Electronic Supplementary Material (ESI) for Faraday Discussions.

Supporting Information for

Vibration-Induced-Emission (VIE) for imaging Amyloid β fibrils

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Contents list:

S1. Additional figures

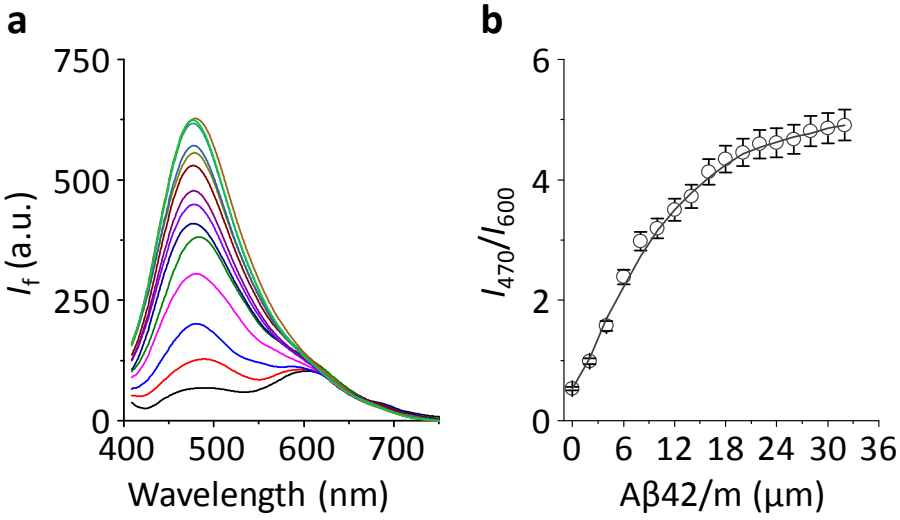


Figure S1. (a) Fluorescence ratiometric titration of **VN** (10 μM) in the presence of increasing Aβ42 peptide monomer (Aβ42/m, 0-32 μM) in Tris-HCl buffer solution (0.01 M, pH 7.4) with an excitation of 365 nm. (b) Plotting the ratiometric change of **VN** in the presence of increasing Aβ42/m.

