Electronic Supplementary Information (ESI)

Synthesis and evaluation of near-infrared probes with barbituric acid acceptors for in vivo detection of amyloid plaques

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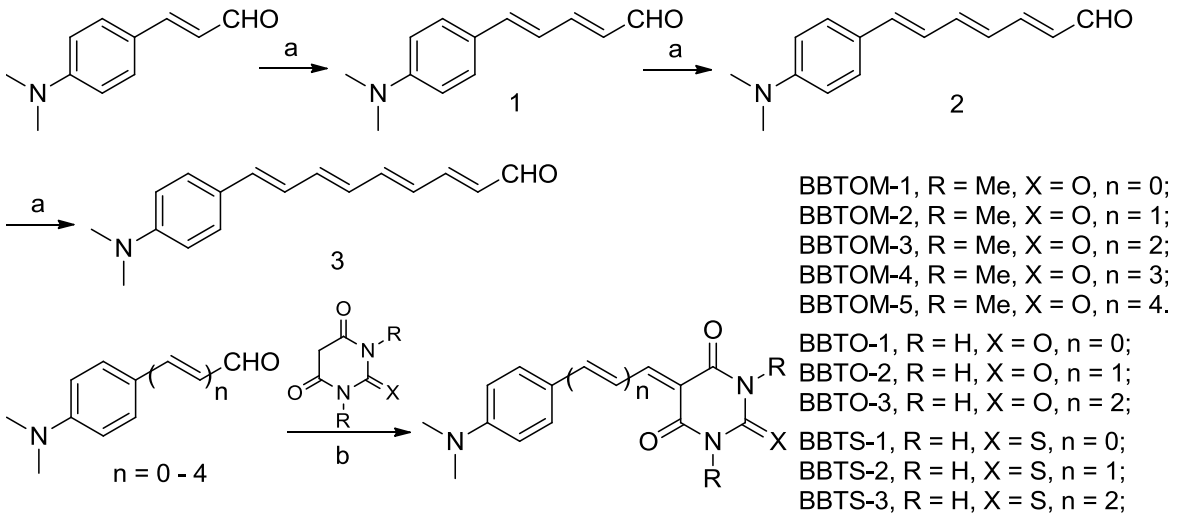
Table of Contents

General Procedures.

All solvents and chemicals were purchased from commercial products and used without further purification unless otherwise stated. Reactions were monitored by thin layer chromatography: Silica gel 60 F_{254} plates (Merck). Flash column chromatography was manipulated using silica gel (45 - 75 μm, Yantai Industry Research Institute). Synthetic A$\beta_{1\text{-}42}$ and A$\beta_{1\text{-}40}$ were purchased from PEPTIDE INSTITUTE, Inc. (Japan), and the aggregation was performed using the procedures reported previously[1] for *in vitro* assays. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III (400 MHz or 100 MHz) NMR spectrometer in $CDCl_3$ or DMSO-d_6 solutions at room temperature. Chemical shift (δ) is reported in ppm downfield from tetramethylsilane and coupling constants (J) are reported in Hertz (Hz), and the multiplicity is defined by s (singlet), d (doublet), t (triplet), or m (multiplet). MS and HRMS spectra were obtained by the Surveyor MSQ Plus (ESI) (Waltham, MA, USA) instrument. Fluorescence spectra were measured on a SHIMADZU RF-5301PC spectrofluorophotometer (Japan). Rhodamine 6G was used as a standard (Φ = 0.76 in H_2O)[2] to measure fluorescence quantum yields of all of the samples. UV-visible spectra were carried out on the SHIMADZU UV-3600 UV-Vis spectrophotometer (Japan). HPLC was conducted on an Agilent 1260 Infinity Quaternary LC (Agilent Technologies) system. The sample was analyzed on a Venusil MP C18 reverse column (Agela Technologies, 5 μm, 4.6 mm×250 mm) eluted with an isocratic system at a flow rate of 1.0 mL/min. The mobile phase A was water while B was acetonitrile. The purity of the compounds were analyzed by HPLC, and were found to be more than 95%. Fluorescence observation was performed by the Axio Observer Z1 inverted fluorescence microscope (Zeiss, Germany) equipped with DAPI, AF488, AF546 and CY5 filter sets. Male ICR

mice (5 weeks, 18-22 g) used for brain uptake studies were purchased from Vital River Laboratories (China). Double transgenic mice (C57BL6, APPsw/PSEN1, 22-month old) used as an Alzheimer's model and age matched control mice (C57BL6, 22-month old) were purchased from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences. Postmortem brain tissues from autopsy confirmed cases of AD patients (91-year old, male; 68-year old, female; 64-year old, female; 70-year old, male; temporal lobe) were obtained from the Chinese Brain Bank Center (CBBC). All protocols requiring the use of animals were approved by the animal care committee of Beijing Normal University.

Chemistry.



Scheme S1. Reagents and conditions: (a) (1) ((1,3-Dioxolan-2-yl)methyl)triphenylphosphonium bromide, anhydrous THF, 18-crown-6, NaH, r.t, 12 h; (2) 1 M HCl, r.t., 30 min. (b) For BBTO 1-3 and BBTS 1-3, DMF, reflux; for BBTOM-1 and BBTOM-2, piperidine, EtOH, r.t., 15 min; for BBTOM-3, BBTOM-4 and BBTOM-5, K_2CO_3, EtOH, reflux.

(2*E*,4*E*)-5-(4-(Dimethylamino)phenyl)penta-2,4-dienal (1)

(*E*)-3-(4-(dimethylamino)phenyl)acrylaldehyde (371.0 mg, 2.1 mmol) was completely dissolved by anhydrous THF (20 mL) in a 100 mL round bottom flask charged a stirrer, the solution was added with ((1,3-Dioxolan-2-yl)methyl)triphenylphosphonium bromide (1.37 g, 3.2 mmol), NaH (254.0 mg, 10.6 mmol) and 18-crown-6 (50.0 mg) successively. The reaction mixture was stirred at room temperature overnight. Then 20 mL of aqueous HCl (1 M) was added to quench the reaction, the mixture was stirred for 30 min and neutralized with ammonia water then extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4. The solvents were evaporated under vacuum and the crude product was purified by flash column chromatography (petroleum ether/dichloromethane = 1 : 3, v/v) to yield compound **1** as yellow crystalline solids (250.3 mg, 58.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.56 (d, *J* = 8.1 Hz, 1H), 7.41 (d, *J* = 8.9 Hz, 2H), 7.32 - 7.21 (m, 1H), 6.95 (d, *J* = 15.3 Hz, 1H), 6.82 (dd, *J* = 15.3, 10.9 Hz, 1H), 6.68 (d, *J* = 8.8 Hz, 2H), 6.18 (dd, *J* = 15.0, 8.1 Hz, 1H), 3.03 (s, 6H).

(2*E*,4*E*,6*E*)-7-(4-(Dimethylamino)phenyl)hepta-2,4,6-trienal (2)

The same reaction as described above to prepare compound **1** was used, and the crude product was purified by flash column chromatography (petroleum ether/dichloromethane = 1 : 3, v/v). Compound **2** was obtained as dark red crystalline solids (116.8 mg, 41.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.56 (d, *J* = 8.1 Hz, 1H), 7.35 (d, *J* = 8.8 Hz, 2H), 7.18 (dd, *J* = 15.0, 11.3 Hz, 1H), 6.86 - 6.80 (m, 1H), 6.78 – 6.72 （m， 2H）, 6.68 (d, *J* = 8.6 Hz, 2H), 6.47 (dd, *J* = 15.0, 8.1 Hz, 1H), 6.14 (dd, *J* = 15.0, 8.0 Hz, 1H), 3.00 (s, 6H). MS: m/z calcd for $C_{15}H_{18}NO$ 228.1; found 228.1, $M + H^+$.

(2*E*,4*E*,6*E*,8*E*)-9-(4-(dimethylamino)phenyl)nona-2,4,6,8-tetraenal (3)

The same reaction as described above to prepare compound **1** was used, and the crude product was

purified by flash column chromatography (petroleum ether/dichloromethane = 1 : 3, v/v). Compound **3** was obtained as purple crystalline solids (30.0 mg, 35.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.56 (d, *J* = 8.1 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.16 (dd, *J* = 15.0, 11.3 Hz, 1H), 6.79 - 6.61 (m, 6H), 6.48-6.35 (m, 2H), 6.14 (dd, *J* = 15.0, 8.0 Hz, 1H), 3.01 (s, 6H). MS: m/z calcd for $C_{17}H_{20}NO$ 254.1; found 254.1, $M + H^+$.

