Supplementary Material (ESI) for Molecular BioSystems

Supporting Information

Highly Sensitive Amyloid Detection Enabled by Thioflavin T Dimers

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I. General Methods

All Fmoc-protected amino acids were purchased from Advanced Chemtech (Louisville, KY). All other chemical reagents and the bovine serum stock were purchased from Sigma-Aldrich (Milwaukee, WI). 96 well and 384 well microplates were purchased from Corning (Corning, NY). Peptide synthesis was carried out on a Tribute peptide synthesizer (Protein Technologies, Tucson, AZ). ^{1}H-NMR and ^{13}C-NMR data were collected on a Varian Gemini 400MHz NMR spectrometer. HR-MS data were generated by Boston College Mass-Spec facilities. Circular dichroism measurements were performed on an AVIV CD spectrometer (Aviv Biomedical Inc. Lakewood, NJ). The protein concentration of all samples used in this study was determined by measuring their absorption at 280 nm on a Lambda 25 UV-Vis spectrometer (PerkinElmer, Waltham, MA). The extinction coefficient ε (280 nm) of Aβ40 was calculated using ExPASy ProtParam Tool (http://ca.expasy.org/tools/protparam.html) to be 1280 $M^{-1}cm^{-1}$. Fluorescence excitation and emission measurements in binding affinity tests were performed on a Fluorolog-3 fluorometer and a MicroMax 384 microwell plate reader (Jobin Yvon Inc. Edison, NJ). Fluorescence emmision measurements in binding specificity tests were performed on a Spectra Max M5 microwell plate reader (Molecular Devices, Sunnyvale, CA).

II. Synthesis of ThT derivatives.

2-amino-5-methoxythiophenol (1, structure see Scheme-1)

2-amino-6-methoxybenzothiazole (10.0 g, 55.5 mmol) was suspended in 50% KOH aq (70 ml), and then ethylene glycol (13.5 ml) was added. The mixture was heated to reflux for 48h. Upon cooling to room temperature, toluene (100 ml) was added and then the mixture was neutralized with acetic acid (70 ml). The organic layer was separated, and the aqueous layer was extracted with toluene (2 ×100 ml). The combined organic layers were washed with water and dried over Na_2SO_4. The solvent was then evaporated under vacuum, and the residue was washed with hexanes to give 6.6g (77%) of 2-amino-5-methoxythiophenol as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.81 (d, J = 2.8 Hz, 1H), 6.70 (d, J = 8.8 Hz, 1H), 6.49 (dd, J_1 = 8.8 Hz, J_2 = 2.8 Hz, 1H), 5.60 (b, 3H), 3.62 (s, 3H). (The NMR data agrees well with the previous report: Rutkauskas, K.; *Polish Journal of Chemistry* **2008**, 82, 2341-2347)

2-(4'-(Dimethylamino)phenyl)-6-methoxybenzothiazole (2; 6-MeO-BTA-2)

2-amino-5-methoxythiophenol **(1)** (9.85 g, 63.5 mmol) and 4-(dimethylamino)benzaldehyde (9.60 g, 64.4 mmol) were dissolved in DMSO (80 ml). The mixture was heated to 180 ^{o}C for 20 min. After cooled to room temperature, the reaction mixture was poured into 500 ml water. Ethyl acetate (100 ml) was then added and the whole mixture was stirred thoroughly. 2-(4'-(Dimethylamino)phenyl)-6-methoxylbenzothiazole as pale-yellowish precipitate was isolated by vacuum filtration, washed with ethyl acetate (3 × 10 ml) and anhydrous ethanol (3 × 10 ml), and dried under vacuum. Yield: 6.75g (72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, J = 8.8 Hz, 2H), 7.84 (d, J = 8.8 Hz, 1H), 7.30 (d, J = 2.8 Hz, 1H), 7.01 (dd, J_1 = 8.8 Hz, J_2 = 2.8 Hz, 1H), 6.72 (d, J = 8.8 Hz, 2H), 3.86 (s, 3H), 3.03 (s, 6H).). (The NMR data agrees well with the previous report: Bose, D. Subhas; *Synthesis* **2007**, 819-823)

2-(4'-(Dimethylamino)phenyl)-6-hydroxybenzothiazole (3; 6-HO-BTA-2)

To a solution of 2-(4'-(Dimethylamino)phenyl)-6-methoxylbenzothiazole **(2)** (1.0g, 3.5mmol) in anhydrous DCM (100ml) was added neat BBr_3 (4.6ml, 49mmol) dropwise at -78 ^{o}C under Ar protection. The reaction mixture was warmed to room temperature slowly and then stirred for 16 h. The reaction was quenched with water (100 ml) and the pH was adjusted to 4-7 with NaOH aq. 2-(4'-(Dimethylamino)phenyl)-6-hydroxylbenzothiazole as yellowish precipitate was isolated by vacuum filtration, washed with water (3 × 10 ml), methanol (10 ml), DCM (10ml) and anhydrous ethanol (3 × 10 ml), and dried under vacuum. Yield: 0.89g (94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.71 (s, 1H) 7.80 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 8.8 Hz, 1H), 7.33 (d, J = 2.4 Hz, 1H), 6.91 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 6.80 (d, J = 8.8

Hz, 2H), 3.01 (s, 6H). Chester A. Mathis; *J. Med. Chem*. **2003**, (46), P2740-2754

Typical procedure of BTA-linker coupling

4,4'-(6,6'-(3,6,9,12-tetraoxatetradecane-1,14-diylbis(oxy))bis(benzo[d]thiazole-6,2-diyl))bis(N,N-dimethylaniline) (5e; diBTA-PEG5)

To a suspension of NaH (10.5 mg, 0.44 mmol) and anhydrous DMF (4 ml) in a pressure vial was added 2-(4'-(Dimethylamino)phenyl)-6-hydroxybenzothiazole **(3)** (120 mg, 0.44 mmol) in one portion. The mixture was stirred for 5 min, and hydrogen gas was released. Then 2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) dimethanesulfonate **(4e)** (80 mg, 0.20 mmol) was added, and the resulting mixture was stirred for 16 h at 90 ^{o}C in the screw-capped vial. The reaction mixture was then quenched with saturated NH_4Cl aq. (5 ml), poured to water (30 ml), and extracted with DCM (3 × 20 ml). After the solvent was evaporated, the product was isolated with silica gel column (EtOAc/hexane=3:1). Yield: 123mg (82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, J = 8.8 Hz, 4H), 7.84 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 2.4 Hz, 2H) 7.04 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 2H), 6.72 (d, J = 8.8 Hz, 4H), 4.17 (t, J = 4.8 Hz, 4H), 3.87 (t, J = 4.8 Hz, 4H), 3.68-3.74 (m, 8H), 3.67 (s, 4H), 3.04 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.7, 156.4, 152.1, 149.3, 135.9, 128.7, 122.9, 121.9, 115.7, 111.9, 105.7, 71.1, 70.9, 70.0, 68.4, 40.4. HRMS (ESI+): *m/z* calculated for $C_{40}H_{47}N_4O_6S_2$ $[M]^+$, 743.29370; found 743.29457.

