Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Degradation of amyloid peptide aggregates by targeted singlet oxygen delivery from a benzothiazole functionalized naphthalene endoperoxide

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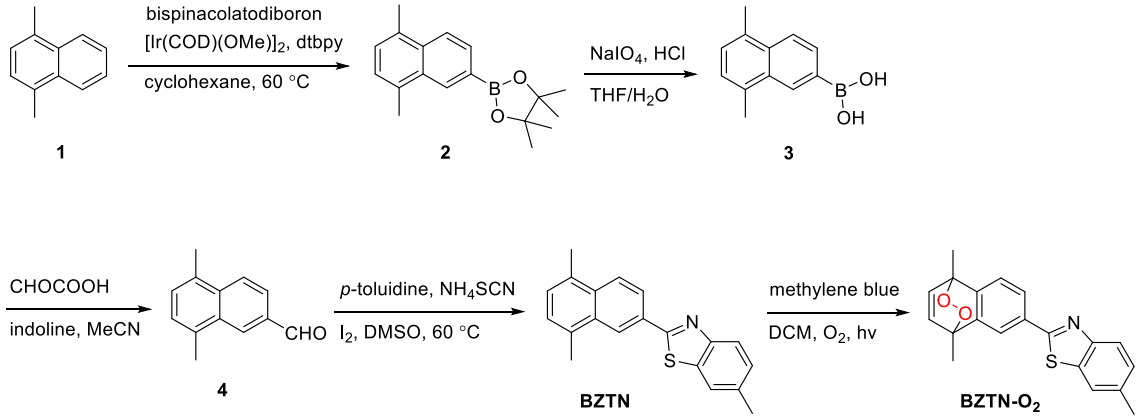
Synthesis of intermediates



*Synthesis of **2*:** [Ir(COD)(OMe)]$_2$ (331 mg, 0.5 mmol), 4,4'-ditertbutyl-2,2'-bipyridine (268 mg, 1 mmol), bispinacolatodiboron (2.79 g, 11 mmol) and 1,4-dimethylnaphthalene (1.54 mL, 10 mmol) were added to a flask with 38 mL cyclohexane and the mixture was stirred at 80 °C for 16 h. After cooled to room temperature, the reaction solvent was evaporated in vacuo and the borylated product **2** (2.72 g, 96%) was isolated by column chromatography (Hexane: EtOAc, 9:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 2.71 (s, 3H), 2.63 (s, 3H), 1.37 (s, 12H).

*Synthesis of **3*:** Compound **2** (500 mg, 1.77 mmol) and sodium periodate (1.136 g, 5.31 mmol) were stirred in THF: H_2O (4:1, v/v) for 30 min, then aqueous HCl (1N, 1.24 mL) was added to the suspension. The reaction mixture stirred at room temperature for 24 h. The reaction mixture was diluted with water and extracted with EtOAc for three times. The combined organic phases were washed with water and brine, respectively. Then, the organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography using DCM: MeOH (98:2, v/v) as the eluent. Compound **3** was obtained as white solid (326 mg, 92%). ^{1}H NMR (400 MHz,

DMSO-d_6) δ 8.54 (s, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 7.3 Hz, 1H), 7.22 (d, J = 7.2 Hz, 1H), 2.65 (s, 3H), 2.60 (s, 3H).

*Synthesis of **4*:** Compound **3** (200 mg, 1 mmol) and glyoxylic acid monohydrate (0.21 mL, 1.05 mmol) were placed in a 50 mL flask. Then, acetonitrile (5 mL) was injected into the vial and 30 mol% indoline (30 μL, 0.3 mmol) was added. The mixture was stirred at room temperature for 24 h and the reaction was monitored by TLC. The solvent was evaporated in vacuo and the residue was purified by column chromatography (100:1, Hexanes: EtOAc, v/v) to afford compound **4** as white solid (144 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20 (s, 1H), 8.52 (d, J = 1.7 Hz, 1H), 8.11 (d, J = 8.7 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.37 (d, J = 7.1 Hz, 1H), 7.31 (d, J = 7.1 Hz, 1H), 2.76 (s, 3H), 2.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.6, 135.9, 134.3, 133.4, 132.7, 132.2, 131.0, 129.6, 127.5, 125.9, 122.5, 19.4, 19.3.

*Synthesis of **BZTN*:** An oven-dried reaction vessel was charged with compound **4** (184 mg, 1 mmol), *p*-toluidine (107 mg, 1 mmol), NH_4SCN (152mg, 2 mmol), and I_2 (360 mg, 1 mmol) in DMSO (3 mL), and the reaction mixture was stirred at 110 °C for 12 h. After completion, the reaction mixture was allowed to cool to room temperature and quenched with saturated $Na_2S_2O_3$ solution. Then the reaction mixture was extracted with ethyl acetate, and the organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography using petroleum ether: EtOAc (9:1, v/v) to afford the pure product (242 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.25 (d, J = 10.7 Hz, 1H), 8.13 (d, J = 8.8 Hz, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.75 (s, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.30 (m, 2H), 2.81 (s, 3H), 2.72 (s, 3H), 2.55 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 152.33, 135.5, 135.3, 134.0, 133.5, 132.7, 132.5, 130.4, 128.0,

127.9, 127.3, 125.7, 124.3, 123.9, 122.7, 121.4, 21.6, 19.4, 19.3; HRMS-ESI *m*/*z* calcd for $C_{20}H_{18}NS$ $[M + H]^+$ 304.1082, found 304.1161.

Synthesis of ***BZTN-O₂*****:** Compound **BZTN** (100 mg, 0.33 mmol) was dissolved in 3 mL DCM and the reaction mixture was cooled down to 0 °C. Catalytic amount of methylene blue was added and the reaction mixture was stirred under oxygen atmosphere with the irradiation using a 18 W red light (630 nm). When the reaction completed, the solvent was removed in vacuo and the crude product was purified by silica gel column chromatography with DCM as the eluent (110 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.00 – 7.93 (m, 2H), 7.72 (s, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 7.8 Hz, 1H), 2.53 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 151.3, 142.5, 141.1, 138.4, 138.0, 134.6, 131.5, 127.0, 125.3, 122.9, 121.8, 120.4, 119.7, 118.0, 77.7, 77.5, 20.5, 15.2, 15.1.

Detection of singlet oxygen release

To detect singlet oxygen release from endoperoxide, 1,3-diphenylisobenzofuran (DPBF) was used as singlet oxygen probe. Briefly, 500 µM of endoperoxide **BZTN-O₂** was mixed with DPBF (50 µM) in DMSO. The measurements were taken at 20 minutes intervals at 37 °C in dark conditions. Absorbance decrease at 414 nm was monitored which revealing the release of singlet oxygen from endoperoxide.

Preparation of monomeric $A\beta_{1–42}$ Solution: Human $A\beta_{1–42}$ (1 mg) was dissolved in hexafluoro-2-propanol (HFIP) and kept at room temperature overnight.[1-2] The solution was divided into microcentrifuge tubes (1/10 mg aliquots), and put into a vacuum desiccator to

afford film-like lyophilized $A\beta_{1-42}$. Monomeric $A\beta_{1-42}$ films were stored at -20 °C and used for further experiments.

