

Crown ethers attenuate aggregation of Amyloid beta of Alzheimer's disease

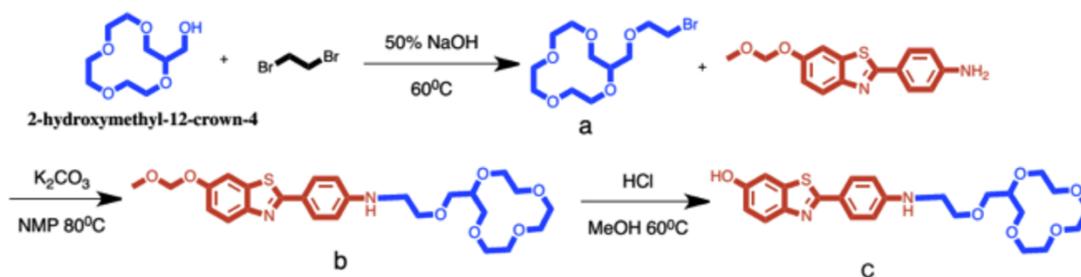
Yanli Tian^{1,2§}, Xueli Zhang^{1,3§}, Yuyan Li^{1,3}, Timothy M. Shoup⁴, Xin Teng⁴, David R. Elmaleh⁴, Anna Moore^{1*}, and Chongzhao Ran^{1*}

Supplemental information

Experimental section

Reagents used for the synthesis were purchased from Aldrich and used without further purification. Column chromatography was performed on silica gel (SiliCycle Inc., 60 Å, 40-63 mm) slurry packed into glass columns. Synthetic A β peptides (1-40) were purchased from rPeptide (Bogart, GA, 30622, Cat. No. A-1001). ¹H and ¹³C NMR spectra were recorded at 500 MHz and 125 MHz respectively, and reported in ppm downfield from tetramethylsilane. Fluorescence measurements were carried out using an F-4500 Fluorescence Spectrophotometer (Hitachi). Mass spectra were obtained at Harvard University, Department of Chemistry Instrumentation Facility. Transgenic female APP-PS1 mice were purchased from Jackson Laboratory. All animal experiments were approved by the Institutional Animal Use and Care Committee at Massachusetts General Hospital.

Synthesis of PiB-C



Compound (a): To a solution of dibromoethane (20 mL), hydroxymethyl-12-crown-4 (500 mg, 2.4 mmol) and triethylbenzyl ammonium chloride (150 mg, 0.8 mmol) were added, followed by the addition an aqueous solution of 50% sodium hydroxide (10 mL). The resulting mixture was stirred at 60°C for about 12 h. After cooling, dichloromethane

(20 mL) and H₂O (20 mL) were added for extraction. The aqueous phase was extracted with dichloromethane (3x20 mL), and the organic fractions were combined and evaporated to give the crude product as yellow oil, which was purified by column chromatography with ethyl acetate as the eluent. 83 mg, yield 11%. ¹H NMR (CDCl₃) δ(ppm) 3.39-3.81(m, 21H); ¹³C NMR (CDCl₃) δ(ppm) 30.36, 70.12, 70.29, 70.57, 70.67, 70.91, 71.20, 71.30, 71.38, 78.51; ESI-MS (M+H) m/z = 313.2.

Compound (b): To the solution of (a) (80 mg, 0.25 mmol) from the above step in NMP (1.5 mL), 4-(5-(methoxymethoxy) benzo[*d*]thiazol-2-yl)aniline (70 mg, 0.25 mmol) and K₂CO₃ (40 mg, 0.29 mmol) were added. The mixture was stirred at 80°C for 8-10 h. A yellow residue was obtained after removing the solvent and was subjected to flash column chromatography (ethyl acetate) to give a yellow powder. 27 mg, yield 20.8%. ¹H NMR (CDCl₃) δ(ppm) 3.35-3.88(m, 24H), 5.23(s, 2H), 6.66(d, 2H, J = 8.5Hz), 7.13(dd, 1H, J = 2, 9 Hz), 7.52(d, 1H, J = 2 Hz), 7.85-7.87(m, 3H); ¹³C NMR (CDCl₃) δ(ppm) 43.09, 56.06, 69.69, 70.00, 70.31, 70.56, 70.76, 71.01, 71.24, 71.40, 78.52, 95.09, 107.78, 112.64, 116.56, 122.74, 122.83, 128.82, 135.59, 149.61, 150.40, 154.66, 167.07; ESI-MS (M+H) m/z = 518.6.

c) PiB-C: To the solution of (b) (27 mg, 0.05 mmol) from the above step in methanol (4 mL), concentrated HCl (37%) (100 uL) was added. The mixture was stirred at 80 °C for 12 h. A yellow residue was obtained after removing the solvent and was subjected to flash column chromatography (CH₂Cl₂ : MeOH = 20:1) to give a light yellow powder 16 mg, yield 67%. ¹H NMR (d-DMSO) δ(ppm) 3.39-3.81(m, 21H), 6.31(t, 1H, J = 6 Hz), 6.69(d, 2H, J = 9 Hz), 6.89(dd, 1H, J = 2.5, 8.5 Hz), 7.30(d, 1H, J = 2.5 Hz), 7.67-7.71(m, 3H), 9.68(s, 1H); ¹³C NMR (d-DMSO) δ(ppm) 42.75, 63.53, 69.80, 70.15, 70.42, 70.48, 70.82, 71.06, 71.45, 78.21, 107.16, 112.35, 115.86, 121.02, 122.77, 128.57, 135.54, 147.82, 151.51, 155.32, 164.87; ESI-MS (M+H) m/z = 475.5.