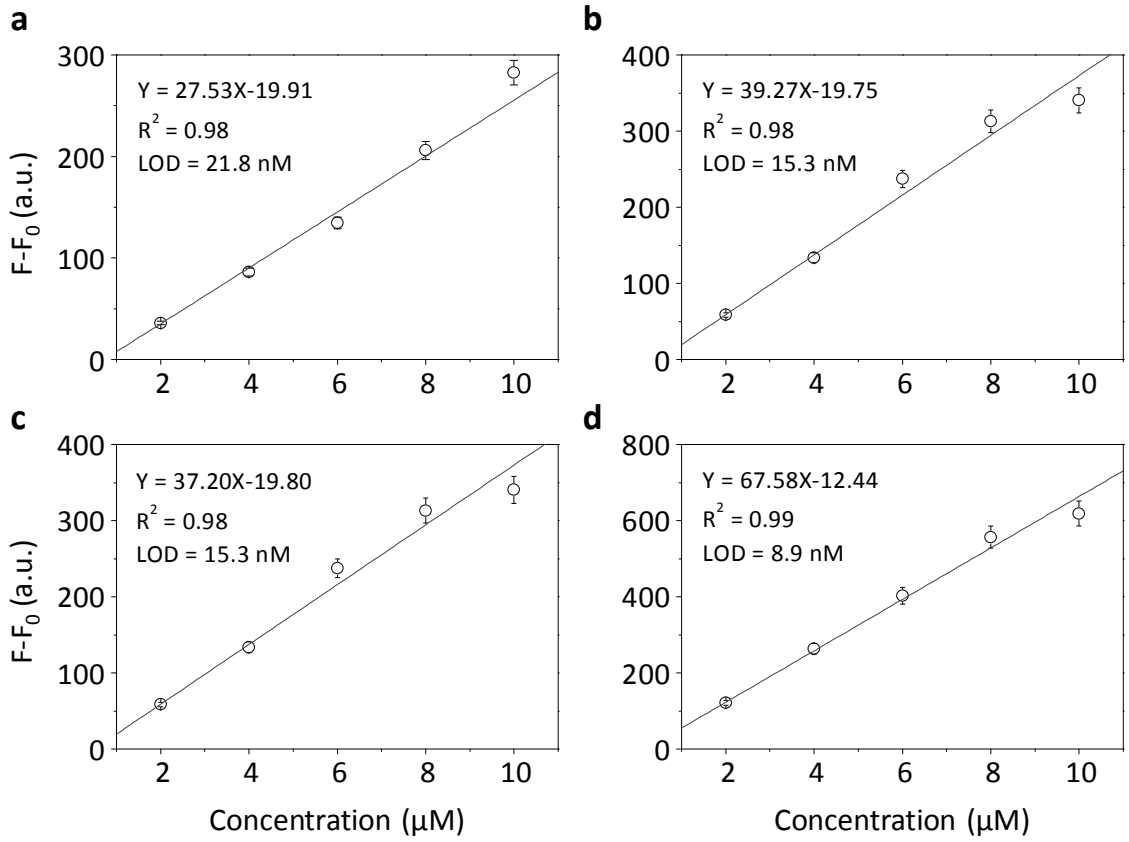


Figure S2. Plotting the fluorescence intensity change (where F and F_0 are the fluorescence intensity of **VN** at I_{470} in the presence and absence of analyte, respectively) as a function of increasing (a) Aβ40 peptide monomer, (b) Aβ40 peptide fibril, (c) Aβ42 peptide monomer and (d) Aβ42 peptide fibril in Tris-HCl buffer solution (0.01 M, pH 7.4) with an excitation of 365 nm. The limit of detection (LOD) was calculated by $3\sigma_b/k$, where σ_b is the standard deviation of blank buffer solution and k the slope of plots.

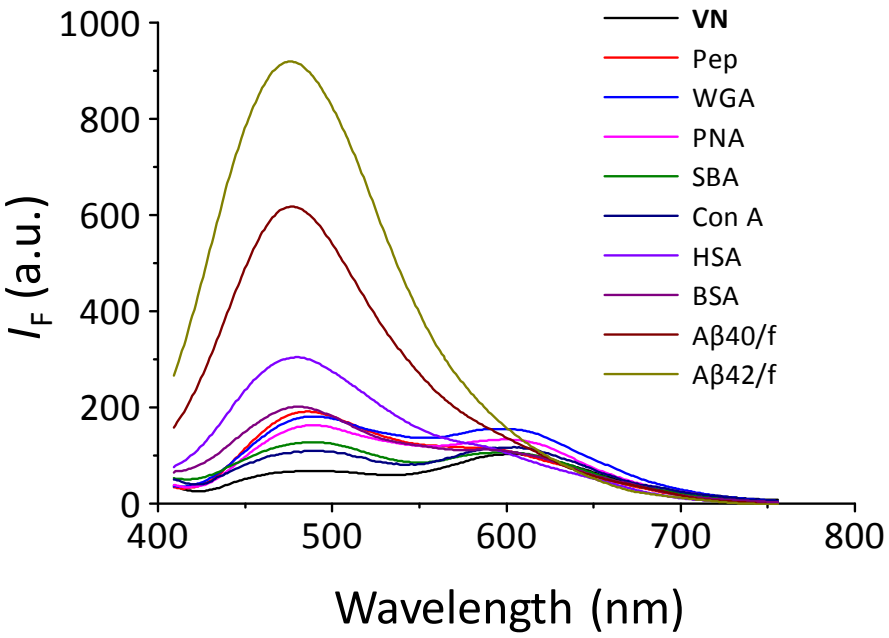


Figure S3. Stacked fluorescence spectra of **VN** (10 μM) in the absence and presence of 20 μM of different proteins in Tris-HCl buffer solution (0.01 M, pH 7.4) with an excitation of 365 nm (WGA = wheat germ agglutinin; Pep = pepsin; SBA = soybean agglutinin; PNA = peanut agglutinin; Con A = Concanavalin A; HSA = human serum albumin; BSA = bovine serum albumin; Aβ40 = amyloid β 40 fibril; Aβ42 = amyloid β 42 fibril).