Thioflavin T (ThT) assay

The $A\beta_{1-42}$ film was re-dissolved in a freshly prepared ammonium hydroxide (20 μL) by brief vortexing and the solution was further diluted with 50 mM phosphate buffer (pH 7.4) to 50 μM before use. A mixture of the peptide (20 μL, 50 μM, final concentration) with or without compound **BZTN** or **BZTN-O$_2$** (100 μM) was incubated at 37 °C for 48 h. After incubation, the sample was diluted to a final volume of 200 μL with 50 mM glycine–NaOH buffer (pH 8.0) containing thioflavin T (5 μM). Then the fluorescence intensities were recorded after five minutes (excitation 450 nm).

DNPH Assay

Peptide $A\beta_{1-42}$ (20 μL, 50 μM, final concentration) with or without the tested compound (20 μL, 100 μM, final concentration) was incubated at 37 °C for 48 h. Then, 500 μL of each 50 μM $A\beta_{1-42}$ solution was treated with a trichloroacetic acid (TCA, 20% final concentration) solution for 15 min in an ice bath, which was then collected by centrifuging (5 min at 14 000 rpm). After that, 400 μL of 10 mM DNPH in 2 M of HCl was added into each tube and the mixture was incubated at RT for 1 hour. Then, each sample was precipitated with 20% TCA solution and washed by 600 μL of ethanol-ethyl acetate (1:1, v/v) solution. The samples were then resuspended in a guanidine hydrochloride solution (600 μL, 6 M, pH 2.3) for 20 min at 37 °C, and the mixture were measured by UV-vis spectroscopy (300 – 600 nm).

For control experiment: 50 μM BSA (Bovine Serum Albumin) pre-treated with or without **BZTN-O$_2$** was precipitated with a trichloroacetic acid (TCA, 20% final concentration)

solution for 15 min in an ice bath and then collected as a form of pellet in a tube by centrifuging (5 min at 14 000 rpm). After that, 400 μL of 10 mM DNPH in 2 M of HCl was added into each tube for 1 h at room temperature. Each sample was precipitated with 20% TCA solution and remains were washed one time with 600 μL of ethanol-ethyl acetate (1:1, v/v) solution. Washed samples were resuspended in a guanidine hydrochloride solution (600 μL, 6 M, pH 2.3) for 20 min at 37 °C. Then, the samples were measured by spectrophotometer to get UV-vis absorbance spectrum (300 – 600 nm).

Inhibition of $A\beta_{1-42}$ aggregation using TEM analysis

$A\beta_{1-42}$ peptide stock was diluted with 10 mM phosphate buffer (pH 7.4) to 100 μM before use. For the inhibition of $A\beta_{1-42}$ aggregation, monomeric $A\beta_{1-42}$ was incubated with endoperoxide **BZTN-O2**, compound **BZTN** or curcumin at 37 ℃ for 48 h, respectively. Monomeric $A\beta_{1-42}$ and aggregated $A\beta_{1-42}$ were used as control groups. The concentration of $A\beta_{1-42}$ and compounds were set as 25 μM (final concentration). Aliquots (10 μL) of the samples were placed on a carbon coated copper/rhodium grid for 2 min at room temperature. Each grid was stained with 0.5% phosphotungstate (PTA) solution for 2 min. The excess staining solution was removed and the specimen was transferred for imaging with transmission electron microscopy.

For the inhibition of aggregated $A\beta_{1-42}$, $A\beta_{1-42}$ was first incubated at 37 ℃ for 48 h, and then one of the tubes was added with **BZTN-O2** and incubated at 37 ℃ for another 48 h. The final concentration of $A\beta_{1-42}$ and compounds were 25 μM. Aliquots (10 μL) of the samples were placed on a carbon coated copper/rhodium grid for 2 min at room temperature. Each grid was stained with 0.5% phosphotungstate (PTA) solution for 2 min. The excess

staining solution was removed and the specimen was transferred for imaging with transmission electron microscopy.

Dynamic Light Scattering (DLS) Measurement

$A\beta_{1-42}$ peptide stock was diluted with 10 mM phosphate buffer (pH 7.4) to 100 μM before use. For the inhibition of $A\beta_{1-42}$ aggregation experiment, $A\beta_{1-42}$ was incubated in the presence of **BZTN-O2** or **BZTN** at 37 ℃ for 48 h. The final concentration of $A\beta_{1-42}$ and compounds were 25 μM. Monomeric $A\beta_{1-42}$ and aggregated $A\beta_{1-42}$ were used as control groups. The equal sample (100 μL) was diluted to 2 mL with PBS. Then, the samples were measured by Zetapotential analyzer to obtain DLS.

Atomic Force Microscopy (AFM)

$A\beta_{1-42}$ peptide stock was diluted with 10 mM phosphate buffer (pH 7.4) to 100 μM before use. For the inhibition of $A\beta_{1-42}$ aggregation experiment, $A\beta_{1-42}$ was incubated in the presence and absence of **BZTN-O2** at 37 ℃ for 48 h. Monomeric $A\beta_{1-42}$ was used as control group. The final concentration of $A\beta_{1-42}$ and compounds were 25 μM. For the AFM measurements, 10 μl aliquots of the $A\beta_{1-42}$ sample solutions were deposited onto a cleaved mica substrate for 10 min and were rinsed several times with DI water to remove any remaining salts and unbound peptides. After the mica was fully dried, the samples were measured by AFM instrument to obtain images (Nanowizard4XP, Bruker).

Safety test

Human umbilical vein endothelial cells (HUVEC) and human normal liver cells (L02) were seeded (3000 cells per well of a 96-well plate) and incubated at 37℃ for 12 h in DMEM medium. And then, after incubation with various concentration of **BZTN-O2 or BZTN** for

