

Supplementary Information

Novel D-A-D Based Near-Infrared Probe for Detection of β -Amyloid and Tau Fibrils in Alzheimer's Disease

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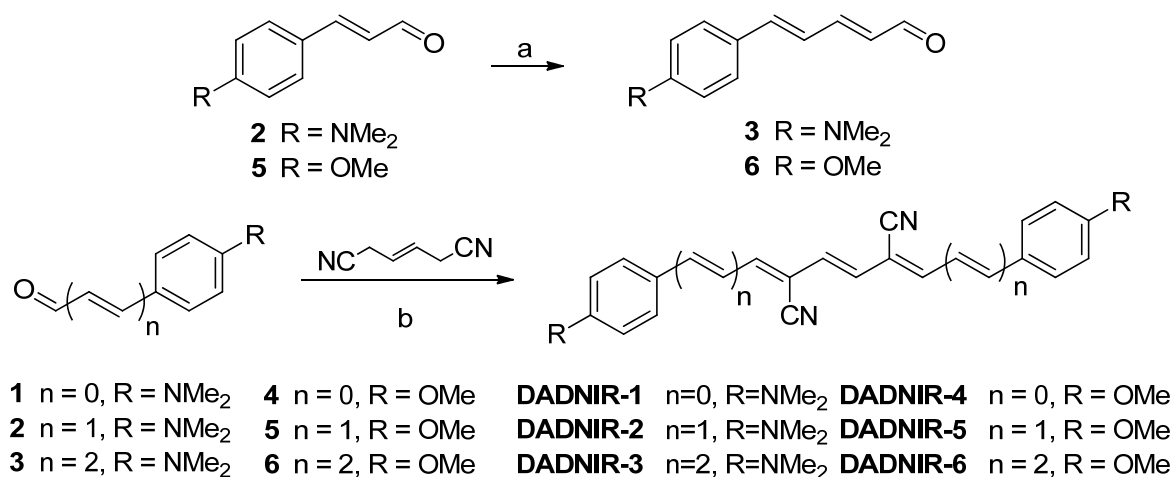
General information

All chemical reagents and solvents were purchased without further purification. Reactions were monitored by thin layer chromatography (TLC) of silica gel 60 F254 plates (Merck, Greman) under UV light (254 nm). Silica gel of 45-70 μm (Yantai Industry Research Institute, China) was used for column chromatography. ^1H NMR and ^{13}C NMR spectra were obtained by JEOL JNM-ECZ400R/S1 (Japan, 400 MHz or 100 MHz) NMR spectrometer in CF_3COOD or CDCl_3 solvents at room temperature. The internal standard is tetramethylsilane (TMS), chemical shift (δ) and coupling constants (J) are recorded in ppm and hertz (Hz), respectively. High resolution mass spectra were measured by Thermo scientific Q-Exactive (ESI) (USA) mass spectrometer. UV-visible spectra were measured by Shimadzu UV-2450 UV-vis spectrophotometer (Japan). Fluorescence spectra were measured by Edinburgh Instruments FS5 (UK) spectrofluorometer. The fluorescence quantum yields were measured by the Absolute PL Quantum Yield Spectrometer C11347 (Hamamatsu, Japan). Fluorescent images were acquired by EVOS FL imaging system (Life, USA) and Axio Observer Z1 inverted fluorescence microscope (Zeiss, Germany), equipped with DAPI, GFP, RFP and Cy5 filter sets. Confocal imaging was processed by laser-scanning confocal microscope (Nikon A1, Japan). Saturation binding constants were measured by Infinite[®] M200 pro (Tecan, Switzerland) microplate readers using black 96-well plate (costar[®], USA). High performance liquid chromatography (HPLC) of Primaide system (Hitachi, Japan) was used to analyze samples. The samples were analyzed by using Venusil MP C18 reverse column (Bonna -Agela Technologies, 5 μm , 4.6 mm \times 250 mm), eluted with an isocratic system at flow rate of 1.0 mL/min and mobile phase A and B were water and acetonitrile. The frozen sections were prepared by CM1900 (Leica, German) freezing microtome. The MTT assay was measured by 1420 Multiabel counter (PerkinElmer, USA). The bio-distribution was captured by IVIS Lumina III system (PerkinElmer, USA).

Synthetic $A\beta_{1-42}$ (peptide) was purchased from Peptide Institute. Inc. (Osaka, Japan). Synthetic peptide fragment tau (k18) was obtained from Wuhan University. The primary antibody of Anti-Human PHF-Tau monoclonal antibody (AT8, Cat. No. MN1020) was purchased from Thermo Fisher Scientific (USA). The secondary antibody of Donkey Anti-Mouse IgG H&L (Alexa Fluor[®] 488) (Cat. No. ab150105) was purchased from Abcam plc (Cambridge, UK).

ICR mice (male, 5 weeks, 18-22 g) used for BBB penetrating experiment were purchased from Vital River Laboratories (Beijing, China). The Alzheimer's model of double transgenic mice (C57BL6, APP^{sw}/PSEN1, 24-month old, male; C57BL6, rTg4510, female) and age-matched control mice (C57BL6, 24-month old, male) were purchased from Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences. The brain tissue sections of AD patients (91-year-old, male, temporal lobe; 85-year-old, male, entorhinal cortex; 70-year-old, male, temporal lobe; 64-year-old, female, temporal lobe; 95-year-old, female, hippocampus) and healthy control (68-year-old, male, temporal lobe), were obtained from the Chinese Brain Bank Center. Procedures related to animal experiments were implemented in accordance with our institutional guidelines and approved by the Beijing Normal University Animal Care Committee.

Chemistry



Scheme 1. Synthesis of **DADNIRs**. Reagents and conditions: (a) (1) ((1,3-dioxolan-2-yl)methyl) triphenylphosphonium bromide, anhydrous THF, NaH (60% in mineral oil), 18-crown-6, r.t., 12 h; (2) 1M HCl, r.t., 30 min; (b) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), methanol, 90 °C, 6 h.

(2E,4E)-5-(4-(dimethylamino)phenyl)penta-2,4-dienal (3)

The same reaction reported previously was used to prepared compound **3**¹. Finally, compound **3** (161 mg, 39%) was obtained by column chromatography (ethyl acetate/ petroleum ether = 1:4, v/v). The ¹H NMR spectrum was in agreement with reported previously¹.

(2E,4E)-5-(4-methoxyphenyl)penta-2,4-dienal (6)

The above method was also used for preparing compound **6** (122 mg, 32%). ¹H NMR (400 MHz, CDCl₃) δ 9.68 (d, *J* = 8.0 Hz, 1H), 7.45-7.40 (m, 2H), 7.25-7.20 (m, 1H), 6.98-6.82 (m, 4H), 6.22 (dd, *J* = 15.1, 8.0 Hz, 1H), 3.83 (s, 3H).

(E)-2,5-bis((Z)-4-(dimethylamino)benzylidene)hex-3-enedinitrile (DADNIR-1)

The same reaction reported previously was used to prepared **DADNIR-1**², as the crimson solid (30 mg, 8.2%). ¹H NMR (400MHz, CF₃COOD) δ 8.05 (d, *J* = 8.7 Hz, 4H), 7.70 (d, *J* = 8.6 Hz, 4H), 7.51 (s, 2H), 7.03 (s, 2H), 3.43 (s, 12H). ¹³C NMR (100MHz, CF₃COOD) δ 144.03, 141.68, 134.90, 130.36, 129.43, 119.34, 111.15, 110.26, 45.79. HRMS: m/z calculated for [C₂₄H₂₄N₄+H]⁺ 369.2074; found 369.2072.

(2Z,3E,5Z)-2,5-bis((E)-3-(4-(dimethylamino)phenyl)allylidene)hex-3-enedinitrile (DADNIR-2)

The above method was also used for preparing **DADNIR-2**, as the purple solid (20 mg, 4.8%). ¹H NMR (400MHz, CF₃COOD) δ 7.80 (d, *J* = 8.4 Hz, 4H), 7.59 (d, *J* = 8.4 Hz, 4H), 7.42 (dd, *J* = 15.0, 11.7 Hz, 2H), 7.26 (d, *J* = 11.5 Hz, 2H), 7.11 (d, *J* = 15.2 Hz, 2H), 6.83 (s, 2H), 3.43 (s, 12H). ¹³C NMR (100MHz, CF₃COOD) δ 145.67, 140.46, 138.90, 137.82, 128.30, 127.85, 125.64, 118.85, 111.71, 110.75, 45.81. HRMS: m/z calculated for [C₂₈H₂₈N₄+H]⁺ 421.2387; found 421.2386.

(2Z,3E,5Z)-2,5-bis((2E,4E)-5-(4-(dimethylamino)phenyl)penta-2,4-dien-1-ylidene)hex-3-enedini

trile (DADNIR-3)

The above method was also used for preparing **DADNIR-3**, as the black solid (48 mg, 20.68%). ¹H NMR (400MHz, CF₃COOD) δ 7.70 (d, *J* = 8.2 Hz, 4H), 7.53 (d, *J* = 8.0 Hz, 4H), 7.20-7.04 (m, 4H), 6.99-6.88 (m, 4H), 6.87 (d, *J* = 15.5 Hz, 2H), 6.75 (s, 2H), 3.43 (s, 12H). ¹³C NMR (100MHz, CF₃COOD) δ 146.84, 142.73, 140.59, 140.08, 135.45, 130.83, 129.77, 128.89, 128.52, 119.66, 115.16, 112.35, 46.96. HRMS: m/z calculated for [C₃₂H₃₂N₄+H]⁺ 473.2700; found 473.2701.

(E)-2,5-bis((Z)-4-methoxybenzylidene)hex-3-enedinitrile (DADNIR-4)

The above method was also used for preparing **DADNIR-4**, given as the yellow solid (141 mg, 20.6%). ¹H NMR (400MHz, CDCl₃) δ 7.84-7.80 (m, 4H), 7.09 (s, 2H), 6.97-6.93 (m, 4H), 6.76 (s, 2H), 3.86 (s, 6H). ¹³C NMR(100MHz, CDCl₃) δ 161.85, 144.62, 131.44, 129.55 126.42, 116.74, 114.62, 107.00, 55.54. HRMS: m/z calculated for [C₂₂H₁₉O₂N₂+H]⁺ 343.1441; found 343.1436.

(2Z,3E,5Z)-2,5-bis((E)-3-(4-methoxyphenyl)allylidene)hex-3-enedinitrile (DADNIR-5)

The above method was also used for preparing **DADNIR-5**, as the brick-red solid (162 mg, 20.6%). ¹H NMR (400MHz, CDCl₃) δ 7.47 (d, *J* = 8.8 Hz, 4H), 7.10-7.17 (m, 2H), 6.97-6.92 (m, 4H), 6.90 (d, *J* = 8.6 Hz, 4H), 6.64 (s, 2H), 3.84 (s, 6H). ¹³C NMR (100MHz, CDCl₃) δ 161.19, 145.55, 141.72, 129.40, 128.72, 128.64, 122.27, 115.60, 114.58, 110.62, 55.30. HRMS: m/z calculated for [C₂₆H₂₃O₂N₂+H]⁺ 395.1754; found 395.1748.

(2Z,3E,5Z)-2,5-bis((2E,4E)-5-(4-methoxyphenyl)penta-2,4-dien-1-ylidene)hex-3-enedinitrile (DADNIR-6)

The above method was also used for preparing **DADNIR-6**, as the black solid (45 mg, 25.3%). ¹H NMR (400MHz, CDCl₃) δ 7.45-7.40 (m, 1H), 7.39-7.37 (m, 6H), 6.87-6.89 (m, 8H), 6.77-6.81 (m, 4H), 6.61 (s, 1H), 3.83 (s, 6H). ¹³C NMR (100MHz, CDCl₃) δ 160.45, 144.90, 142.74, 139.90,

138.39, 129.21, 128.92, 128.66, 127.99, 126.17, 124.18, 117.25, 114.46, 110.80, 100.00, 55.46, 29.77. HRMS: m/z calculated for $[C_{30}H_{27}O_2N_2+H]^+$ 447.2067; found 447.2061.

Fluorescence spectral

The **DADNIRs** were configured into dimethyl sulfoxide (3 mL) with gradient concentration (1-10 μ M). These solutions were used for measuring UV-absorption maximum wavelength (λ_{abs} , nm) and molar absorption coefficient (ϵ , $L \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$). The solvents of **DADNIRs** (10 μ M) in dichloromethane (CH_2Cl_2) and phosphate-buffered saline (PBS) were prepared for the fluorescence excitation/emission spectra and fluorescence quantum yields (Φ , %) measurement. The 3 mL CH_2Cl_2 of **DADNIR-2** (10 μ M) were prepared avoiding the light. Then photostability of **DADNIR-2** was performed with constant illumination over 30 minutes by using spectrofluorometer. The emission/excitation wavelength was 622/562 nm.

As shown in Table S1, the UV-absorption maximum wavelength (λ_{abs}) increased with lengthening of polyenic chains, and distributed in 393-563 nm. The λ_{abs} of probes with methoxy group was shorter than *N,N*-dimethylamino substitutes, distributed in 393-490 nm. The molar absorption coefficients (ϵ) were calculated between 70151-139717 $L \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. The fluorescence excitation/emission maxima ($\lambda_{\text{ex}}/\lambda_{\text{em}}$) increased with lengthening of polyenic chains. Emission maxima of **DADNIR 1-3** distributed in 650-828 nm, and emission maxima of **DADNIR 4-6** distributed in 540-668 nm in PBS (pH = 7.4, 10% ethanol). The fluorescence quantum yields (Φ , %) exhibited that the **DADNIR-2** in dichloromethane possessed a highest value of 1.9%. In addition, from the quantum yields measured on solid state, **DADNIR-1** and **DADNIR-4** displayed aggregation-induced emission (AIE) properties.

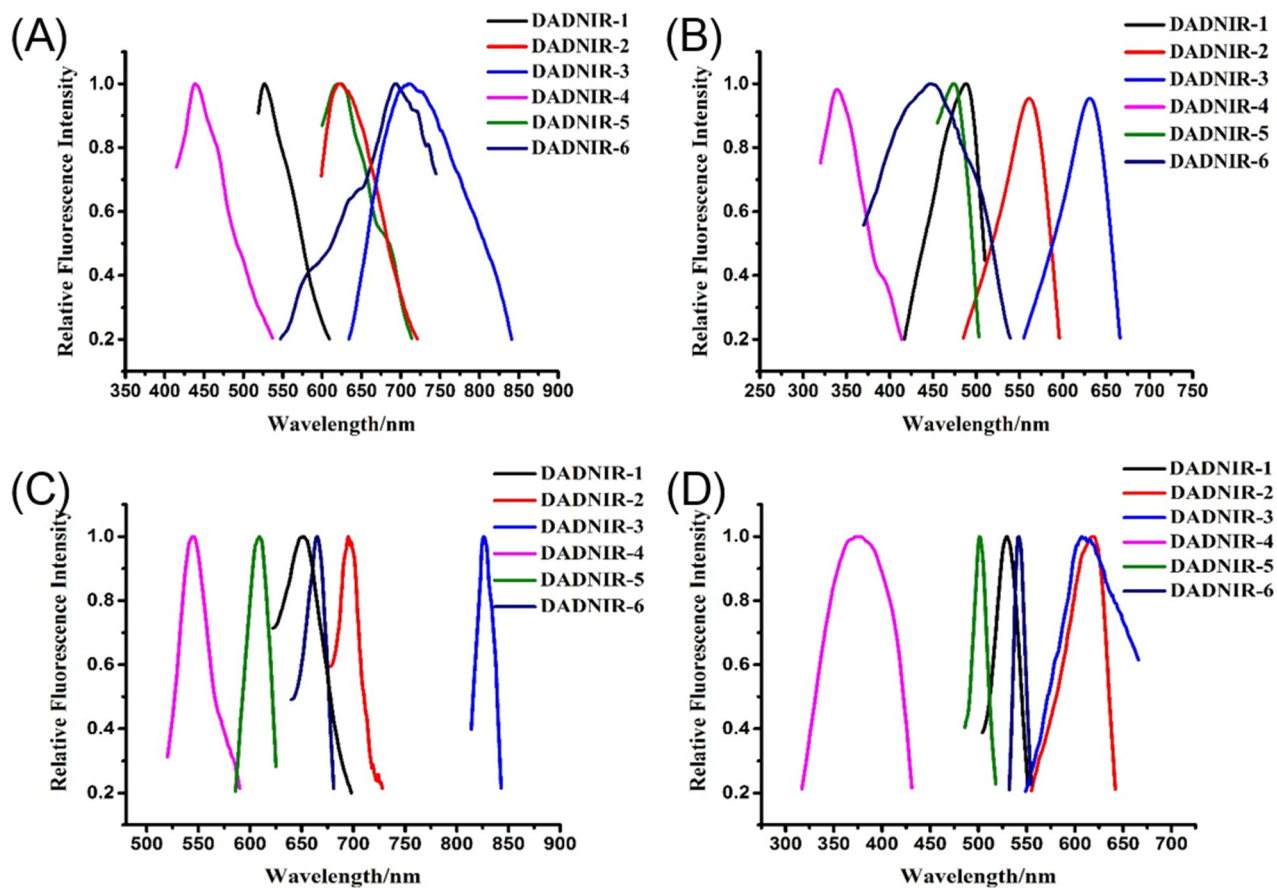


Figure S1. The emission (left panel) and excitation (right panel) spectrum of **DADNIR 1-6** (10 μM) in different solutions: dichloromethane (A, B), and phosphate-buffered saline (PBS) (10% ethanol, pH = 7.4) (C, D).