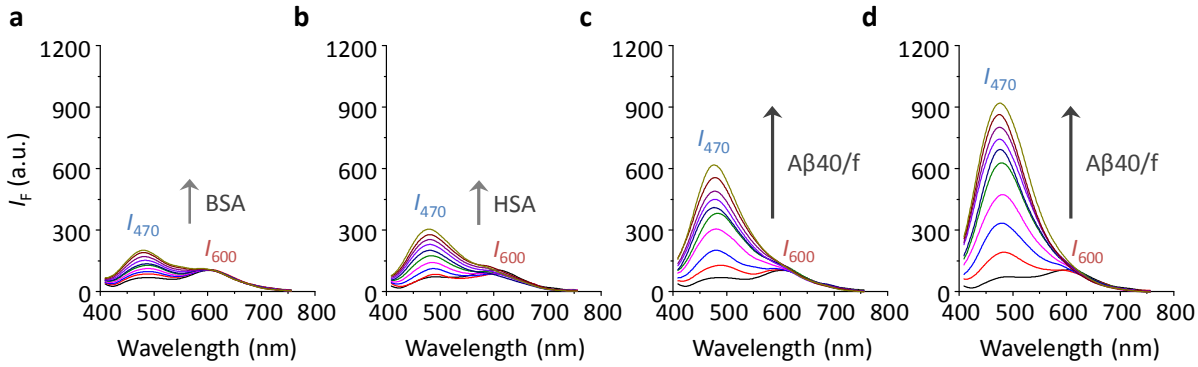


Figure S4. Fluorescence ratiometric titration of **VN** (10 μM) in the presence of increasing (a) BSA (0-20 μM), (b) HSA (0-20 μM), (c) Aβ40/f (0-20 μM) and (d) Aβ42/f (0-20 μM) in Tris-HCl buffer solution (0.01 M, pH 7.4) with an excitation of 365 nm.