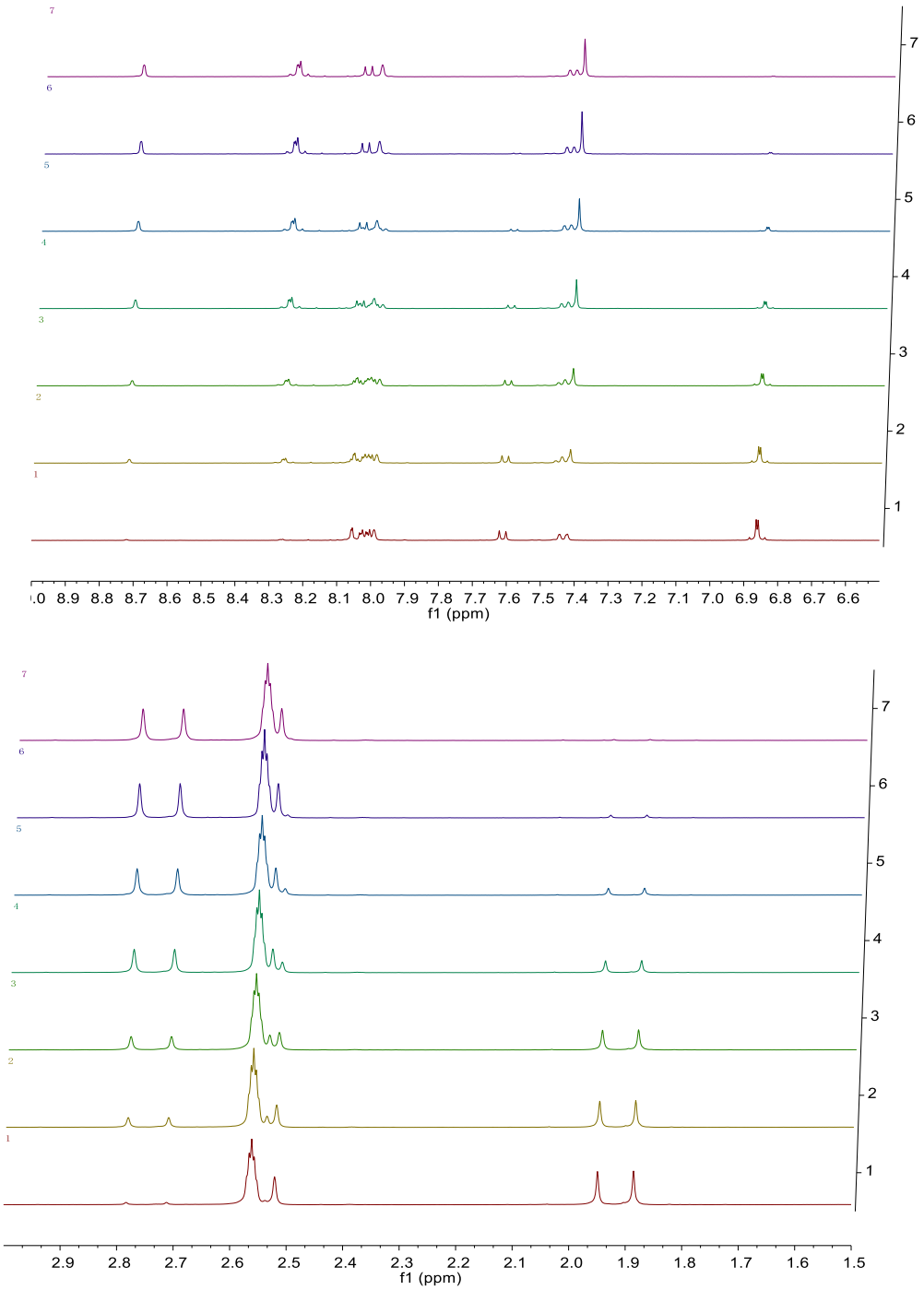
another 24 h at 37℃, 3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) in PBS (5 mg/mL, 20 μL) were added. With further incubation at 37 ℃ for 4 h, the medium was removed. DMSO (200 μL) was added to each well and the absorbance at 570 nm was measured with a microplate reader.

Reference

1. You Jung Chung, Byung Il Lee, Jong Wan Ko, and Chan Beum Park, *Adv. Healthcare Mater*., **2016**, *5*, 1560–1565.

2. Minkoo Ahn, Byung Il Lee, Sean Chia, Johnny Habchi, Janet R. Kumita, Michele Vendruscolo, Christopher M. Dobson and Chan Beum Park, *Chem. Commun*., **2019**, *55*, 1152–1155.

Fig. S1A Temporal evolution of **BZTN-O$_2$** ^{1}H NMR in D_2O:DMSO-d_6 (3:7) at 37 °C



Spectra 1- 0 hours, Spectra 2- 0.5 hours, Spectra 3- 1 hours, Spectra 4- 2 hours, Spectra 5- 3 hours, Spectra 6- 5 hours, Spectra 7- 7 hours.

The rate constant and half-life calculations were done in accordance to the first-order reaction rate equations. Cycloreversion reaction rate of **BZTN-O2** is calculated based on the ^{1}H NMR integral ratios. Peak integrals between 1.8-2.0 ppm (**BZTN-O2**) and 2.7 to 2.8 ppm (**BZTN**) were used for this calculation. The equations are given below:

$$\ln[A] = -kt + \ln[A]_0 \quad , \quad t_{1/2} = 0.693/k$$

Fig. S1B Half-life calculated at 37 ºC in $CDCl_3$ $t_{1/2}$ = 1.29 h

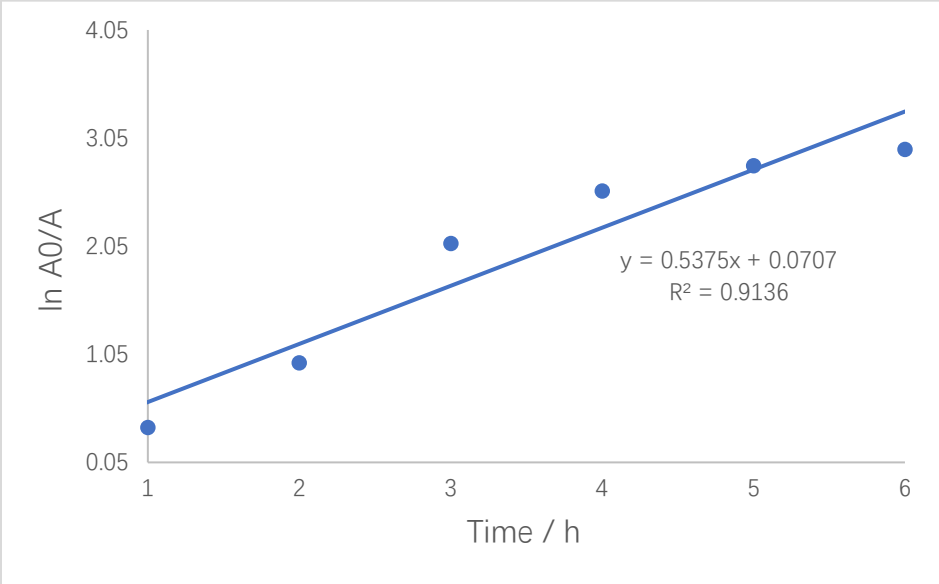


Fig. S1C Half-life calculated at 37 °C in D_2O:DMSO-d_6 (3:7) $t_{1/2}$ = 1.37 h

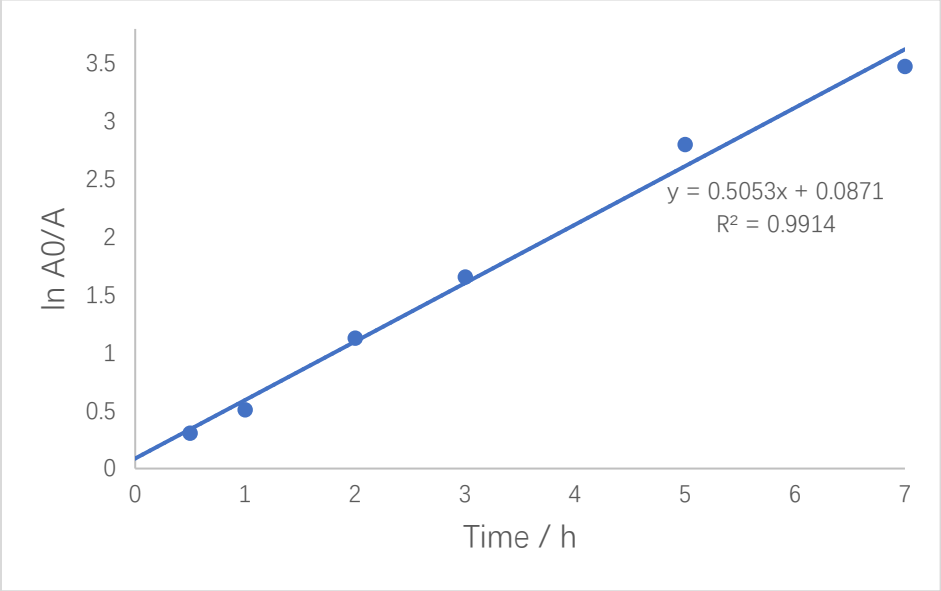


Fig. S2 DNPH Assay of BSA treated with **BZTN-O_2**

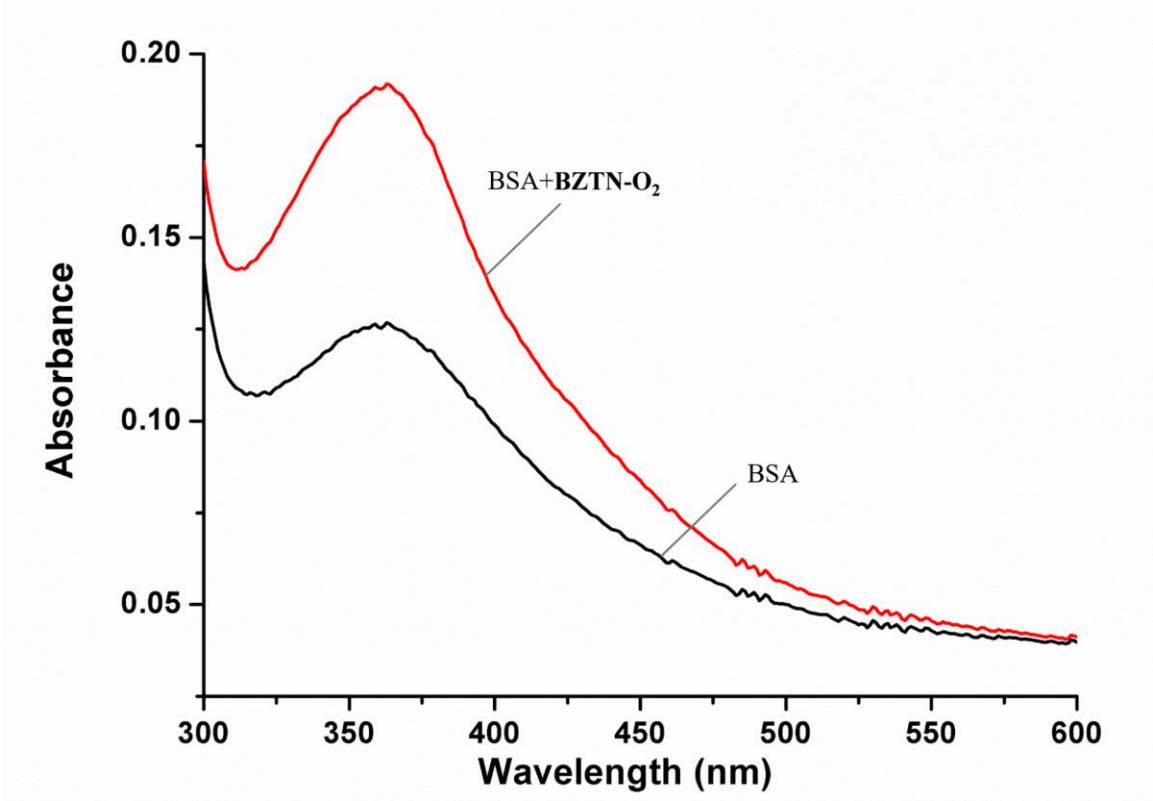


Fig. S3 DLS size distribution of monomeric $A\beta_{1-42}$

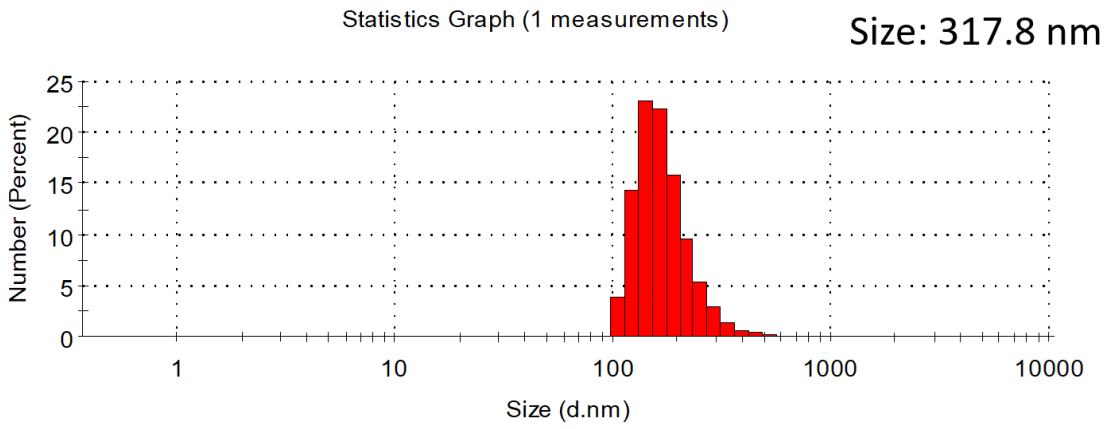


Fig. S4 DLS size distribution of $A\beta_{1-42}$ incubated at 37 ℃ for 48 h

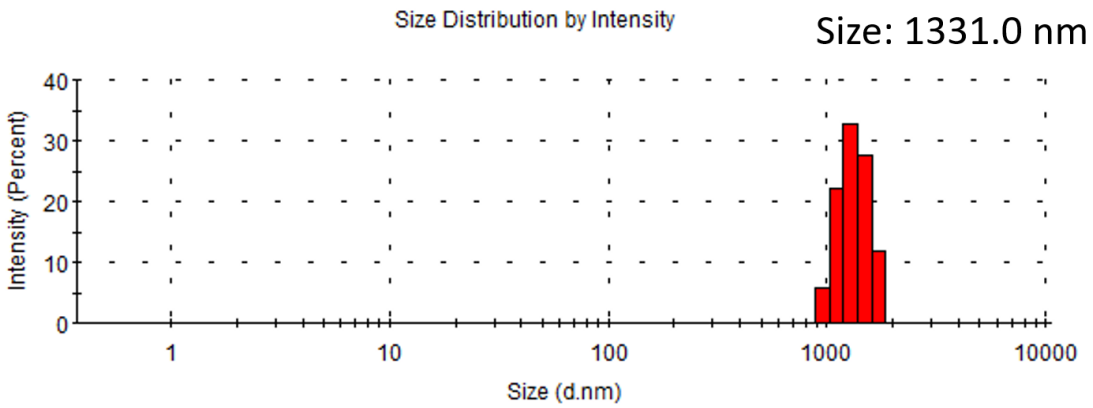


Fig. S5 DLS size distribution of $A\beta_{1-42}$ incubated with **BZTN-O$_2$** at 37 ℃ for 48 h