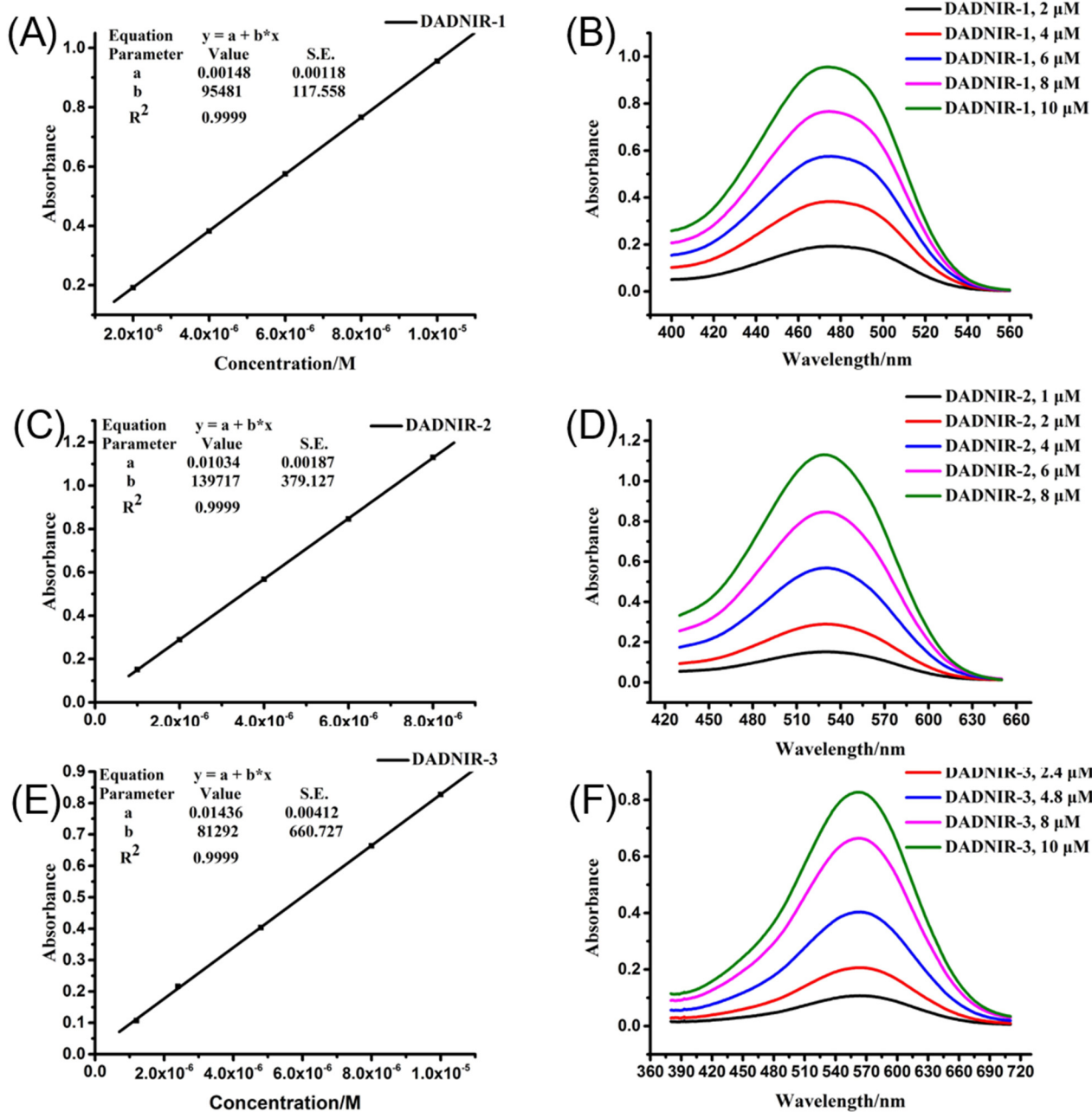


Figure S2. Molar absorption coefficients (ϵ , left panel) and absorption spectra (right panel) of the **DADNIR-1** (A, B), **DADNIR-2** (C, D), **DADNIR-3** (E, F) measured in dimethyl sulfoxide.

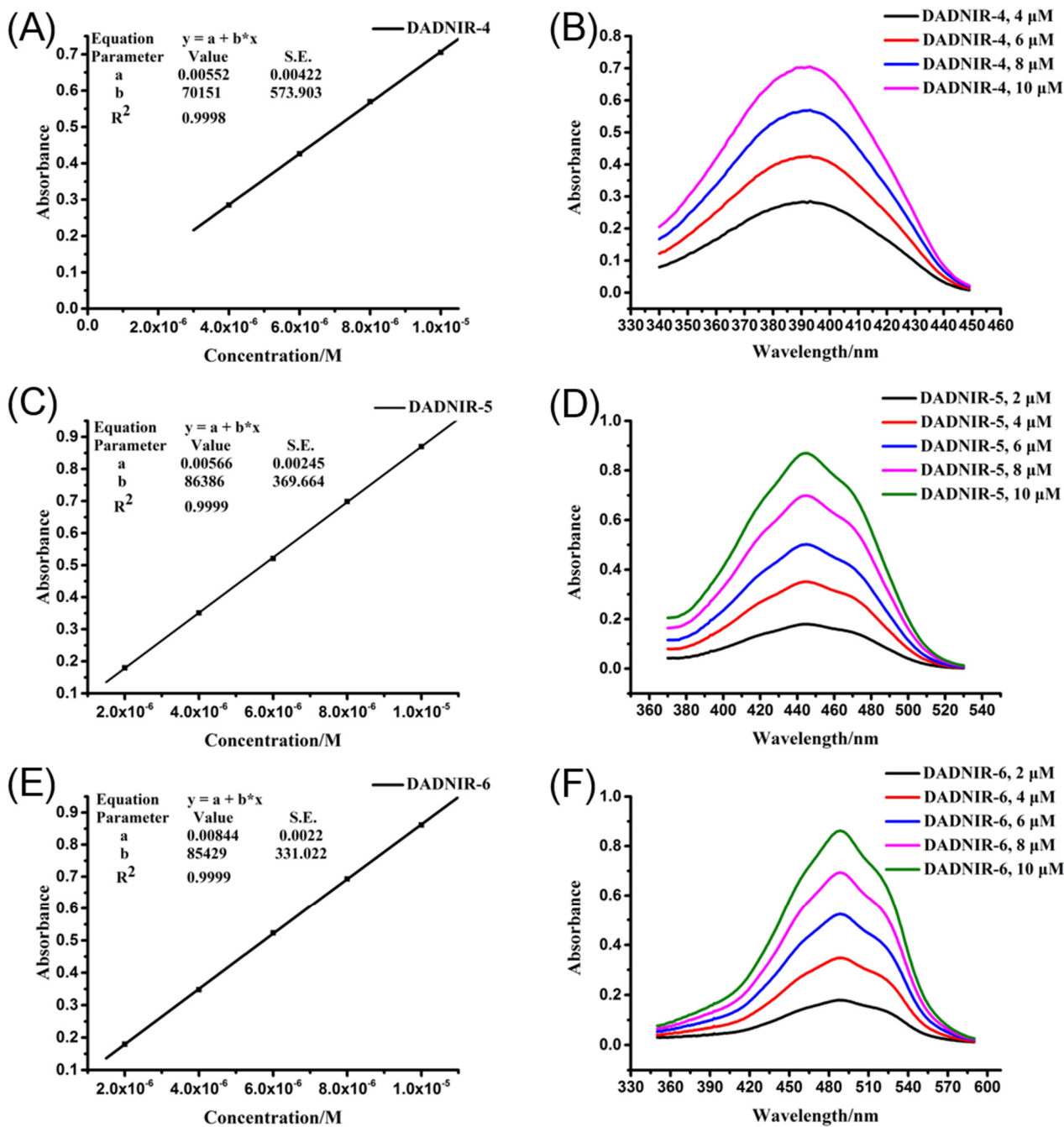


Figure S3. Molar absorption coefficients (ϵ , left panel) and absorption spectra (right panel) of the **DADNIR-4** (A, B), **DADNIR-5** (C, D), **DADNIR-6** (E, F) measured in dimethyl sulfoxide.

Table S1. The spectroscopic properties (absorption, excitation and emission wavelength, Stokes Shifts, fold of increase, molar absorption coefficient, and fluorescence quantum yield) of **DADNIRs**.

Probe	Solvents	Fluorescence Spectrum				Φ_a %		UV-vis Spectrum	
		$\lambda_{em}(nm)$	$\lambda_{ex}(nm)$	Stokes shift	Fold	CH_2Cl_2	Solid	$\lambda_{abs}(nm)$	$\epsilon(L \cdot mol^{-1} \cdot cm^{-1})$
DADNIR-1	CH_2Cl_2	527	491	36	/	0.3	20.0	475	95481
	PBS	650	530	120	/				
	with A β	546	462	84	93				
	with TAU	546	463	83	2				
DADNIR-2	CH_2Cl_2	622	562	60	/	1.9	0.6	529	139717
	PBS	690	617	73	/				
	with A β	642	538	106	70				
	with TAU	651	530	121	48				
DADNIR-3	CH_2Cl_2	710	603	107	/	0.8	0.1	563	81292
	PBS	828	610	218	/				
	with A β	650	530	120	1				
	with TAU	650	530	120	1				
DADNIR-4	CH_2Cl_2	450	350	100	/	0.5	56.6	393	70151
	PBS	540	395	145	/				
	with A β	470	430	40	82				
	with TAU	465	400	65	2				
DADNIR-5	CH_2Cl_2	650	481	69	/	0.8	0.3	445	86386
	PBS	610	500	110	/				
	with A β	577	460	117	19				
	with TAU	550	460	90	17				
DADNIR-6	CH_2Cl_2	700	460	240	/	0.3	0.2	490	85429
	PBS	668	541	127	/				
	with A β	600	500	100	1				
	with TAU	600	500	100	1				

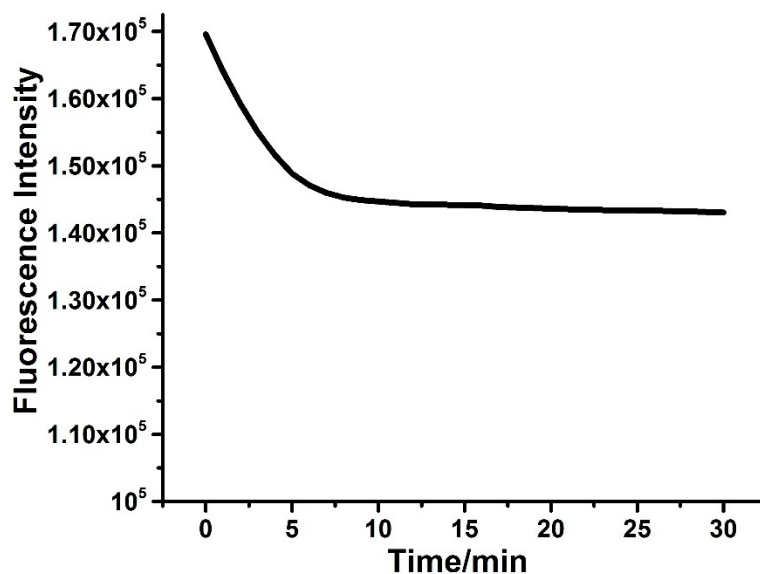


Figure S4. Photobleaching curve of 10 μM DADNIR-2 in CH_2Cl_2 .

A β ₁₋₄₂ aggregation in vitro

A β ₁₋₄₂ aggregates were attained by dissolving the *A β ₁₋₄₂* peptide (0.55 mg) in 2.5 mL buffer solution, including 10 mM NaH_2PO_4 and 1 mM EDTA. Then the solution was incubated at 37 °C with constant shaking (120 r/min) for 48 h. Aggregated *A β ₁₋₄₂* fibers (final concentration of 48.74 μM) were stored at -20 °C (avoid freeze/thaw cycle).

Tau aggregation in vitro

Recombinant fragments of tau (k18, 1.66 mg) were dissolved in 2.5 mL HEPES buffer solution (pH = 7.4) to prepare tau aggregates. The buffer solution include 0.05 mM heparin sodium, 1 mM DL-Dithiothreitol (DTT) and 0.1 mM NaCl. Then the solution was incubated at 37 °C with constant shaking (120 r/min) for 8 h. Aggregated tau fibers (final concentration of 48.74 μM) were stored at -20 °C (avoid freeze/thaw cycle).

Fluorescence enhanced experiment

A solution of probes (50 nM, final concentration), proteins (tau or *A β* or BSA, 10 $\mu\text{g}/\text{mL}$, final concentration) in 3 mL PBS (pH = 7.4, 10% ethanol) were incubated at 37 °C with constant shaking (100 r/min) for 1 h, respectively. Next, the fluorescent excitation/emission spectra were measured by

fluorescence spectrophotometer. The solvents with only probes (50 nM) in PBS as blank control and only PBS as background were also measured in the same conditions. The fold increase of the fluorescence intensity was the ratio of the fluorescence intensity of the probe bound to tau aggregates (or $A\beta$ aggregates) and the intensity of the blank control.

In addition, the fluorescent interaction of **DADNIR-2** with $A\beta$ and Tau monomers in solution were tested as above described.

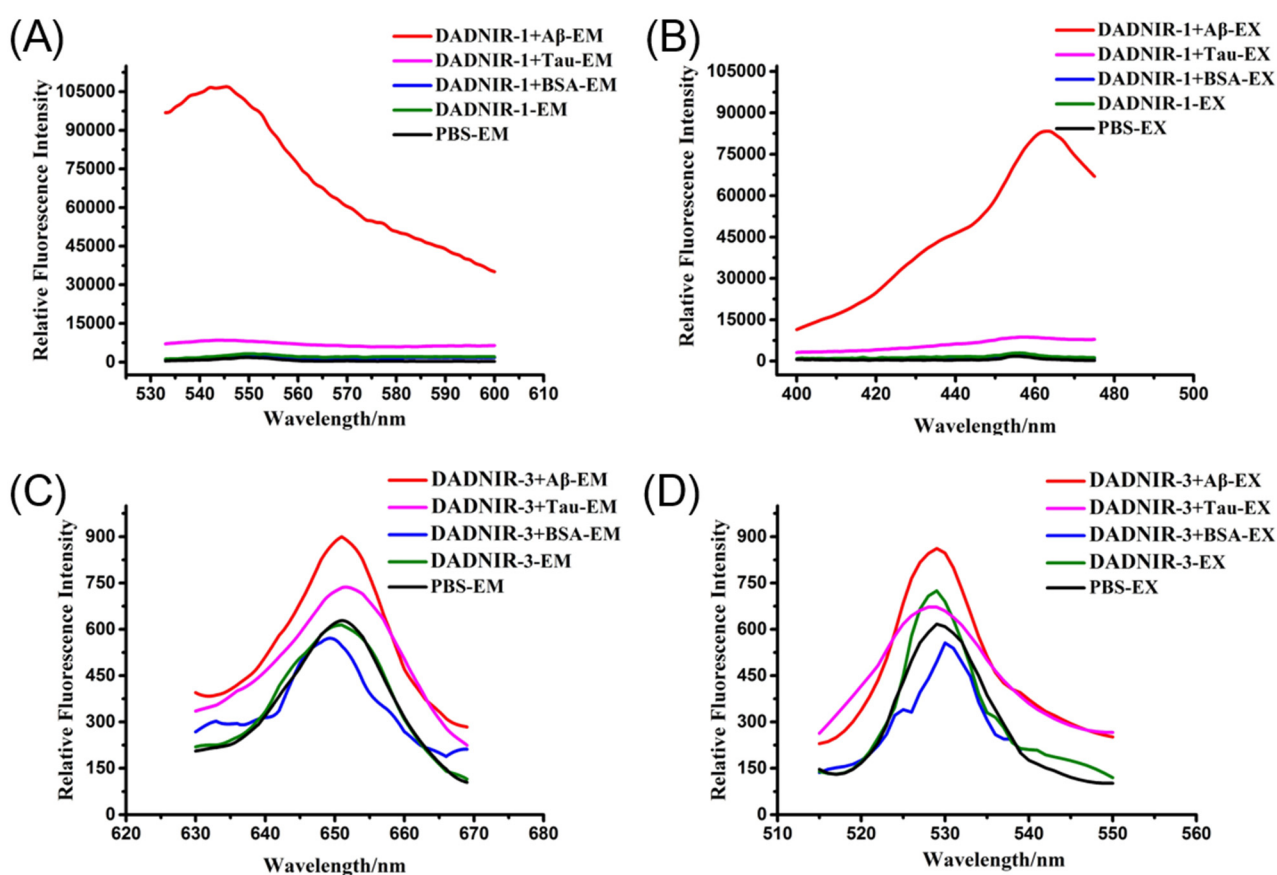


Figure S5. The emission (left panel) and excitation (right panel) spectra of the **DADNIR-1** (A, B) and **DADNIR-3** (C, D) at concentration of 50 nM upon interaction with $A\beta_{1-42}$ aggregates (10 $\mu\text{g/mL}$), tau aggregates (10 $\mu\text{g/mL}$) and BSA (10 $\mu\text{g/mL}$) in PBS (10% ethanol).

