3.83-3.82 (m, 2H); 3.72-3.60 (m, 9H). 3.42-3.40 (m, 4H); 2.84 (t, J = 6.8 Hz, 4H); 2.13-2.09 (m, 2H); 2.05 (s, 3H); 2.01 (s, 3H); 1.74-1.68 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.3, 155.8, 152.1, 149.8, 148.7, 145.5, 144.8, 141.6, 139.4, 138.0, 137.7, 137.0, 136.8, 136.4, 135.0, 133.7, 132.8, 132.3, 130.8, 127.4, 126.4, 125.8, 125.4, 121.2, 119.4,119.3, 116.6, 108.3, 98.0, 70.9, 70.5, 70.4, 70.3, 69.7, 68.9, 65.4, 59.9, 49.3, 38.6, 38.5, 29.7, 25.5, 24.4, 22.9, 15.2, 14.7. HRMS (ESI, m/z): calcd for $C_{52}H_{55}N_4O_5S_2$ $[M\text{-}Br^-]^+$, 879.3614; found: 879.3621. FT-IR (cm^{-1}): 2925, 2854, 1719, 1637, 1578, 1502, 1378.

Synthesis of T2

The synthesis of **T2** is the same with that of **T1** except that the molar ratio of **5** and **6**

changed to 7: 3. **T2** was obtained as a yellow solid, 33% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.22 (m, 4H); 8.25 (m, 2H); 8.16 (m, 2H); 8.04 (d, J = 8.8 Hz, 2H);7.91 (m, 4H); 7.74 (d, J = 8.8 Hz, 2H); 7.64-7.59 (m, 4H); 7.29 (m, 2H); 7.04 (s, 2H); 6.96 (m, 4H); 4.46 (s, 4H); 4.01 (s, 4H); 3.82 (s, 4H); 3.71-3.59 (m, 16H); 3.41 (m, 8H); 2.82 (m, 4H); 2.11 (m, 8H); 1.73-1.68 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.3, 155.6, 148.6, 145.4, 144.4, 138.7, 137.8, 135.4, 134.9, 133.0, 132.6, 130.7, 127.3, 125.8, 125.4, 121.5, 119.2, 116.5, 108.3, 98.1, 70.7, 70.4, 70.3, 70.2, 68.9, 65.3, 59.9, 49.3, 38.1, 29.6, 29.3, 25.4, 24.2, 22.9, 15.1, 14.2. HRMS (ESI, m/z): calcd for $C_{79}H_{88}N_6O_{10}S_2$ $[M\text{-}2Br^-]^{2+}$, 879.3608; found: 879.3639. FT-IR (cm^{-1}): 3406, 2923, 2852, 2215, 1718, 1636, 1577, 1378.

3. Preparation of Aβ aggregates

Aβ (1-42) monomer was dissolved in PBS (pH = 7.31). This solution was magnetically stirred at 1200 rpm for three days at room temperature. Then pre-aggregated Aβ solution was obtained.

4. Confocal laser microscopy images

Confocal fluorescence imaging was performed with an OLYMPUS ZX81 laser scanning microscopy. Excitation at 405 nm was carried out with a semiconductor laser, and emission was collected from 500 to 600 nm. In the scan mode, the lamp power of 405 nm for fluorescence is 0.15 mW on the focus plane of CLMS, which are applied to achieve the fluorescence image. The lamp powers of 405 and 633 nm for switching the fluorescence of brain slices are 2 mW and 0.7 mW, respectively.

5. AD transgenic mouse model

AD transgenic model for amyloid plaques, 5XFAD mouse, was used to examine the possible co-localization of **T1** or **T2** staining and Aβ immunoreactivity. The 5XFAD transgenic mice [4] were obtained from The 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. Expression of both transgenes is regulated by neural-specific elements of the mouse Thy1 promoter to drive overexpression in the brain. Mice were genotyped by PCR using primers for the APP transgene (forward 5'-GAC TGA CCA CTC GAC CAG GTT CTG-3', reverse 5'-CTT GTA AGT TGG ATT CTC ATA TCC G-3') or/and the PS1 transgene (forward 5'-AAT AGA GAA CGG CAG GAG CA-3', reverse 5'-GCC ATG AGG GCA CTA ATC AT-3'). Male or female mice at age of 9-12 months old were used in this study. Animals were handled in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

6. 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 7.4). 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.

7. Cytotoxicity Assay

MTT assay. The cytotoxicity was performed by 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay with Hela cell lines. Cells growing in log

phase were planted into a 96-well cell culture plate at 1 × 10^5/ well. The cells were incubated for 12 h at 37 °C under 5% CO_2 in an incubator. A solution of **T1** and **T2** (100.0 μL/well) at concentrations of 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0 μM in nutrient with 10‰ DMSO was added to the wells of the treatment group, respectively. The cells were subsequently incubated for 2 h at 37 °C under 5% CO_2. Thereafter, MTT (0.5 mg/mL) was added to each well and the plate was incubated for an additional 4 h at 37 °C under 5% CO_2. The optical density OD490 value (Abs.) of each well was measured by means of a Tecan Infinite M200 monochromator-based multifunction microplate reader, which was used as cell viability.

8. Additional absorption and fluorescent spectra

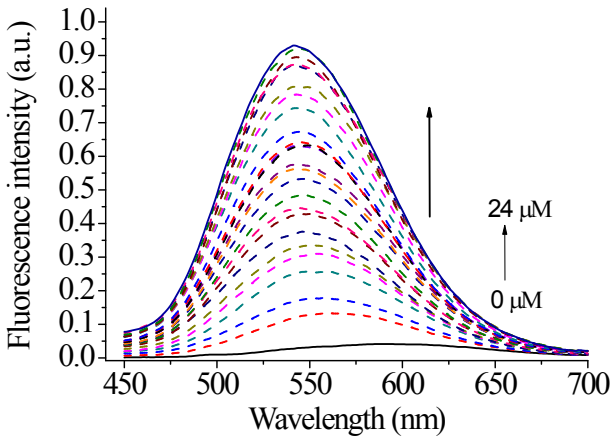


Fig. S1. Fluorescence changes of **T1** with varied concentration of Aβ aggregates

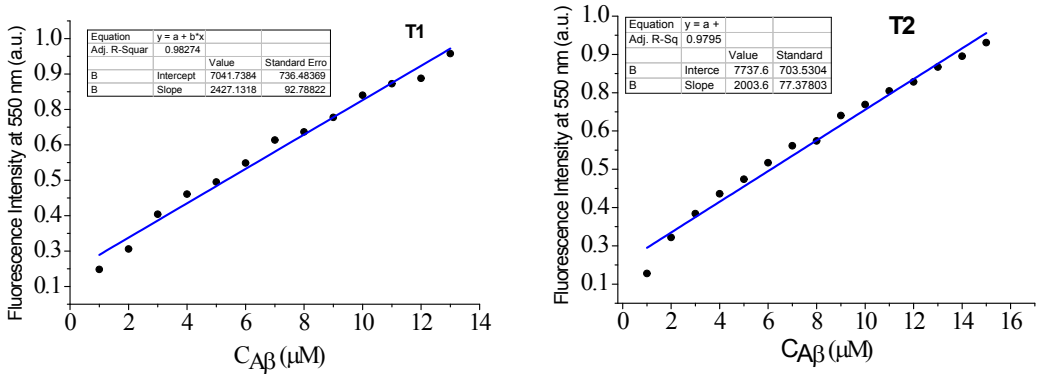


Fig. S2. Fluorescence intensity changes of (a) **T1** and (b) **T2** at 550 nm with varied concentration of Aβ aggregates

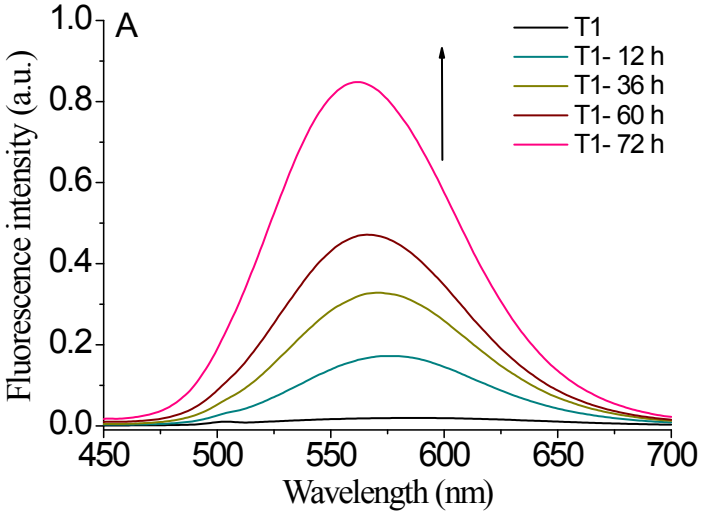


Fig. S3. The fluorescence spectra of **T1** (2.0×10^{-6} M, 5% DMSO in PBS) with different incubation time (within 72 hours) of Aβ monomer.