4,4'-(6,6'-(ethane-1,2-diylbis(oxy))bis(benzo[d]thiazole-6,2-diyl))bis(N,N-dimethylaniline) (5a; diBTA-PEG1)

The product was directly isolated by vacuum filtration after the reaction was quenched, washed with DMF (10 ml), water (3 × 10 ml), ethanol (3 × 10 ml) and ether (3 × 10 ml), dried under vacuum, and directly used in the followed step. Yield: 87%. The product was insoluble in all common solvents and improper for NMR analysis. HRMS (ESI+): *m/z* calculated for $C_{32}H_{31}N_4O_2S_2$ $[M]^+$, 567.18884; found 567.18879.

4,4'-(6,6'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(benzo[d]thiazole-6,2-diyl))bis(N,N-dimethylaniline) (5b;

diBTA-PEG2)

The product was directly isolated by vacuum filtration after the reaction was quenched, washed with DMF (10 ml), water (3 × 10 ml), ethanol (3 × 10 ml) and ether (3 × 10 ml), dried under vacuum, and directly used in the followed step. Yield: 76%. The product was only slightly soluble in $CDCl_3$ and improper for ^{13}C NMR analysis. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.87 (d, J = 8.8 Hz, 4H), 7.84 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 2.8 Hz, 2H) 7.04 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 2H), 6.70 (d, J = 8.8 Hz, 4H), 4.22 (t, J = 4.8 Hz, 4H), 3.97 (t, J = 4.8 Hz, 4H), 3.02 (s, 12H). HRMS (ESI+): *m/z* calculated for $C_{32}H_{31}N_4O_2S_2$ $[M]^+$, 611.2151; found 611.2158.

4,4'-(6,6'-(2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(benzo[d]thiazole-6,2-diyl))bis(N,N-dimethylaniline) (5c; diBTA-PEG3)

The product was directly isolated by vacuum filtration after the reaction was quenched, washed with DMF (10 ml), water (3 × 10 ml), ethanol (3 × 10 ml) and ether (3 × 10 ml), dried under vacuum, and directly used in the followed step. Yield: 70%. The product was only slightly soluble in $CDCl_3$ and improper for ^{13}C NMR analysis. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.86 (d, J = 9.0 Hz, 4H), 7.82 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 2.4 Hz, 2H) 7.03 (dd, J_1 = 9.0 Hz, J_2 = 2.7 Hz, 2H), 6.70 (d, J = 8.7 Hz, 4H), 4.17 (t, J = 4.8 Hz, 4H), 3.89 (t, J = 4.8 Hz, 4H), 3.77 (s, 4H), 3.02 (s, 12H). HRMS (ESI+): *m/z* calculated for $C_{36}H_{39}N_4O_4S_2$ $[M]^+$, 655.24127; found 655.23766.

4,4'-(6,6'-(2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(benzo[d]thiazole-6,2-diyl))bis(N,N-dimethylaniline) (5d; diBTA-PEG4)

The product was directly isolated by vacuum filtration after the reaction was quenched, recrystallized with EtOAc, and directly used in the followed step. Yield: 56%. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, J = 8.8 Hz, 4H), 7.84 (d, J = 8.8 Hz, 2H), 7.30 (d, J = 2.4 Hz, 2H) 7.05 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 2H), 6.72 (d, J = 8.8 Hz, 4H), 4.17 (t, J = 4.8 Hz, 4H), 3.89 (t, J = 4.8 Hz, 4H), 3.71-3.76 (m, 8H), 3.04 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.6, 152.1, 128.9, 122.7, 115.6, 112.0, 105.7, 71.1, 70.0, 68.4, 40.5. HRMS (ESI+): *m/z*

calculated for $C_{40}H_{47}N_4O_6S_2$ $[M]^+$, 699.2675; found 699.2656.

4,4'-(6,6'-(pentane-1,5-diylbis(oxy))bis(benzo[d]thiazole-6,2-diyl))bis(N,N-dimethylaniline) (5f; diBTA-C5)

The product was directly isolated by vacuum filtration after the reaction was quenched, washed with DMF (10 ml), water (3 × 10 ml), ethanol (3 × 10 ml) and ether (3 × 10 ml), dried under vacuum, and directly used in the followed step. Yield: 91%. The product was insoluble in all common solvents and improper for NMR analysis. HRMS (ESI+): *m/z* calculated for $C_{38}H_{43}N_4O_2S_2$ $[M]^+$,651.28274; found 651.28372.

4-(6-(2-(2-(tert-butyldimethylsilyloxy)ethoxy)ethoxy)benzo[d]thiazol-2-yl)-N,N-dimethylaniline (7; TBSO-PEG2-BTA)

The product was isolated with silica gel column (EtOAc/hexane=1:3). Yield: 91%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.87 (d, J = 8.8 Hz, 2H), 7.84 (d, J = 9.2 Hz, 1H), 7.29 (d, J = 2.8 Hz, 1H) 7.04 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 6.68 (d, J = 9.2 Hz, 2H), 4.14 (t, J = 4.8 Hz, 2H), 3.86 (t, J = 4.8 Hz, 2H), 3.79 (t, J = 5.2 Hz, 2H), 3.63 (t, J = 5.2 Hz, 2H), 2.98 (s, 6H), 0.88 (s, 9H), 0.07 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.5, 156.3, 151.9, 149.1, 135.8, 128.5, 122.7, 121.6, 115.5, 111.8, 105.4, 73.0, 69.9, 68.3, 62.9, 40.2, 26.1, 18.5, -5.1. HRMS (ESI+): *m/z* calculated for $C_{25}H_{37}N_2O_3SiS$ $[M]^+$, 473.2294; found 473.2295.