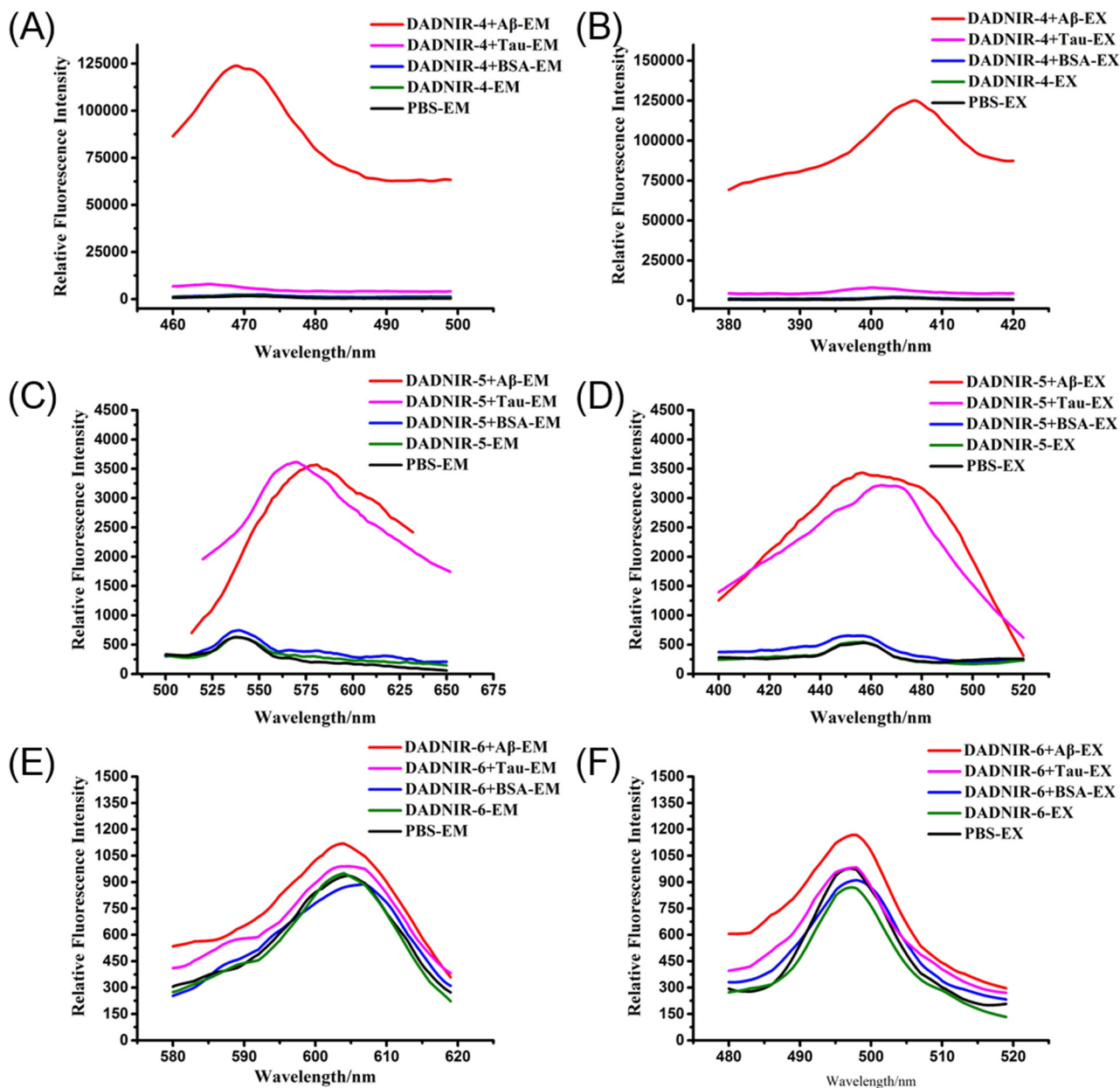


Figure S6. The emission (left panel) and excitation (right panel) spectra of the **DADNIR-4** (A, B), **DADNIR-5** (C, D) and **DADNIR-6** (E, F) at concentration of 50 nM upon interaction with $A\beta_{1-42}$ aggregates (10 $\mu\text{g}/\text{mL}$), tau aggregates (10 $\mu\text{g}/\text{mL}$) and BSA (10 $\mu\text{g}/\text{mL}$) in PBS (10% ethanol).

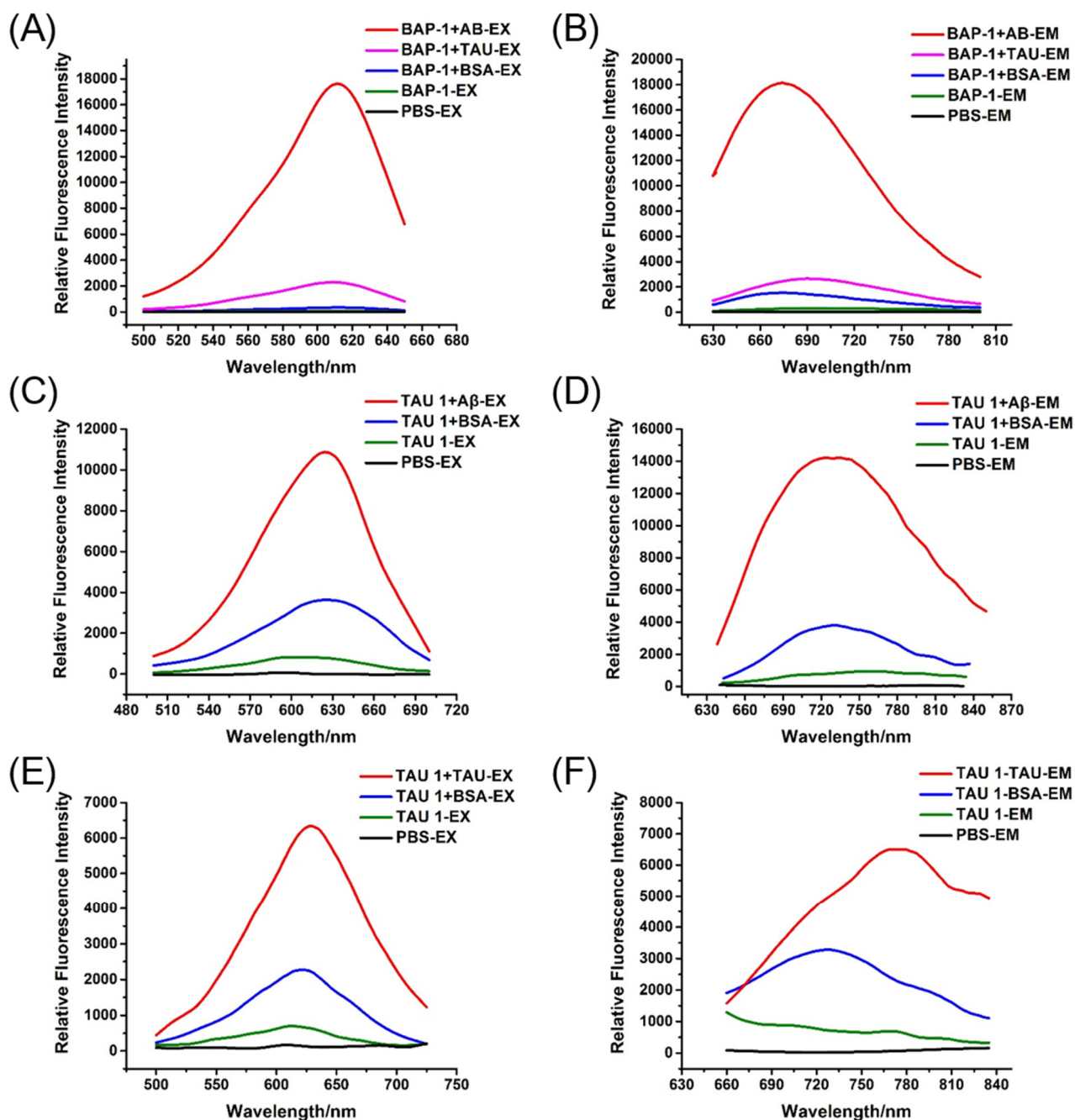


Figure S7. The excitation (left panel) and emission (right panel) spectra of the **BAP-1**, **TAU 1** at concentration of 50 nM upon interaction with $A\beta_{1-42}$ aggregates (10 $\mu\text{g}/\text{mL}$), tau aggregates (10 $\mu\text{g}/\text{mL}$) and BSA (10 $\mu\text{g}/\text{mL}$) in PBS (10% ethanol)

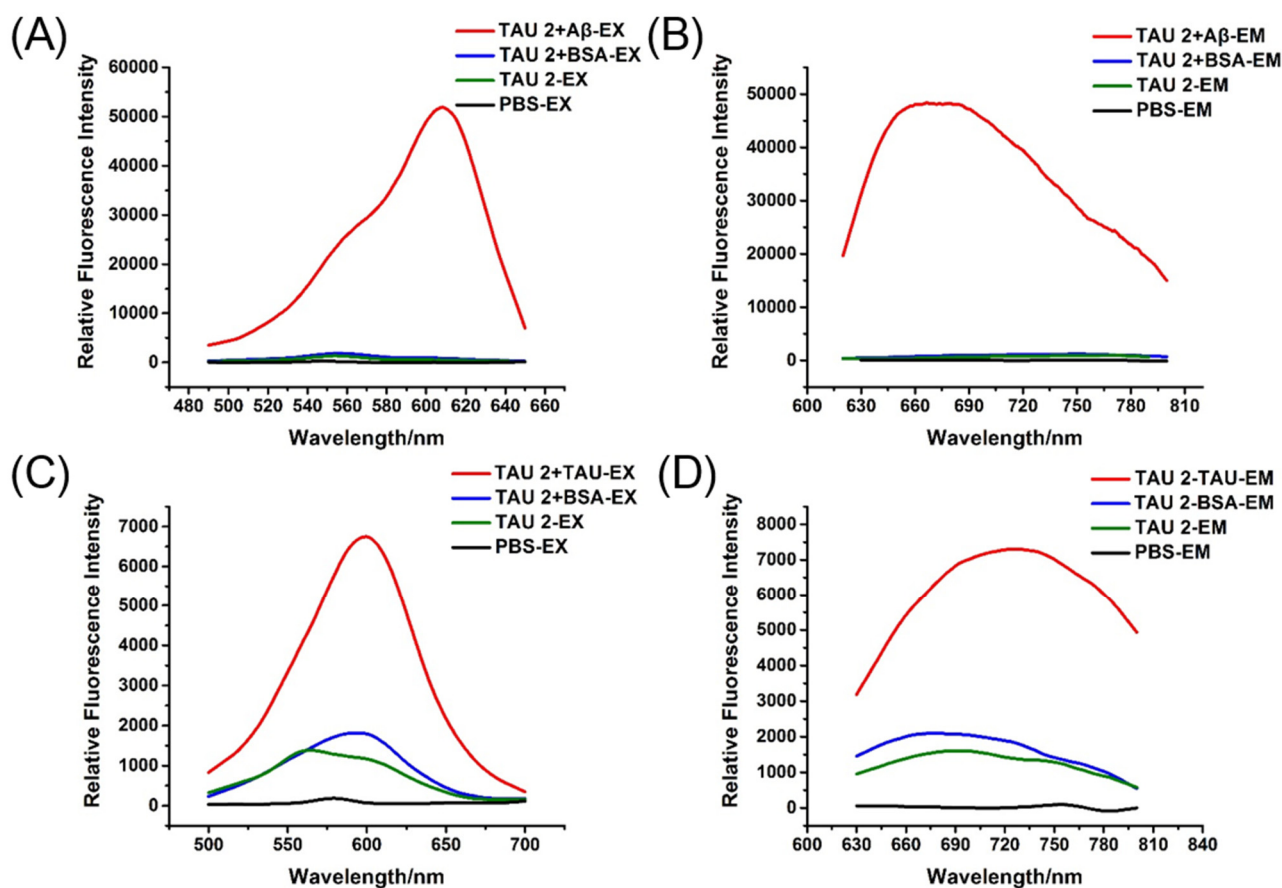


Figure S8. The excitation (left panel) and emission (right panel) spectra of the TAU 2 at concentration of 50 nM upon interaction with $A\beta_{1-42}$ aggregates (10 $\mu\text{g}/\text{mL}$), tau aggregates (10 $\mu\text{g}/\text{mL}$) and BSA (10 $\mu\text{g}/\text{mL}$) in PBS (10% ethanol).

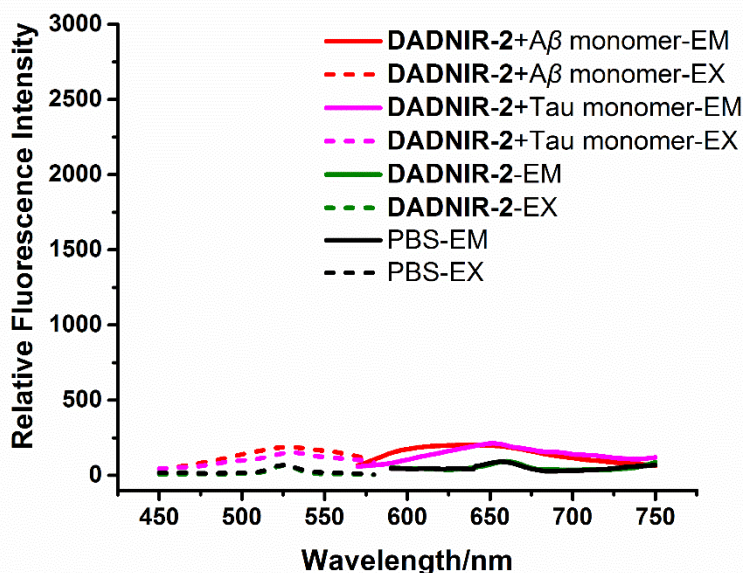


Figure S9. The excitation and emission spectra of the DADNIR-2 at concentration of 50 nM upon interaction with $A\beta_{1-42}$ monomer (10 $\mu\text{g}/\text{mL}$), tau monomer (10 $\mu\text{g}/\text{mL}$) and in PBS (10% ethanol).

Saturation binding constants

A solution of probes (concentration gradient), proteins (tau or $A\beta$, 2 $\mu\text{g}/\text{mL}$, the final test

concentration) in 1 mL PBS (pH = 7.4, 10% ethanol) were incubated at 37 °C with constant shaking (100 r/min) for 1 h, respectively. And the solvents with only probes (50 nM) in PBS as blank control were also prepared in the same conditions. The reaction mixture and the blank control were transferred to black 96-well plate, and measured by microplate readers. The data were analyzed by GraphPad Prism 5.0, and calculated the K_d with nonlinear regression. All of the experiments were tested in triplicate. Saturation binding curves were shown in Figure S8 and Figure S9.

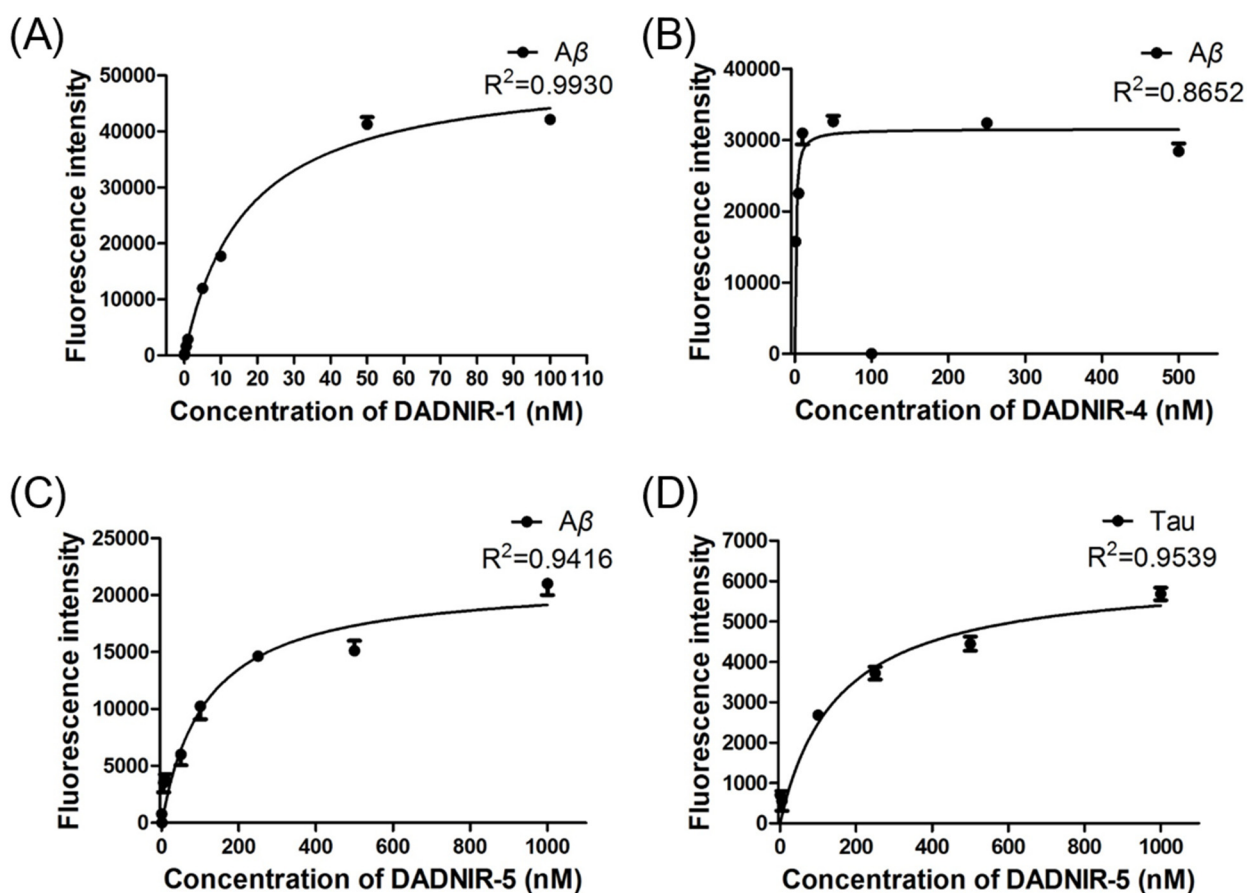


Figure S10. Saturation binding curve of DADNIR-1 (A), DADNIR-4 (B) and DADNIR-5 (C and D) for $A\beta$ and tau aggregates.