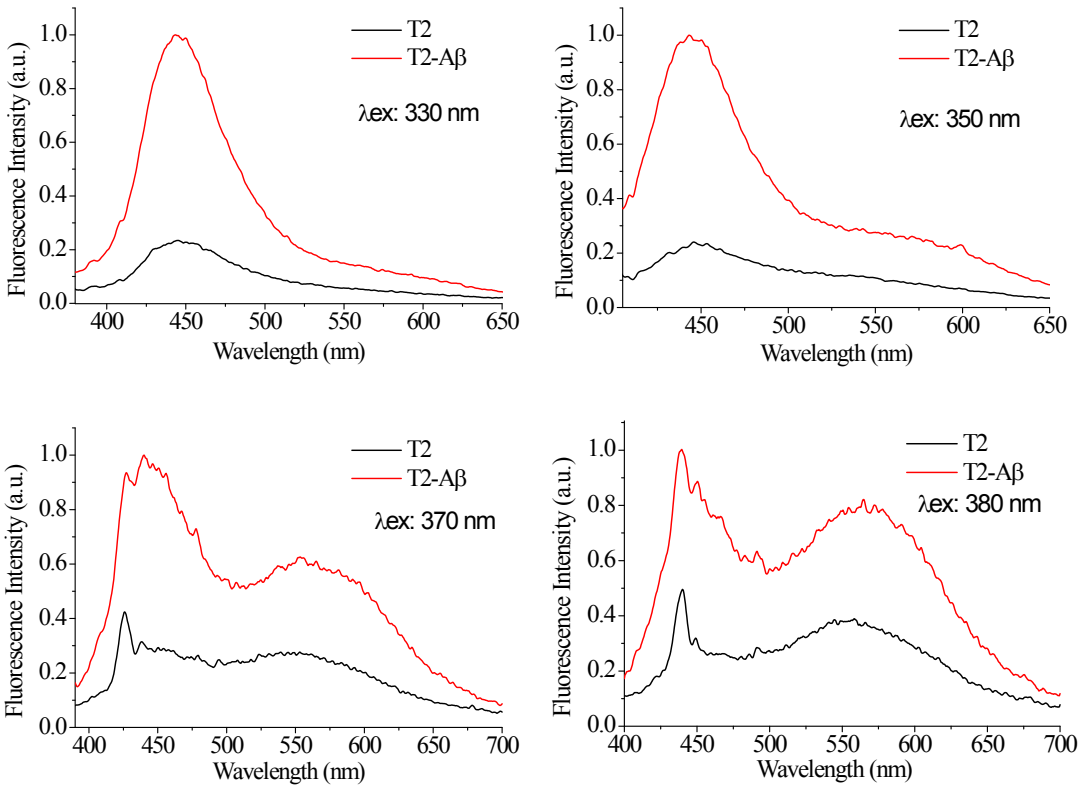


Fig. S4. Normalized fluorescent spectra of **T2** (2.0 μM) absent and in the present of Aβ aggregates (5.0 μM) with different excitation wavelength. λ_{ex} = 330, 350, 370 and 380 nm, respectively.

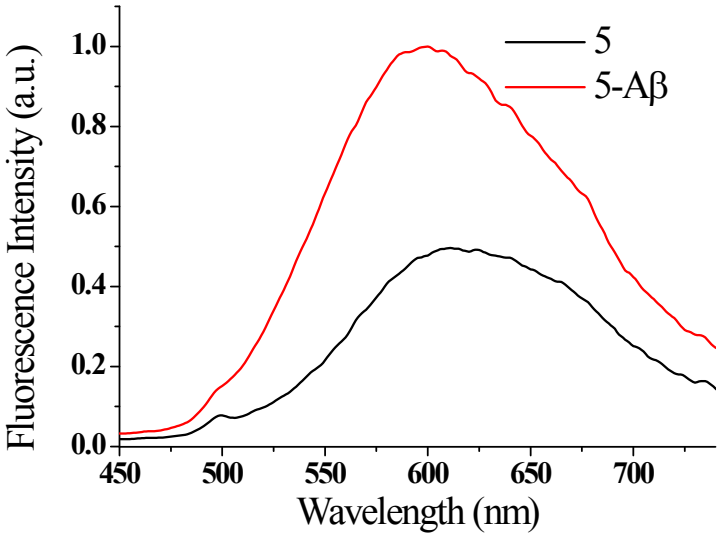


Fig. S5. Normalized fluorescent spectra of **5** (2.0 μM) absent and in the present of Aβ aggregates (5.0 μM) with excitation at 430 nm.

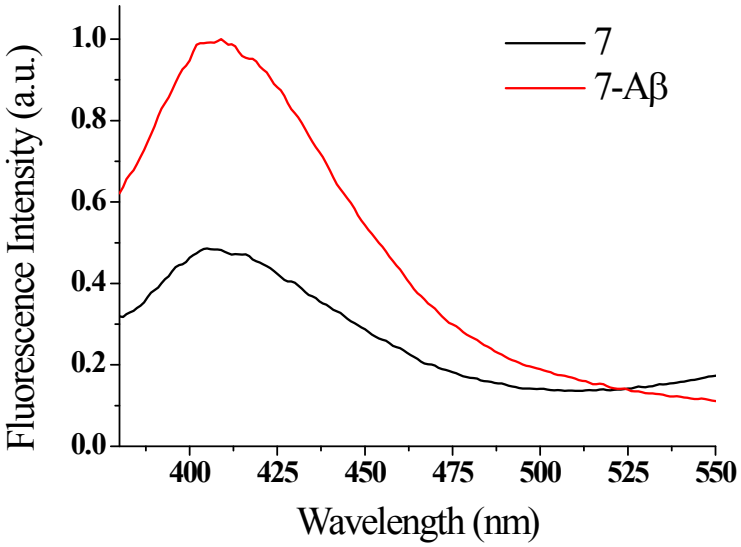


Fig. S6. Normalized fluorescent spectra of **7** (2.0 μM) **without and** with Aβ aggregates (5.0 μM), λ_{ex} = 330 nm.

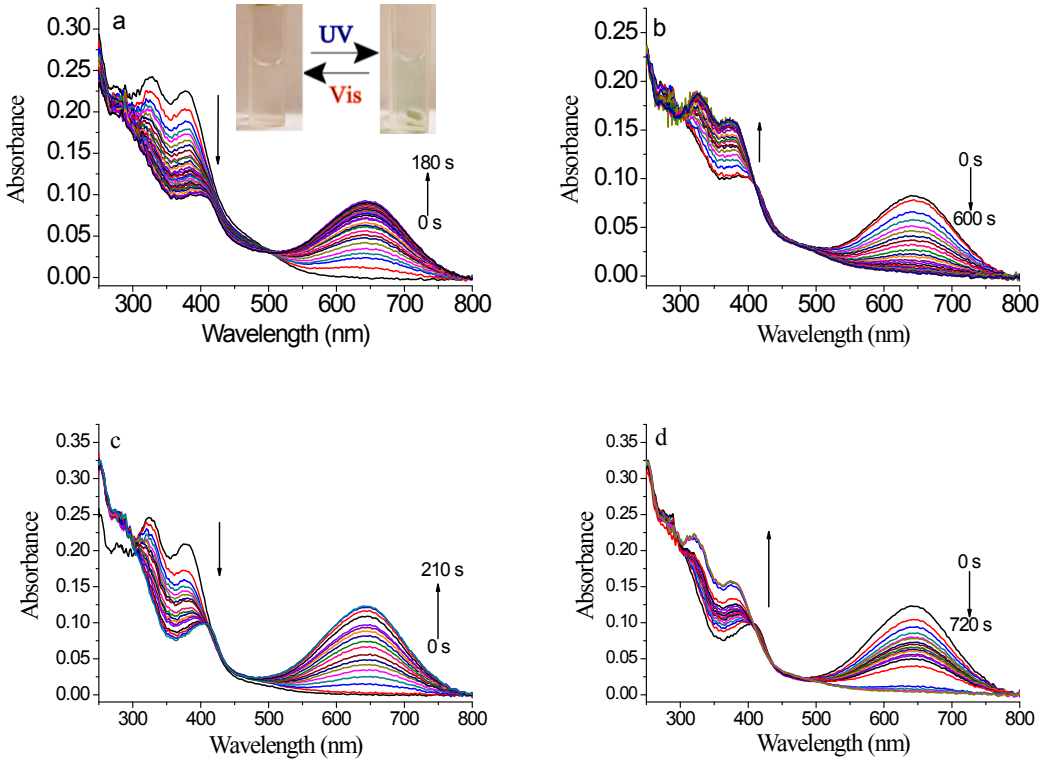


Fig. S7. The absorption spectral change of (a, b) **T1** and (c, d) **T1**+Aβ aggregates (1.0×10^{-5} M, 5% DMSO in PBS) under alternate irradiation of (a, c) UV and (b, d) visible light. Inset in a shows the colour change with the photochromic process.