2-(2-(2-(4-(dimethylamino)phenyl)benzo[d]thiazol-6-yloxy)ethoxy)ethanol (8)

4-(6-(2-(2-(tert-butyldimethylsilyloxy)ethoxy)ethoxy)benzo[d]thiazol-2-yl)-N,N-dimethylaniline **(7)** (302 mg, 0.64 mmol) was dissolved in 1 M TBAF in THF (5% water) (1 ml, 1.0 mmol) and stirred for 1 h at rt. The reaction was quenched with water, extracted with DCM (3 × 15 ml), and the combined organic layers were dried over Na_2SO4. The product was isolated with silica gel column (EtOAc/hexane=1:5). Yield: 206 mg (90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.83 (d, J = 9.2 Hz, 2H), 7.81 (d, J = 9.2 Hz, 1H), 7.64 (d, J = 2.4 Hz, 1H), 7.07 (dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 1H), 6.81 (d, J = 8.8 Hz, 2H), 4.64 (t, J = 9.2 Hz, 1H), 4.17 (m, 2H), 3.78 (m, 2H), 3.52 (m, 4H) 3.01 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.3, 155.9, 151.8, 148.2, 135.1, 128.0,

122.3, 120.4, 115.5, 111.8, 105.6, 72.4, 68.8, 67.8, 60.2. High-resolution mass spectrometry (HRMS) (ESI+): *m/z* calculated for $C_{19}H_{23}N_2O_3S_1$ $[M]^+$, 359.14294; found 359.14300.

2-(2-(2-(4-(dimethylamino)phenyl)benzo[d]thiazol-6-yloxy)ethoxy)acetaldehyde (9)

To a solution of 2-(2-(2-(4-(dimethylamino)phenyl)benzo[d]thiazol-6-yloxy)ethoxy)ethanol **(8)** (88 mg, 0.24 mmol) in DCM (5 ml) was added Dess-Martin periodinane (126 mg, 0.29 mmol) in one portion. The mixture was stirred at rt for 2 h. The reaction was quenched with sat. $NaHCO_3$ and $Na_2S_2O_3$, the organic layer was separated, and the aqueous layer was extracted with DCM (2 × 15 ml). The combined organic layers were washed with brine and dried over Na_2SO4. After the solvent was evaporated, the product was isolated with silica gel column (EtOAc/hexane=3:1). Yield: 63 mg (72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.75 (d, J = 0.4 Hz, 1H), 7.89 (d, J = 8.8 Hz, 2H), 7.86 (d, J = 8.8 Hz, 1H), 7.32 (d, J = 2.4 Hz, 1H), 7.04 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 6.72 (d, J = 8.8 Hz, 2H), 4.25 (s, 2H), 4.22 (t, J = 4.8 Hz, 2H), 3.95 (t, J = 4.8 Hz, 2H), 3.03 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 200.5, 156.1, 152.1, 149.4, 135.9, 128.7, 122.3, 121.7, 115.4, 111.9, 105.6, 76.9, 70.6, 68.4, 40.4, 29.9. HRMS (ESI+): *m/z* calculated for $C_{19}H_{21}N_2O_3S_1$ $[M]^+$, 357.12729; found 357.12885.

4-(6-(2-(2-(4-(6-methoxybenzo[d]thiazol-2-yl)phenylamino)ethoxy)ethoxy)benzo[d]thiazol-2-yl)-N,N-dimethylaniline (11)

To a solution of 2-(2-(2-(4-(dimethylamino)phenyl)benzo[d]thiazol-6-yloxy)ethoxy)acetaldehyde **(9)** (44mg, 0.13 mmol) and 4-(6-methoxybenzo[d]thiazol-2-yl)aniline **(10)** (40 mg, 0.16 mmol) in anhydrous 1,2-dichloroethane (10 ml) was added $NaBH(OAc)_3$ (40 mg, 0.19 mmol). The mixture was stirred at rt for 24 h. Precipitate was generated gradually. The reaction was quenched with sat. $NaHCO_3$, and the whole mixture was filtered. The filtrate (product) was washed with water, MeOH and ether, dried under vacuum, and directly used in the followed step without further purification.

Typical procedure of methylation of BTA derivatives.

6,6'-(3,6,9,12-tetraoxatetradecane-1,14-diylbis(oxy))bis(2-(4-(dimethylamino)phenyl)-3-methylbenzo[d]thiazol-3-ium) chloride (6e; diThT-PEG5)

4,4'-(6,6'-(3,6,9,12-tetraoxatetradecane-1,14-diylbis(oxy))bis(benzo[d]thiazole-6,2-diyl))bis(N,N-dimethylanilin e) **(5e)** (56 mg, 0.075 mmol), MeI (94 μl, 1.5 mmol) and nitrobenzene (2ml) were mixed in a pressure vial and stirred 48 h at 110 ^{o}C. The solvent was evaporated under vacuum, and the residue was dissolved in DMSO (5 ml), filtered, and purified on HPLC. The isolated product was anion-exchanged with 1 M HCl in methanol (5 × 20 ml) and lyophilized. Yellow solid was finally obtained. Yield: 44 mg (69%). ^{1}H NMR (400 MHz, CD_3CN) δ: 7.88 (d, J = 9.6 Hz, 2H), 7.69 (d, J = 9.2 Hz, 4H), 7.64 (d, J = 2.4 Hz, 2H), 7.39 (dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 6.90 (d, J = 9.2 Hz, 4H), 4.22 (t, J = 4.8 Hz, 4H), 4.13 (s, 6H), 3.84 (t, J = 4.8 Hz, 4H), 3.58-3.66 (m, 8H), 3.57 (s, 4H), 3.12 (s, 12H). ^{13}C NMR (100 MHz, CD_3CN) δ: 173.5, 160.1, 155.5, 138.5, 133.4, 131.3, 120.2, 113.3, 112.2, 108.2, 71.8, 71.6, 70.4, 70.1, 40.8, 39.6. HRMS (ESI+): *m/z* calculated for $C_{42}H_{52}N_4O_6S_2$ $[M]^+$,386.1658; found 386.1668.