5-(4-(Dimethylamino)benzylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (BBTOM-1)

4-(Dimethylamino)benzaldehyde (34.0 mg, 0.2 mmol) and 1,3-dimethylbarbituric acid (34.0 mg, 0.2 mmol) were dissolved in 10 mL ethanol. Piperidine (50 μL) was added as catalyst and the reaction mixture was stirred for 15 min at room temperature. The precipitate was collected by filtration, dried, and weighed. The final product was obtained as red crystal (43.2 mg, 72.3%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.41 (d, *J* = 9.2 Hz, 2H), 8.22 (s, 1H), 6.80 (d, *J* = 9.2 Hz, 2H), 3.21 (s, 6H), 3.13 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ:163.2, 161.1, 156.3, 154.2, 151.2, 139.0, 120.0, 111.2, 109.3, 28.4, 27.8. MS: m/z calcd for $C_{15}H_{18}N_3O_3$ 288.1; found 287.9, $M + H^+$.

(*E*)-5-(3-(4-(Dimethylamino)phenyl)allylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (BBTOM-2)

The same reaction as described above to prepare BBTOM-1 was used, and the final product was obtained as purple crystalline solids (56.8 mg, 46.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.42 (dd, *J* = 14.8, 12.5 Hz, 1H), 8.20 (d, *J* = 12.4 Hz, 1H), 7.60 (d, *J* = 8.9 Hz, 2H), 7.40 (d, *J* = 14.8 Hz, 1H), 6.69 (d, *J* = 8.9 Hz, 2H), 3.38 (s, 6H), 3.10 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.0, 162.4,

158.6, 157.1, 153.0, 151.8, 132.1, 123.6, 120.6, 112.0, 110.0, 40.2, 28.5, 27.8. MS: m/z calcd for (M + H^+) 314.2; found 314.0.

5-((2*E*,4*E*)-5-(4-(Dimethylamino)phenyl)penta-2,4-dien-1-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (BBTOM-3)

Compound 1 (30.2 mg, 0.2 mmol) and 1,3-dimethylbarbituric acid (45.3 mg, 0.3 mmol) were dissolved in 10 mL ethanol, 1 mg K_2CO_3 was added as catalyst. The reaction mixture was refluxed for 15 min, then the solvents were evaporated under vacuum and the crude product was purified by flash column chromatography (petroleum ether/dichloromethane = 1 : 2, v/v) and the final product was obtained as brown crystaline solids (74.3 mg, 53.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (d, *J* =12.5 Hz, 1H), 8.04 - 7.98 (m, 1H), 7.42 (d, *J* = 8.9 Hz, 2H), 7.31 - 7.28 (m, 1H), 7.00 - 6.92 (m, 2H), 6.69 (d, *J* = 8.8 Hz, 2H), 3.38 (s, 3H) , 3.37 (s, 3H) , 3.06 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.8, 162.0, 157.3, 151.8, 151.7, 145.9, 130.0, 126.9, 123.9, 123.8, 112.0, 111.1, 40.1, 28.5, 27.9. MS: m/z calcd for $C_{19}H_{22}N_3O_3$ 340.1661; found 340.1664, M + H^+.

5-((2*E*,4*E*,6*E*)-7-(4-(Dimethylamino)phenyl)hepta-2,4,6-trien-1-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (BBTOM-4)

The same reaction as described above to prepare BBTOM-3 was used. The crude product was purified by flash column chromatography (petroleum ether/dichloromethane = 2 : 1, v/v) and the final produce was obtained as black crystaline solids (16.3 mg, 30.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (d, *J* =12.6 Hz, 1H), 8.00 - 7.93 (m, 1H), 7.38 (d, *J* = 8.9 Hz, 2H), 7.20 (dd, *J* = 14.0, 11.6 Hz, 1H), 6.94 - 6.87 (m, 1H), 6.81 (s, 1H), 6.79 (d, *J* = 5.4 Hz, 1H), 6.67 (d, *J* = 8.9 Hz, 2H), 6.58 (dd, *J*

= 14.0, 11.6 Hz, 1H), 3.37 (s, 3H) , 3.36 (s, 3H) , 3.03 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.7, 162.0, 157.0, 156.2, 151.7, 151.2, 150.2, 146.6, 141.1, 130.0, 129.0, 128.4, 127.8, 126.1, 124.5, 124.0, 119.2, 112.1, 111.7, 40.2, 28.6, 28.0. MS: m/z calcd for $C_{21}H_{24}N_3O_3$ 366.1818; found 366.1818, M + H^+.

5-((2*E*,4*E*,6*E*,8*E*)-9-(4-(Dimethylamino)phenyl)nona-2,4,6,8-tetraen-1-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (BBTOM-5)

The same reaction as described above to prepare BBTOM-3 was used. The crude product was purified by flash column chromatography (petroleum ether/dichloromethane = 1 : 3, v/v) and the final produce was obtained as atropurpureus solid (21.0 mg, 89.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.96 (d, *J* =12.4 Hz, 1H), 7.90 - 7.83 (m, 1H), 7.49 - 7.43 (m, 1H), 7.38 (d, *J* = 8.8 Hz, 2H), 7.00 - 6.77 (m, 4H), 6.74 - 6.64 (m, 3H), 6.57 - 6.50 (m, 1H), 3.19 (s, 3H) , 3.18 (s, 3H) , 2.96 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.6, 161.9, 156.7, 155.5, 151.6, 150.7, 145.5, 141.9, 138.2, 131.0, 130.2, 128.5, 128.3, 125.0, 124.4, 112.2, 112.1, 40.2, 28.6, 28.0. MS: m/z calcd for $C_{23}H_{26}N_3O_3$ 392.1974; found 393.1968, M + H^+.

5-(4-(Dimethylamino)benzylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (BBTO-1)

4-(Dimethylamino)benzaldehyde (33.6 mg, 0.2 mmol) and barbituric acid (28.8 mg, 0.2 mmol) were were dissolved in 3 mL DMF. The reaction mixture was refluxed for 10 min, then cooled to precipitate BBTO-1 as red crystaline solids (17.2 mg, 30.1%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.42 (d, *J* = 9.2 Hz, 2H), 8.15 (s, 1H), 7.80 (d, *J* = 9.2 Hz, 2H), 3.12 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.6, 162.7, 155.4, 154.1, 150.3, 139.0, 120.0, 111.1, 109.5. MS: m/z

calcd for $C_{13}H_{13}N_3O_3Na$ 282.1; found 282.1, M + Na^+.

(*E*)-5-(3-(4-(Dimethylamino)phenyl)allylidene)pyrimidine-2,4,6-(1H,3H,5H)-trione (BBTO-2)

The same reaction as described above to prepare BBTO-1 was used, and the final product was obtained as atropurpureus crystaline solids (53.0 mg, 80.1%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.22 (dd, *J* = 15.0,12.4 Hz, 1H), 7.98 (d, *J* = 12.3 Hz, 1H), 7.62 (d, *J* = 15.0 Hz, 1H), 7.55 (d, *J* = 8.9 Hz, 2H), 6.80 (d, *J* = 8.9 Hz, 2H), 3.06 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.6, 163.3, 156.0, 155.4, 152.8, 150.42, 131.5, 122.7, 119.0, 112.0, 110.4. MS: m/z calcd for $C_{15}H_{16}N_3O_3$ 286.1; found 285.9, M + H^+.

5-((2*E*,4*E*)-5-(4-(Dimethylamino)phenyl)penta-2,4-dien-1-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (BBTO-3)

The same reaction as described above to prepare BBTO-1 was used, and the final product was obtained as atropurpureus crystaline solids (40.0 mg, 64.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.93 - 7.82 (m, 2H), 7.53 (d, *J* = 8.9 Hz, 2H), 7.51 - 7.45 (m, 1H), 7.13 - 7.11 (m, 2H), 6.73 (d, *J* = 8.9 Hz, 2H), 3.01 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.6, 156.0, 152.8, 150.4, 150.4, 131.5, 130.0, 125.6, 123.7, 122.7, 119.0, 112.0, 112.0. MS: m/z calcd for $C_{17}H_{17}N_3O_3Na$ 334.1; found 334.1, M + Na^+.

5-(4-(Dimethylamino)benzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (BBTS-1)

The same reaction as described above to prepare BBTO-1 was used, and the final product was obtained as crimson crystaline solids (13.4 mg, 21.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.12 (s,

1H), 12.02 (s, 1H), 8.46 (d, *J* = 9.1 Hz, 2H), 8.15 (s, 1H), 6.82 (d, *J* = 9.1 Hz, 2H), 3.16 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 177.4, 162.9, 160.4, 156.1, 154.7, 139.6, 120.4, 111.4, 109.2. MS: m/z calcd for $C_{13}H_{14}N_3O_2S$ 276.1; found 275.8, M + H^+.