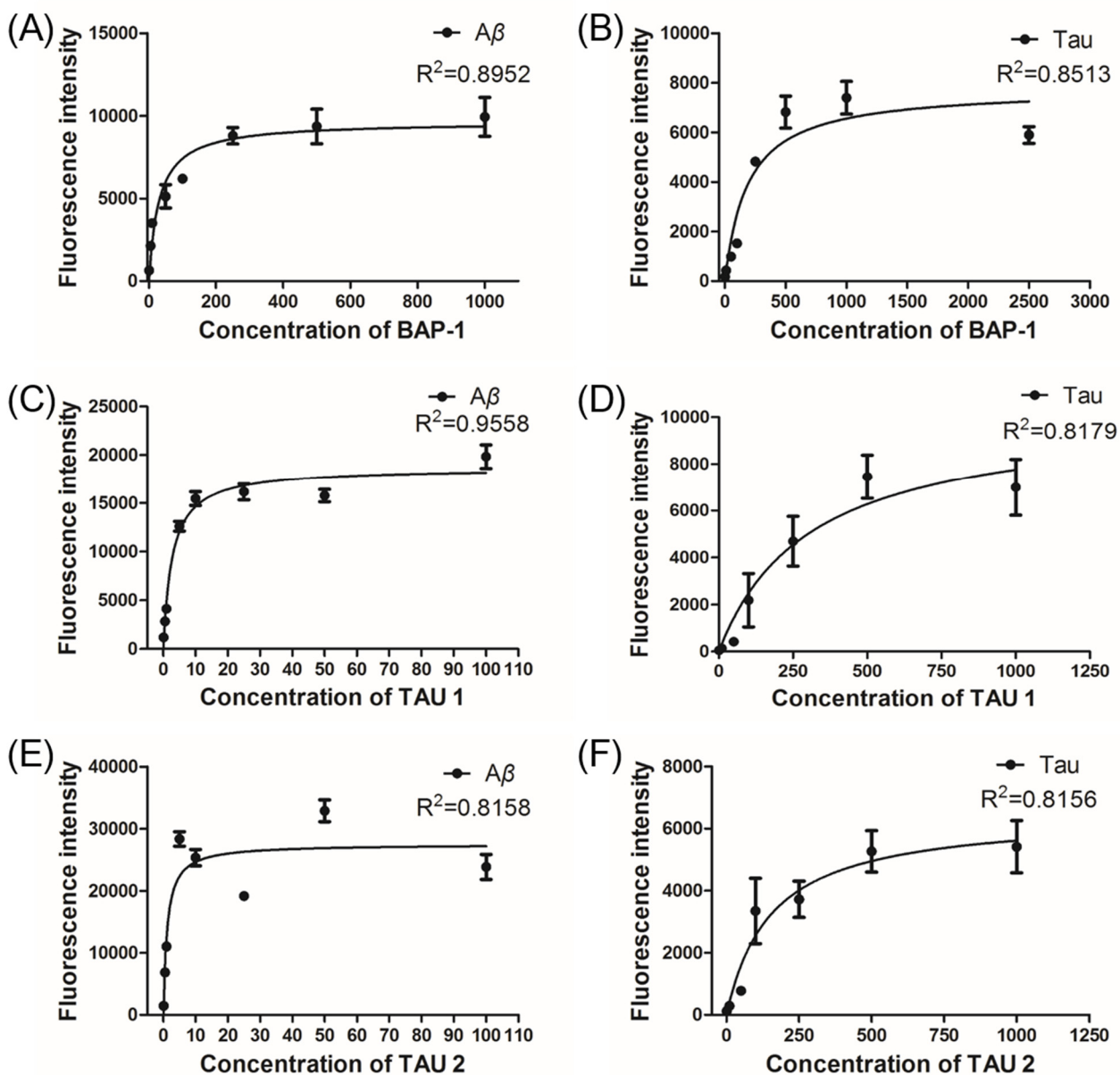


Figure S11. Saturation binding curve of **BAP-1** (A and B), **TAU 1** (C and D) and **TAU 2** (E and F) for A β and tau aggregates.

***In vitro* fluorescent staining**

Paraffin embedded brain sections were deparaffinized by immersing in xylene for 5 min, then washed by ethanol for 2 min and water for 5 min. Next, the brain sections were incubated with solutions of **DADNIRs** (1 μ M, 50% ethanol) for 5 min, then washed by 40% ethanol and water for 5 min. Fluorescent images were observed by fluorescence microscope using appropriate filter sets. ThS (10 μ M, 20% ethanol) and **DANIR 3b³** (1 μ M, 20% ethanol) were counterstained on the same brain

section. In addition, **BAP-1⁴** (1 μ M, 40% ethanol), **TAU 1** (1 μ M, 40% ethanol) and **TAU 2⁵** (1 μ M, 40% ethanol) were also used for staining of AD brain sections under the same conditions.

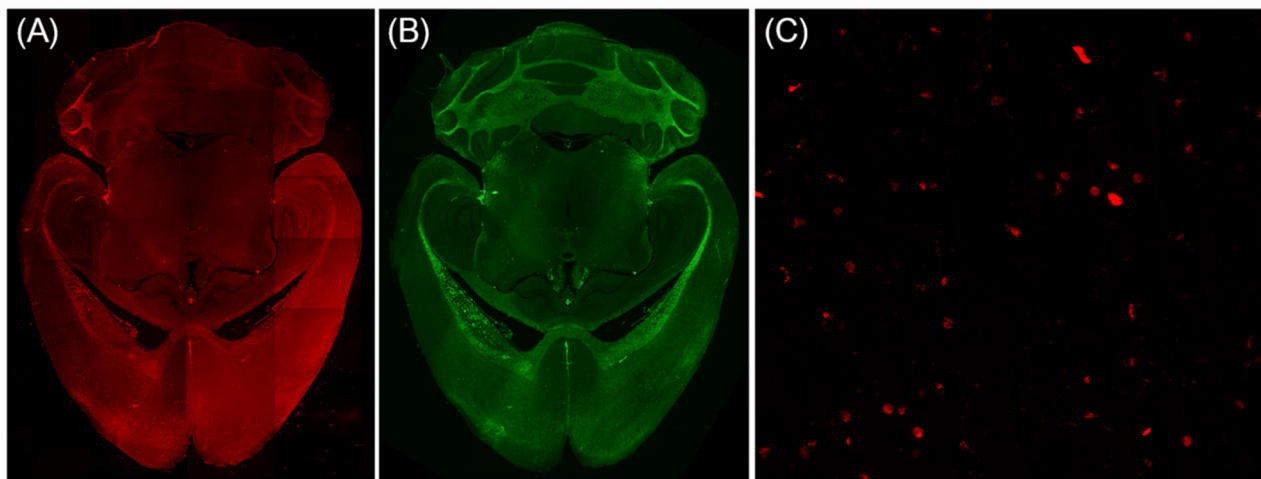


Figure S12. *In vitro* fluorescent images of **DADNIR-2**. (A) WT mice (C57BL6, 22-month old, male), 5 \times ; (B) Counterstaining using ThS, 5 \times ; (C) healthy control (temporal lobe, 68-year-old, male), 20 \times . RFP filter was used for **DADNIR-2**, and GFP filter for ThS.

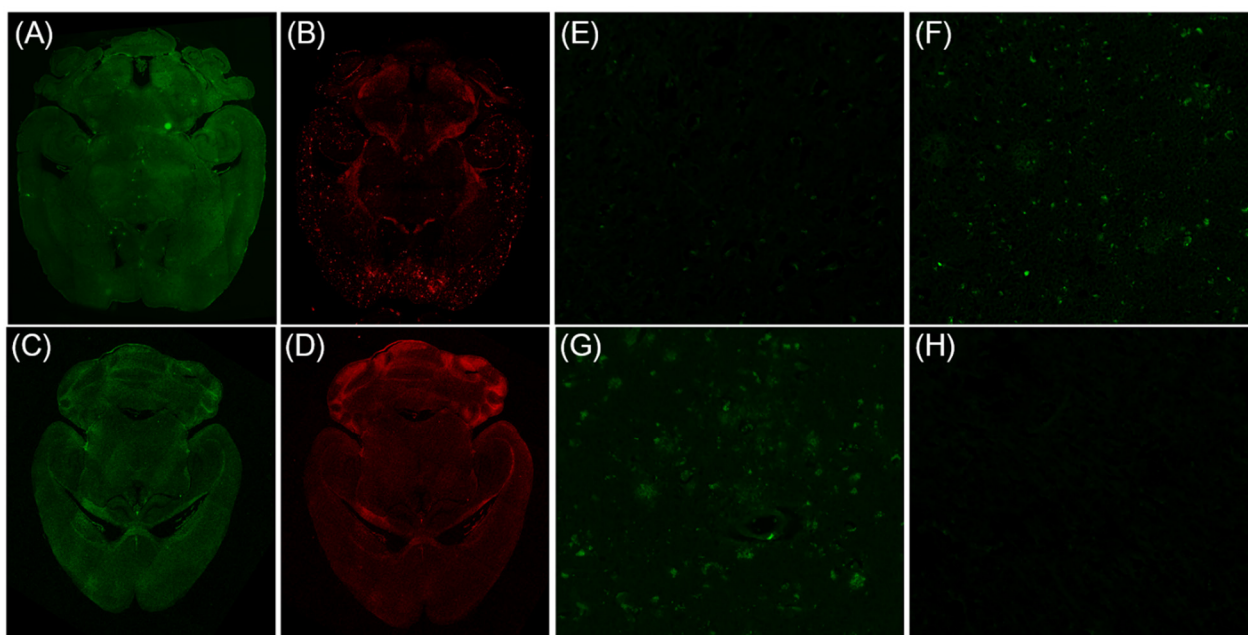


Figure S13. *In vitro* fluorescent images of **DADNIR-1**. (A) Tg mice (C57BL6, APPsw/PSEN1, 22-month old, male), 5 \times ; (C) WT mice (C57BL6, 22-month old, male), 5 \times ; Counterstaining using **DANIR 3b** (B and D, 5 \times); (E) AD patient (temporal lobe, 64-year-old, female), 20 \times ; (F) AD patient (temporal lobe, 91-year-old, male), 20 \times ; (G) AD patient (entorhinal cortex, 85-year-old, male), 20 \times ; (H) healthy control (temporal lobe, 68-year-old, male), 20 \times . RFP filter was used for **DANIR 3b**, and GFP filter for **DADNIR-1**.

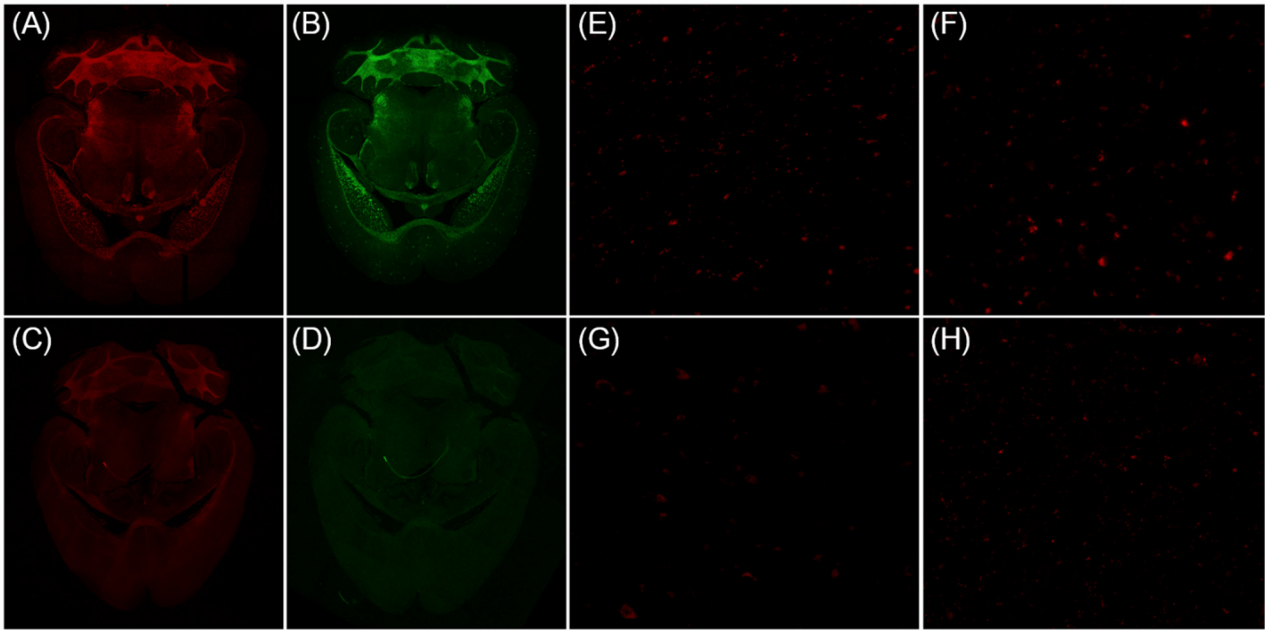


Figure S14. *In vitro* fluorescent images of **DADNIR-3**. (A) Tg mice (C57BL6, APPsw/PSEN1, 22-month old, male), 5×; (C) WT mice (C57BL6, 22-month old, male), 5×; Counterstaining using ThS (B and D, 5×); (E) AD patient (temporal lobe, 64-year-old, female), 20×; (F) AD patient (temporal lobe, 91-year-old, male), 20×; (G) AD patient (entorhinal cortex, 85-year-old, male), 20×; (H) healthy control (temporal lobe, 68-year-old, male), 20×. RFP filter was used for **DADNIR-3**, and GFP filter for ThS.

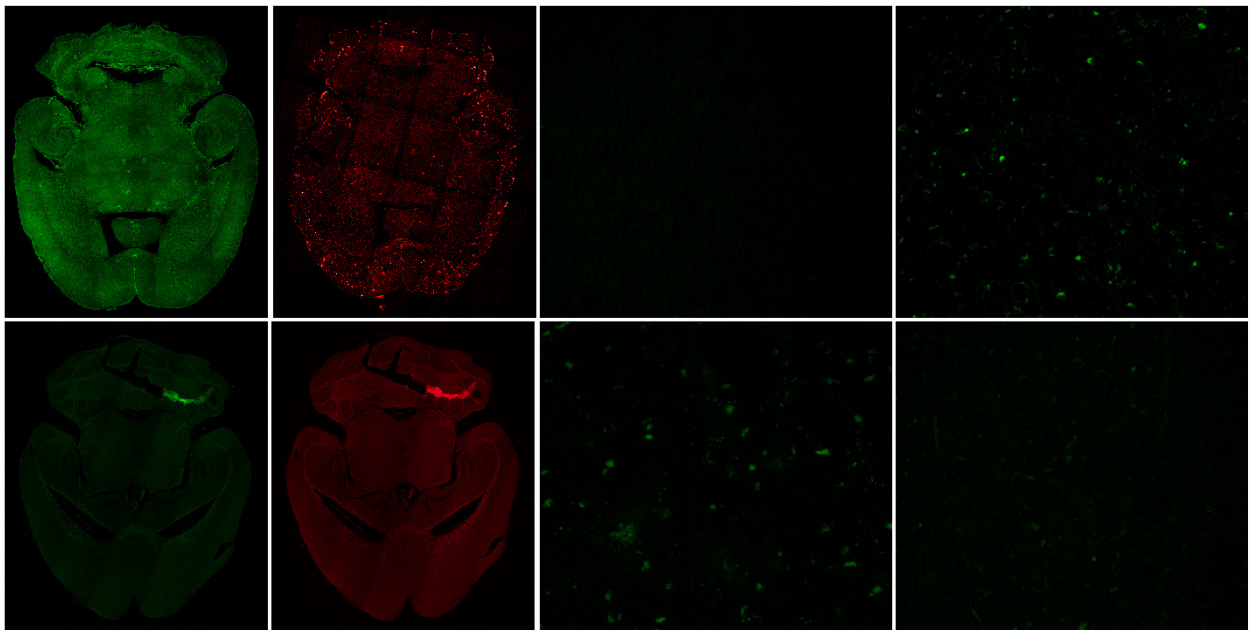


Figure S15. *In vitro* fluorescent images of **DADNIR-4**. (A) Tg mice (C57BL6, APPsw/PSEN1, 22-month old, male), 5×; (C) WT mice (C57BL6, 22-month old, male), 5×; Counterstaining using **DANIR 3b** (B and D, 5×); (E) AD patient (temporal lobe, 64-year-old, female), 20×; (F) AD patient (temporal lobe, 91-year-old, male), 20×; (G) AD patient (entorhinal cortex, 85-year-old, male), 20×; (H) healthy control (temporal lobe, 68-year-old, male), 20×. RFP filter was used for **DANIR 3b**, and GFP filter for **DADNIR-4**.

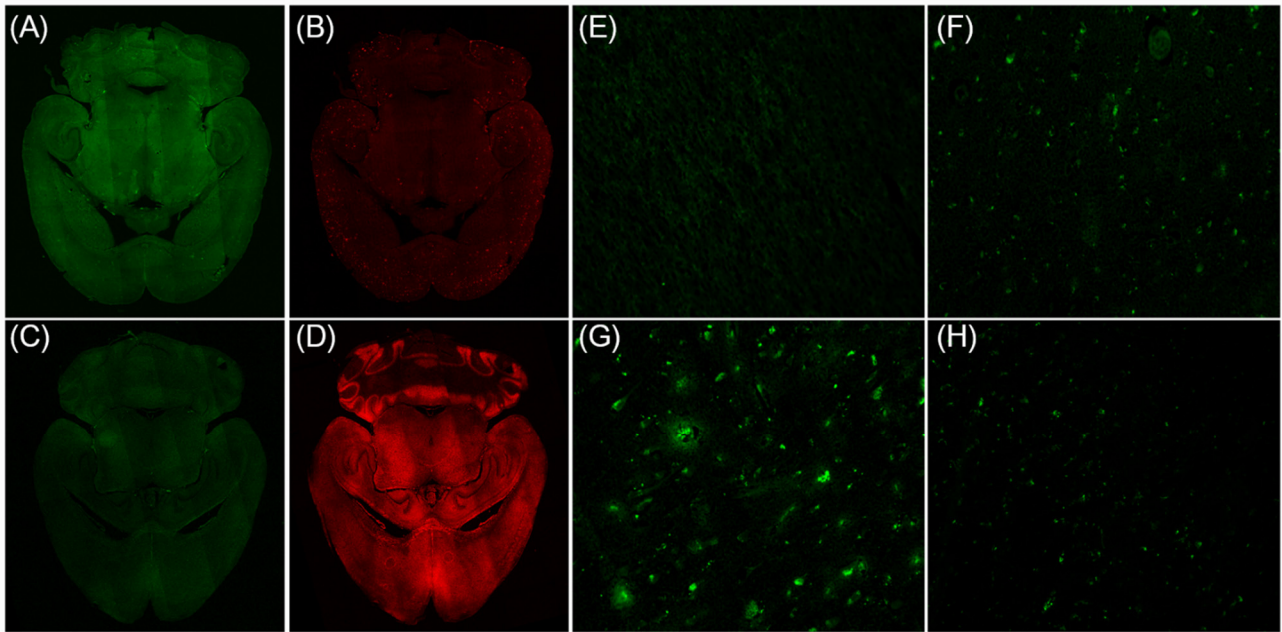


Figure S16. *In vitro* fluorescent images of **DADNIR-5**. (A) Tg mice (C57BL6, APPsw/PSEN1, 22-month old, male), 5×; (C) WT mice (C57BL6, 22-month old, male), 5×; Counterstaining using **DANIR 3b** (B and D, 5×); (E) AD patient (temporal lobe, 64-year-old, female), 20×; (F) AD patient (temporal lobe, 91-year-old, male), 20×; (G) AD patient (entorhinal cortex, 85-year-old, male), 20×; (H) healthy control (temporal lobe, 68-year-old, male), 20×. RFP filter was used for **DANIR 3b**, and GFP filter for **DADNIR-5**.