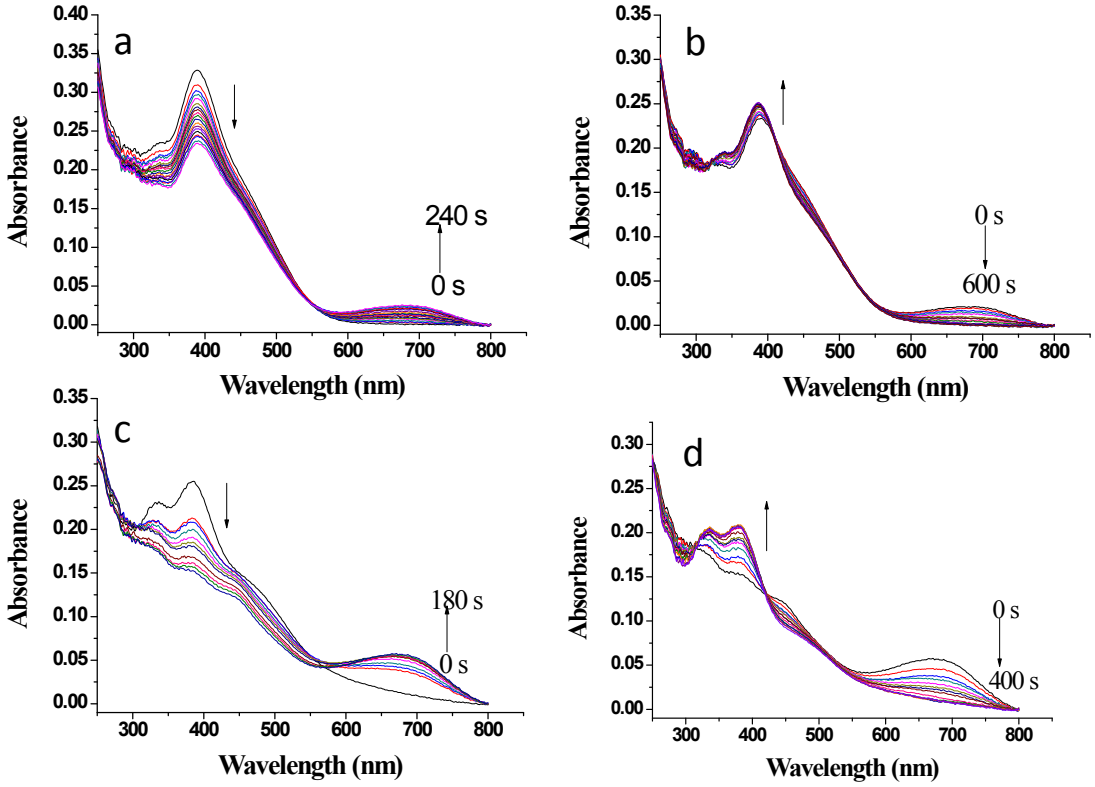


Fig. S8. The absorption spectral change of (a, b) **T2** and (c, d) **T2**+Aβ aggregates (1.0×10^{-5} M, 5% DMSO in PBS) under alternate irradiation of (a, c) UV and (b, d) visible light.

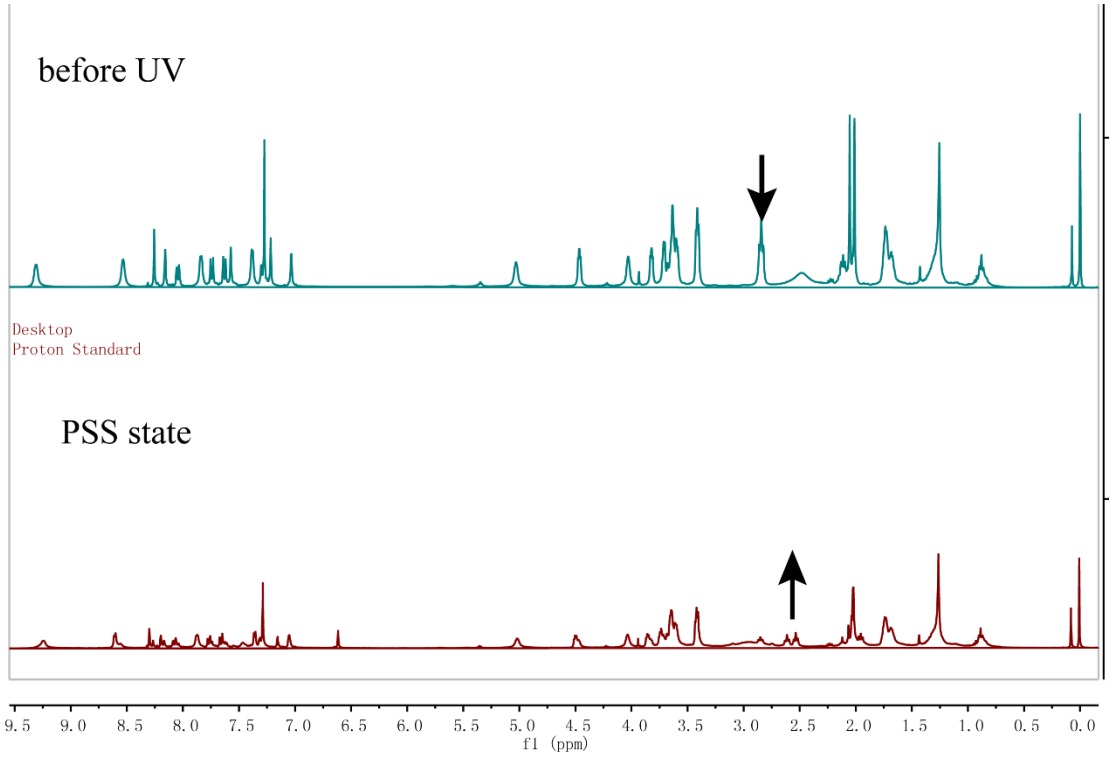


Fig. S9. ^{1}H NMR spectra of **T1** before and after UV irradiation.

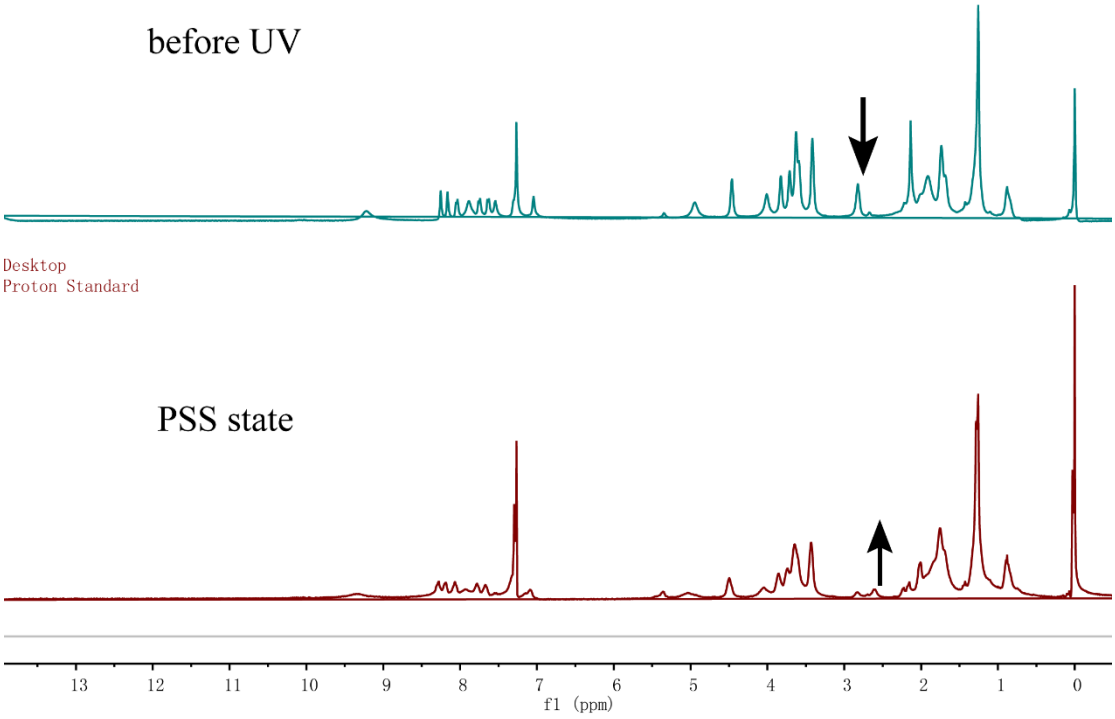


Fig. S10. ^{1}H NMR spectra of **T2** before and after UV irradiation.