6,6'-(ethane-1,2-diylbis(oxy))bis(2-(4-(dimethylamino)phenyl)-3-methylbenzo[d]thiazol-3-ium) chloride (6a; diThT-PEG1)

Yield: 63%. ^{1}H NMR (400 MHz, CD_3CN) δ: 7.93 (d, J = 9.2 Hz, 2H), 7.74 (d, J = 2.4 Hz, 2H), 7.71 (d, J = 8.8 Hz, 4H), 7.45 (dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 6.93 (d, J = 8.8 Hz, 4H), 4.54 (s, 4H), 4.16 (s, 6H), 3.13 (s, 12H). ^{13}C NMR (100 MHz, CD_3CN) δ: 173.8, 159.8, 155.5, 138.9, 133.5, 131.4, 120.2, 113.4, 112.3, 108.4, 69.0, 40.8, 39.6. HRMS (ESI+): *m/z* calculated for $C_{34}H_{36}N_4O_2S_2$ $[M]^+$,298.1134; found 298.1140.

6,6'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(2-(4-(dimethylamino)phenyl)-3-methylbenzo[d]thiazol-3-ium) chloride (6b; diThT-PEG2)

Yield: 63%. ^{1}H NMR (400 MHz, CD_3CN) δ: 7.88 (d, J = 9.2 Hz, 2H), 7.67 (d, J = 9.2 Hz, 4H), 7.64 (d, J = 2.4 Hz, 2H), 7.40 (dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 6.90 (d, J = 9.6 Hz, 4H), 4.28 (t, J = 4.4 Hz, 4H), 4.13 (s, 6H),

3.94 (t, J = 4.4 Hz, 4H), 3.12 (s, 12H). ^{13}C NMR (100 MHz, CD_3CN) δ: 173.5, 160.1, 155.4, 138.6, 133.4, 131.2, 120.1, 113.3, 112.2, 108.2, 70.7, 70.1, 40.8, 39.6. HRMS (ESI+): *m/z* calculated for $C_{36}H_{40}N_4O_3S_2$ $[M]^+$,320.1265; found 320.1275.

6,6'-(2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-(4-(dimethylamino)phenyl)-3-methylbenzo[d]thiazol-3-ium) chloride (6c; diThT-PEG3)

Yield: 59%. ^{1}H NMR (400 MHz, CD_3CN) δ: 7.88 (d, J = 9.2 Hz, 2H), 7.66 (d, J = 8.8 Hz, 4H), 7.64 (d, J = 2.4 Hz, 2H), 7.39 (dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 6.89 (d, J = 9.2 Hz, 4H), 4.22 (t, J = 4.4 Hz, 4H), 4.13 (s, 6H), 3.86 (t, J = 4.8 Hz, 4H), 3.70 (s, 4H), 3.11 (s, 12H). ^{13}C NMR (100 MHz, CD_3CN) δ: 172.2, 158.9, 154.2, 137.3, 132.2, 130.0, 119.0, 112.1, 111.0, 106.9, 70.6, 69.2, 68.9, 39.6, 38.3. HRMS (ESI+): *m/z* calculated for $C_{38}H_{44}N_4O_4S_2$ $[M]^+$,342.1396; found 342.1405.

6,6'-(2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-(4-(dimethylamino)phenyl)-3-methylbenzo[d]thiazol-3-ium) chloride (6d; diThT-PEG4)

Yield: 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.14 (d, J = 9.2 Hz, 2H), 7.97 (d, J = 2.4 Hz, 2H), 7.77 (d, J = 9.2 Hz, 4H), 7.47 (dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 6.96 (d, J = 9.2 Hz, 4H), 4.22 (m, 4H), 4.20 (s, 6H), 3.82 (m, 4H), 3.58-3.63 (m, 8H), 3.12 (s, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.6, 158.4, 154.0, 137.3, 132.4, 130.0, 119.0, 118.1, 112.4, 111.2, 107.3, 70.3, 69.1, 68.8, 38.5. HRMS (ESI+): *m/z* calculated for $C_{40}H_{48}N_4O_5S_2$ $[M]^+$,364.1527; found 364.1534.

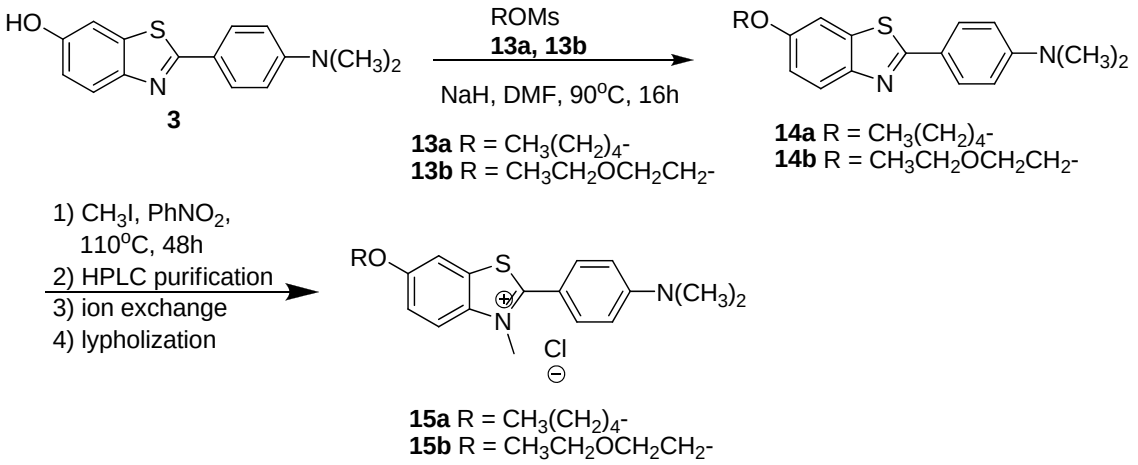
6,6'-(pentane-1,5-diylbis(oxy))bis(2-(4-(dimethylamino)phenyl)-3-methylbenzo[d]thiazol-3-ium) chloride (6f; diThT-C5)

Yield: 67%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.15 (d, J = 9.6 Hz, 2H), 7.97 (d, J = 2.8 Hz, 2H), 7.79 (d, J = 8.8 Hz, 4H), 7.47 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 2H), 6.97 (d, J = 9.2 Hz, 4H), 4.21 (s, 6H), 4.17 (t, J = 6.4 Hz, 4H), 3.12 (s, 12H), 1.90 (m, 4H), 1.65 (m, 2H). ^{13}C NMR (100 MHz, CD_3CN) δ: 160.3, 155.4, 138.4, 133.4,

131.4, 120.2, 113.3, 112.3, 107.9, 70.4, 40.8, 39.5, 29.8, 23.6. HRMS (ESI+): *m/z* calculated for $C_{37}H_{42}N_4O_2S_2$ $[M]^+$,319.1369; found 319.1378.