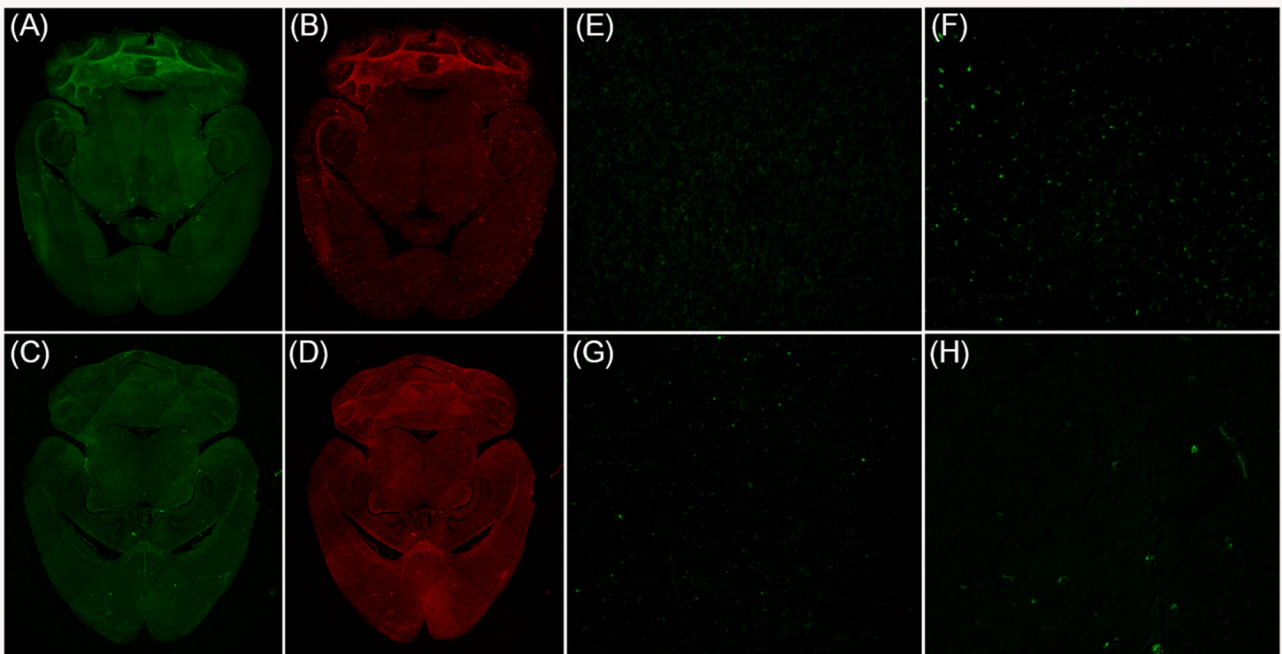


Figure S17. *In vitro* fluorescent images of **DADNIR-6**. (A) Tg mice (C57BL6, APPsw/PSEN1, 22-month old, male), 5×; (C) WT mice (C57BL6, 22-month old, male), 5×; Counterstaining using **DANIR 3b** (B and D, 5×); (E) AD patient (temporal lobe, 64-year-old, female), 20×; (F) AD patient (temporal lobe, 91-year-old, male), 20×; (G) AD patient (entorhinal cortex, 85-year-old, male), 20×; (H) healthy control (temporal lobe, 68-year-old, male), 20×. RFP filter was used for **DANIR 3b**, and GFP filter for **DADNIR-6**.

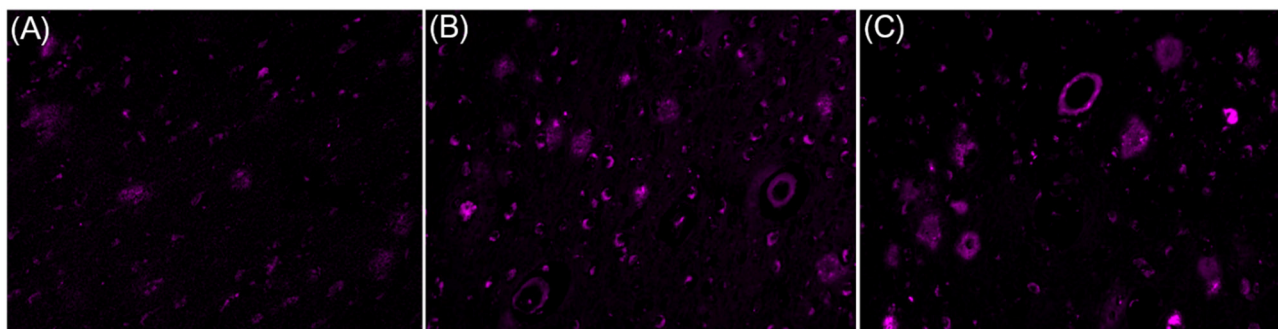


Figure S18. *In vitro* fluorescent images of **BAP-1** (A) on brain section of AD patient (entorhinal cortex, 85-year-old, male), 20 \times ; **TAU 1** (B) and **TAU 2** (C) on brain section of AD patient (temporal lobe, 64-year-old, female), 20 \times . Cy5 filter was used for these probes.

Immunofluorescent staining

Immunofluorescent staining were conducted using the brain section of AD patient (entorhinal cortex, 85-year-old, male). All steps were fulfilled in the moist cassette at room temperature unless otherwise mentioned. Deparaffinization procedures according the methods described before were completed. The antigen of slices were retrieved at 95 °C in 0.01 M sodium citrate-hydrochloric acid buffer (pH = 6.0) for 10 min. After cooling to room temperature, the section was washed by PBS (pH = 7.4, 3 \times 5 min). Next, the antigen was blocked by incubating the sections in 3% BSA for 2 h. After removing blocking buffer by PBS (pH = 7.4, 3 \times 5 min), the slices were incubated at 4 °C with primary antibodies (AT8 (Cat. No. MN1020), 5 μ g/mL, diluted in PBS) for 20 h. The antibody was washed by PBS (pH = 7.4, 3 \times 5 min) after rewarming 1 h. Alexa Fluor[®] 488-conjugated secondary antibody (donkey anti-mouse IgG (Cat. No. ab150105), 4 μ g/mL, diluted in PBS) were incubated in a dark cassette for 2 h, and washed by PBS (pH = 7.4, 3 \times 5 min). After immunofluorescent staining, the same section was stained by **DADNIR-2** (1 μ M, 50% ethanol) for 5 min and washed by ethanol and H₂O. The images were captured by using confocal microscopy, and the FITC-filter for Alexa Fluor[®] 488-conjugated secondary antibody while TRITC filter for **DADNIR-2**.

BBB penetrating experiment of DADNIR-2

The quantitative determination of the brain uptake was measured by the previously reported methods³. The samples were analyzed by HPLC with an isocratic system (10% H₂O and 90% CH₃CN) at flow rate of 1.0 mL/min. The UV detector is 530 nm. The data were quantitated by analyzing retention time and peak area, and the brain uptakes (brain_{2min}) were expressed by % injected dose per gram (% ID/g). % ID/g was measured in triplicate with results given as the mean ± SD³.

Cytotoxicity study of DADNIR-2

The cytotoxicity study of **DADNIR-2** was performed by MTT assay using a human neuronal cell line (SH-SY5Y) at different concentrations as previous reported⁶. SH-SY5Y cells were seeded into a 96-well plate and incubated at 37 °C in incubator with 5% CO₂. Then the cells were treated with at different concentrations of **DADNIR-2** (0.05, 0.1, 0.5 and 1 μM) and PBS control for 1 h incubation and then exposed to a laser of an IVIS Lumina III system (excitation: 652 nm, exposure time: 10s) or not. After further incubation for 23 h, MTT solution (5 mg/mL) was added, and the cells were incubated for another 4 h. Next, removed the culture media by centrifuge. Added 100 μL DMSO to dissolve the purple crystal. The absorbance of each well was measured at 570 nm using 1420 Multiabel counter for assessing the viability of living cells.

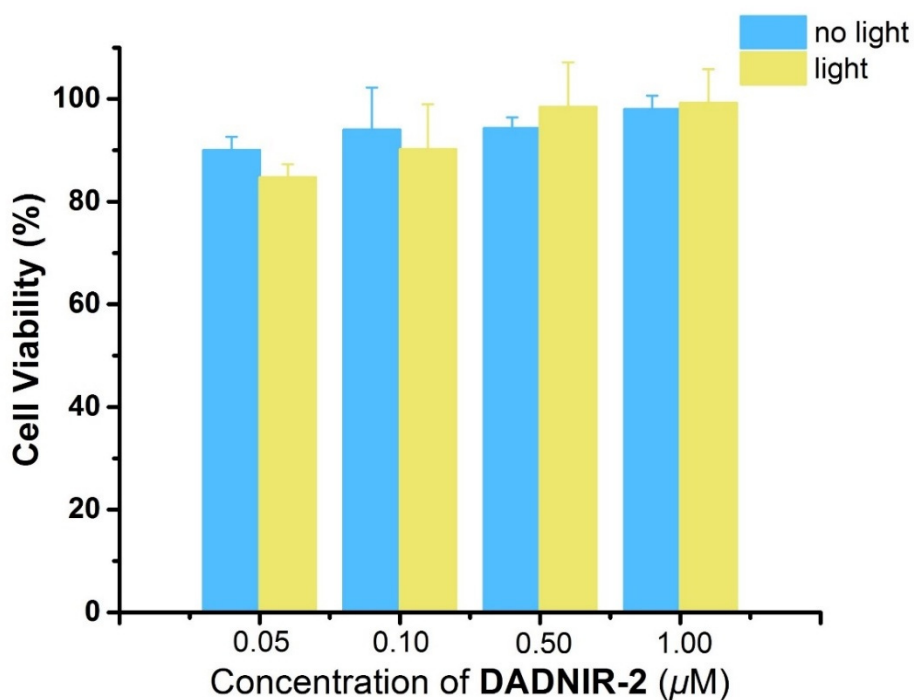


Figure S19. Cell viability after incubation of **DADNIR-2** at different concentrations (each sample was tested in triplicate with results given as the mean \pm SD).

Bio-distribution of DADNIR-2 in nude mice

The ICR mice (3-week-old, male) were intravenously injected with **DADNIR-2** (0.40 mg/kg, 20% DMSO, 80% 1,2-propylene glycol, 100 μ L). The mice were sacrificed after 2 min, 10 min, 30min and 60 min, respectively. Next, the organs were excised and the fluorescent signals were captured by IVIS Lumina III system.

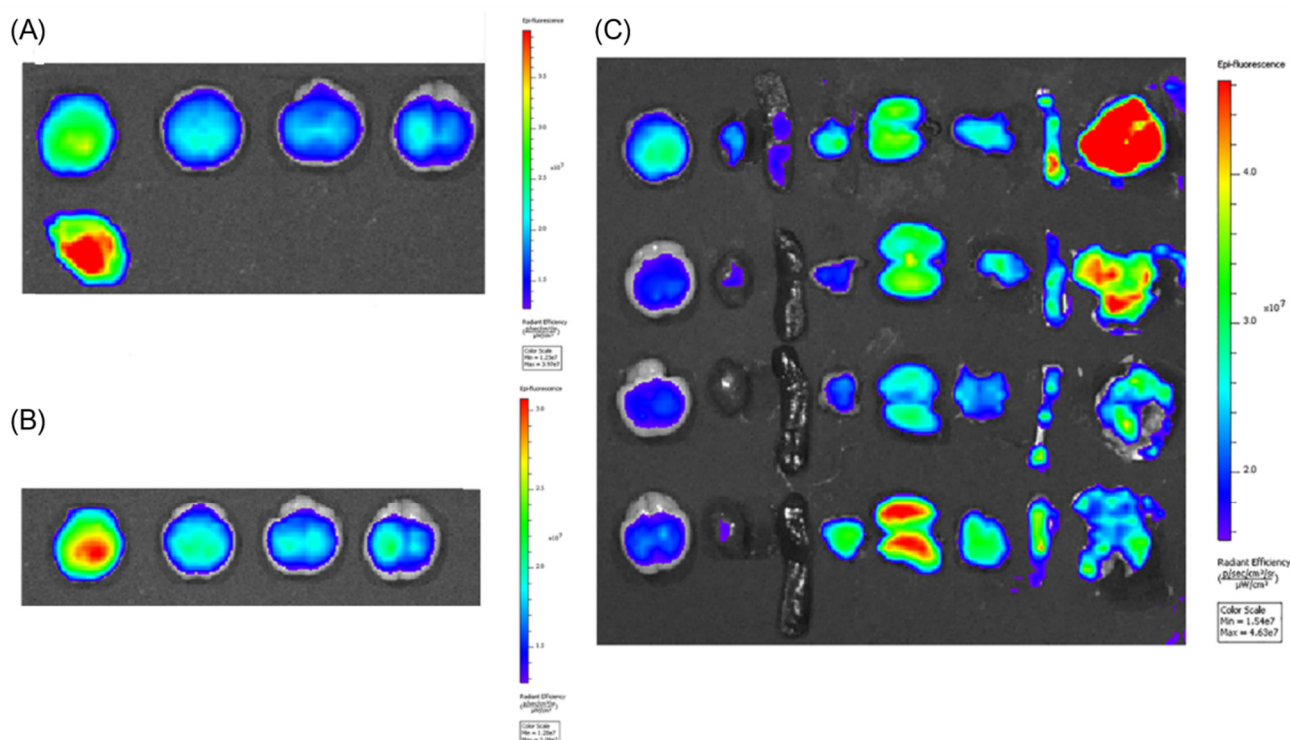
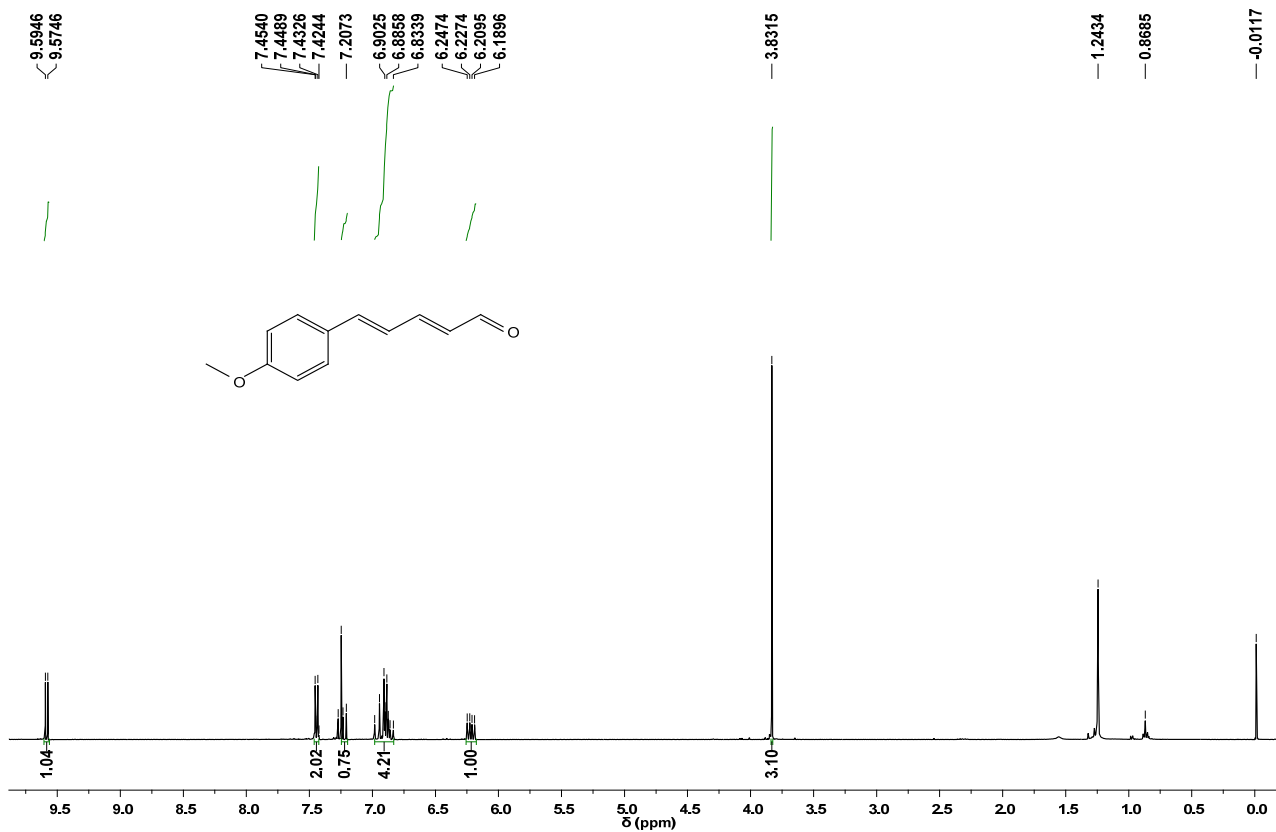


Figure S20. Bio-distribution of **DADNIR-2** in ICR mice. (A) The fluorescent signals of brain in 2 min, 10 min, 30 min and 60 min (top row, successively), and scalp (bottom row). (B) The fluorescent signals of brain in different time points as (A). (C) The fluorescent signals of of organs in different time points as (A) (brain, heart, spleen, pancreas, kidney, lung, bone and liver were arranged in turn).