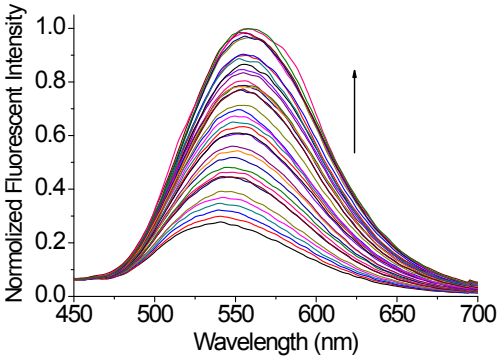


Fig. S11 Fluorescence spectra changes of PSS of **T2** (2.0×10^{-6} M, 5% DMSO in PBS) in presence of Aβ aggregates (5.0×10^{-6} M) with irradiation of visible light.

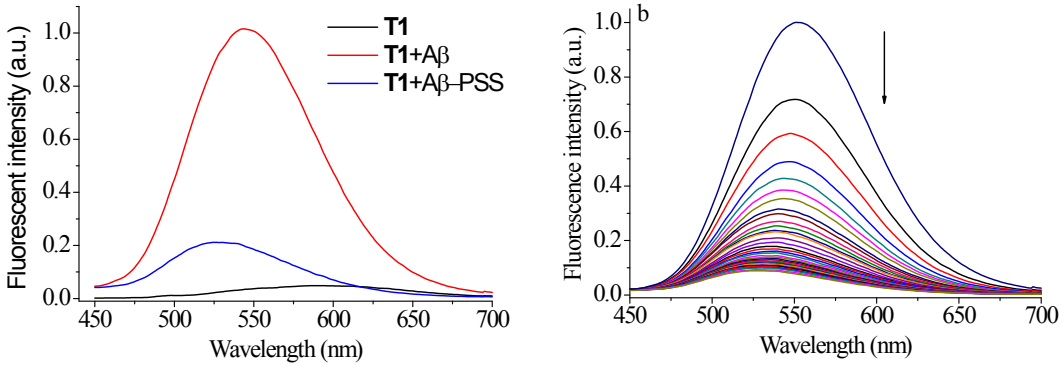


Fig. S12 (a) Fluorescent spectra of **T1**, **T1**+ Aβ aggregates before and after UV light irradiation. (b) Fluorescent spectral change of **T1** (2.0×10^{-6} M, 5% DMSO in PBS) in the presence of Aβ aggregates (5.0×10^{-6} M) with irradiation of UV light;

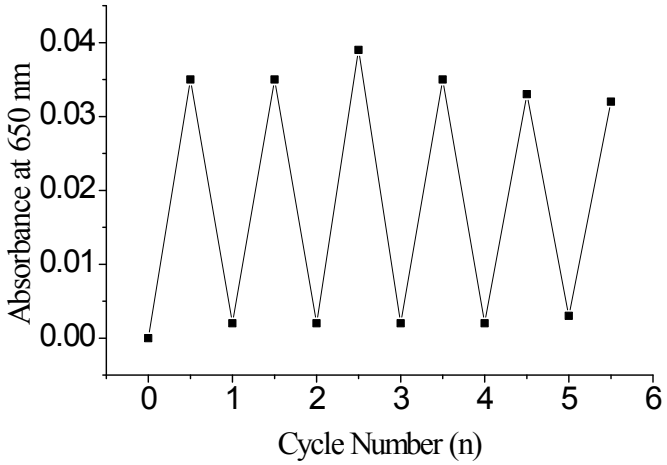


Fig. S13 The change of absorbance at 650 nm of **T1**+Aβ aggregates upon alternating irradiation of UV and visible light.

9. Additional brain section images

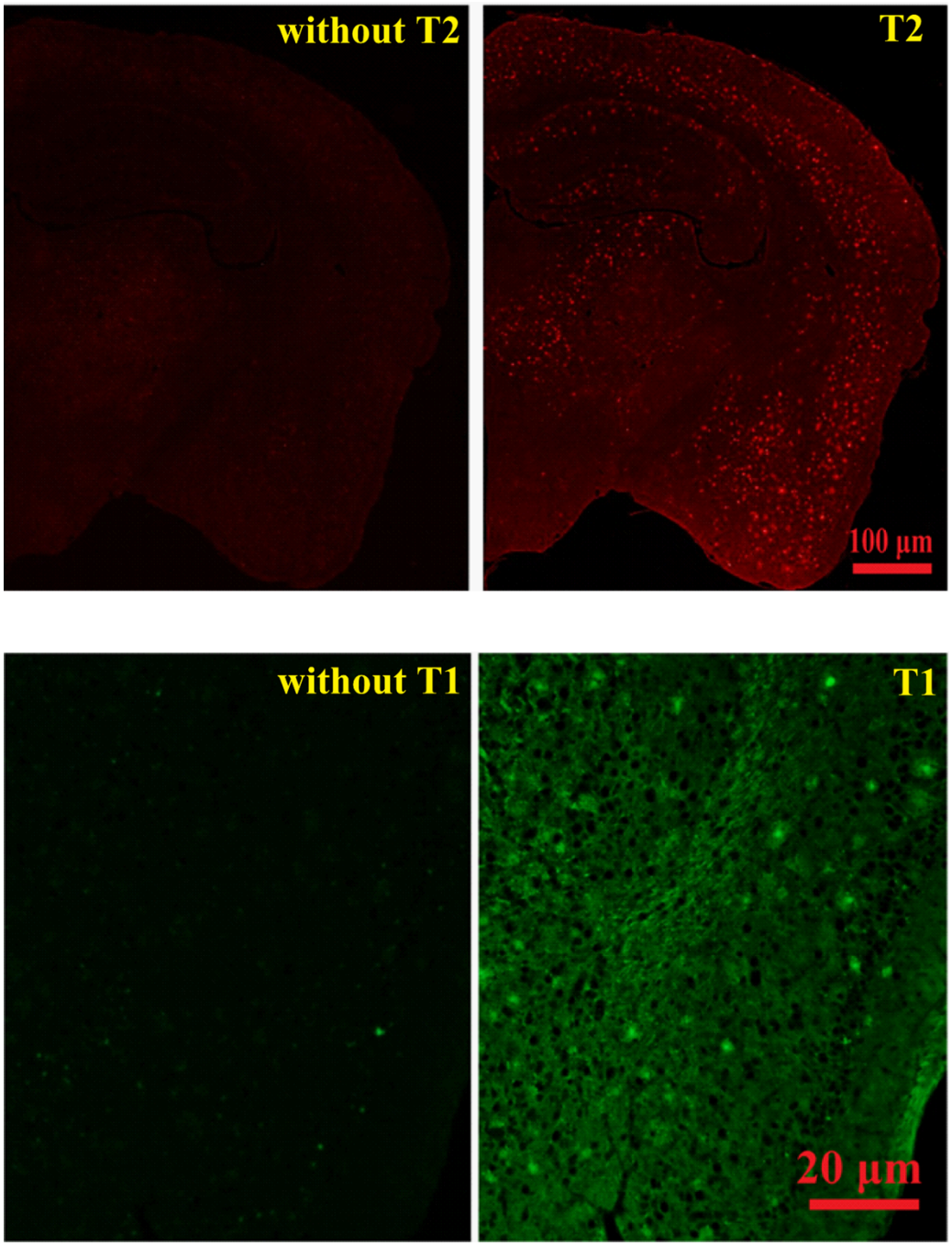


Fig. S14 Fluorescence microscope images of AD brain slices. Top: staining with and without **T2** (60 μM). Bottom: staining with and without **T1** (60 μM).

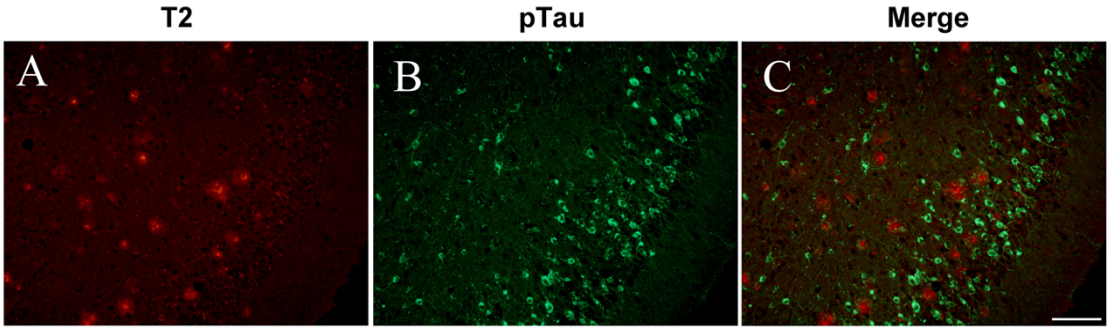


Fig. S15 Fluorescence microscope images of AD brain slices. A: staining with **T2** (60 μM); B: staining with Tau protein antibody; C: the merged image of A and B. Scale bar: 20 μm.