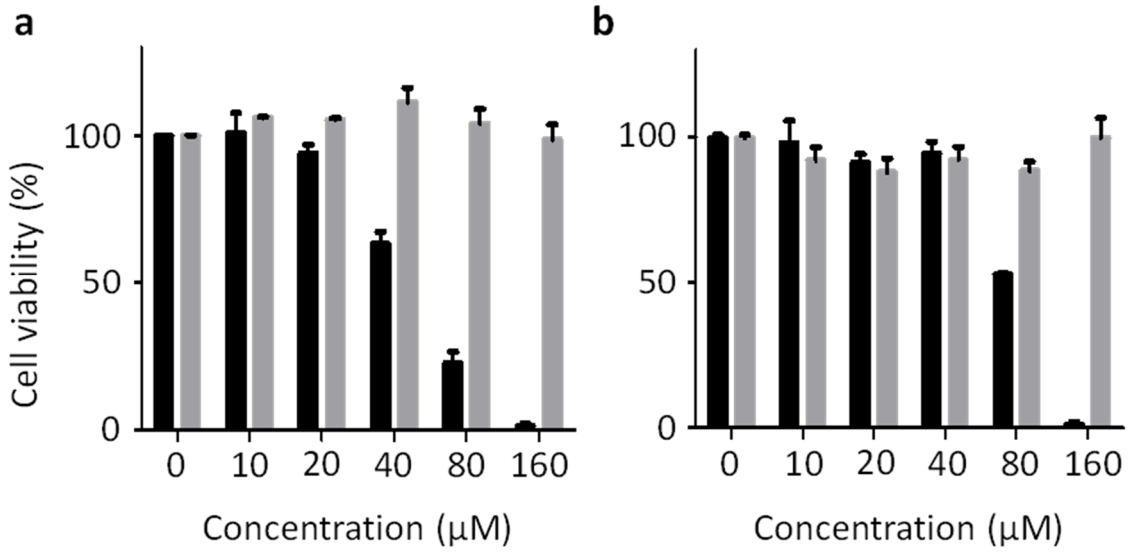
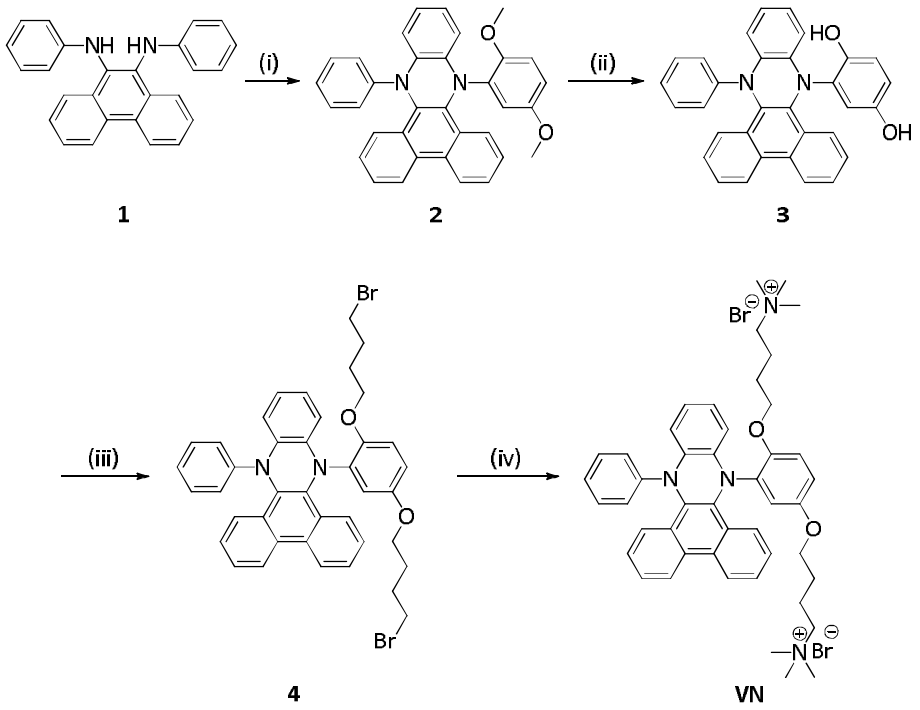


Figure S5. Cell viability of (a) 293T (human embryonic kidney cell line) and (b) L02 (human hepatocyte) measured by an MTS assay; the compounds were incubated with the cells for 30 min prior to a 72 h cell viability assay.[2]

S2. Additional experimental section

General. All purchased chemicals and reagents are of analytical grade. Amyloid- β peptides were purchased from GL Biochem (Shanghai) Ltd. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker AM-400 spectrometer. Chemical shifts were reported in ppm relative to a tetramethylsilane (TMS) standard in $CDCl_3$ or DMSO-d_6. Mass spectra (MS) were obtained on a Waters LCT Premier XE spectrometer. Fluorescence spectra were recorded on a Varian Cray Eclipse. Transmission electron microscopy (TEM) images were obtained on a JEOL 100CX transmission electron microscope operating at an accelerating bias voltage of 100 kV. Dynamic light scattering (DLS) was carried out on a Horiba LB-550 Dynamic Light Scattering Nano-Analyzer.

Synthesis. The synthesis of **VN** is shown in Scheme S1 below. Synthesis of **VR** has been described by us previously.[1]



Scheme S1. Synthetic scheme of compound **VN**. (i) 2-Iodo-1,4-dimethoxybenzene, K_2CO_3, $Cu(CF_3SO_3)_2$ and 1,3,5-trichlorobenzene; (ii) BBr_3 in CH_2Cl_2; (iii) 1,4-Dibromobutane, K_2CO_3 in MeCN; (iv) trimethylamine in THF.