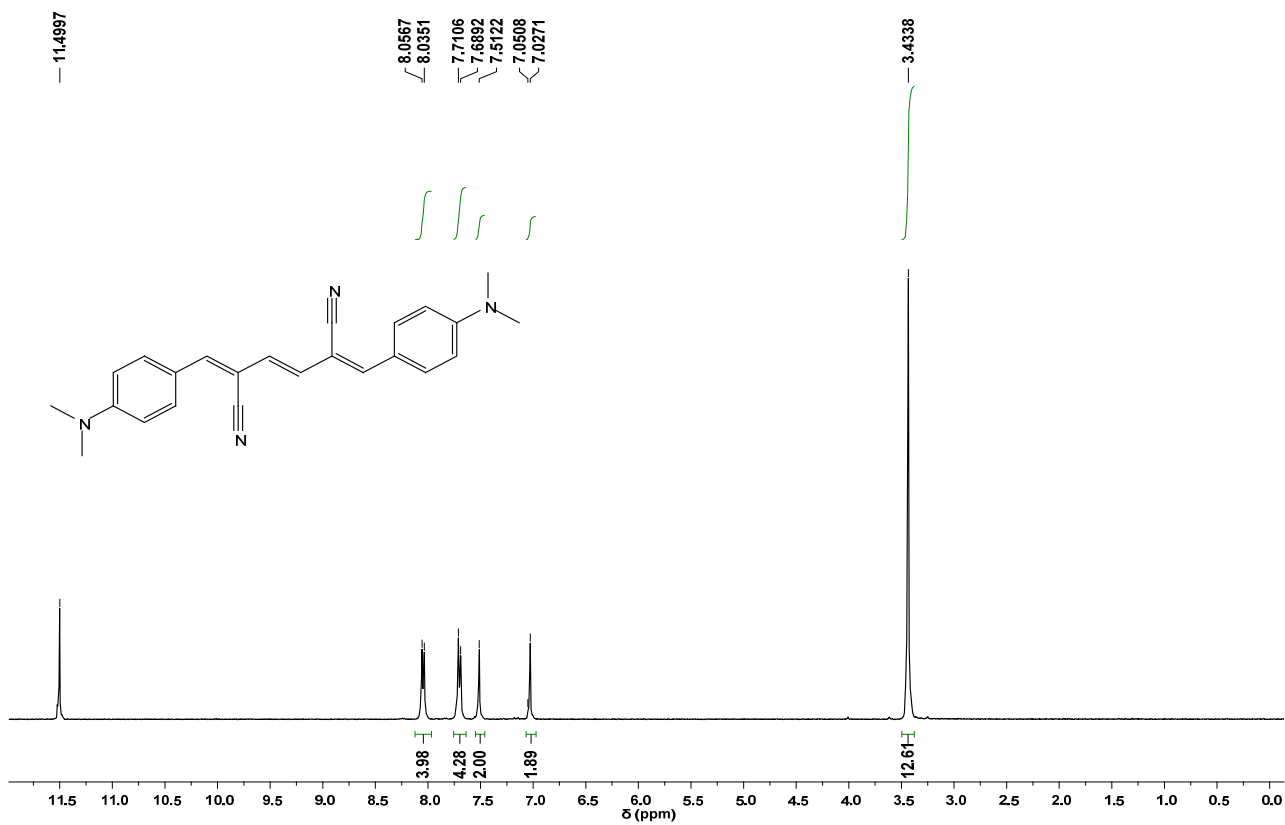
***Ex vivo* fluorescent staining of DADNIR-2**

The transgenic mice (C57BL6, APP^{sw}/PSEN1, 24-month-old, male) and wild-type mice (C57BL6, 24-month-old, male) were intravenously injected with **DADNIR-2** (0.32 mg/kg, 20% DMSO, 80% 1,2-propylene glycol, 150 μ L). The mice were sacrificed after 10 min, and then the brain were excised and embedded in optimum cutting temperature (O.C.T., tissue freezing medium). Frozen sections (15 μ m) were obtained by freezing microtome, and fluorescence images were observed by fluorescence microscope using Cy5 filter sets. Furthermore, the A β plaques were confirmed by the staining with ThS on the same sections using GFP filter sets.

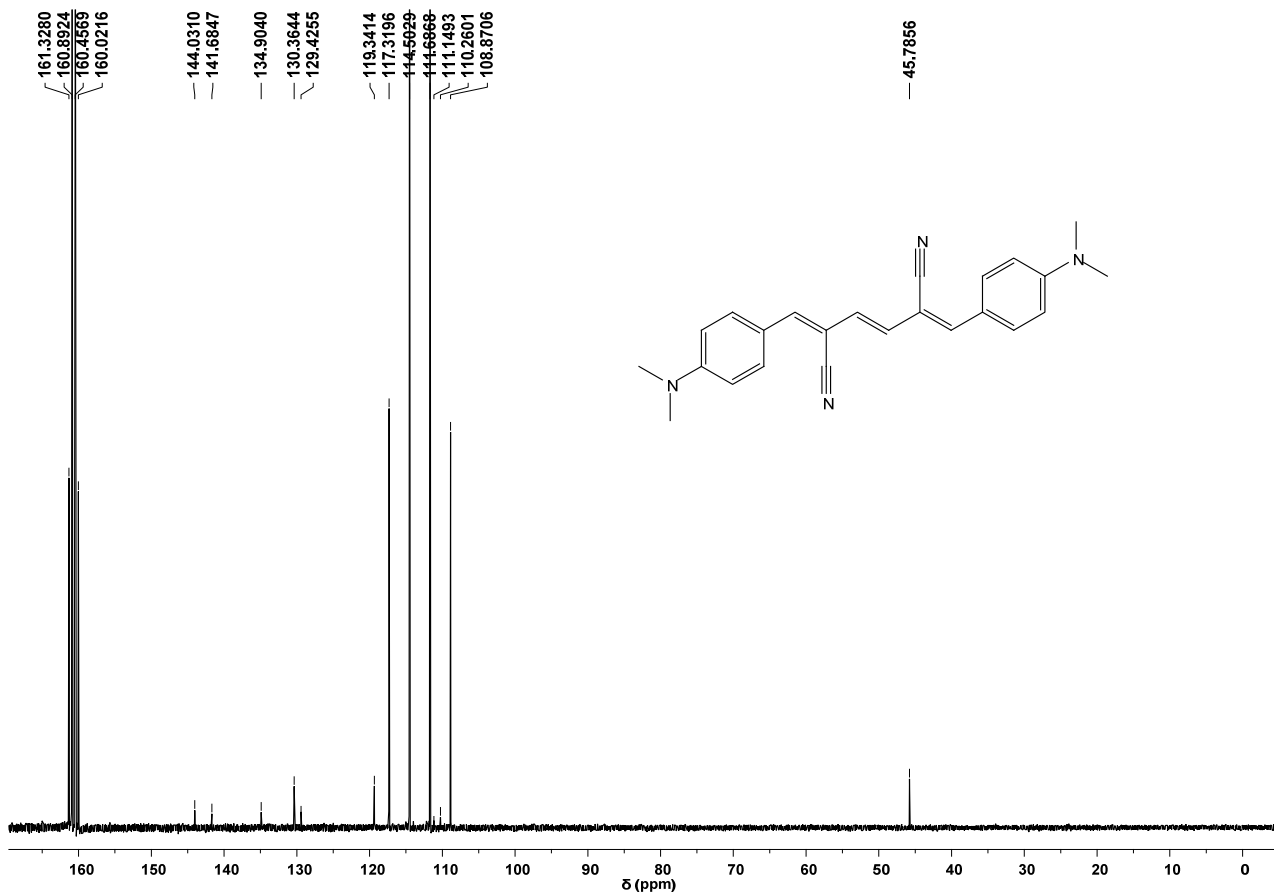
NMR and HRMS spectra of compounds



¹H NMR spectrum of compounds 6



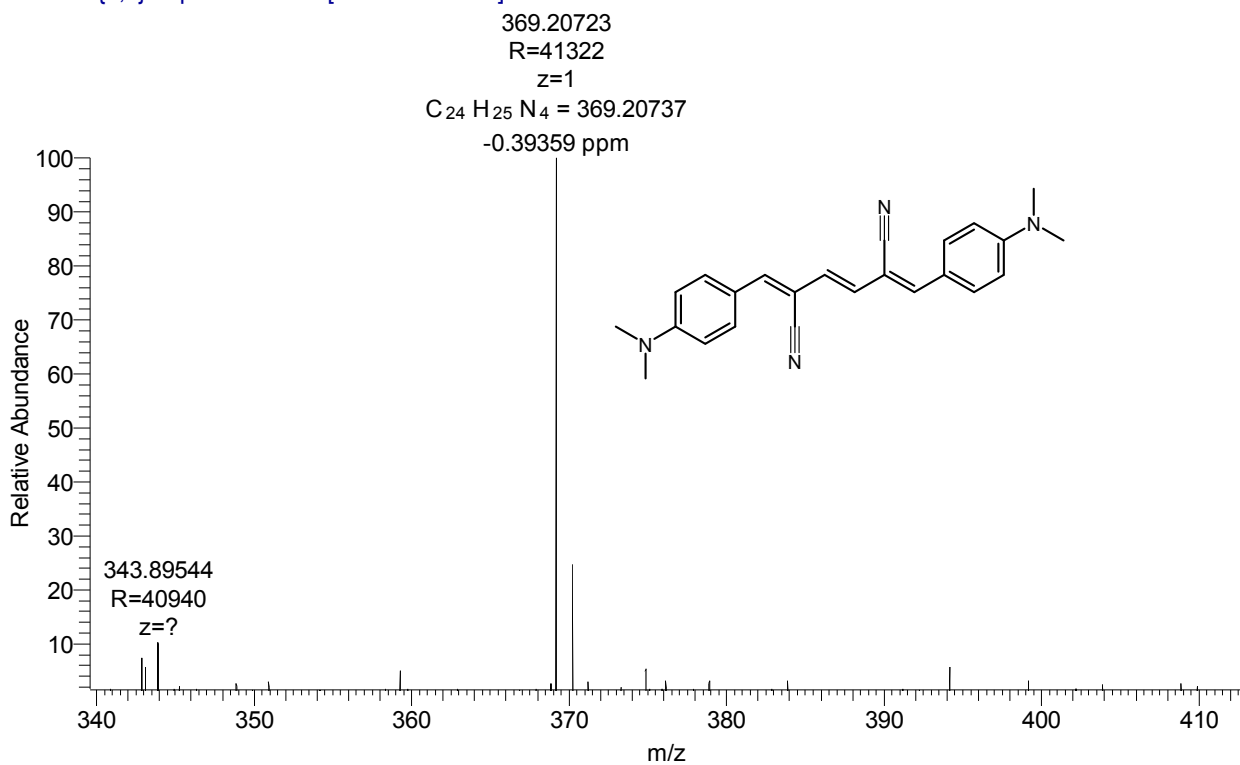
¹H NMR spectrum of DADNIR-1



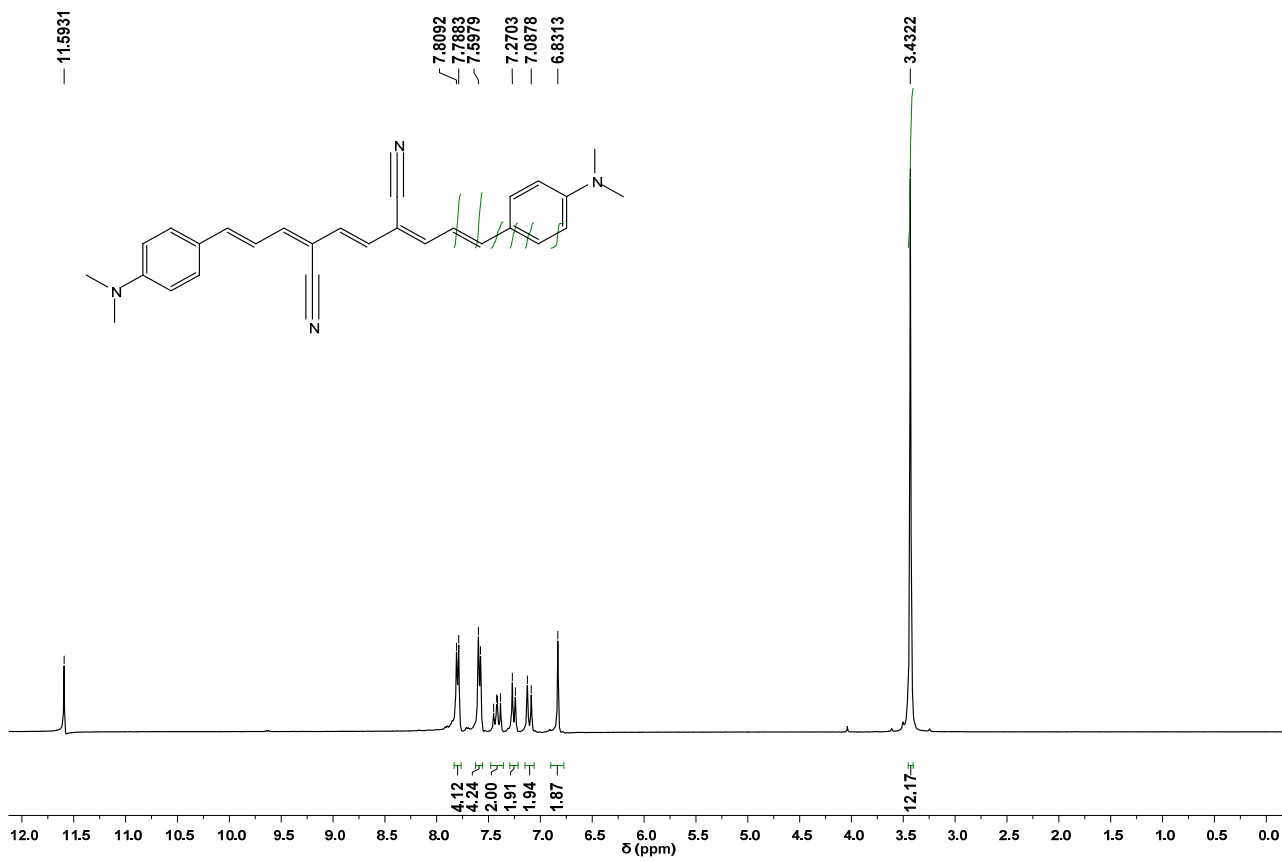
¹³C NMR spectrum of **DADNIR-1**

LYY-20170701-PH1 #33-36 RT: 0.37-0.41 AV: 4 NL: 1.99E4

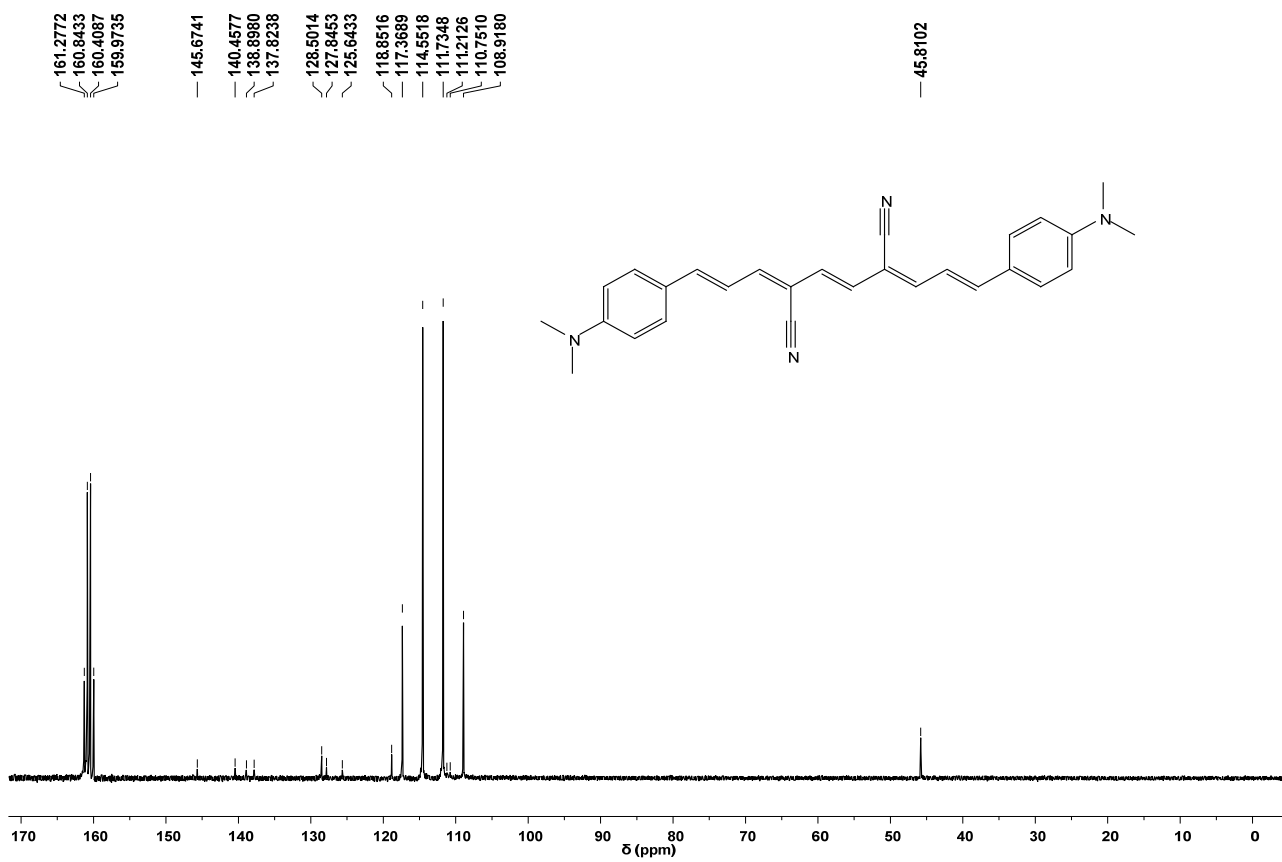
T: FTMS {1,1} + p ESI Full ms [100.00-1000.00]