(*E*)-5-(3-(4-(Dimethylamino)phenyl)allylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (BBTS-2)

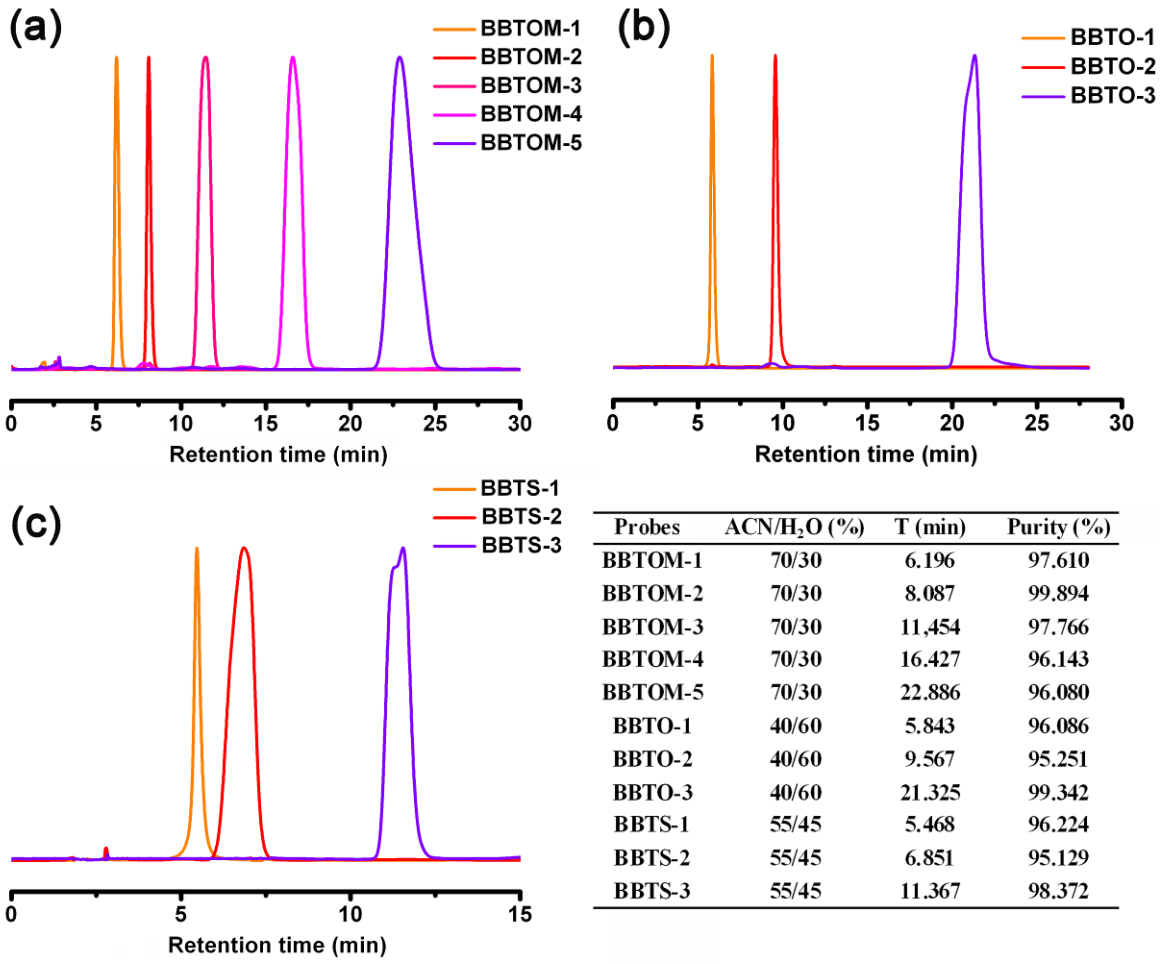
The same reaction as described above to prepare BBTO-1 was used, and the final product was obtained as purple crystaline solids (54.0 mg, 93.6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.25 (dd, *J* = 14.6, 12.6 Hz, 1H), 8.00 (d, *J* = 12.4 Hz, 1H), 7.72 (d, *J* = 14.8 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 3.09 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 177.8, 161.8, 161.2, 157.7, 156.4, 153.3, 132.2, 122.8, 119.3, 112.2, 110.0. MS: m/z calcd for $C_{15}H_{16}N_3O_2S$ 302.1; found 301.8, M + H^+.

5-((2*E*,4*E*)-5-(4-(Dimethylamino)phenyl)penta-2,4-dien-1-ylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (BBTS-3)

The same reaction as described above to prepare BBTO-1 was used. The final product was obtained as dark green crystaline solids (14.5 mg, 29.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.15 (s, 1H), 12.09 (s, 1H), 7.96 - 7.83 (m, 2H), 7.60 - 7.56 (m, 3H), 7.22 - 7.17 (M, 2H), 6.74 (d, *J* = 8.8 Hz, 2H), 3.03(s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 177.9, 161.7, 161.0, 158.6, 155.3, 151.9, 147.4, 130.5, 126.0, 123.8, 123.4, 112.0, 111.1. MS: m/z calcd for $C_{17}H_{18}N_3O_2S$ 327.1; found 328.0, M + H^+.

The Purity Determination.

The purity of the compounds was determined on a HPLC system eluted with acetonitrile/water at a flow rate of 1.0 mL/min. As shown in Fig. S1, the retention time increased with the lengthening of the conjugated double bonds and the purity of these probes was higher than 95%.



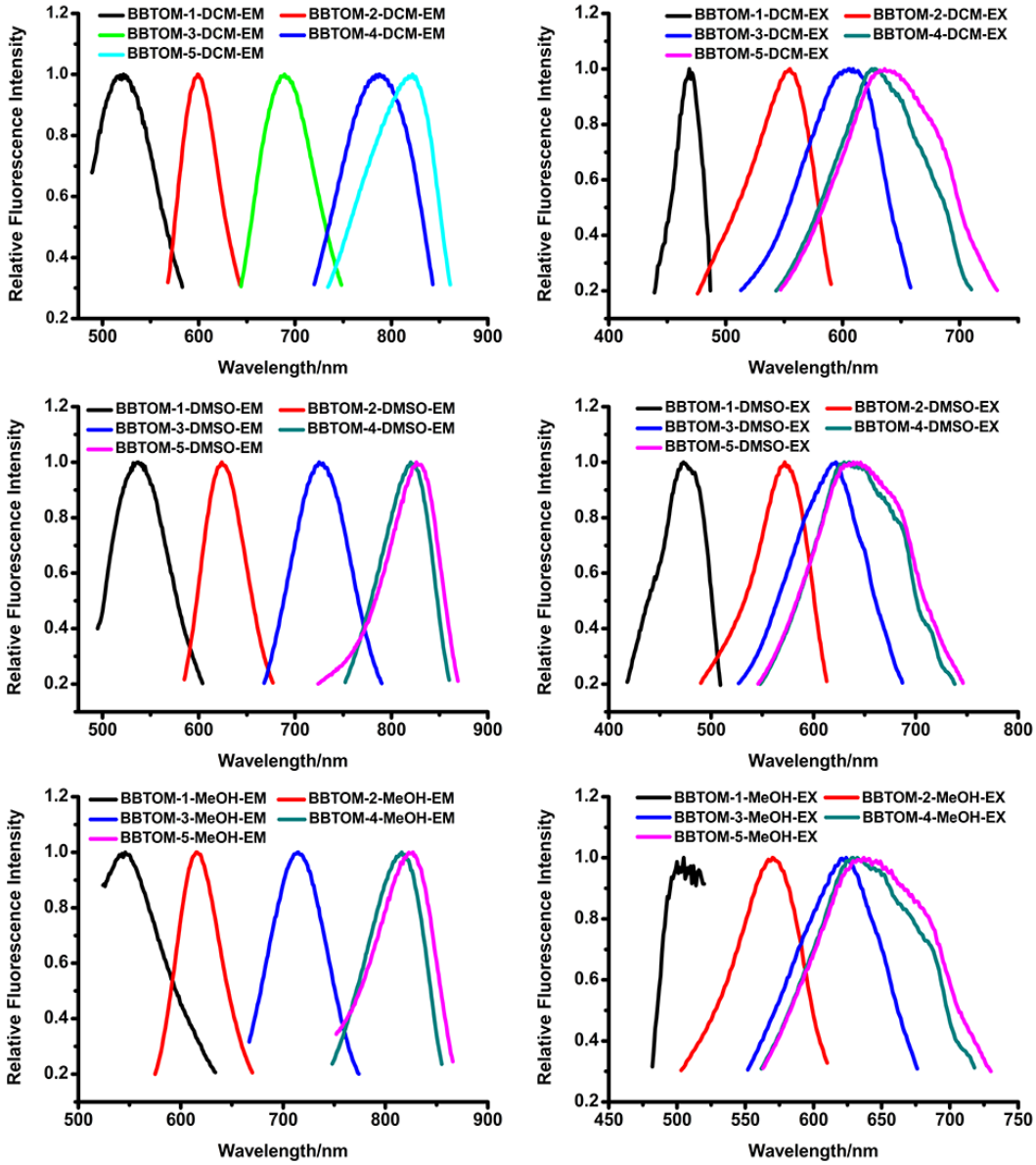
Probes	ACN/H_2O (%)	T (min)	Purity (%)
BBTOM-1	70/30	6.196	97.610
BBTOM-2	70/30	8.087	99.894
BBTOM-3	70/30	11,454	97.766
BBTOM-4	70/30	16.427	96.143
BBTOM-5	70/30	22.886	96.080
BBTO-1	40/60	5.843	96.086
BBTO-2	40/60	9.567	95.251
BBTO-3	40/60	21.325	99.342
BBTS-1	55/45	5.468	96.224
BBTS-2	55/45	6.851	95.129
BBTS-3	55/45	11.367	98.372