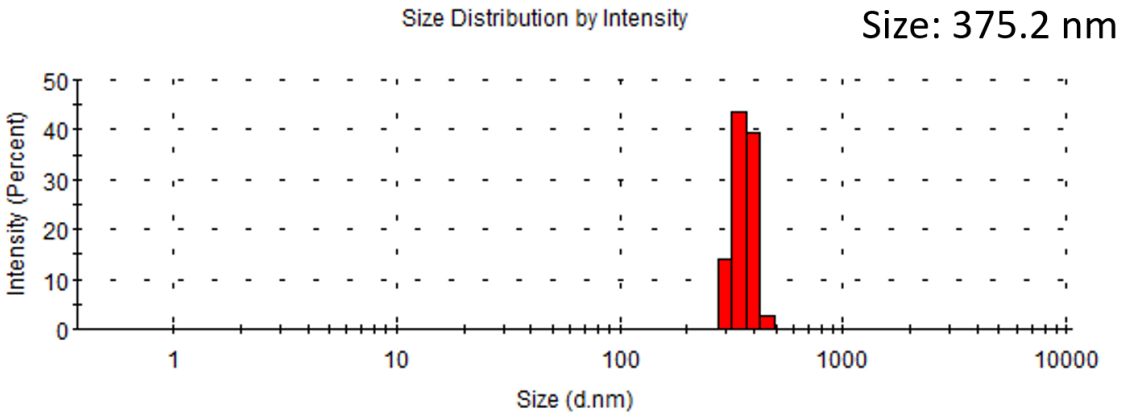


Fig. S6 DLS size distribution of $A\beta_{1-42}$ incubated with **BZTN** at 37 ℃ for 48 h

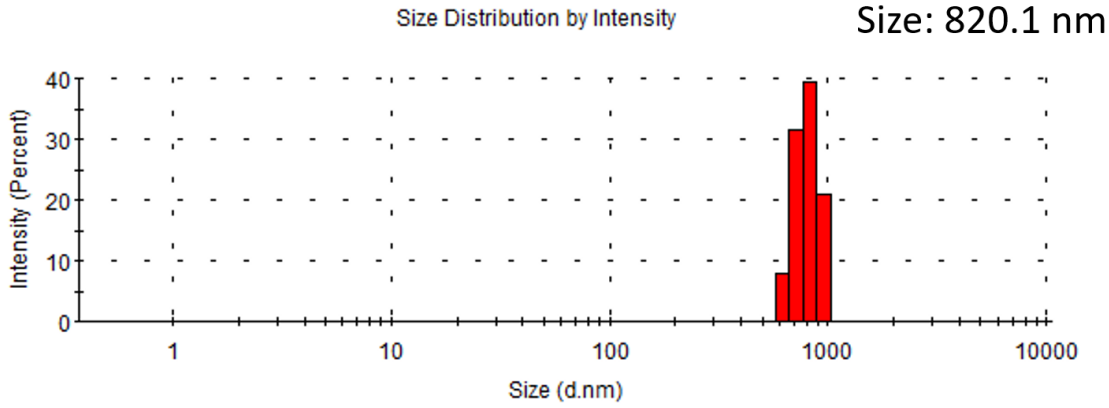
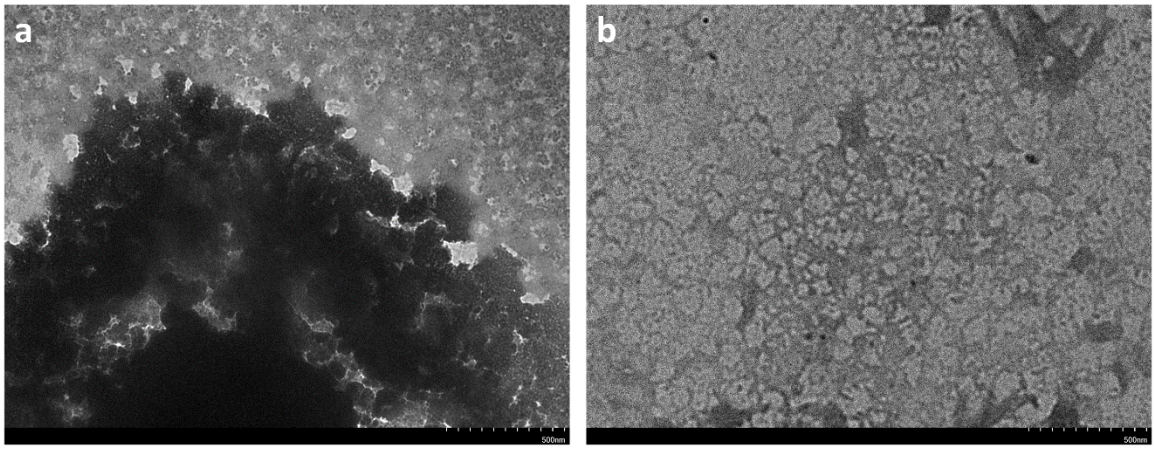


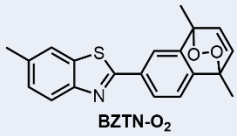
Fig. S7 TEM analysis of aggregated $A\beta_{1-42}$ followed by incubation with **BZTN-O_2**



(a) 25 μM $A\beta_{1-42}$ alone at 96 h, 37 ℃

(b) 25 μM $A\beta_{1-42}$ alone at 48 h, 37 ℃, then incubated with 25 μM **BZTN-O_2** for another 48 h.

Fig. S8 Molecular properties of **BZTN-O_2**

Structure	miLogP	MW	Ha(nON)	Hd(nOHNH)	TPSA	Volume
Lipinski's rule of five	< 5	< 500	< 5	< 10		
 BZTN-O_2	5.63	335.43	3	0	31.36	292.39

Molecular properties were calculated using an on-line services at www.molinspiration.com.