HRMS spectrum of **DADNIR-1**



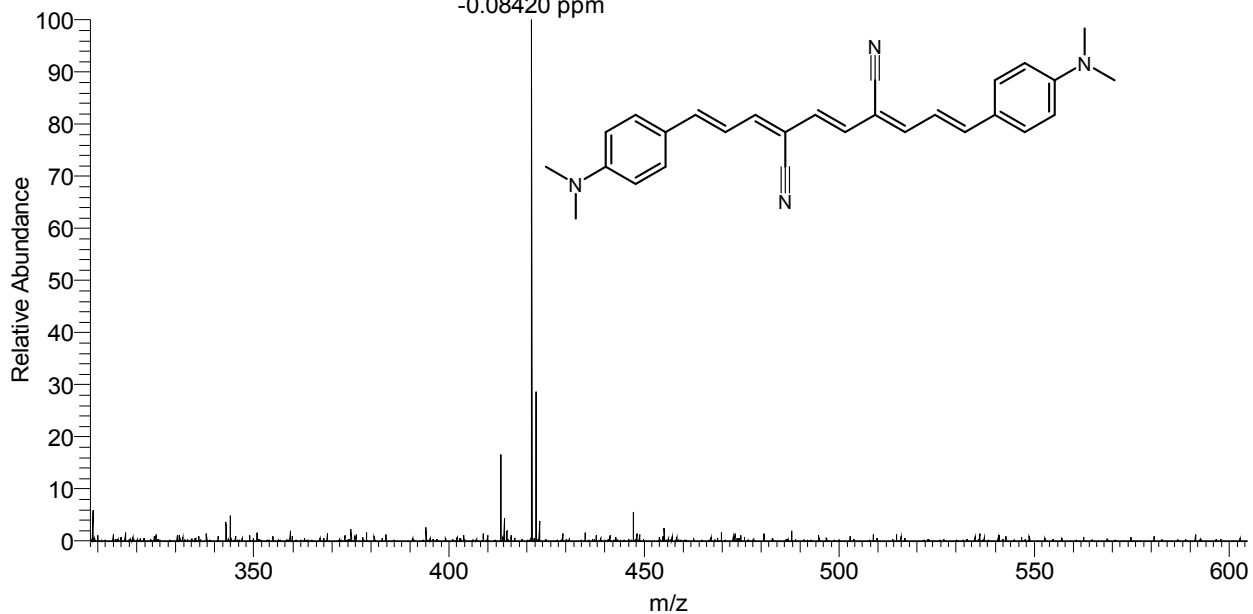
¹H NMR spectrum of DADNIR-2



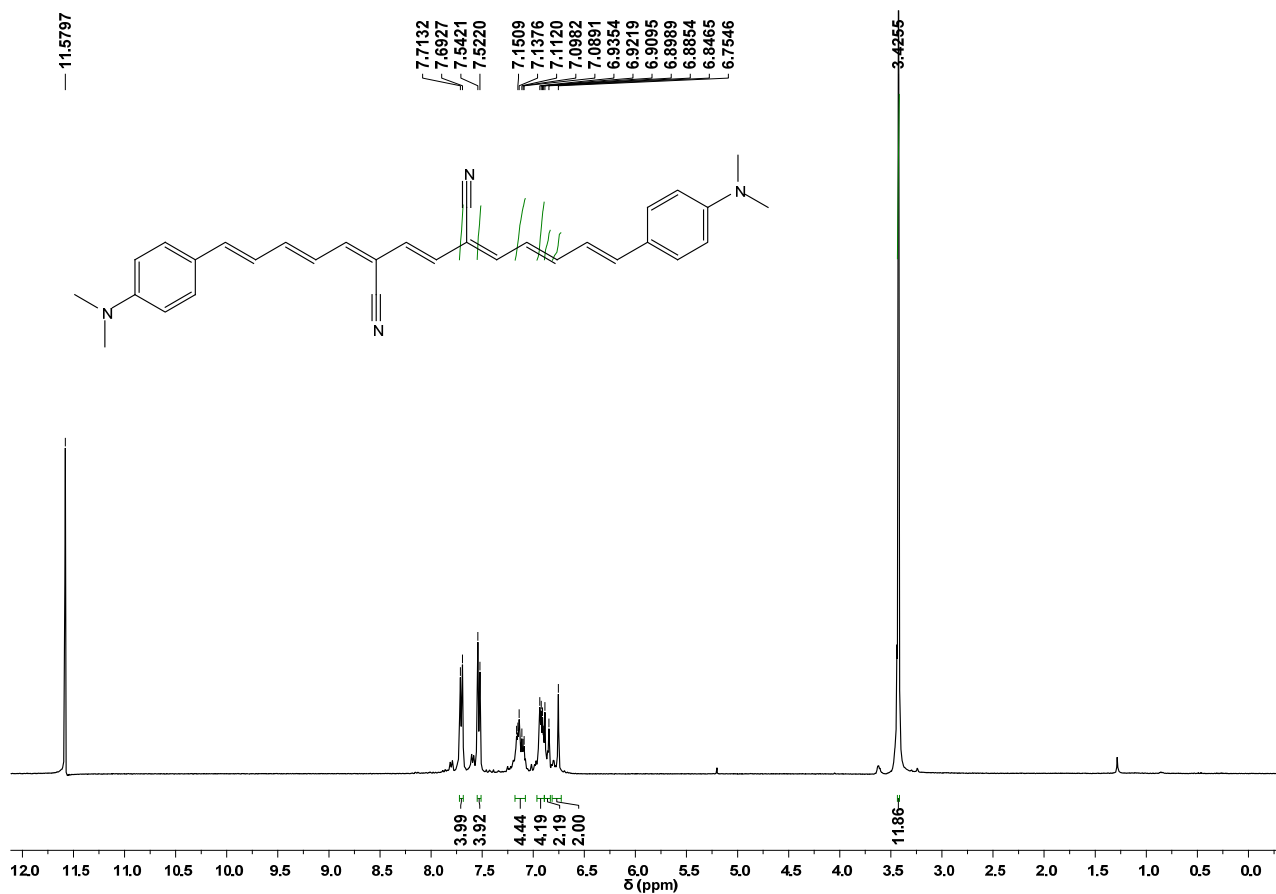
¹³C NMR spectrum of DADNIR-2

LYY-20170701-PH2 #38-41 RT: 0.43-0.47 AV: 4 NL: 4.17E4
T: FTMS {1,1} + p ESI Full ms [100.00-1000.00]

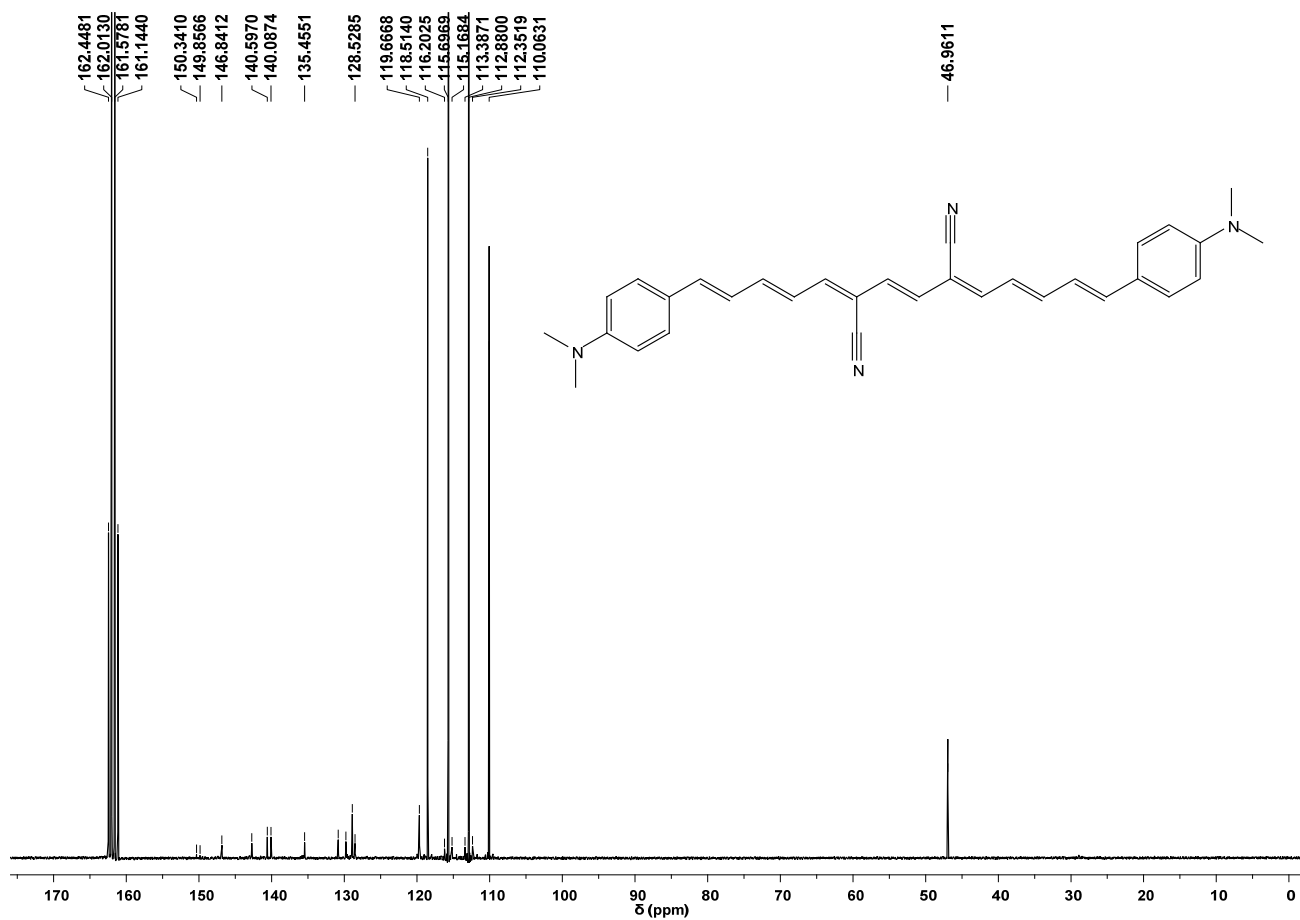
421.23864
R=39184
Z=1
C₂₈ H₂₉ N₄ = 421.23867
-0.08420 ppm



HRMS spectrum of DADNIR-2

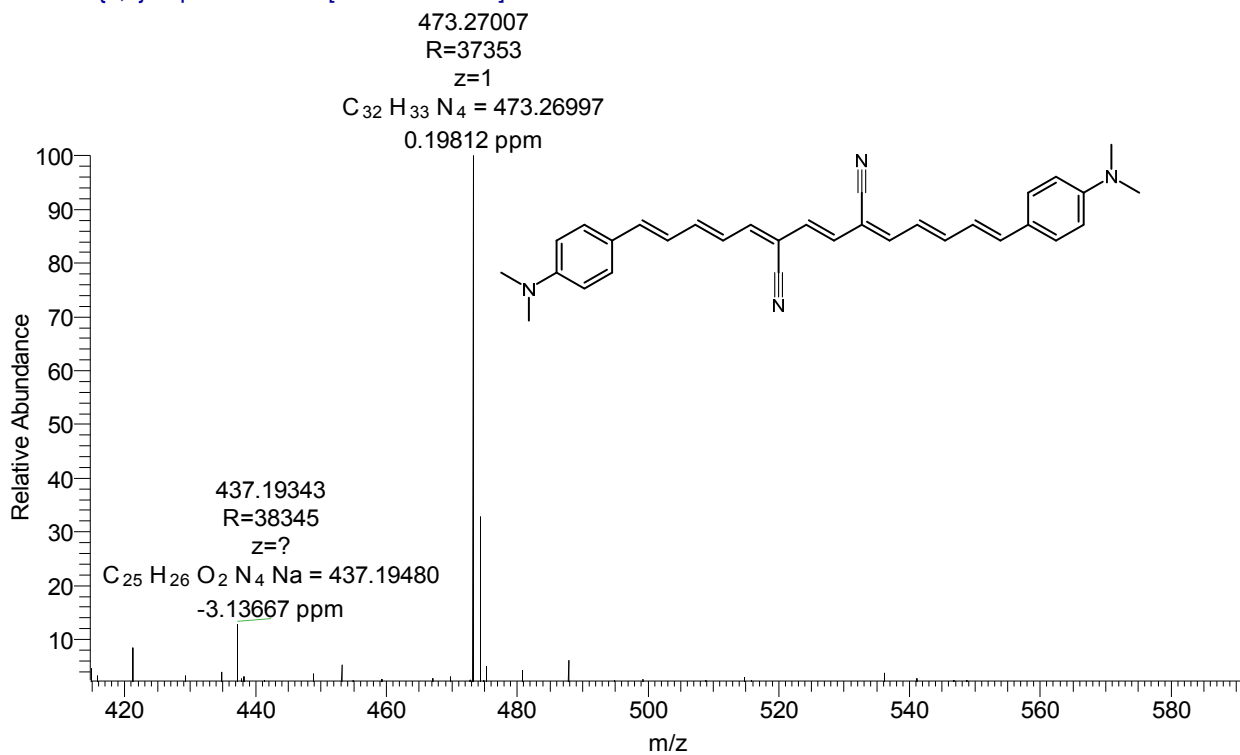


¹H NMR spectrum of DADNIR-3

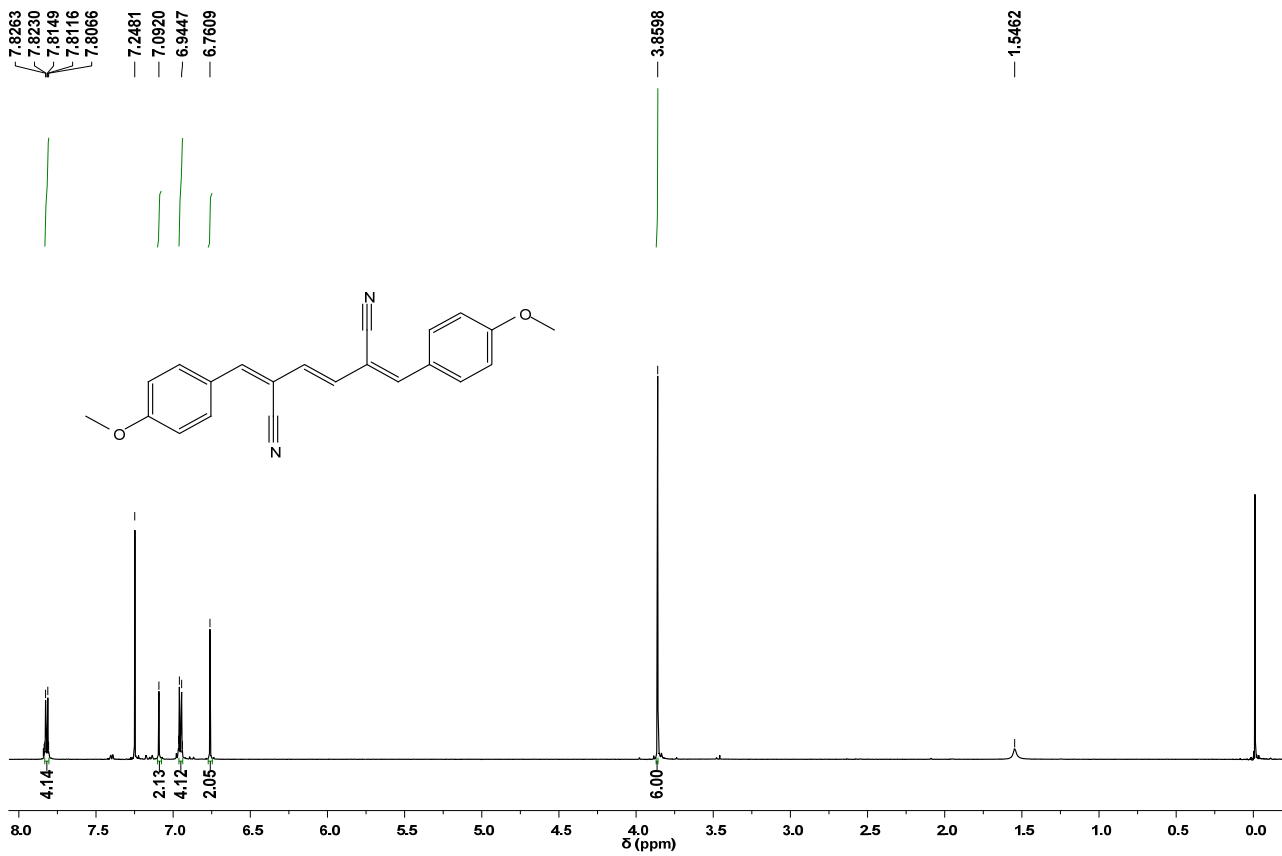


¹³C NMR spectrum of **DADNIR-3**

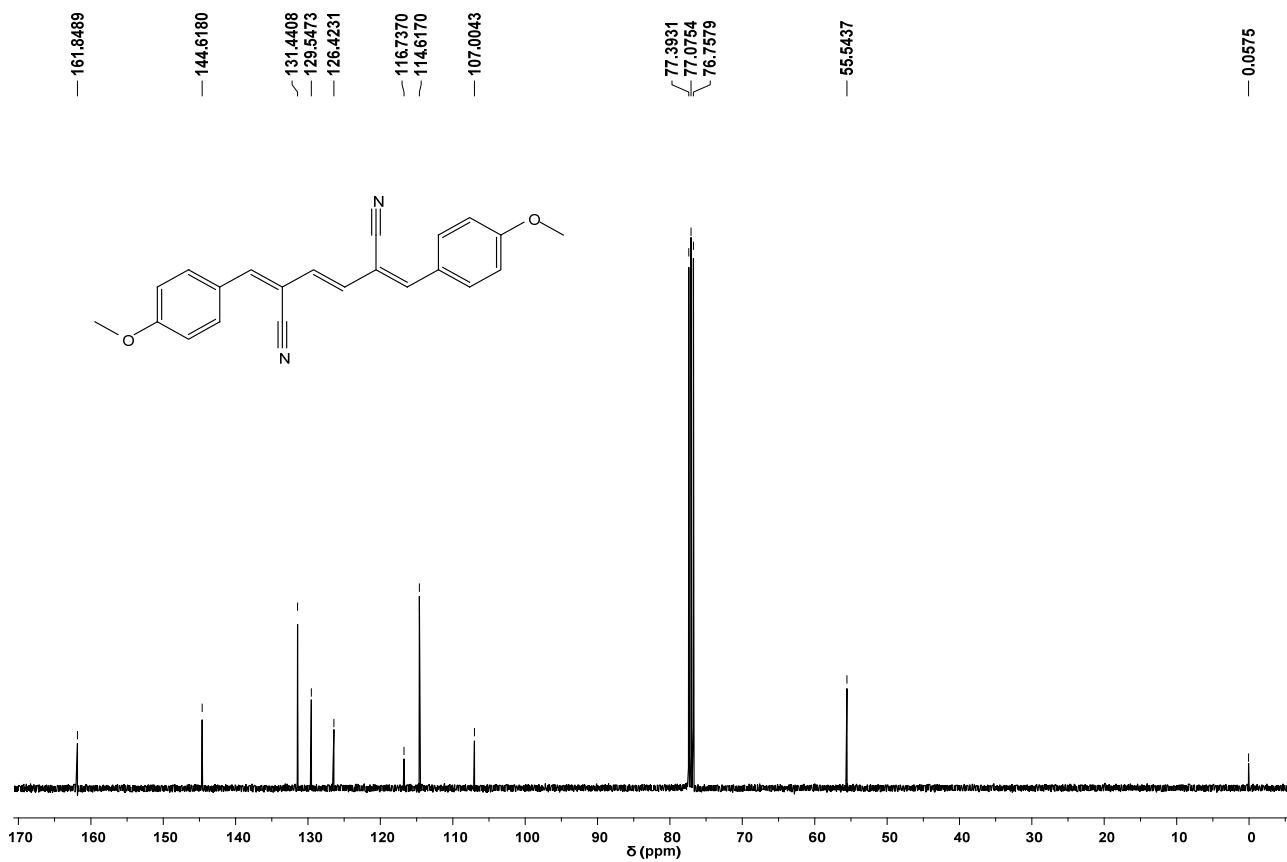
LYY-20170701-PH3 #30-32 RT: 0.34-0.36 AV: 3 NL: 1.74E4
 T: FTMS {1,1} + p ESI Full ms [100.00-1000.00]