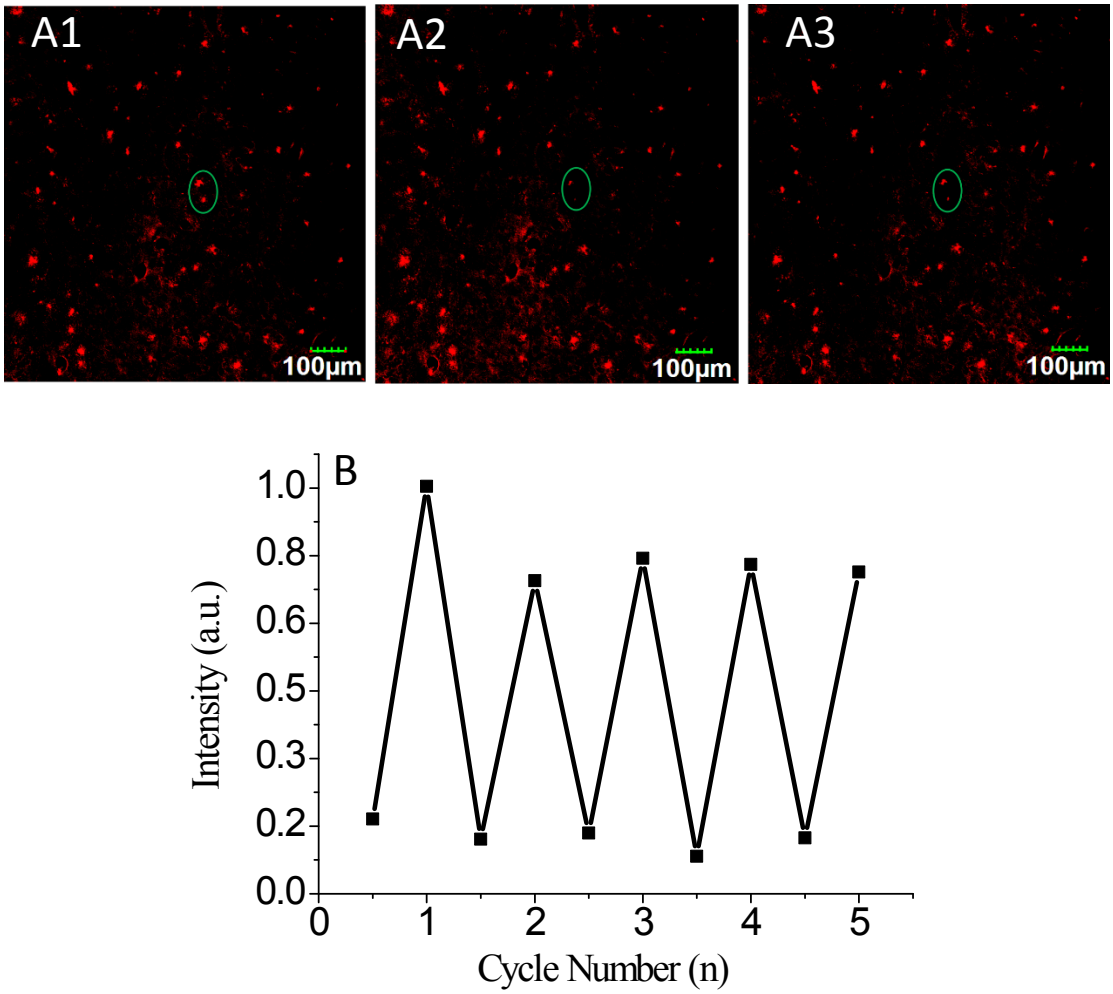


Fig. S16 (A) CLMS images of AD mouse brain slices staining with **T2** (60 μM), A1: original state; A2: irradiated by 458 nm light in the assigned oval region; A3: all region was irradiated by 633 nm light. (B) Fluorescence switching of brain section by alternating 458 nm (2 mW, 240 s/time) and 633 nm (0.7 mW, 20 min/time) illumination.

10. Toxicity of T1 and T2

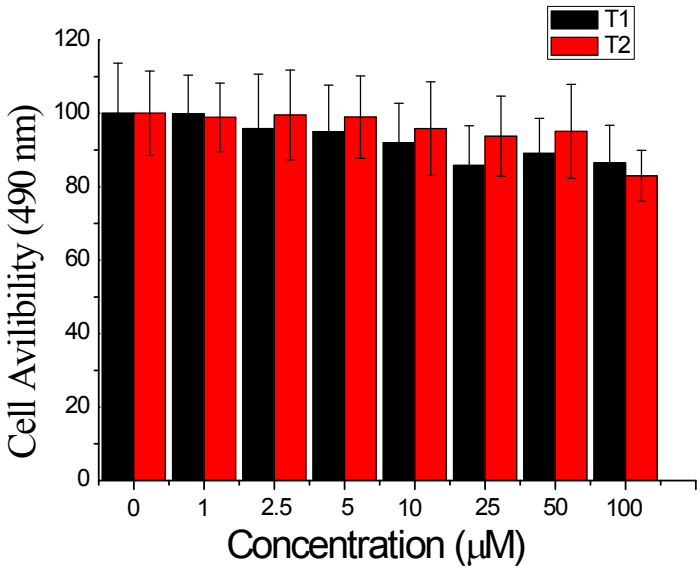


Fig. S17 Cell viability values (%) estimated by MTT assay in Hela cells, which were cultured in the presence of 0-100 μM **1** for 2 h at 37 ^{o}C.