Experimental procedures for the synthesis of compound VN

9-(2,5-Dimethoxyphenyl)-14-phenyl-9,14-dihydrodibenzo[a,c]phenazine (2). Compound **1** (3.6 g, 10 mmol), 2-iodo-1,4-dimethoxybenzene (2.7 g, 10 mmol), K_2CO_3 (2.1 g, 15 mmol), $Cu(CF_3SO_3)_2$ (0.9 g, 2.5 mmol) and 1,3,5-trichlorobenzene (10 g) were added in a 100 mL flask. The mixture was stirred for 10 h under dry atmosphere. Then the solvent was distilled

under reduced pressure and the solid was washed with CH_2Cl_2 (20 mL) three times. The solvent was distilled under reduced pressure and the crude product was purified by column chromatography on silica (Petroleum ether [PE]/EtOAc = 30:1, v/v) to afford a light yellow solid (2.5 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.69 (d, J = 8.8Hz, 2H), 8.23 (t, J = 7.6 Hz, 2H), 7.84-7.79 (m, 1H), 7.64-7.53 (m, 4H), 7.43 (t, J = 8.0 Hz, 1H), 7.24-7.17 (m, 2H), 7.07 (t, J =8.0 Hz, 2H), 6.99 (d, J = 9.2 Hz, 1H), 6.90 (d, J =8.4 Hz, 2H), 6.79 (t, J = 7.2 Hz, 1H), 6.67 (q, J = 3.2 Hz, 1H), 6.38 (d, J = 2.8 Hz, 1H), 4.07 (s, 3H), 2.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 153.49, 150.26, 149.83, 147.26, 140.11, 139.22, 137.01, 133.33, 130.20, 129.55, 129.22, 129.04, 128.61, 128.05, 127.89, 127.38, 127.17, 126.93, 126.70, 124.38, 123.93, 123.65, 122.15, 120.82, 115.57, 115.03, 114.56, 114.02, 56.46, 55.38. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{34}H_{27}N_2O_2$, 495.2073, found 495.2070.

2-(14-Phenyldibenzo[a,c]phenazin-9(14H)-yl)benzene-1,4-diol (3). To a solution of **2** (1.23 g, 2.5 mmol) in 30 mL CH_2Cl_2, BBr_3 (0.7 mL, 7.5 mmol) dissolved in 10 mL CH_2Cl_2 was added at 0 ºC under Ar atmosphere. The mixture was stirred at room temperature for 8 h. Then the reaction was quenched by 100 mL water and the organic layer was purified by column chromatography on silica (PE/EA = 2:1, v/v) to afford a gray solid (0.8 g, 69%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.82 (s, 1H), 8.85 (t, J = 7.8 Hz, 2H), 8.58 (s, 1H), 8.18 (d, J = 7.8 Hz, 1H), 8.01 (dd, J = 7.8, 1.6 Hz, 1H), 7.83 (m, 1H), 7.71-7.66 (m, 1H), 7.62-7.58 (m, 3H), 7.45 (t, J = 7.8 Hz, 1H), 7.24-7.18 (m, 2H), 7.17-7.13 (m, 2H), 6.94-6.87 (m, 4H), 6.52 (dd, J = 8.8, 2.9 Hz, 1H), 6.15 (d, J = 2.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 150.43, 149.96, 147.06, 147.03, 139.51, 138.68, 135.62, 132.13, 130.23, 129.41, 129.04, 128.95, 128.32, 127.73, 127.26, 127.05, 126.91, 126.49, 126.34, 124.11, 123.78, 121.47, 121.37, 118.10, 116.80, 116.15, 115.68. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{32}H_{23}N_2O_2$, 467.1760, found 467.1766.