Aβ40 Aggregates and TEM imaging: According to our previously published protocol ^{1, 2}, a solution of the fibrillar aggregates was prepared, and TEM images were captured.

Zeta potential measurement: To a solution of Aβ40 aggregates (400nM) in 1.0mL double distilled water, a solution of 12-crown-4 ether (32μl) (25μM in water) was added. The resulting mixture was inserted into the cuvette holder of a Nano-sizer (Malvern, Nano-ZS) to measure Zeta potential.

Thioflavin T fluorescence intensity measurement for monitoring the aggregation of

A β 40: A 100 μ L of A β 40 (250 μ M, re-suspended with 1% NH₄OH from the powder) was added to 700 μ L PBS, followed by the addition of 12-crown-4 (200 μ L, 250 μ M in PBS). The resulting mixture was stirred at 37°C for the indicated time (5 minutes to 10 days). To measure fluorescence intensity with Thioflavin T, 10 μ L of the above mixture and 10 μ L of Thioflavin T (25 μ M in PBS) were added to 1.0mL PBS. Fluorescence spectrum was recorded at 450nm excitation. Quantification was based on fluorescence intensity at 505nm. Triplicated samples were measured.

Thermodynamic saturation concentration test: This test was conducted according to the reported procedure³, and the tested concentrations of A β 40 were 1.0, 5.0, 10.0, 15.0, 20.0, 30.0, 50.0 μ M. For PiB-C test, the ratios of PiB-C and A β 40 were 1:1.

Dot blotting with 2H4 A β antibody: 10 μ L of the above mixture was spotted onto a nitrocellulose membrane. After the membrane dried, it was incubated with primary antibody (2H4 antibody, 1:2000 dilution with TBS-T) overnight at 4°C. After washing three times with TBS-T (3 x 5 min), the membrane was incubated with HRP-conjugated secondary antibody (1:2000 dilution with TBS-T) for 60 min at RT. After washing three times with TBS-T (3 x 5 min), the membrane was visualized with ECL reagent using an IVIS®Spectrum imaging system (Perkin Elmer).

MTT test: To a 96-well plate with SH-SY5Y cells (30,000/well) in 80 μ L medium, a 20 μ L solution of A β 42 (100 μ M in 1%DMSO and 10% PBS and 90% cell culture medium) was added (n=4). Next, 40 μ L of 12-crown-4 ether or PiB-C (100 μ M in 1%DMSO and 10% PBS and 90% cell culture medium) was added to the above wells that contained A β 42 (n=4). The plate was incubated for 4 hours, and then subjected to standard MTT testing. The reading from the wells containing 100% cell culture medium was used as 100% cell viability.

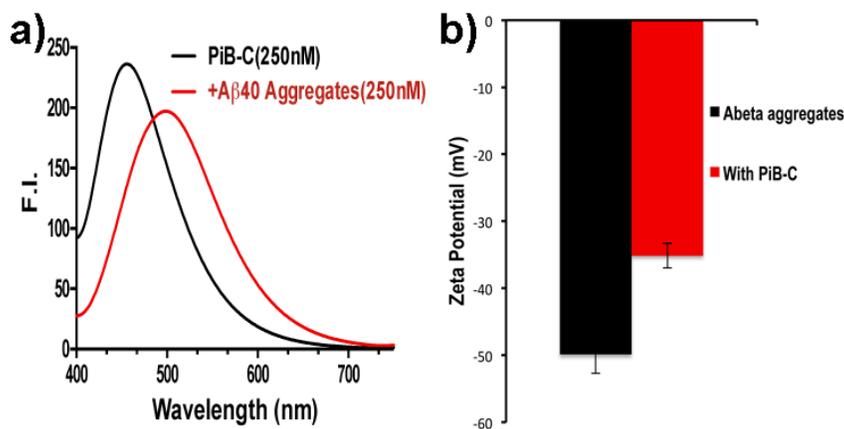
Two-photon imaging: PiB-C (2.0 mg/kg in a fresh solution containing 15% cremorpher, 15% DMSO and 70% PBS) was injected intravenously at time 0 min by a bolus injection during image acquisition. Two-photon fluorescence excitation was accomplished with a 900-nm laser (Prairie Ultima). Imaging was performed using a two-photon microscope (Olympus BX-51) equipped with a 20x water immersion objective (0.45 numerical aperture, Olympus). Images were collected for 15 seconds per frame 512x512 μ m matrix

for 60 min. Taxes red-Dextran (MW:70000) conjugate (50 μ L) was injected in the tail vein 60 min after PiB-C injection, and images outlining the blood vessels were obtained. In BBB penetration study, a wild type mouse (C57BL6) was imaged for 5 min to obtain pre-injection background. Images were analyzed with ImageJ software using 2 ROIs for each data group ⁴.