2-(4-(dimethylamino)phenyl)-6-(2-(2-((4-(6-methoxy-3-methylbenzo[d]thiazol-3-ium-2-yl)phenyl)(methyl) amino)ethoxy)ethoxy)-3-methylbenzo[d]thiazol-3-ium chloride (12; h-t-diThT-PEG2)

Yield: 64% from **(10)**. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.15 (m, 2H), 7.94 (m, 2H), 7.78 (m, 4H), 7.46 (m, 2H), 6.95 (m, 4H), 4.20-4.24 (m, 7H), 3.90-3.91 (m, 3H), 3.85 (m, 2H), 3.74 (s, 3H), 3.11 (m, 11H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.1, 171.0, 158.8, 158.0, 153.4, 152.7, 136.9, 136.8, 131.9, 129.7, 129.6, 118.3, 118.2, 117.8, 112.0, 111.9, 111.0, 110.9, 107.2, 106.4, 68.8, 68.2, 67.9, 56.2, 56.1, 51.1, 38.2. HRMS (ESI+): *m/z* calculated for $C_{40}H_{47}N_4O_6S_2$ $[M]^+$,320.1265; found 320.1259.



Scheme-S1. Synthesis of the monomeric ThT derivatives

N,N-dimethyl-4-(6-(pentyloxy)benzo[d]thiazol-2-yl)aniline (14a; C5-BTA)

The product was isolated with silica gel column (EtOAc/hexane=1:4). Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, J = 9.2 Hz, 2H), 7.77 (d, J = 8.8 Hz, 1H), 7.20 (d, J = 2.8 Hz, 1H) 6.93 (dd, J_1 = 8.8 Hz, J_2 = 2.8 Hz, 1H), 6.63 (d, J = 9.2 Hz, 2H), 3.91 (t, J = 4.8 Hz, 2H), 2.93 (s, 6H), 1.73 (m, 2H), 1.30-1.37 (m, 4H), 0.86 (t, J = 4.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.4, 156.7, 152.0, 148.9, 135.9, 128.6, 122.8, 121.8, 115.5, 111.9,

105.2, 68.8, 40.4, 29.2, 28.4, 22.7, 14.3. HRMS (ESI+): *m/z* calculated for $C_{20}H_{25}N_2OS$ $[M]^+$,341.1687; found 341.1689.

4-(6-(2-ethoxyethoxy)benzo[d]thiazol-2-yl)-N,N-dimethylaniline (14b; EtOEt-BTA)

The product was isolated with silica gel column (EtOAc/hexane=1:3). Yield: 76%. ^{1}H NMR (400 MHz, CD_2Cl_2) δ: 7.81 (d, J = 9.2 Hz, 2H), 7.74 (d, J = 8.8 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H) 6.97 (dd, J_1 = 9.2 Hz, J_2 = 2.8 Hz, 1H), 6.66 (d, J = 9.2 Hz, 2H), 4.07 (m, 2H), 3.71 (m, 2H), 3.51 (q, J = 7.2 Hz, 2H), 2.95 (s, 6H), 1.16 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2) δ: 166.3, 156.5, 152.1, 149.2, 135.9, 128.4, 122.7, 121.6, 115.4, 111.8, 105.4, 69.1, 68.4, 66.9, 40.1, 15.2. HRMS (ESI+): *m/z* calculated for $C_{19}H_{23}N_2O_2S$ $[M]^+$,343.1480; found 343.1483.

2-(4-(dimethylamino)phenyl)-3-methyl-6-(pentyloxy)benzo[d]thiazol-3-ium chloride (15a; C5-ThT)

Yield: 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.14 (d, J = 9.6 Hz, 1H), 7.97 (d, J = 2.4 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.44 (dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 1H), 6.96 (d, J = 9.2 Hz, 2H), 4.21 (s, 3H), 4.11 (t, J = 6.4 Hz, 2H), 3.11 (s, 6H), 1.79 (m, 2H), 1.40 (m, 4H), 0.91 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.1, 158.1, 153.4, 136.7, 131.9, 129.7, 118.4, 117.7, 111.9, 111.0, 107.0, 68.6, 38.2, 28.1, 27.6, 21.9, 13.9. HRMS (ESI+): *m/z* calculated for $C_{21}H_{27}N_2OS$ $[M]^+$,355.1838; found 355.1837.

2-(4-(dimethylamino)phenyl)-6-(2-ethoxyethoxy)-3-methylbenzo[d]thiazol-3-ium chloride (15b; EtOEt-ThT)

Yield: 70%. ^{1}H NMR (400 MHz, CD_3CN) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 9.2 Hz, 2H), 7.66 (d, J = 2.0 Hz, 1H), 7.41 (dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 1H), 6.91 (d, J = 9.6 Hz, 2H), 4.23 (m, 2H), 4.15 (s, 3H), 3.80 (m, 2H), 3.56 (q, J = 8.8 Hz, 2H), 3.12 (s, 6H), 1.18 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.6, 159.3, 154.2, 136.9, 132.1, 129.7, 119.7, 117.3, 112.4, 110.8, 106.7, 68.9, 68.7, 67.0, 58.8, 40.2, 38.6, 23.9. HRMS (ESI+): *m/z* calculated for $C_{20}H_{25}N_2O_2S$ $[M]^+$,357.1631; found 357.1642.