11. Characteristic of the compounds

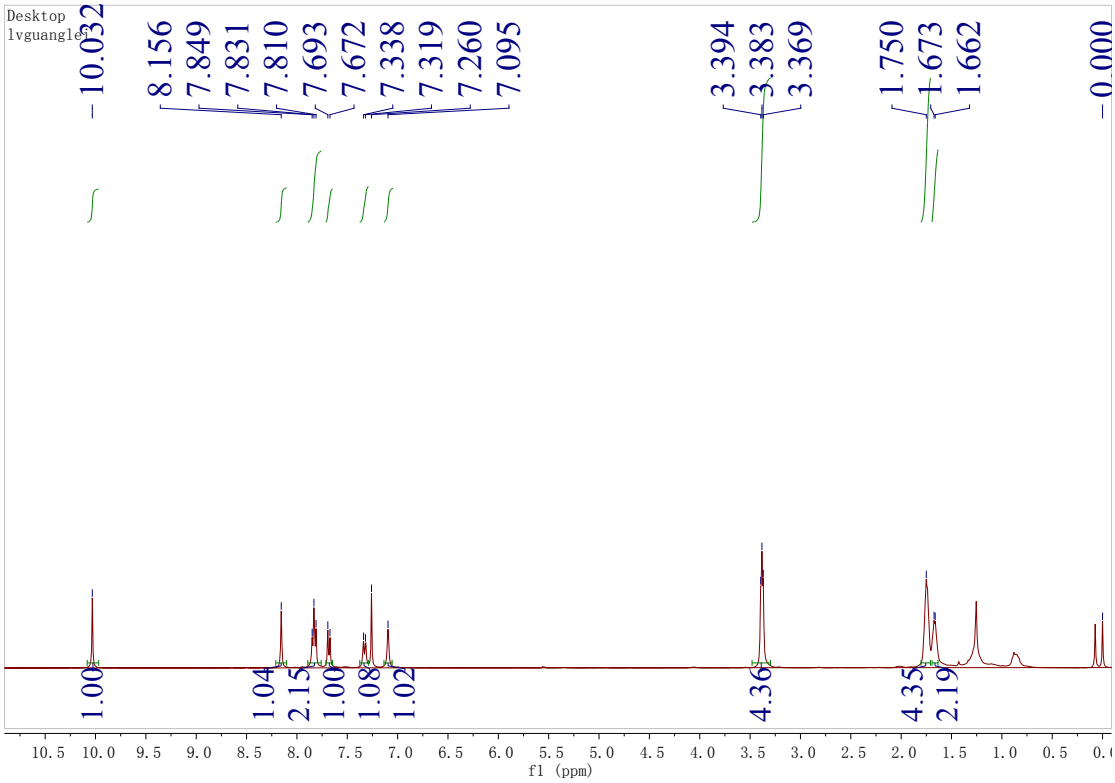


Fig. S18 The ^{1}H NMR spectrum of **2.**

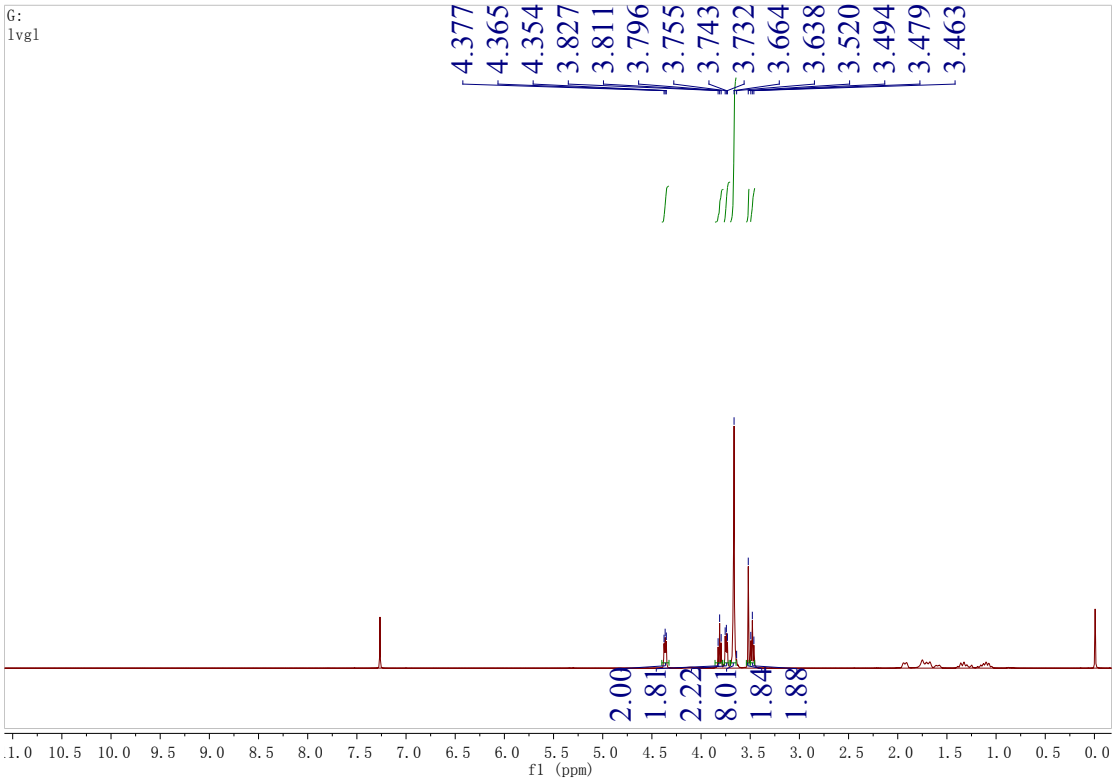


Fig. S19 The ^{1}H NMR spectrum of **4.**

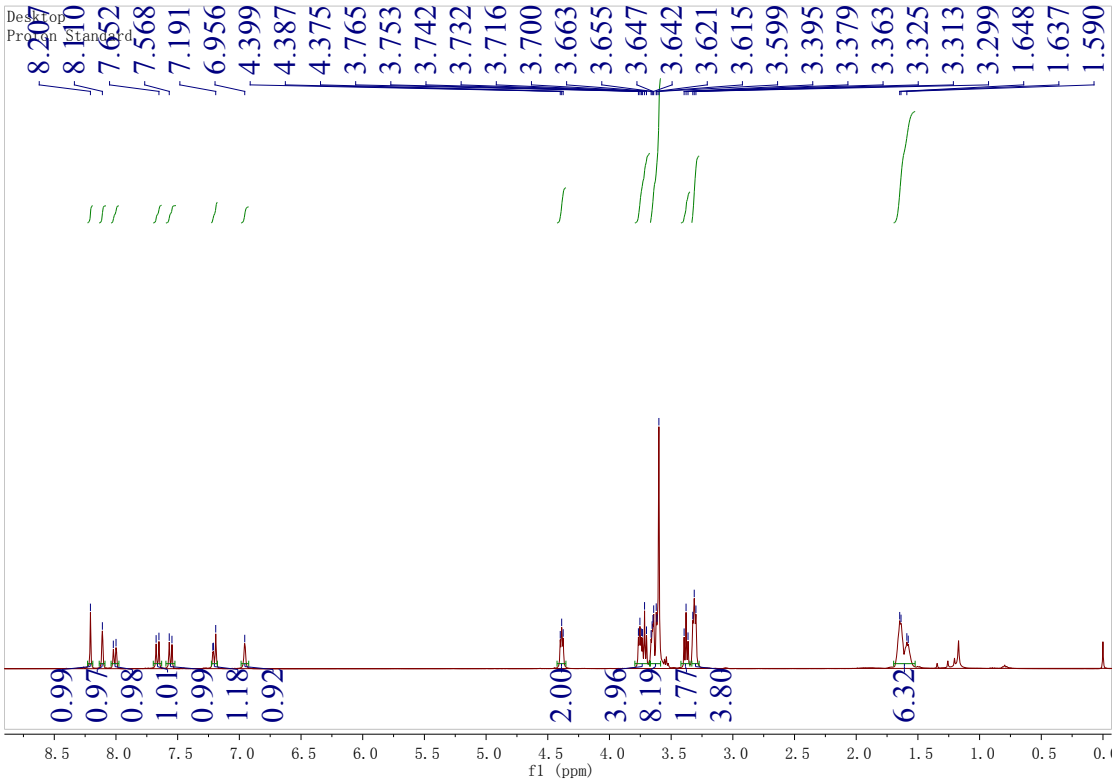


Fig. S20 The ^{1}H NMR spectrum of **5.**

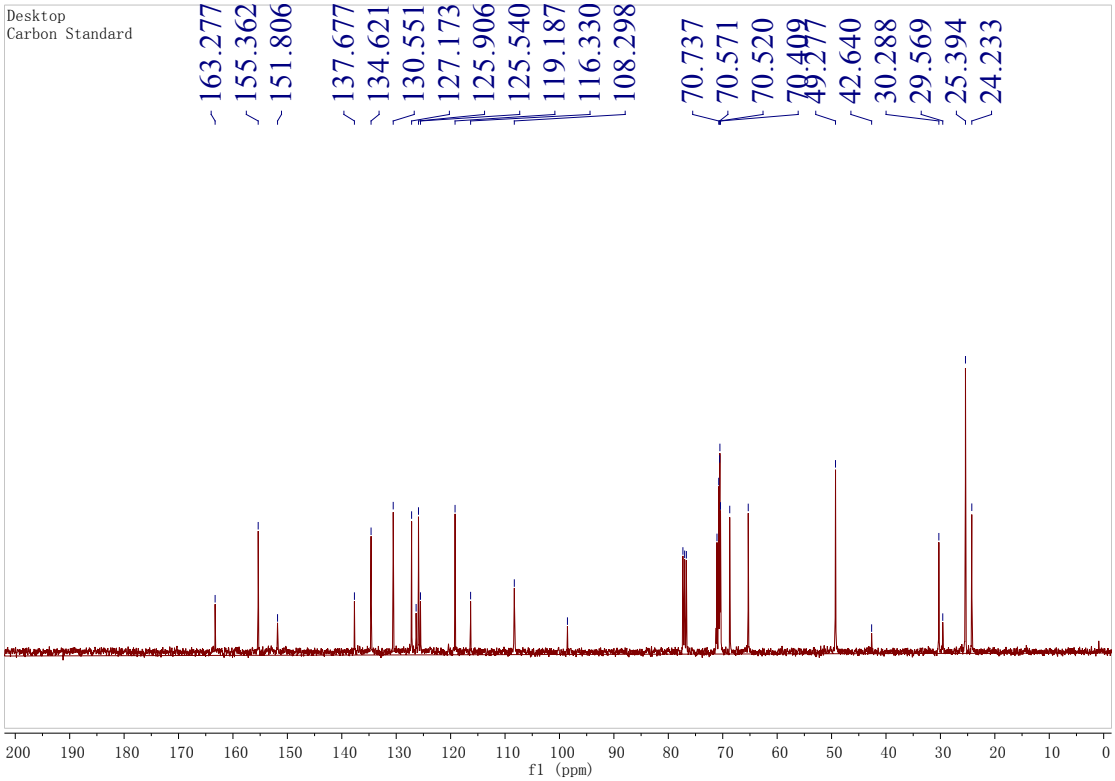


Fig. S21 The ^{13}C NMR spectrum of **5.**