Figure. **S1** HPLC profiles of BBTOMs (A), BBTOs (B), BBTSs (C) and HPLC conditions and purity (D).

Spectroscopic Determinations.

The fluorescence spectra were measured on a SHIMADZU RF-5301PC spectrofluorophotometer (Japan) in different solvents of CH_2Cl_2, DMSO, MeOH, THF and phosphate-buffered saline (PBS).

The absorption spectra are determined on the SHIMADZU UV-3600 UV-Vis spectrophotometer (Japan) in CH_2Cl_2. CH_2Cl_2 is a solvent that can simulate the hydrophobic microenvironment of the Aβ fibril "binding pocket",[3] then it was employed to determine the molar absorption coefficient and quantum yield. The spectroscopic data are shown in Figure S2-7 and Table S1, 2.



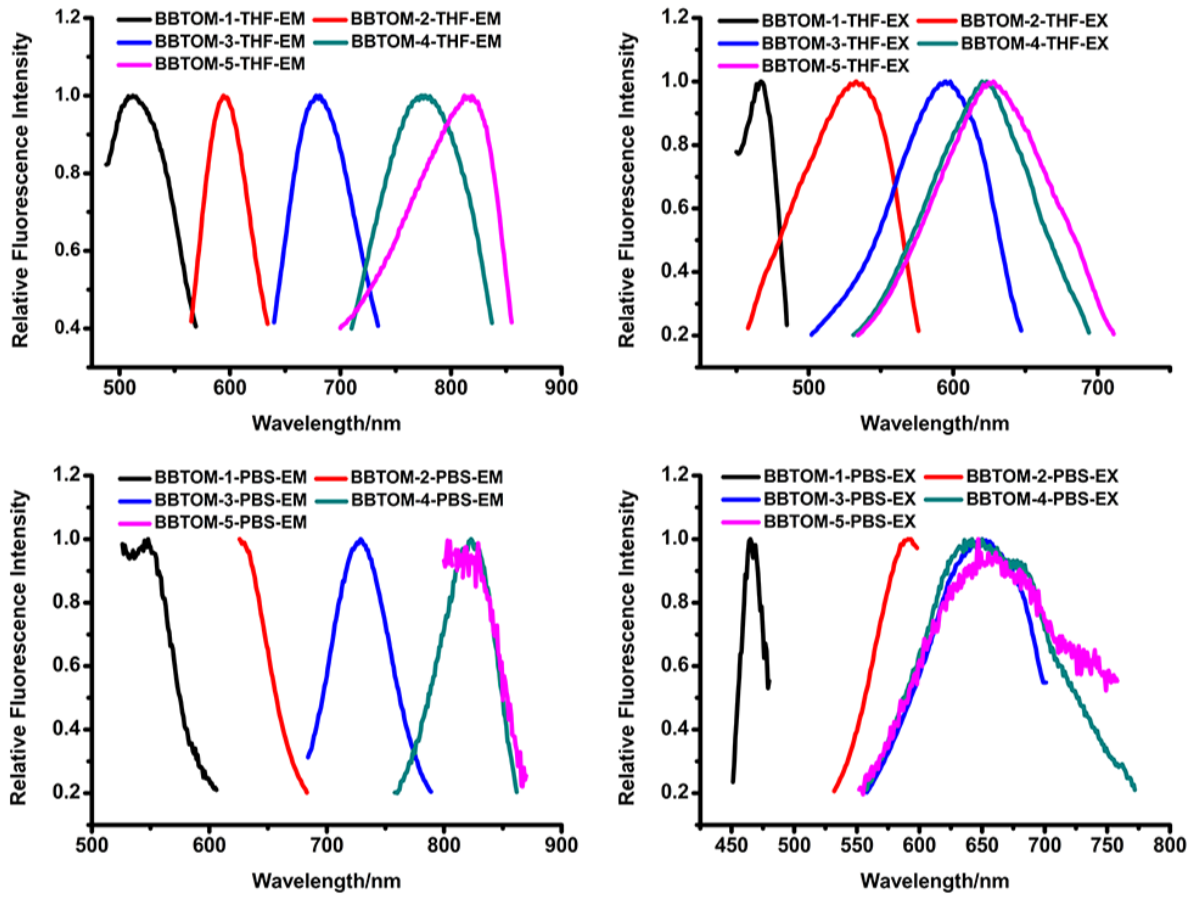
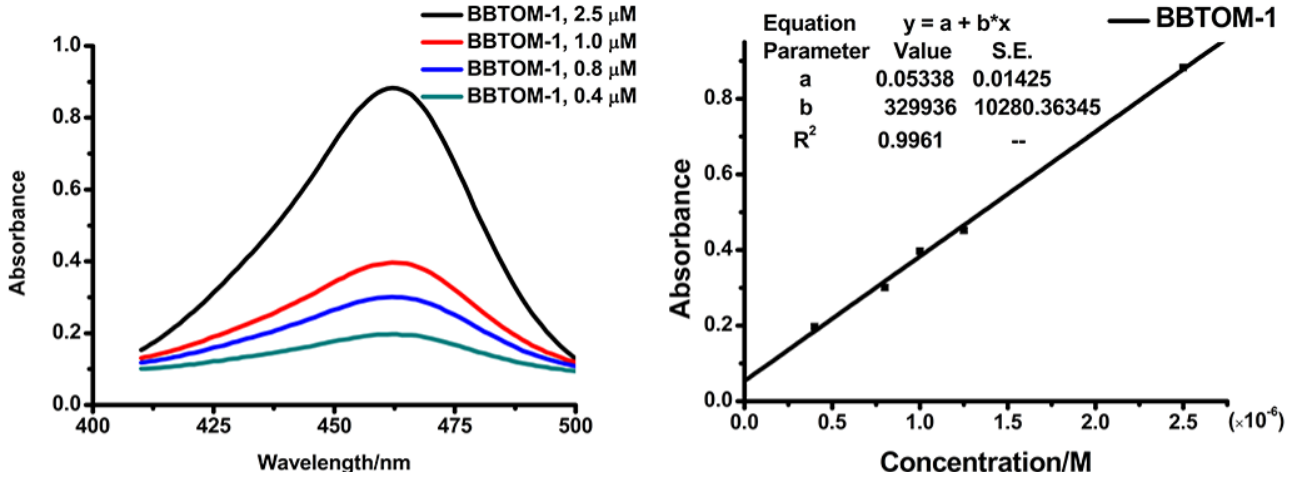
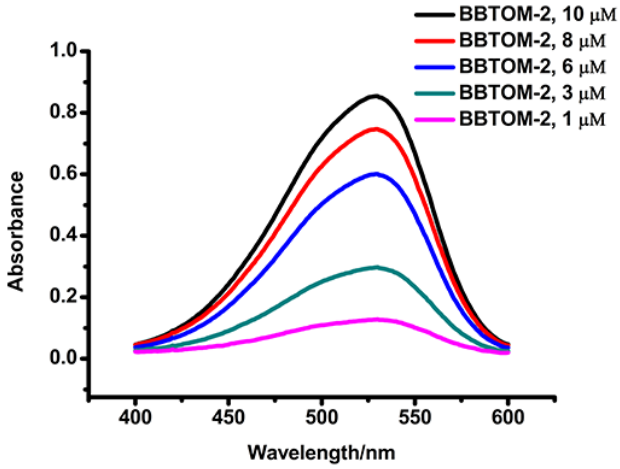
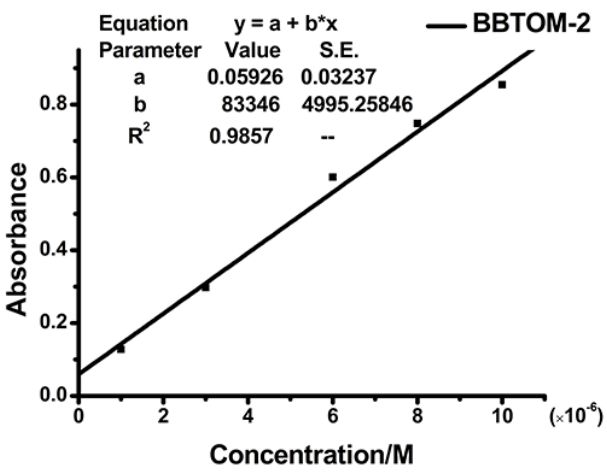
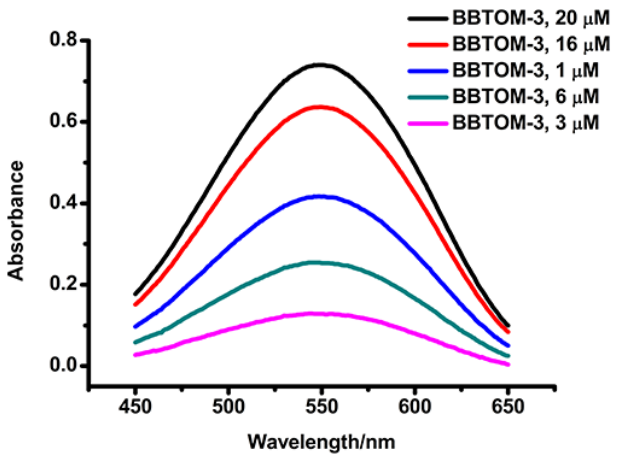