III. Spectroscopic characterization of ThT derivatives

All ThT monomer derivatives were stored in DMSO stock solution at 2.5 mM (dimers at 1.25 mM) and diluted into a phosphate buffer (50 mM phosphates, 300 mM NaCl, pH 7.4) in all tests below. UV/Vis spectra (***Figure S1***) were collected for each ThT monomer at 5 μM (dimer at 2.5 μM) in the phosphate buffer. Except diThT-PEG1, all ThT dimers display no tailing in their absorption spectra, indicating that the dimers are well-soluble at this concentration. The extinction coeffients at 410 nm are listed in ***Table S1*** for all the ThT derivatives.

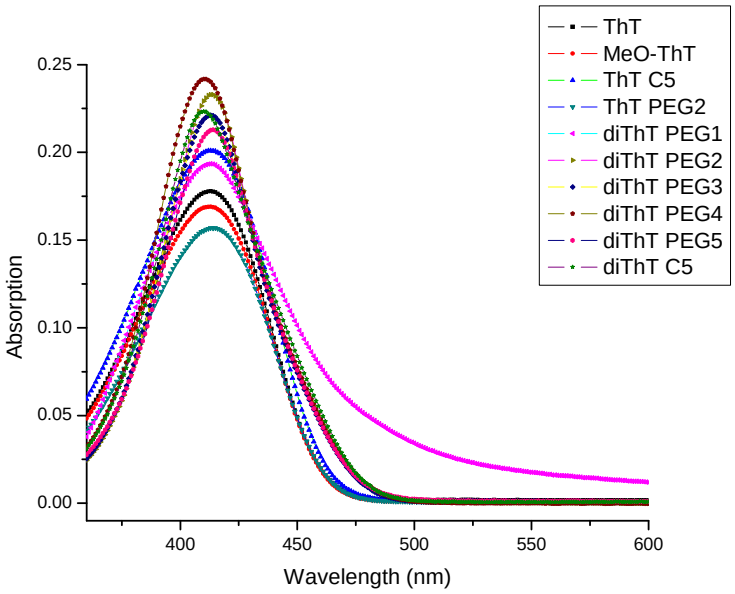


Figure S1. UV/Vis absorption spectra of ThT derivatives

The fluorescence excitation spectra (***Figure S2***) of ThT and diThT-PEG3 were collected in the phosphate buffer with 2 μM Aβ40 fibril on a Fluorolog-3 fluorimeter and a MicroMax 384 microwell plate reader (Jobin Yvon Inc. Edison, NJ). The small molecule concentration used was 2 μM for ThT and 1 μM for diThT PEG3. The result shows that the ThT monomer and dimer share the same excitation profile (λmax = 440 nm). The fluorescence intensity difference is due to the fact that diThT-PEG3 binds better than the ThT monomer under the experimental conditions.

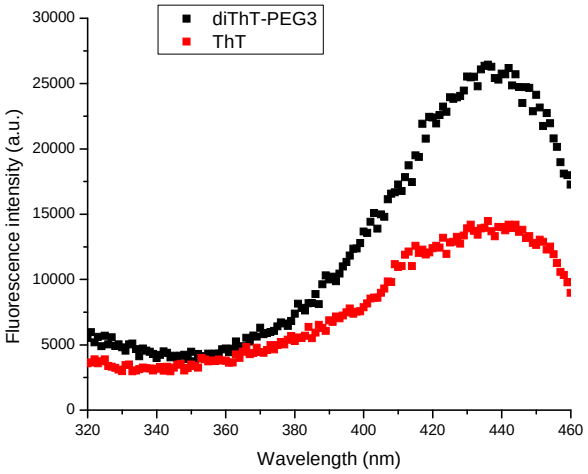


Figure S2. The fluorescence excitation spectra of ThT and diThT-PEG3.

The fluorescence emission spectra (***Figure S3***) of ThT and diThTs were collected in the phosphate buffer with 15μM Aβ-(1-40) fibril on a Fluorolog-3 fluorimeter (Jobin Yvon Inc. Edison, NJ). The small molecule concentration used was 1 μM for ThT and 0.5 μM for the ThT dimers. Both the monomer and the dimers show amyloid induced fluorescence emission. The emission spectra of ThT and diThT-PEG2 are shown in ***Figure S3*** as examples. Due to the improved binding, diThT-PEG2 shows greater fluorescence intensity than ThT under the same condition.

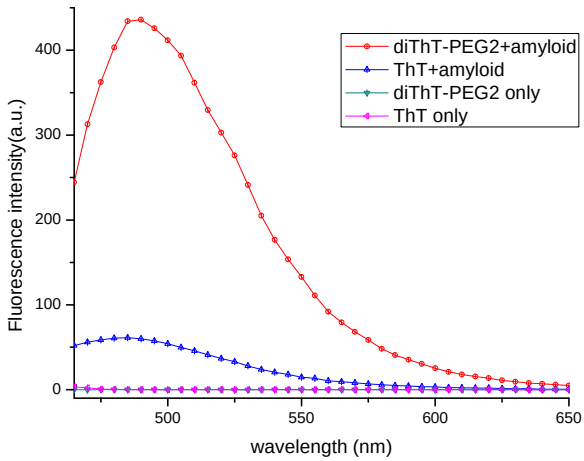


Figure S3. The emission spectra of ThT and diThT-PEG2. In absence of amyloid, both the ThT monomer and dimer exhibit no detectable fluorescence, as shown by the overlapping traces at the bottom.

All the ThT derivatives display no detectable fluorescence emission in absence of Aβ40 amyloid. The quantum yields of the amyloid-bound dye molecules are difficult to determine because an unrealistically high concentration of Aβ40 amyloid is needed to saturate ThT binding. In addition, the protein aggregates at high concentrations give strong light scattering that obscures the measurement of ThT absorption at 440 nm. Therefore, the relative quantum yields of the ThT derivatives are reported (***Table S1***), with the ThT monomer as the standard. These values were obtained by comparing the fluorescence intensities at the dye concentrations that saturate the binding sites on Aβ amyloids. The result shows that the ThT derivatives exhibit a small variation (within two folds) in their quantum yields when bound onto amyloids.

Table S1. The extinction coefficients and relative quantum yields of the ThT derivatives.