NMR spectra

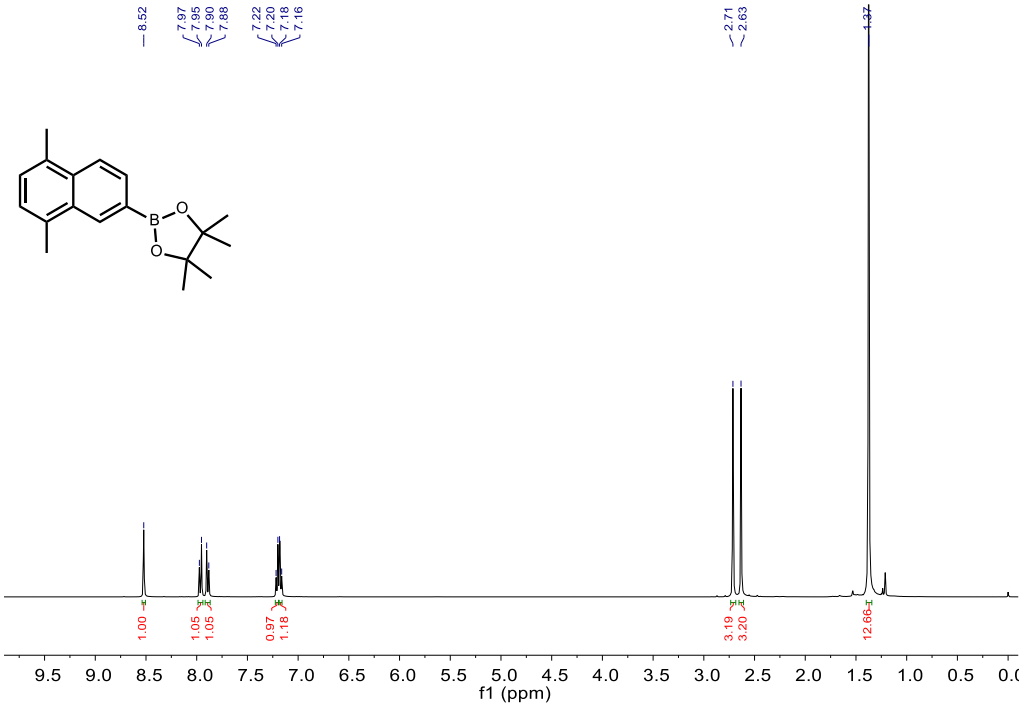


Fig. S9 ^{1}H NMR of compound **2**

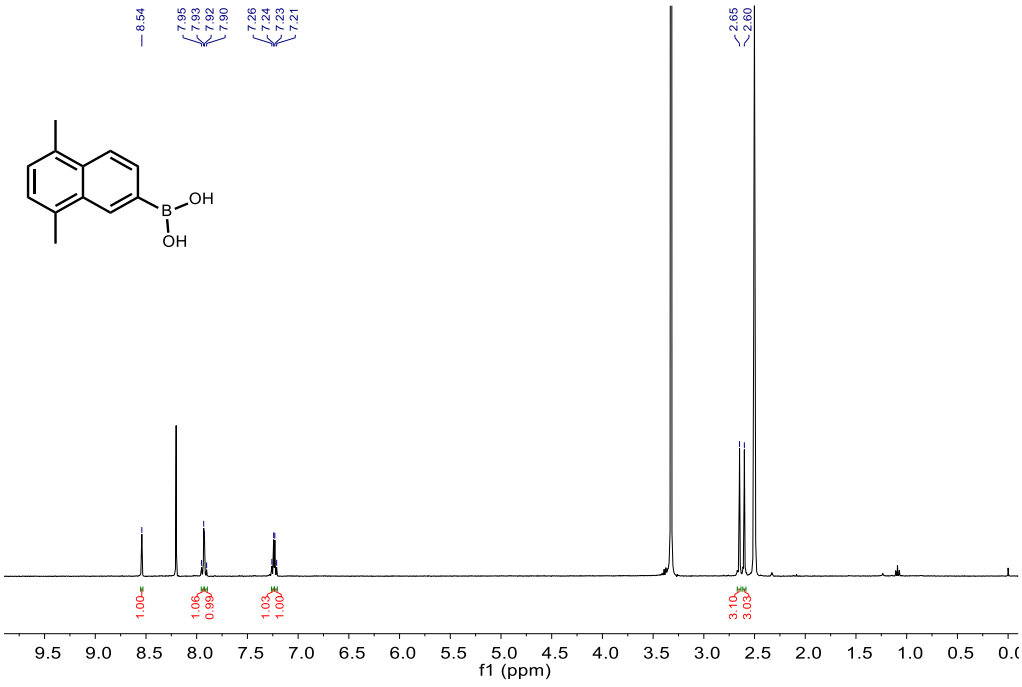


Fig. S10 ^{1}H NMR of compound **3**

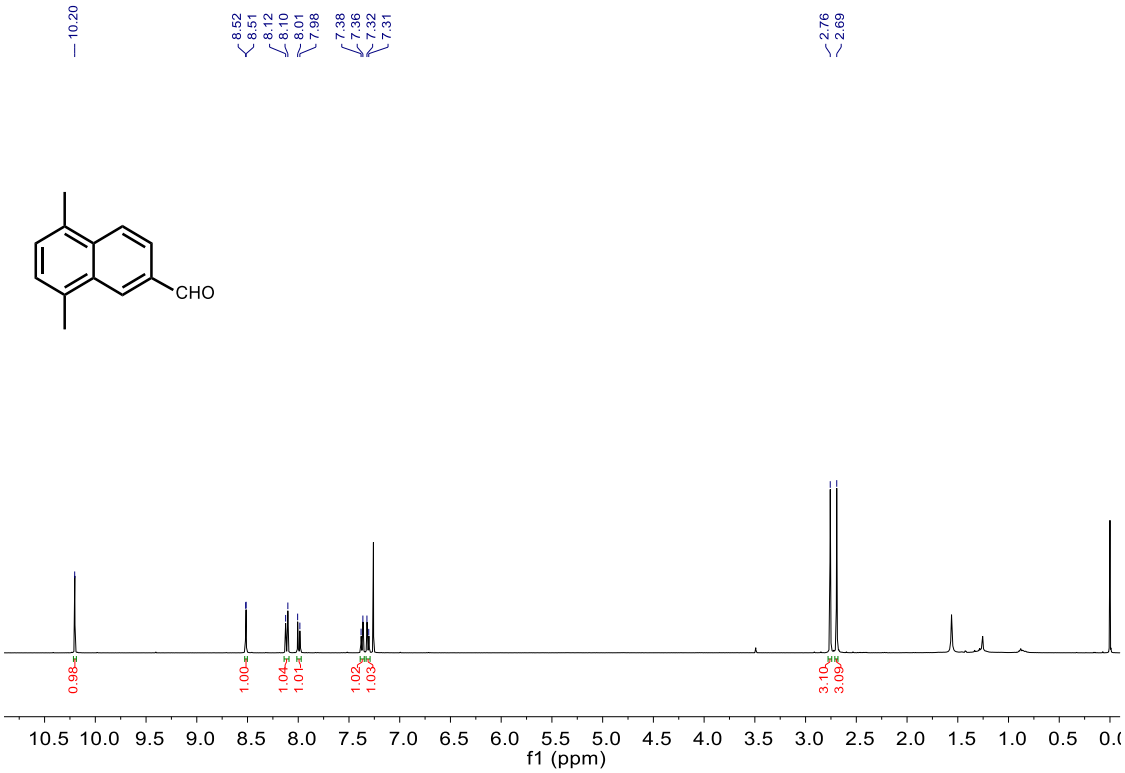


Fig. S11 ^{1}H NMR of compound **4**