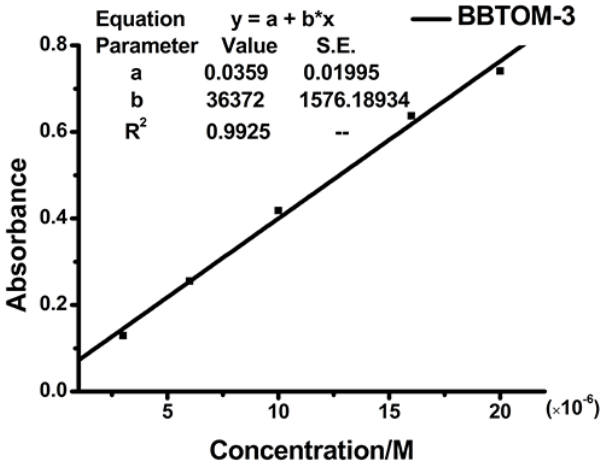
Figure. **S2** The excitation (right panel) and emission (left panel) spectra of the BBTOM1-5 in different solvents: dichloromethane, DMSO, methanol, THF and PBS (10% ethanol). The emission wavelengths of the solutions were measured at a concentration of 10 μM.

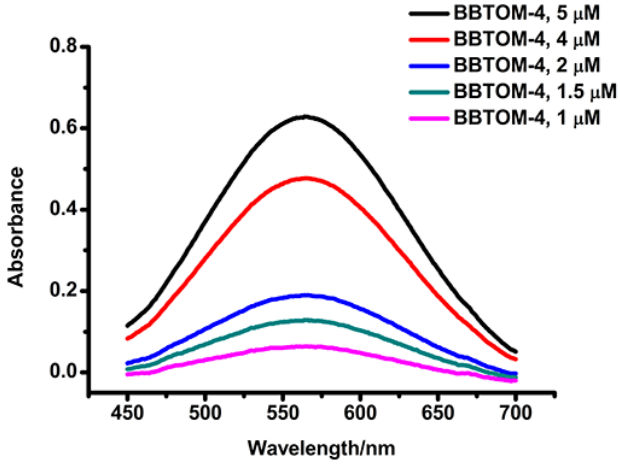


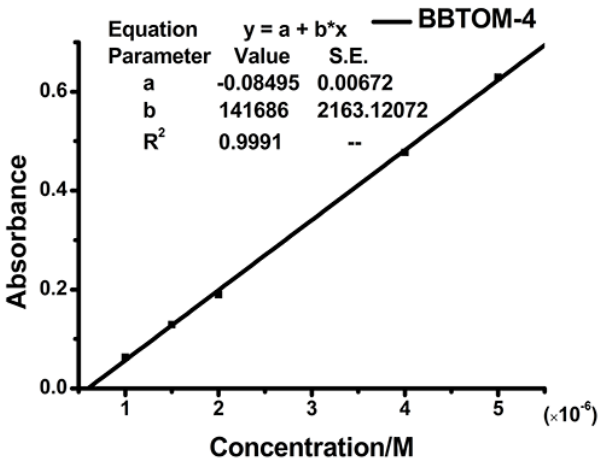

BBTOM-2, 10 μM
BBTOM-2, 8 μM
BBTOM-2, 6 μM
BBTOM-2, 3 μM
BBTOM-2, 1 μM
Absorbance
Wavelength/nm

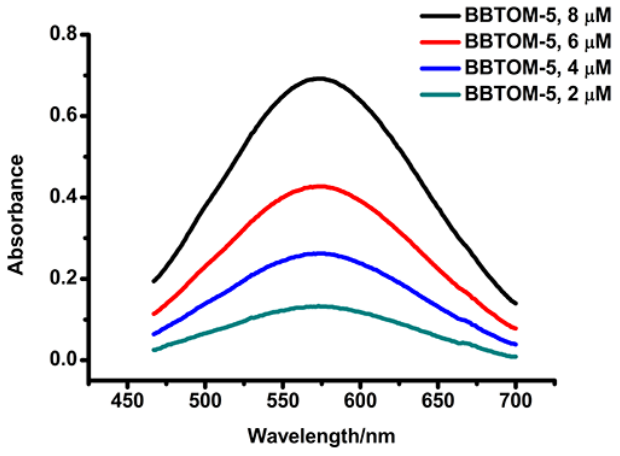

BBTOM-2
Equation y = a + b*x
Parameter Value S.E.
a 0.05926 0.03237
b 83346 4995.25846
R2 0.9857 --
Absorbance
Concentration/M
(×10-6)


BBTOM-3, 20 μM
BBTOM-3, 16 μM
BBTOM-3, 1 μM
BBTOM-3, 6 μM
BBTOM-3, 3 μM
Absorbance
Wavelength/nm


BBTOM-3
Equation y = a + b*x
Parameter Value S.E.
a 0.0359 0.01995
b 36372 1576.18934
R2 0.9925 --
Absorbance
Concentration/M
(×10-6)


BBTOM-4, 5 μM
BBTOM-4, 4 μM
BBTOM-4, 2 μM
BBTOM-4, 1.5 μM
BBTOM-4, 1 μM
Absorbance
Wavelength/nm


BBTOM-4
Equation y = a + b*x
Parameter Value S.E.
a -0.08495 0.00672
b 141686 2163.12072
R2 0.9991 --
Absorbance
Concentration/M
(×10-6)