ThT derivatives	Extinction coefficient [a] (at 410 nm, $M^{-1}cm^{-1}$)	Relative quantum yield (Φ/Φ_{ThT})
ThT	35,300	1
diThT-PEG2	45,800	1.48
diThT-PEG3	43,700	1.35
diThT-PEG4	48,300	1.32
diThT-PEG5	41,700	1.30
diThT-PEG2-HT	44,900	1.30
diThT-C5	44,600	2.04
MeO-ThT	33,700	1.07
PEG2-ThT	31,000	1.01
C5-ThT	40,000	2.04

[a] data calculated based on the absorption spectra shown in ***Figure S1***.

The photobleaching experiments of ThT and diThT-PEG2 were carried out in the phosphate buffer with 2 μM Aβ40 fibril on a Fluorolog-3 fluorimeter (Jobin Yvon Inc. Edison, NJ). The small molecule concentration used was 1 μM for ThT and 0.5 μM for diThT PEG2. The samples were excited at 440 nm, and the emission fluorescence at 490 nm was recorded over a two hour time period (***Figure S4***). The result shows that photobleaching is insignificant for either the monomer or the dimer; less than 10% of fluorescence intensity decrease was observed for diThT-PEG2 after 2 hr irradiation.

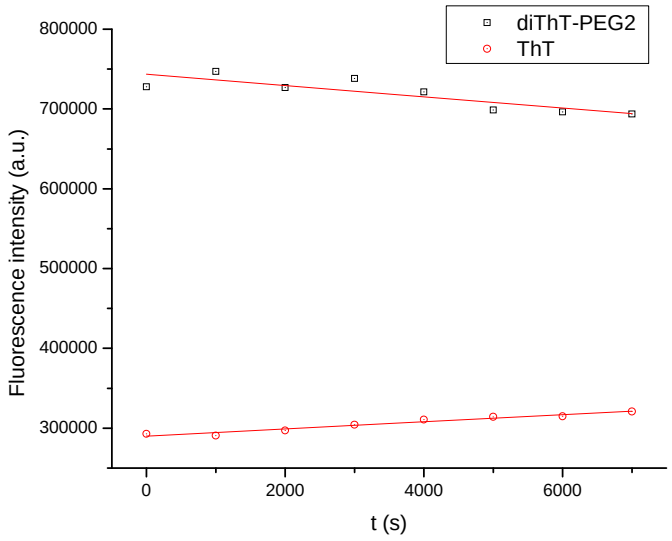


Figure S4. The photobleaching data of ThT and diThT-PEG2

IV. Aβ40 synthesis

Aβ40 was synthesized through the standard Fmoc/tBu chemistry with the Fmoc-Phe-Wang resin (Novabiochem) as the solid support. The synthesis was carried out on 0.1 mmole scale. Five equivalents of amino acids and HBTU were used for the coupling reaction. To ensure the quality of the peptide, double couplings were carried out for residues after all beta-branched amino acids. The peptides were cleaved off the resin and deprotected with TFA (95% TFA, 2.5% H2O, 2.5% triethylsilane). The crude products were purified by RP-HPLC (Waters Prep LC, Jupiter 10u C18 300A Column). The integrity of the peptide was conformed by LC-MS, which showed the purity is greater than 90% (***Figure S5***).

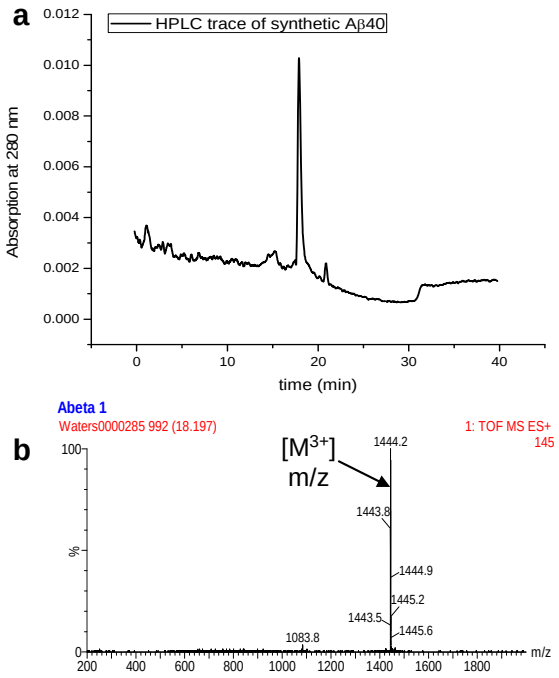


Figure S5. Analytical HPLC trace of the synthetic Aβ40 exhibiting good purity (a) and ESI-MS result confirming the identity of the peptide (b).

V. Preparation and characterization of Aβ40 fibrils

In order to prepare Aβ40 amyloid, the peptide was treated according to a published protocol to obtain fresh Aβ40 monomers. Briefly, the lyophilized Aβ40 peptide was dissolved in 1 mM NaOH solution, filtered through 0.22 μM syringe filter (Millipore, Billerica, MA), and then through a Centricon filter with 10KDa molecular weight cutoff (Millipore, Billerica, MA). The peptide was diluted into the phosphate buffer (50 mM phosphates, 300 mM NaCl, pH 7.4); the concentration was determined by the tyrosine absorption at 280 nm right after the dilution. The sample was put on the rotating wheel at 30 rpm. The aggregation progress was monitored by circular dichroism spectroscopy, which showed complete aggregation after seven days. The formation of beta-sheet rich amyloids was confirmed by CD data (***Figure S6***) and the Congo red binding assay (***Figure S7***). The stock fibril solution was stored at 2 ℃ and used within 2 weeks.

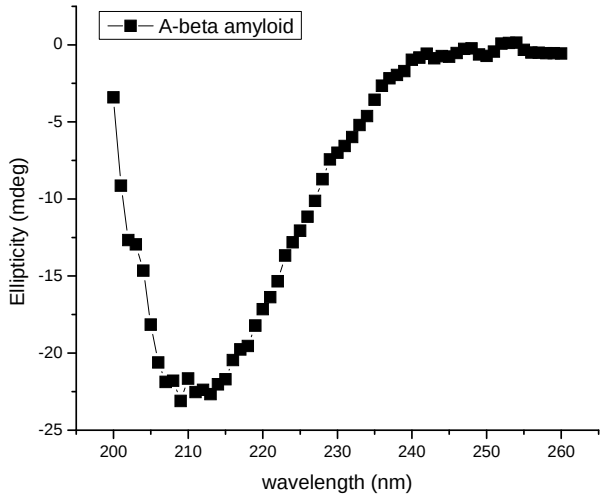


Figure S6. The circular dichroism spectrum of the aggregated Aβ40.