9-(2,5-Bis(4-bromobutoxy)phenyl)-14-phenyl-9,14-dihydrodibenzo[a,c]phenazine (4). Compound **3** (0.47 g, 1 mmol), 1,4-dibromobutane(0.32 g, 1.5 mmol) and K_2CO_3 (0.4 g, 3 mmol) in 30 mL MeCN were refluxed under Ar atmosphere for 6 h. After cooling, the solvent was distilled under reduced pressure and the crude product was purified by column chromatography on silica (PE/CH_2Cl_2 = 20:1, v/v) to afford a light yellow solid (0.7 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.88 (d, J = 8.4 Hz, 2H), 8.21 (d, J = 8.4 Hz, 1H), 8.07-8.11 (m, 1H), 7.62-7.78 (m, 5H), 7.52 (t, J = 8.0 Hz, 1H), 7.20-7.31 (m, 3H), 7.17 (t, J = 16.0 Hz, 2H), 6.84-6.89 (m, 3H), 6.71-6.75 (m, 1H), 6.23 (d, J = 3.2 Hz, 1H), 4.37 (t, J = 6.0 Hz, 2H), 3.65 (t, J = 6.4 Hz, 2H), 3.40 (t, J = 6.4 Hz, 2H), 2.99 (t, J = 6.0 Hz, 2H), 2.04-2.17 (m, 4H), 1.65 (dt, J = 6.8,14.4 Hz, 2H), 1.44 (dt, J = 6.0,13.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 152.72, 149.98, 149.41, 147.62, 140.31, 139.43, 137.07, 133.70, 130.20, 129.58, 129.22, 129.06, 128.75, 128.14, 127.93, 127.36, 127.25, 126.98, 126.76, 124.44,

123.92, 123.68, 122.37, 120.75, 115.69, 115.52, 114.98, 114.40, 67.88, 66.94, 35.29, 35.18, 29.91, 29.30, 28.29, 27.46. HRMS (ESI) *m/z*: $C_{40}H_{37}Br_2N_2O_2$, 735.1222, found 735.1222.