BBTOM-5, 8 μM
BBTOM-5, 6 μM
BBTOM-5, 4 μM
BBTOM-5, 2 μM
Absorbance
Wavelength/nm

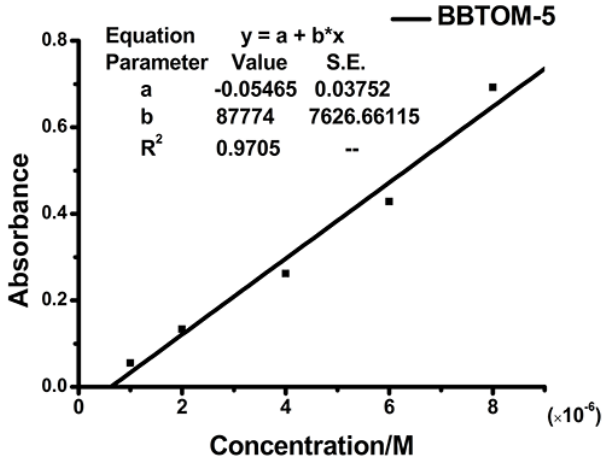
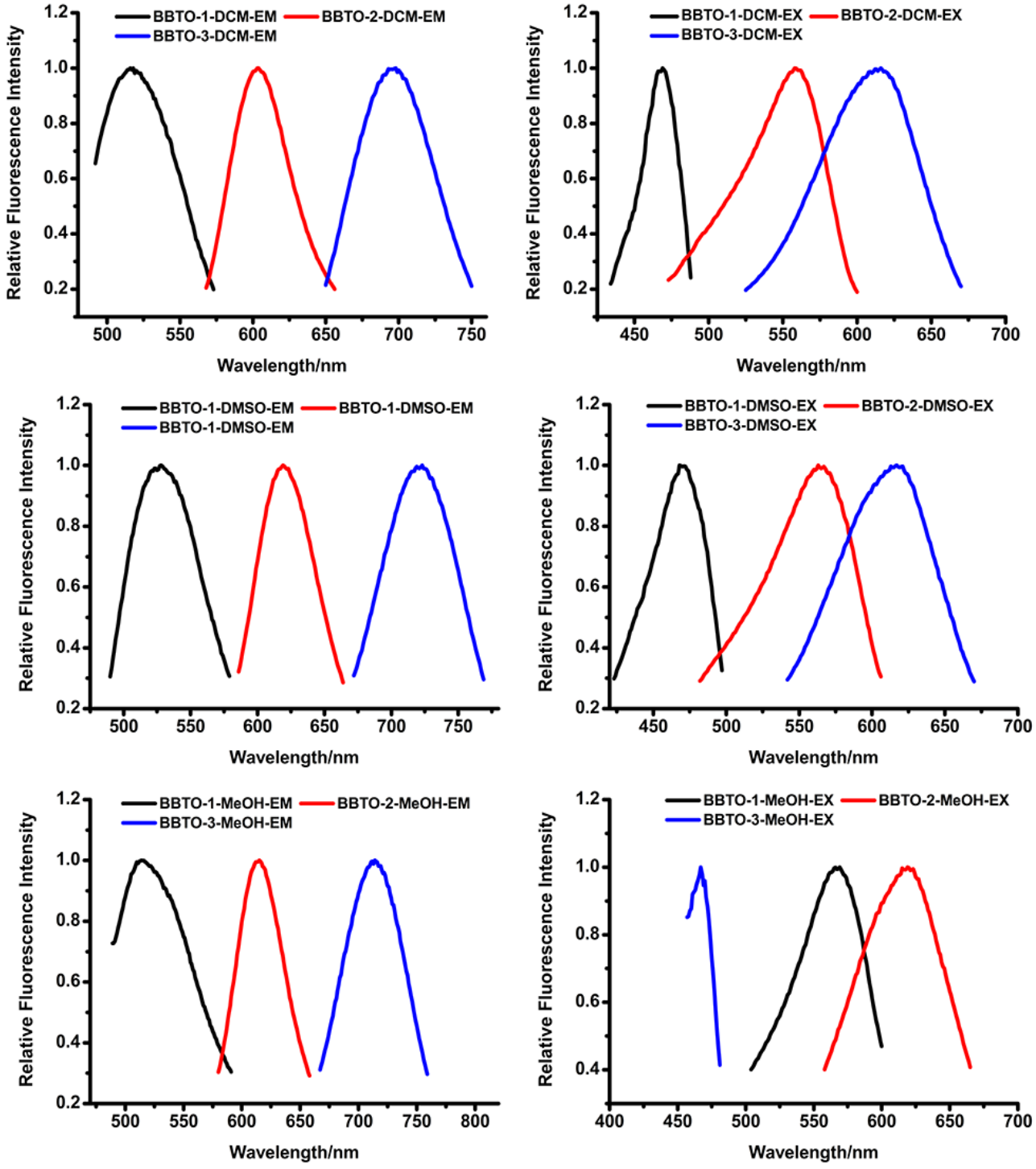

BBTOM-5
Equation y = a + b*x
Parameter Value S.E.
a -0.05465 0.03752
b 87774 7626.66115
R2 0.9705 --
Absorbance
Concentration/M
(×10-6)

Figure. **S3** Absorption spectra (left panel) and linear fitting curve between absorbance and concentration (right panel) of the BBTOM1-5 in CH_2Cl_2, molar absorption coefficients (ε) equals to b.



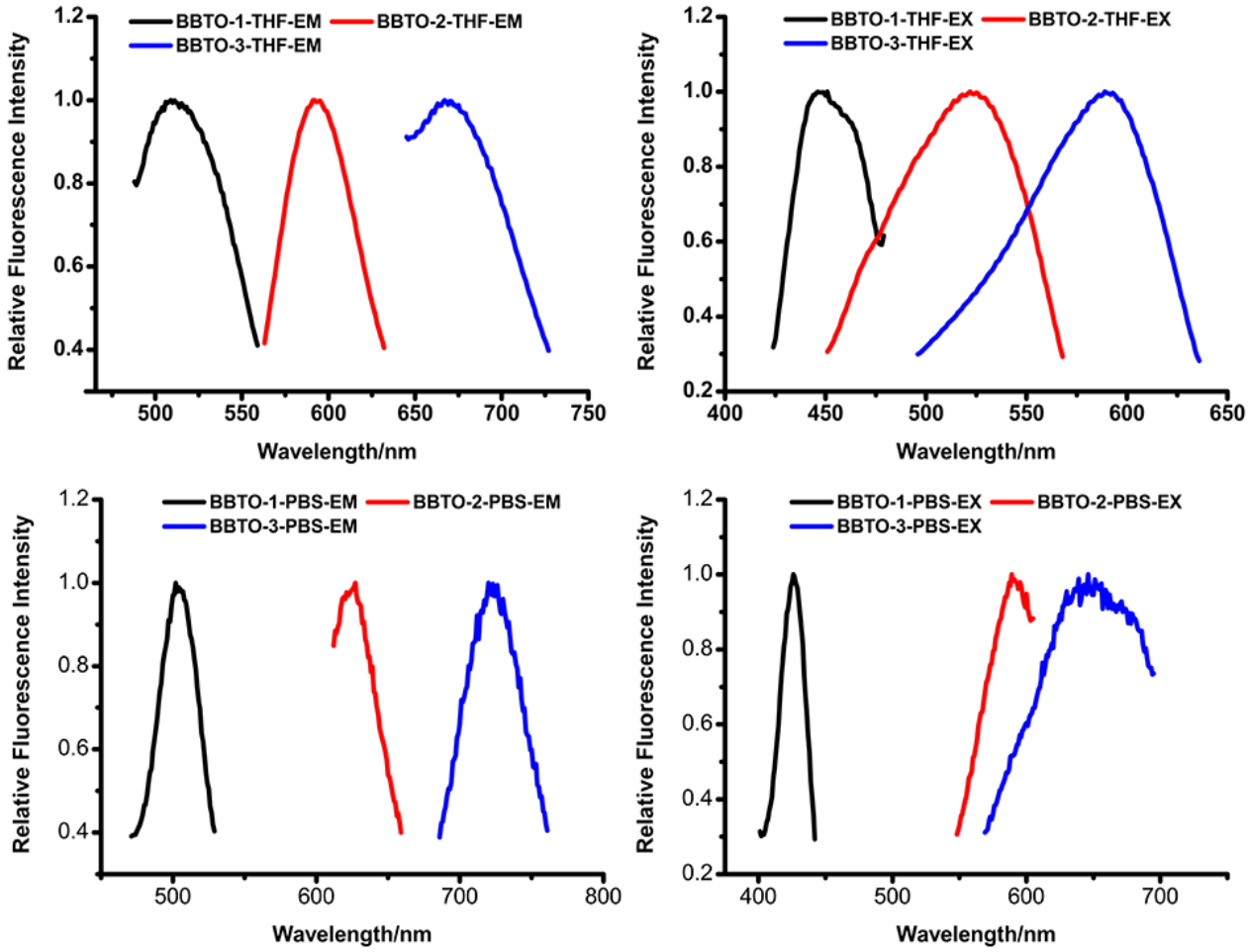
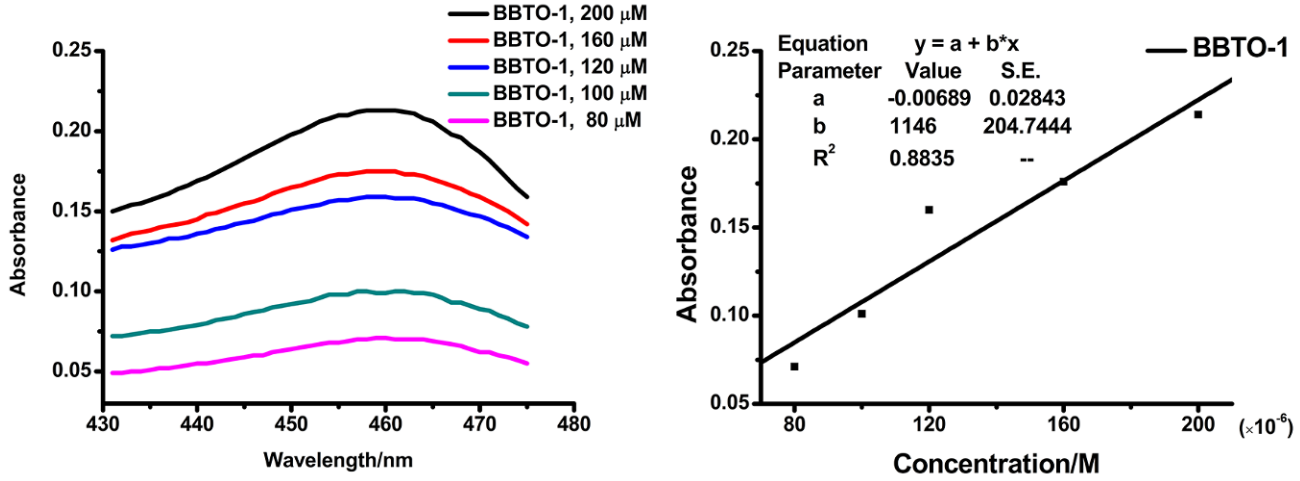