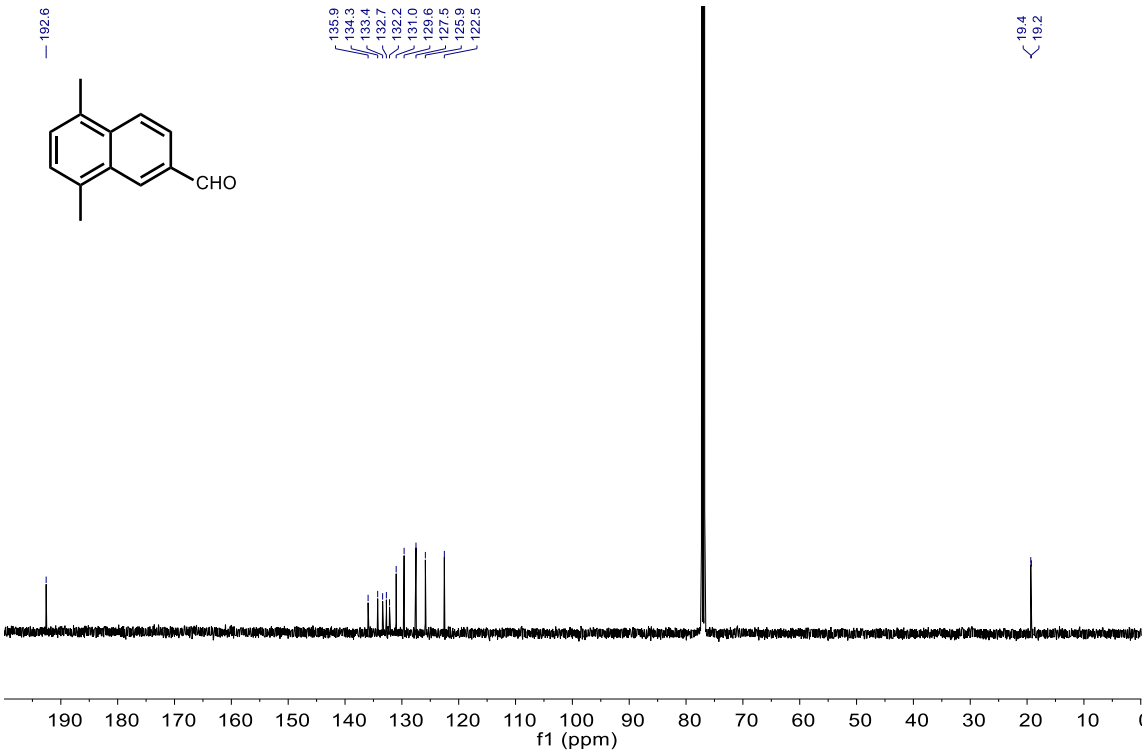


Fig. S12 ^{13}C NMR of compound **4**

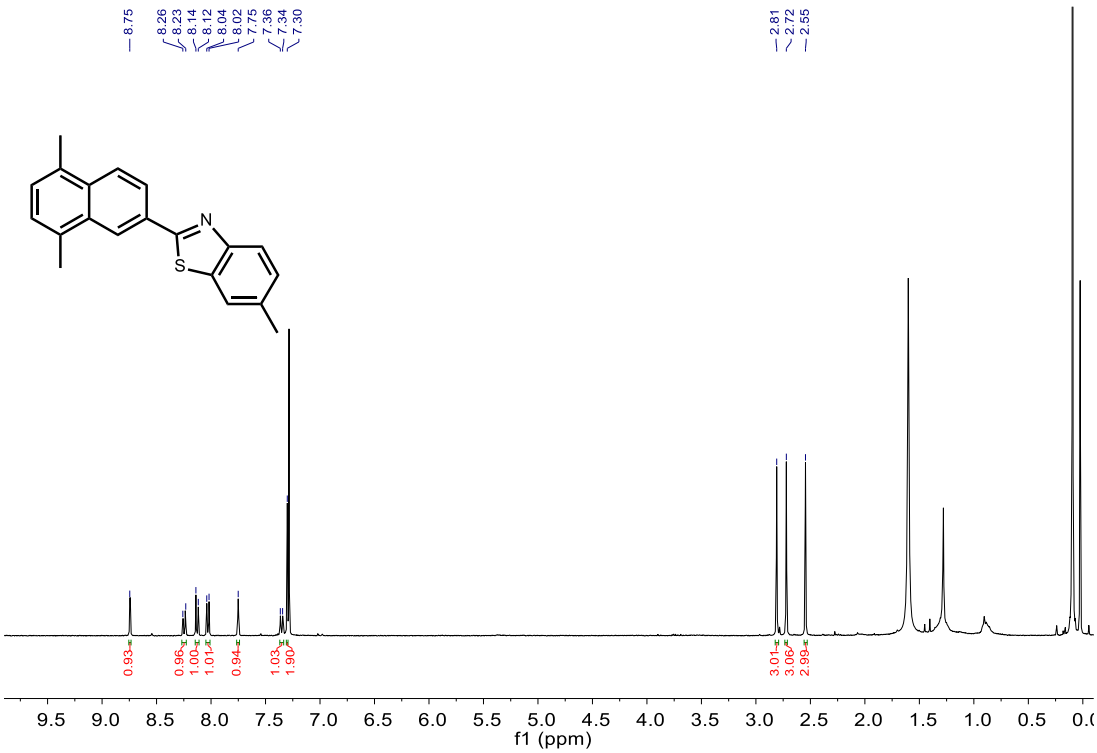


Fig. S13 ^{1}H NMR of compound **BZTN**

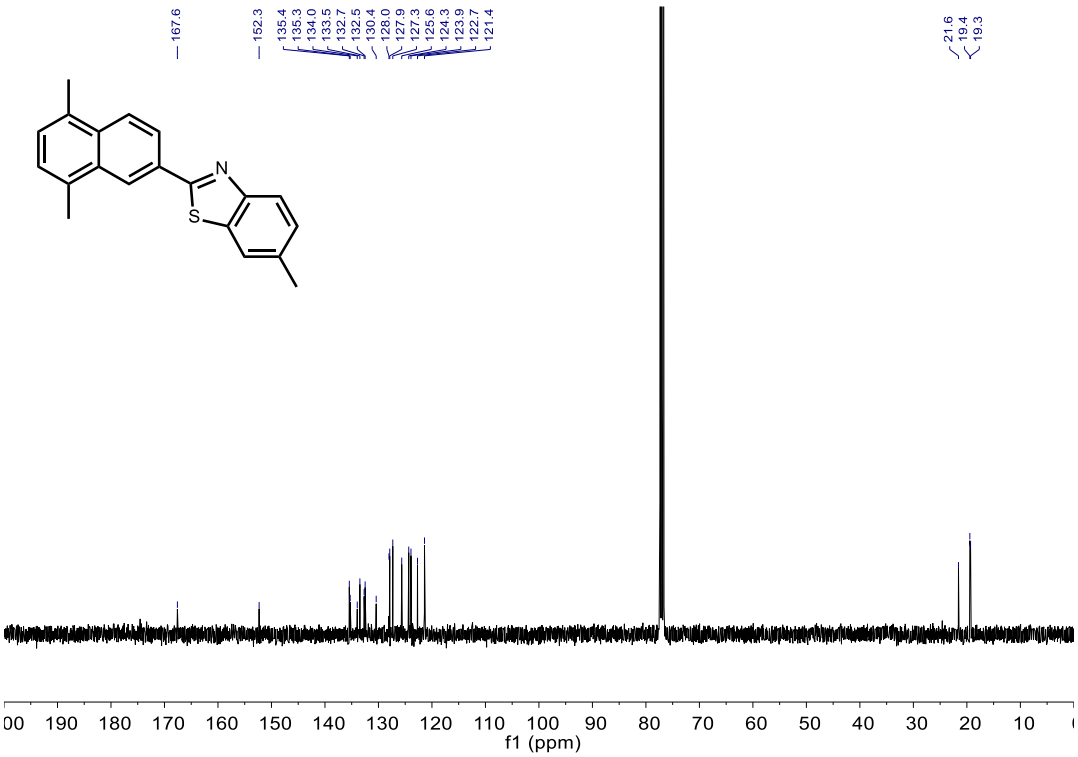


Fig. S14 ^{13}C NMR of compound **BZTN**