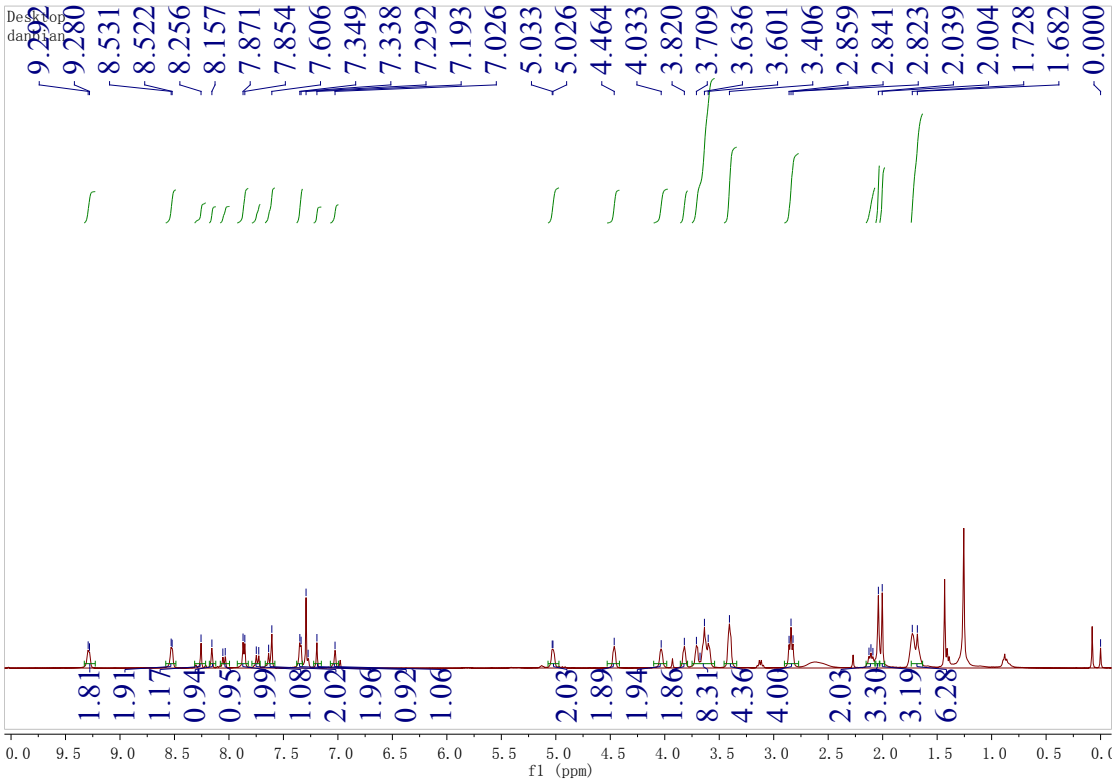


Fig. S22 The ^{1}H NMR spectrum of **T1.**

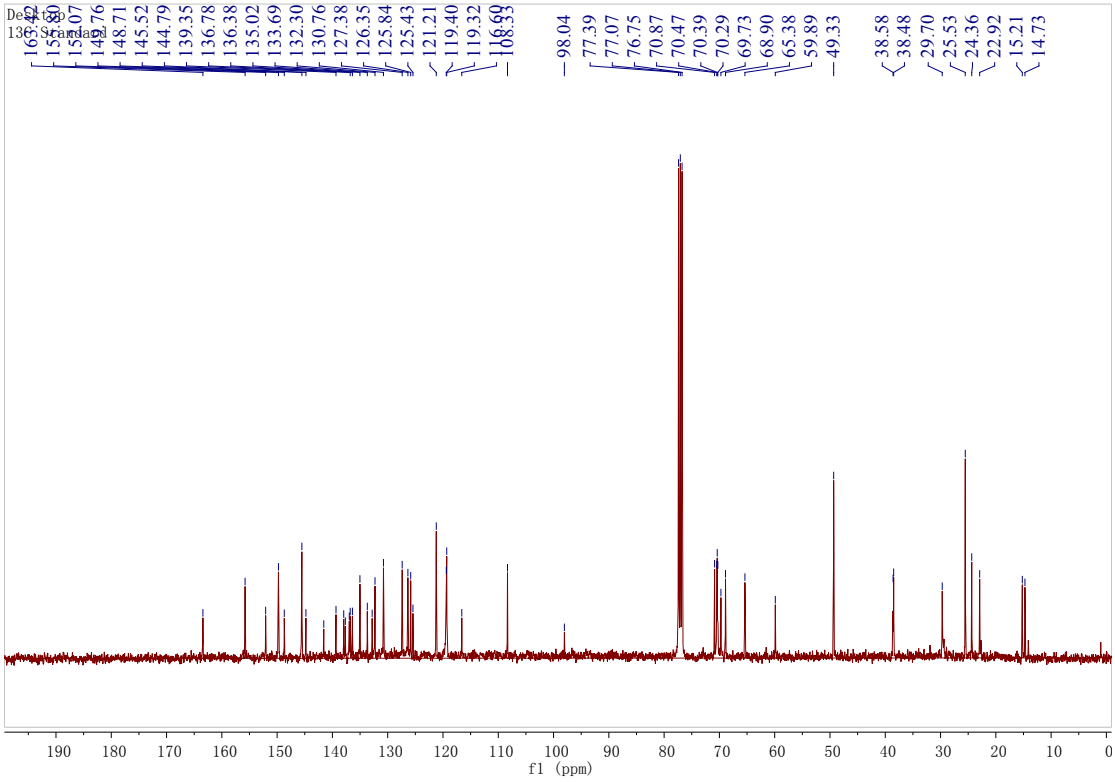


Fig. S23 The ^{13}C NMR spectrum of **T1.**

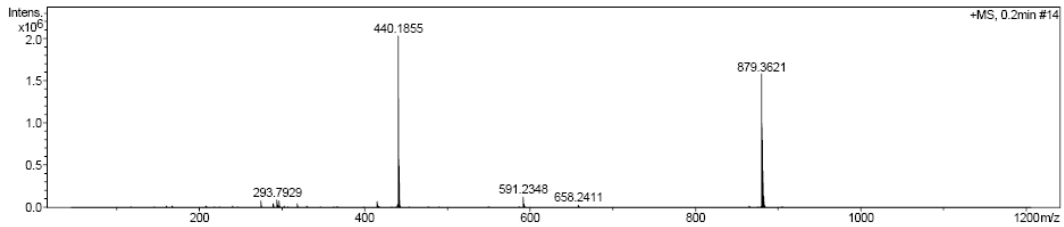


Fig. S24 The HRMS spectrum of **T1.**

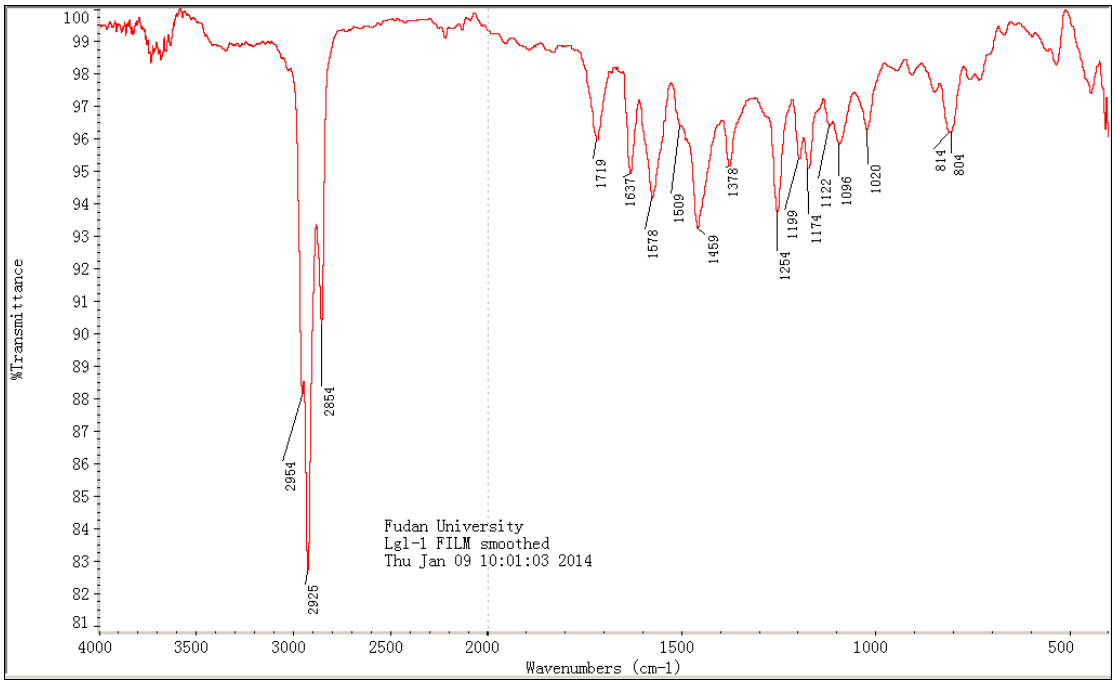


Fig. S25 The IR spectrum of **T1.**