Congo Red assay: [1, 2] The Congo Red stock was prepared in 90% phosphate buffer saline (10 mM phosphate, 2.7 mM KCl, and 137 mM NaCl; pH 7.4) and 10% ethanol. The concentration was determined by measuring the absorbance of a diluted aliquot in a solution of sodium phosphate (1 mM, pH 7.0) and 40% ethanol at 505 nm. The stock solution of Aβ40 amyloid was mixed with the Congo Red stock in phosphate buffer (50 mM phosphates, 300 mM NaCl, pH 7.4) to yield a final concentration of 20 μM Congo Red and 20 μM Aβ-(1-40) fibril. Two control samples were prepared: (1) a solution of 20 μM Congo Red alone and (2) a solution of 20 μM Aβ40 amyloid alone. The UV/Vis absorption spectra of all three samples were collected and shown below. The spectral shift allowed us to back calculate the amyloid concentration, which nicely agreed with the concentration that we prepared it to be. This result indicates Aβ40 aggregation largely yielded beta-sheet structured amyloid, rather than non-specific aggregates.

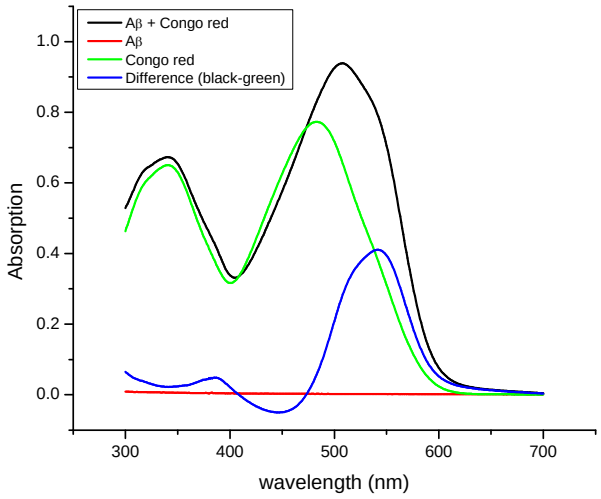


Figure S7. UV/Vis absorption shift of Congo red binding to Aβ amyloid.

VI. Measuring the amyloid binding affinities of the ThT derivatives

The Aβ40 amyloid stock solution was sonicated in ice-water bath for 1 h and added into solutions of a ThT derivative at various concentrations. The final concentration of Aβ40 amyloid was set at 2 μM. The fluorescence emission was scanned immediately in a 384 well microplate (50 μL each sample) at 465-650 nm with the excitation at 440 nm and slits as 6 nm on a Fluorolog-3 fluorometer and a MicroMax 384 microwell plate reader (Jobin Yvon Inc. Edison, NJ). Triplicates of each sample were prepared and measured.

The fluorescence intensity at 485nm was plotted against the small molecule concentration, yielding the binding curves shown in **Figure S8**.

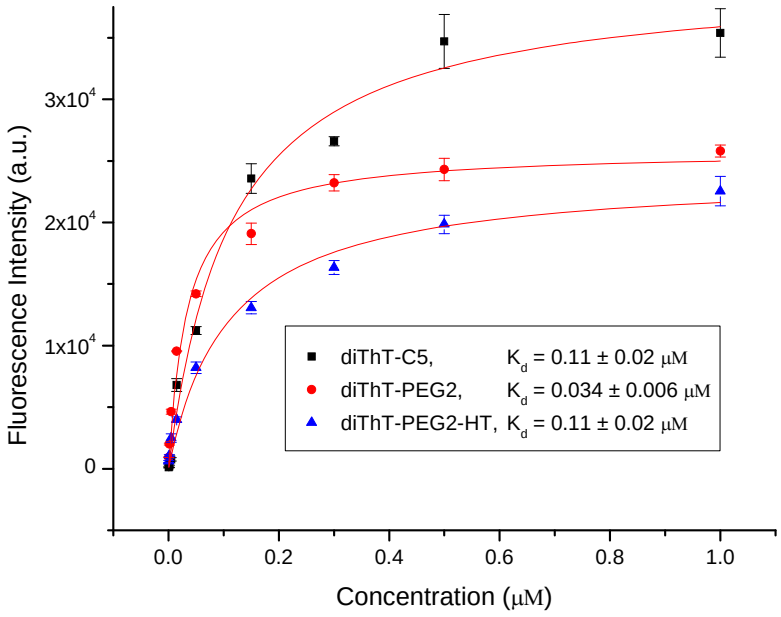


Figure S8. Aβ40 amyloid binding curves of diThT-C5, diThT-PEG2 and diThT-PEG2-HT.

VII. Evaluating the binding specificity of the ThT dimers to Aβ40 amyloid

The stock Aβ40 amyloid fibril solution was sonicated for 1 h and diluted to various concentrations into solutions of 1 μM ThT or 0.5 μM diThT derivatives and 10% (v/v) bovine serum (protein concentration ~60-80 mg/ml) in phosphate buffer. The fluorescence emission was scanned immediately in a 96 well microplate (3 × 150 μL each sample) at 465-650 nm with the excitation at 440 nm on a Spectra Max M5 microwell plate reader (Molecular Devices, Sunnyvale, CA). The data are shown in **Figure 5** in the main text.

1. Klunk, W. E.; Jacob, R. F.; Mason, R. P.,"*Quantifying amyloid beta-peptide (Abeta) aggregation using the Congo red-Abeta (CR-abeta) spectrophotometric assay"*, *Anal Biochem* **1999**, *266*, 66-76.
2. Klunk, W. E.; Jacob, R. F.; Mason, R. P.,"*Quantifying amyloid by congo red spectral shift assay"*, *Methods Enzymol* **1999**, *309*, 285-305.