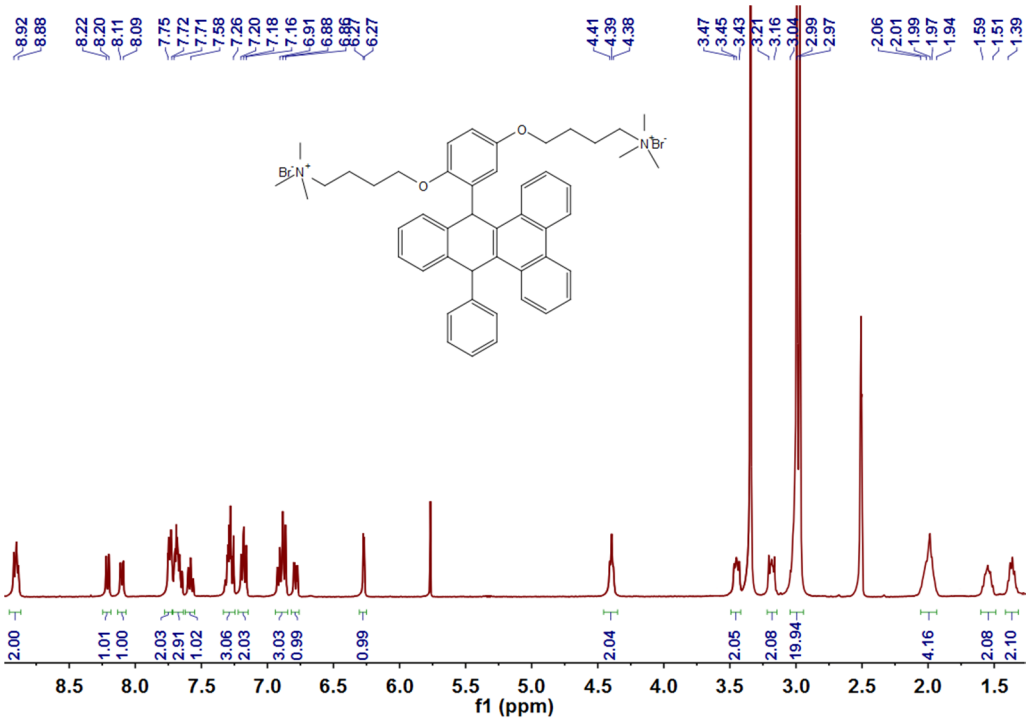
VN. Compound **4** (0.35 g, 0.8 mmol), trimethylamine (excessive) in 30 mL THF were stirred under Ar atmosphere at 35 ºC for three days. After cooling, the product was filtered and washed with THF to afford **VN** as a light green solid (0.3 g, 75%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.90 (d, J = 14.0 Hz, 2H), 8.21 (d, J = 7.7 Hz, 1H), 8.10 (d, J = 9.5 Hz, 1H), 7.74 (d, J = 11.5 Hz, 2H), 7.67 (d, J = 24.5 Hz, 3H), 7.58 (t, J = 7.4 Hz, 1H), 7.29 (d, J = 26.4 Hz, 3H), 7.21-7.15 (m, 2H), 6.89 (dd, J_1= 16.9 Hz, J_2= 7.8 Hz, 3H), 6.79 (dd, J_1= 9.1 Hz, J_2= 3.0 Hz, 1H),6.27 (d, J = 3.0 Hz, 1H), 4.39 (t, J = 5.2 Hz, 2H), 3.49-3.41 (m, 2H), 3.18 (d, J = 16.9 Hz,2H), 3.00 (t,J = 14.4 Hz, 20H), 2.07-1.92 (m, 4H), 1.55 (d, J = 31.7 Hz,2H), 1.36 (d, J = 26.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 152.29, 149.59, 149.03, 147.00, 139.67, 138.77, 136.55, 133.06, 129.73, 129.17, 128.70, 128.53, 128.16, 127.65, 127.53, 126.95, 126.78, 126.56, 126.35, 123.98, 123.54, 123.13, 121.67, 120.37, 115.43, 115.18, 114.64, 113.86, 67.68, 66.58, 64.92, 64.76, 52.02, 26.20, 25.32, 19.59, 19.02. HRMS (ESI) *m/z*: $C_{46}H_{54}N_4O_2^{2+}$, 347.2123, found 347.2111.

Sample preparation. Aβ40 and Aβ42 peptide powder was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at a concentration of 1 mg mL^{-1}, which was stored at -20 °C as a stock solution. Prior to use, HFIP was removed by evaporation under a gentle stream of nitrogen and the peptide was redissolved in a Tris-HCl buffer solution (0.01 M, pH 7.4). Aβ40 and Aβ42 fibrogenesis was accomplished by mixing an aliquot of the peptides from the stock solution into a buffer (0.01 M Tris in 0.01 M NaCl, pH 7.4) at 37 °C for one to seven days.