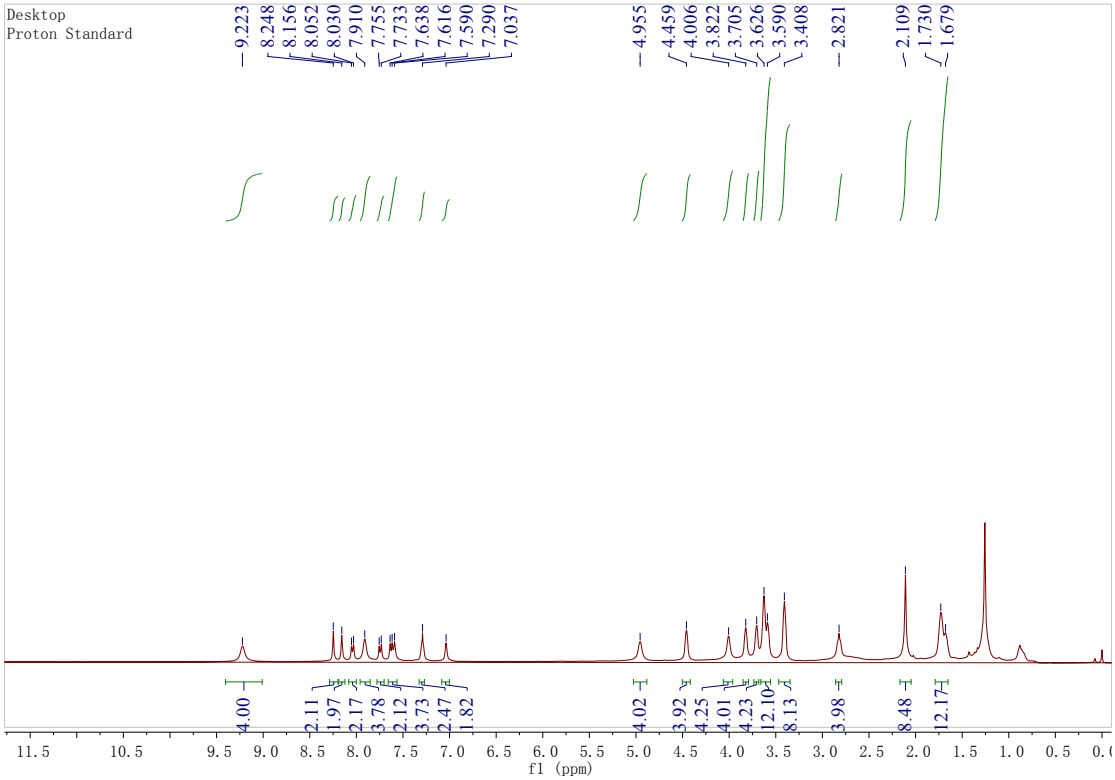


Fig. S26 The ^{1}H NMR spectrum of **T2.**

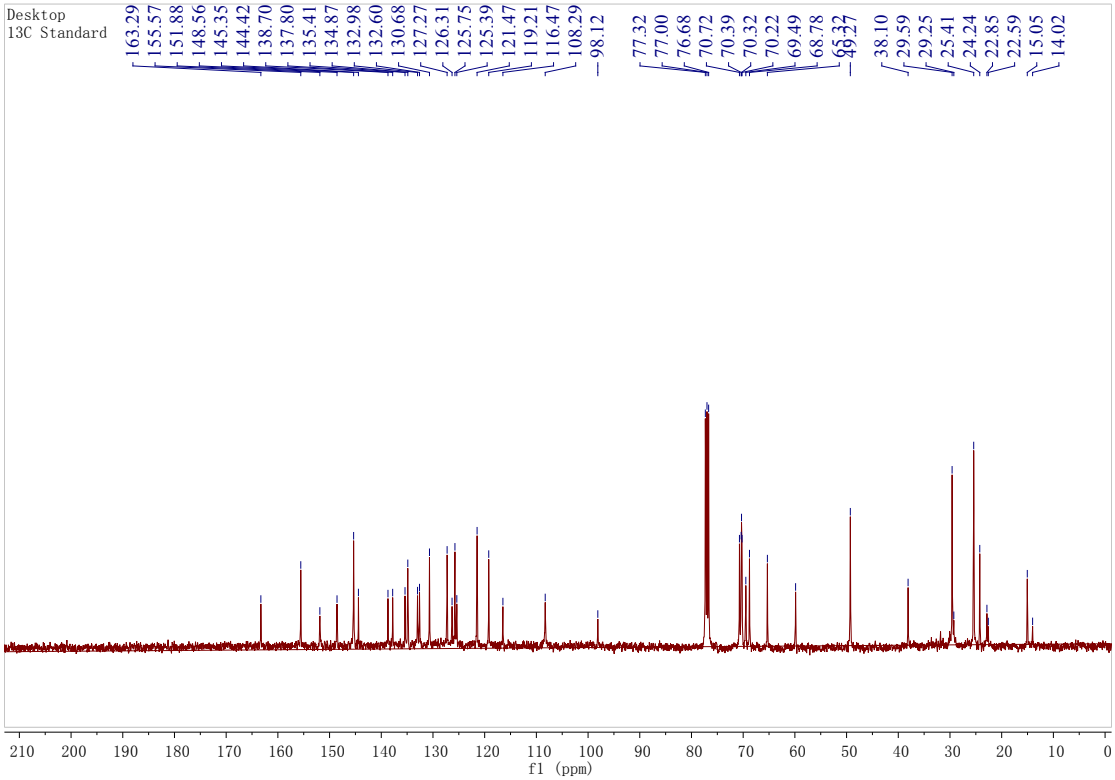


Fig. S27 The ^{13}C NMR spectrum of **T2.**

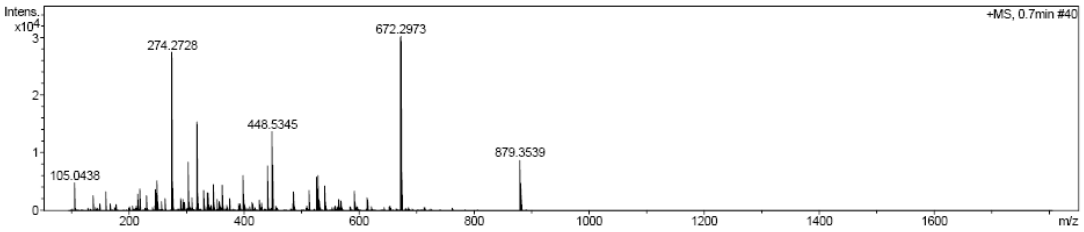


Fig. S28 The HRMS spectrum of **T2.**

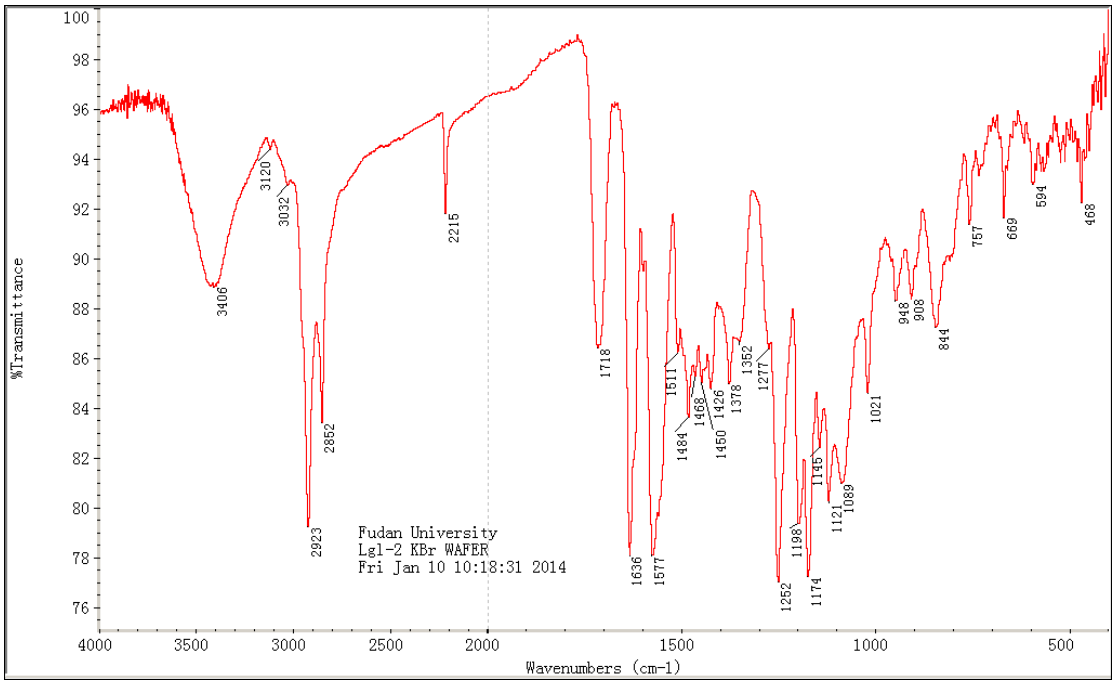


Fig. S29 The IR spectrum of **T2**.

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