Figure. **S4** The excitation (right panel) and emission (left panel) spectra of the BBTO1-3 in different solvents: dichloromethane, DMSO, methanol, THF and PBS (10% ethanol). The emission wavelengths of the solutions were measured at a concentration of 10 μM.



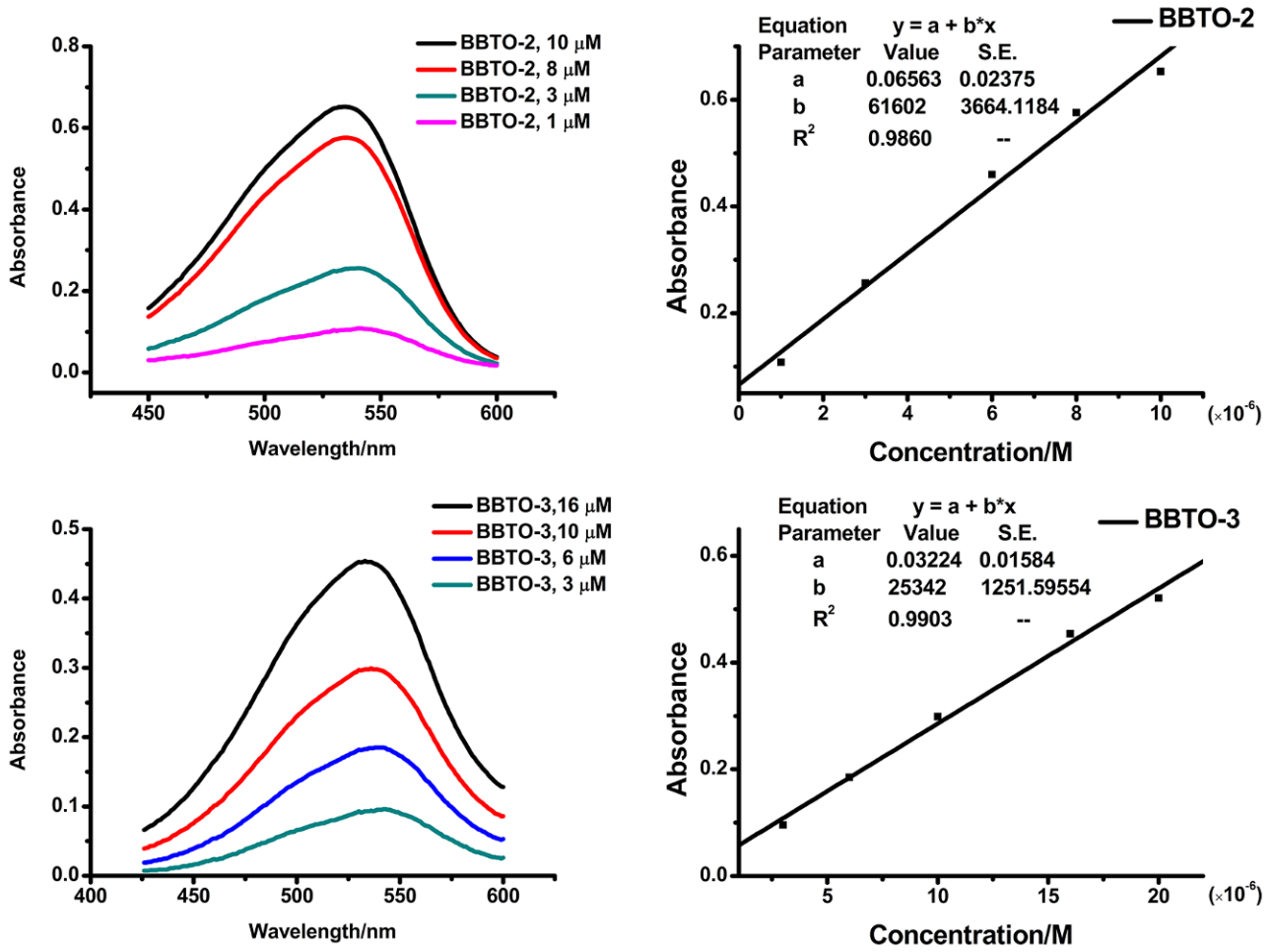
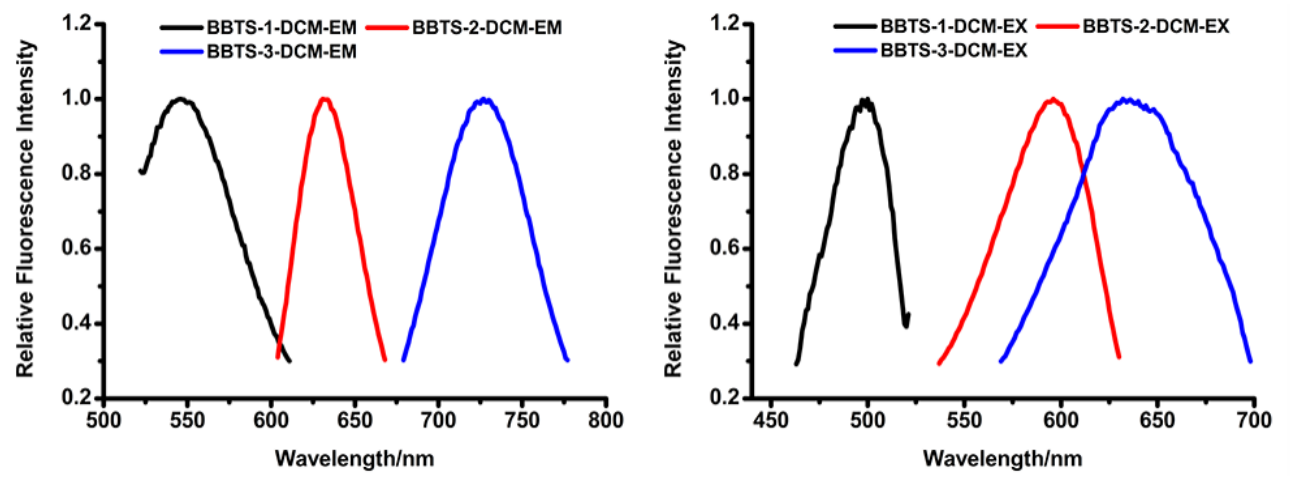
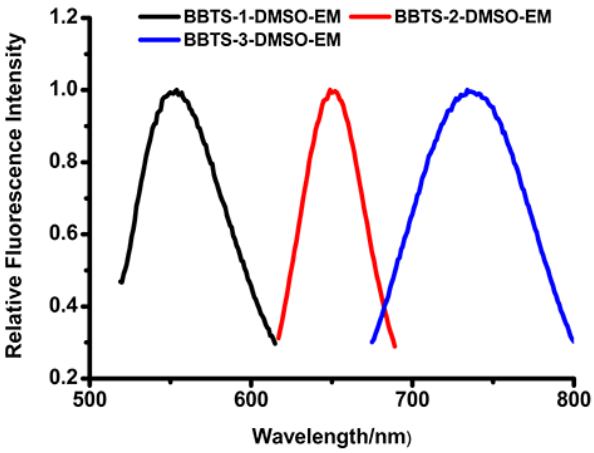
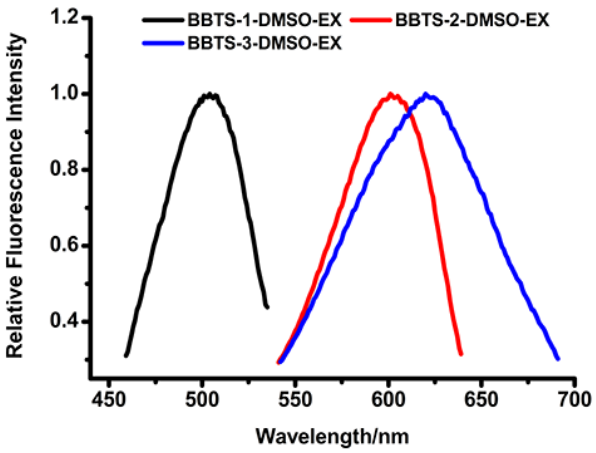
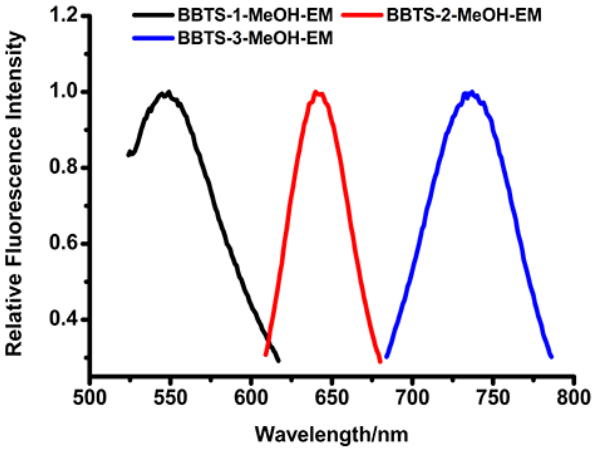