HRMS spectrum of **DADNIR-3**

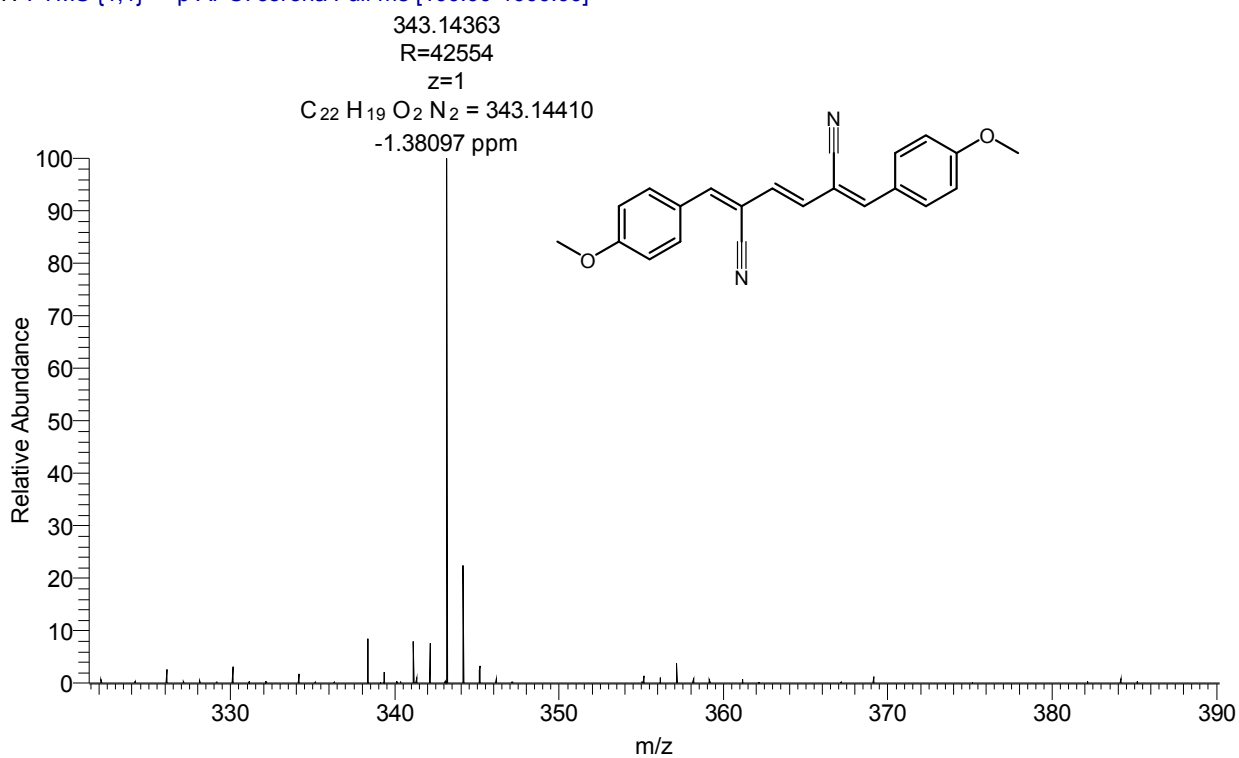


¹H NMR spectrum of DADNIR-4

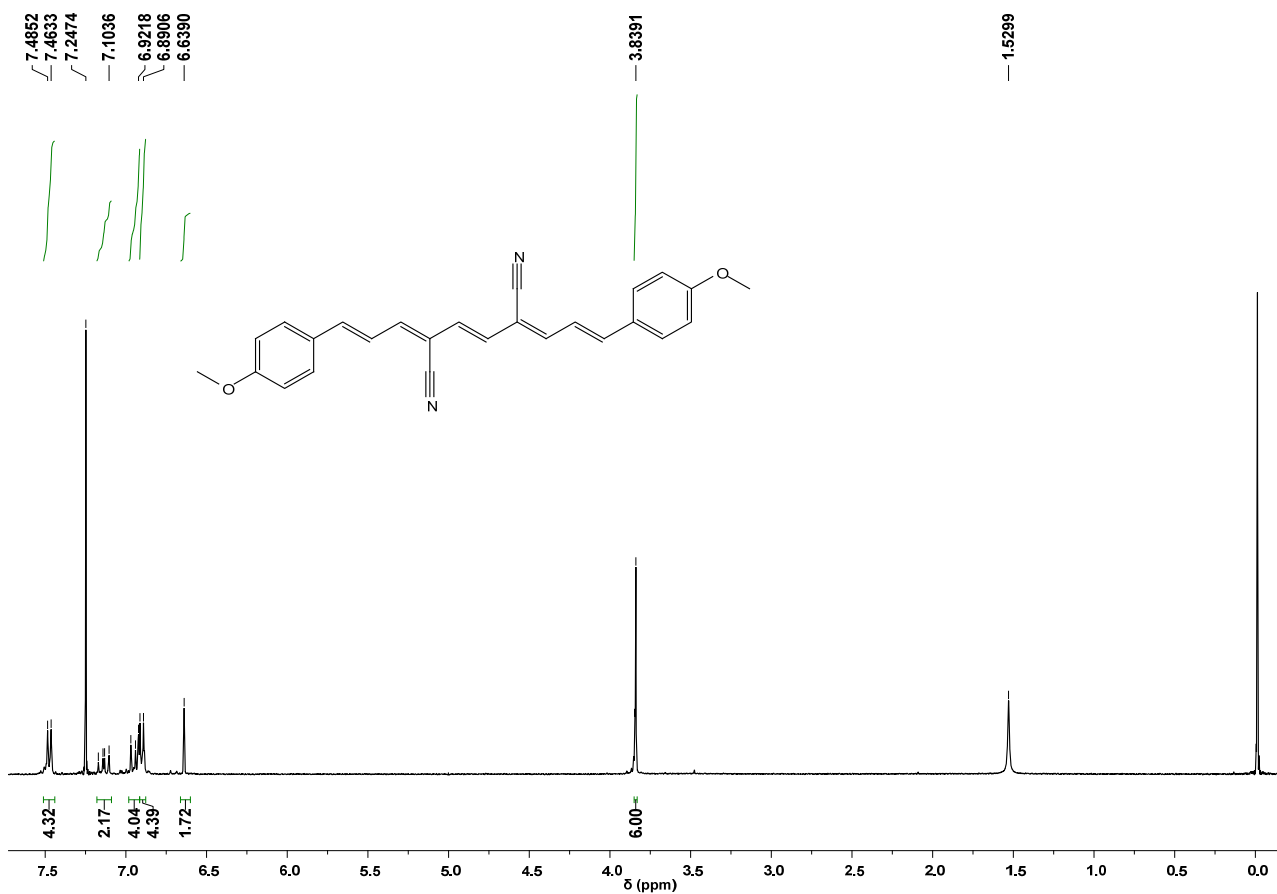


¹³C NMR spectrum of DADNIR-4

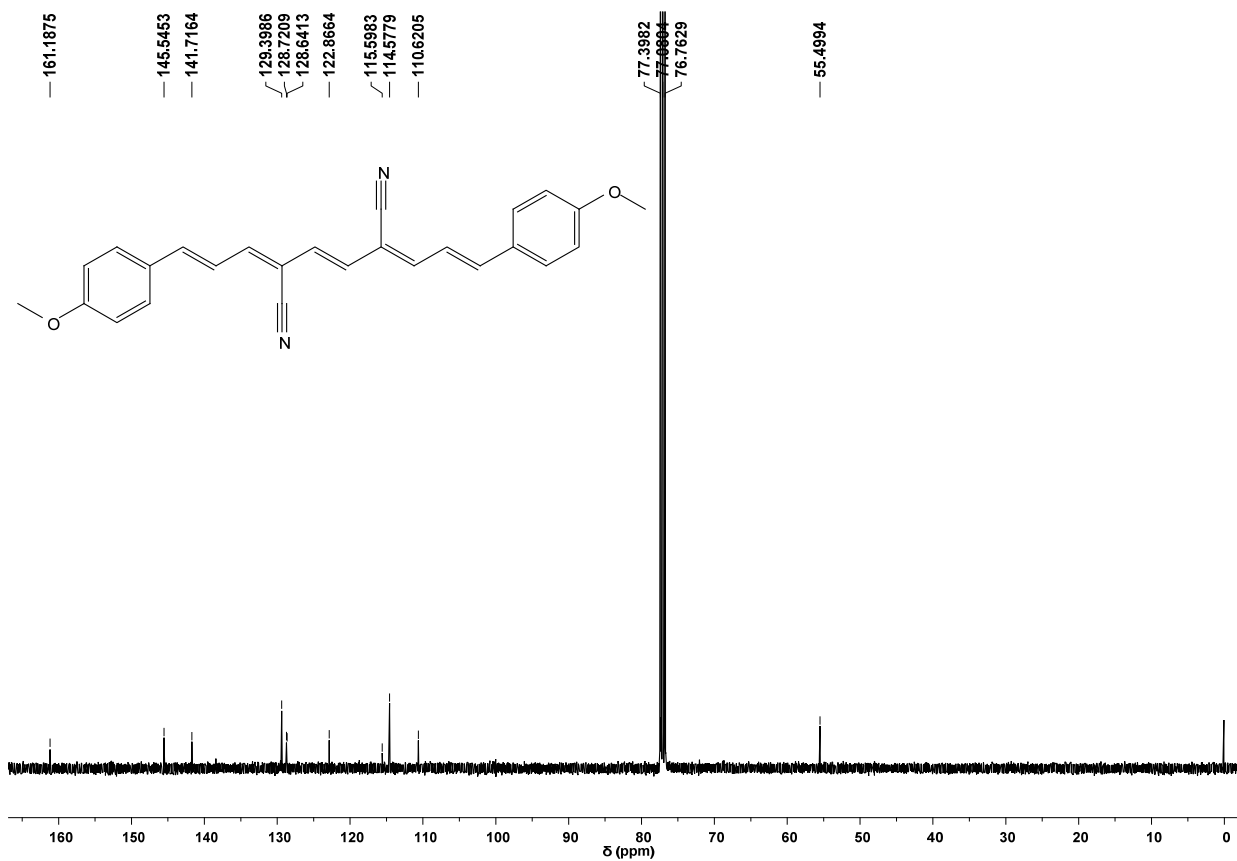
LYY-OMe-PH1 #27 RT: 0.20 AV: 1 SB: 47 0.00-0.04 , 0.69-1.01 NL: 2.02E6
T: FTMS {1,1} + p APCI corona Full ms [100.00-1000.00]



HRMS spectrum of DADNIR-4



¹H NMR spectrum of DADNIR-5



^{13}C NMR spectrum of DADNIR-5

LYY-OMe-PH2 #12 RT: 0.09 AV: 1 SB: 39 0.01-0.03, 0.72-1.02 NL: 7.64E7

T: FTMS {1,1} + p APCI corona Full ms [100.00-1000.00]

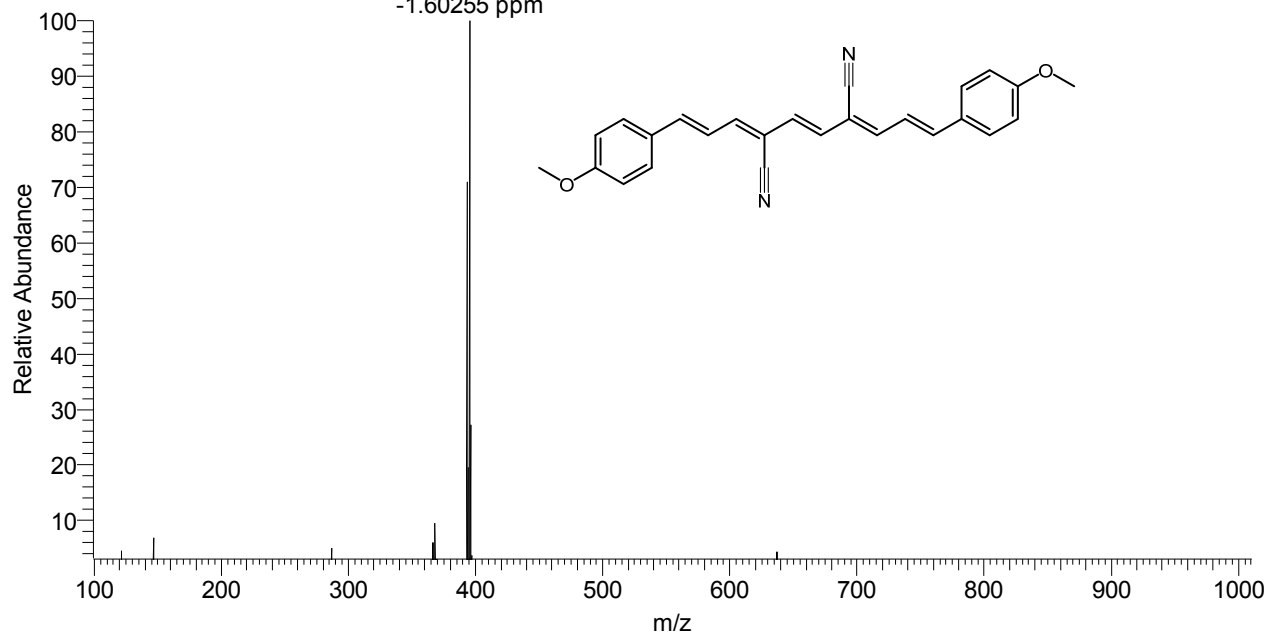
395.17477

R=41667

z=1

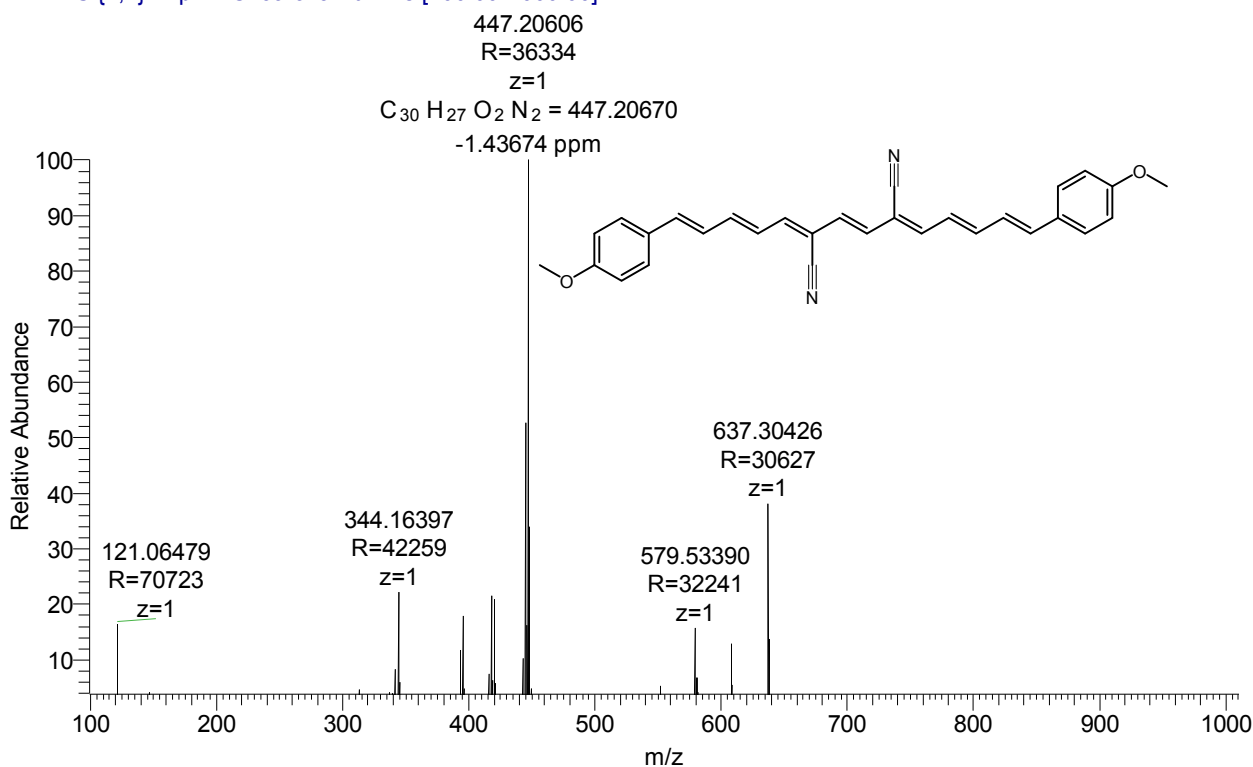
$\text{C}_{26}\text{H}_{23}\text{O}_2\text{N}_2 = 395.17540$

-1.60255 ppm



HRMS spectrum of DADNIR-5

LYY-OMe-PH3 #10 RT: 0.08 AV: 1 SB: 31 0.01-0.03 , 0.72-1.02 NL: 1.64E7
T: FTMS {1,1} + p APCI corona Full ms [100.00-1000.00]



HRMS spectrum of **DADNIR-6**

References

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- 6 M. Cui, M. Ono, H. Watanabe, H. Kimura, B. Liu and H. Saji, *J. Am. Chem. Soc.*, 2014, **136**, 3388-3394.