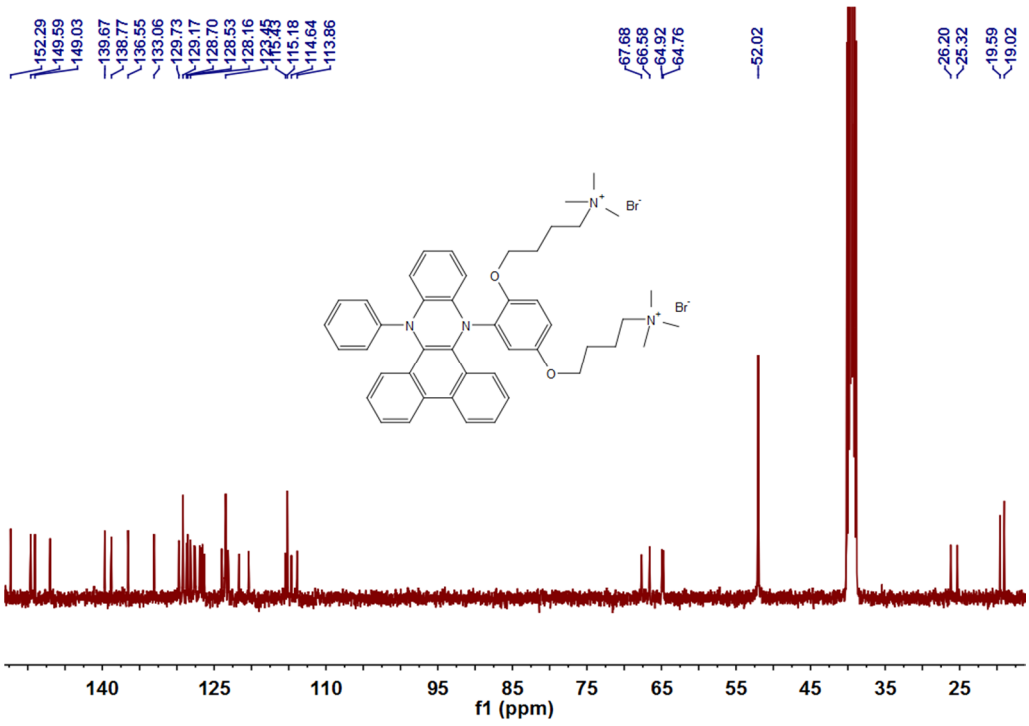
Fluorescence spectroscopy. In a typical fluorescence assay, a VIEgen dissolved in Tris-HCl (0.01 M, pH 7.4) was incubated with Aβ peptides/fibrils of different concentrations for 2 min at room temperature. Then, fluorescence spectrum was recorded with a Varian Cary Eclipse fluorescence spectrophotometer with an excitation of 365 nm.

Staining of transgenic mouse section. Double transgenic mice (APPsw/PS1De9, 5 months old, male) were perfused in cold saline and 4% paraformaldehyde, then sacrificed. Brains were taken and fixed in 4% paraformaldehyde overnight, and dehydrated in 30% sucrose. Then OCT (optimum cutting temperature compound)-embedded tissues were sliced into serial sections (20 μm thick) in frozen section machine. Sections were treated with PBS for 10 min, and then incubated with **VN** (40 μM) for 30 min. Then, the sections were gently washed with PBS three times and mounted by 50% glycerin (dissolved in PBS). The fluorescence images were recorded using a fluorescence microscope (Olympus, Japan).

S3. Original NMR and MS copies of VN



^{1}H NMR spectrum of **VN**



^{13}C NMR spectrum of **VN**

Elemental Composition Report Page 1

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
168 formula(e) evaluated with 5 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-46 H: 0-60 N: 0-4 O: 0-2 Br: 0-2

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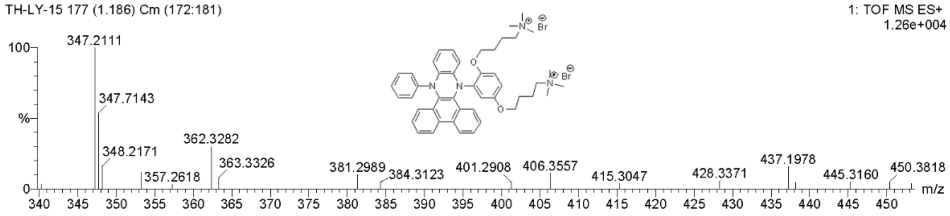
TH-LY-15 177 (1.186) Cm (172:181) 1: TOF MS ES+ 1.26e+004



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347.2111	347.2123	-1.2	-3.5	11.5	47.4	0.0	C23 H27 N2 O

HRMS spectrum of **VN**

S4. Additional reference

1. Z. Zhang, Y.-S. Wu, K.-C. Tang, C.-L. Chen, J.-W. Ho, J. Su, H. Tian and P.-T. Chou, *J. Am. Chem. Soc.*, 2015, **137**, 8509-8520.
2. X.-P. He, Q. Deng, L. Cai, C.-Z. Wang, Y. Zang, J. Li, G.-R. Chen and H. Tian, *ACS Appl. Mater. Interfaces*, 2014, **6**, 5379-5382.