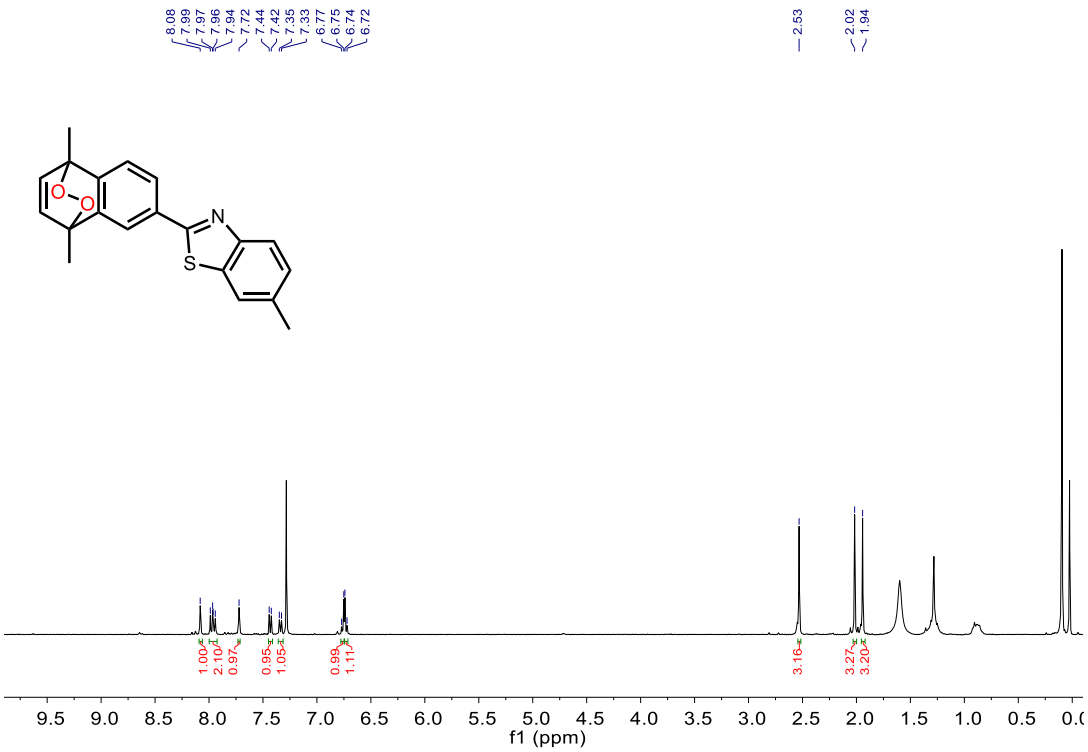


Fig. S15 ^{1}H NMR of compound **BZTN-O$_2$**

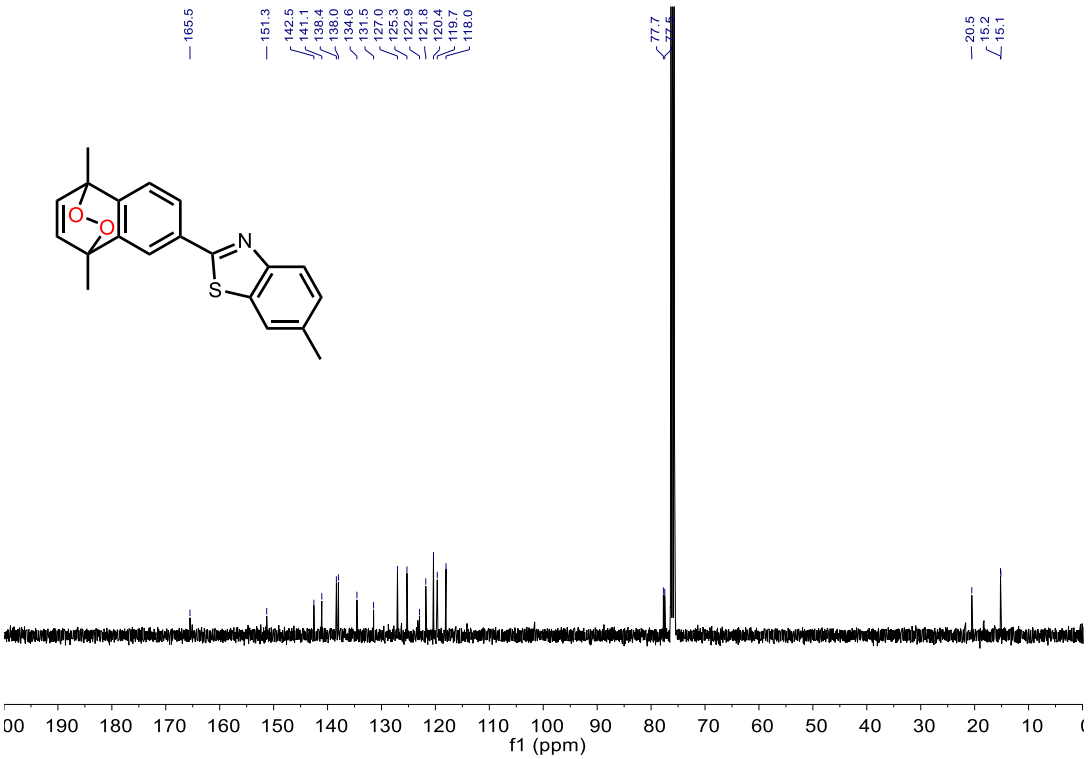


Fig. S16 ^{13}C NMR of compound **BZTN-O$_2$**

Fig. S17 HRMS spectra of key intermediate