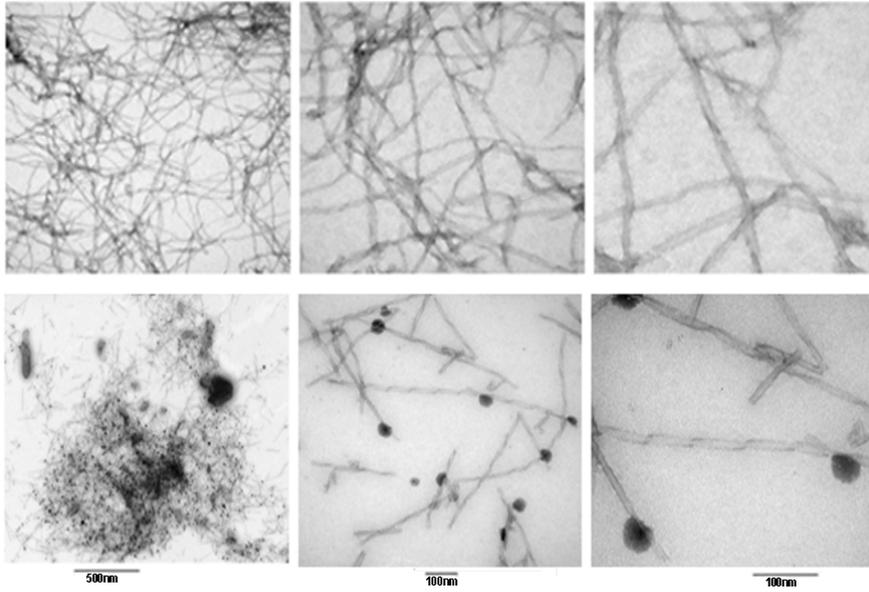
References:

1. C. Ran, X. Xu, S. B. Raymond, B. J. Ferrara, K. Neal, B. J. Bacskai, Z. Medarova and A. Moore, *J Am Chem Soc*, 2009, **131**, 15257-15261.
2. C. Ran, Zhao, W., Moir, RD, Moore, A. , *PLoS One*, 2011, **6**, e19362.
3. P. Sengupta, K. Garai, B. Sahoo, Y. Shi, D. Callaway and S. Maiti, *Biochemistry*, 2003, **42**, 10506-10513.
4. X. Zhang, Y. Tian, P. Yuan, Y. Li, M. A. Yaseen, J. Grutzendler, A. Moore and C. Ran, *Chem Comm*, 2014, **50**, 11550-3.

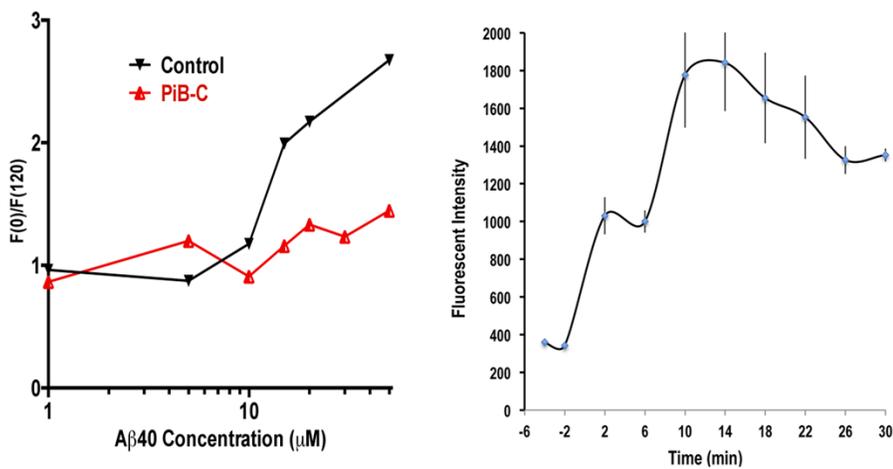
Supplemental figures



SI Fig.1 (a) Fluorescence spectra of PiB-C with and without A β 40 aggregates. (b) Zeta potential measurement of A β 40 aggregates with and without PiB-C (n=3).



SI Fig.2 Representative TEM images of A β 40 without (upper) and with (bottom) PiB-C at day 6. Three images are shown with different scale bars.



SI Fig.3 (a) Thermodynamic saturation concentration measurements for control samples (without PiB-C) and samples with PiB-C treatment. (b) BBB penetrating time course of PiB-C after i.v. injection in a wild type mouse. Three ROIs were used for the analysis.