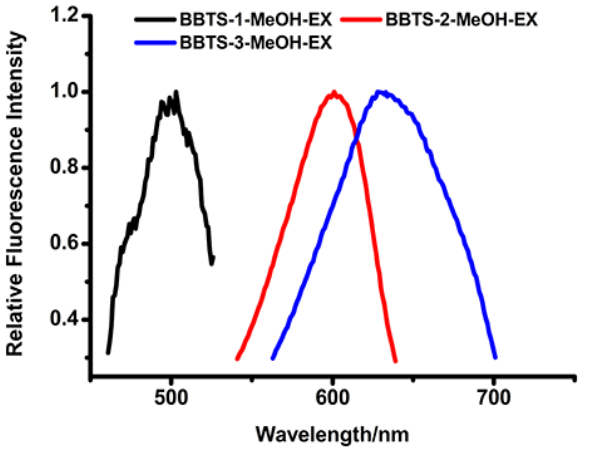
Figure. **S5** Absorption spectra (left panel) and linear fitting curve between absorbance and concentration (right panel) of the BBTO1-3 in CH_2Cl_2, molar absorption coefficients (ε) equals to b.

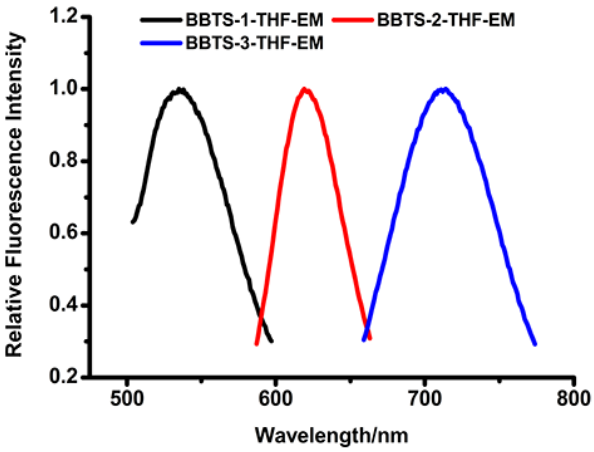


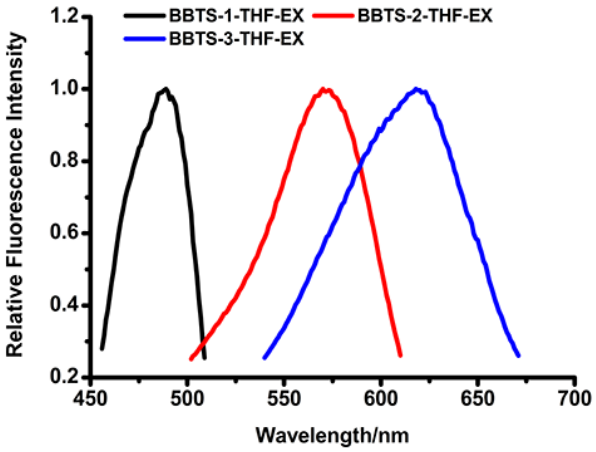

BBTS-1-DMSO-EM
BBTS-2-DMSO-EM
BBTS-3-DMSO-EM
Relative Fluorescence Intensity
Wavelength/nm)

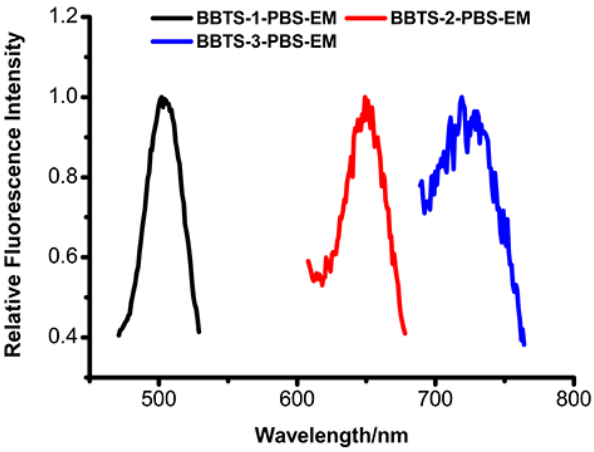

BBTS-1-DMSO-EX
BBTS-2-DMSO-EX
BBTS-3-DMSO-EX
Relative Fluorescence Intensity
Wavelength/nm


BBTS-1-MeOH-EM
BBTS-2-MeOH-EM
BBTS-3-MeOH-EM
Relative Fluorescence Intensity
Wavelength/nm


BBTS-1-MeOH-EX
BBTS-2-MeOH-EX
BBTS-3-MeOH-EX
Relative Fluorescence Intensity
Wavelength/nm


BBTS-1-THF-EM
BBTS-2-THF-EM
BBTS-3-THF-EM
Relative Fluorescence Intensity
Wavelength/nm


BBTS-1-THF-EX
BBTS-2-THF-EX
BBTS-3-THF-EX
Relative Fluorescence Intensity
Wavelength/nm


BBTS-1-PBS-EM
BBTS-2-PBS-EM
BBTS-3-PBS-EM
Relative Fluorescence Intensity
Wavelength/nm

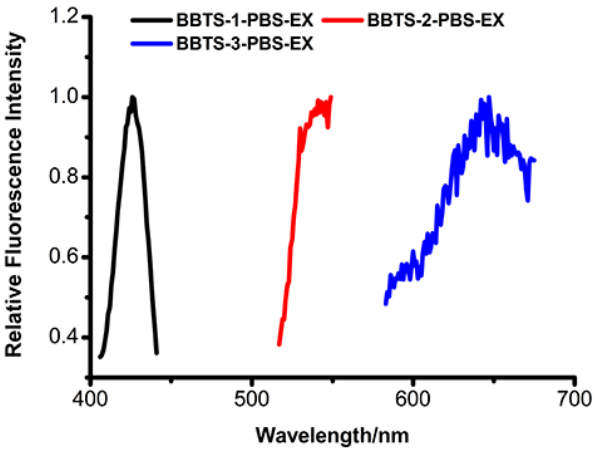

BBTS-1-PBS-EX
BBTS-2-PBS-EX
BBTS-3-PBS-EX
Relative Fluorescence Intensity
Wavelength/nm

Figure. **S6** The excitation (right panel) and emission (left panel) spectra of the BBTS1-3 in different solvents: dichloromethane, DMSO, methanol, THF and PBS (10% ethanol). The emission wavelengths of the solutions were measured at a concentration of 10 μM.

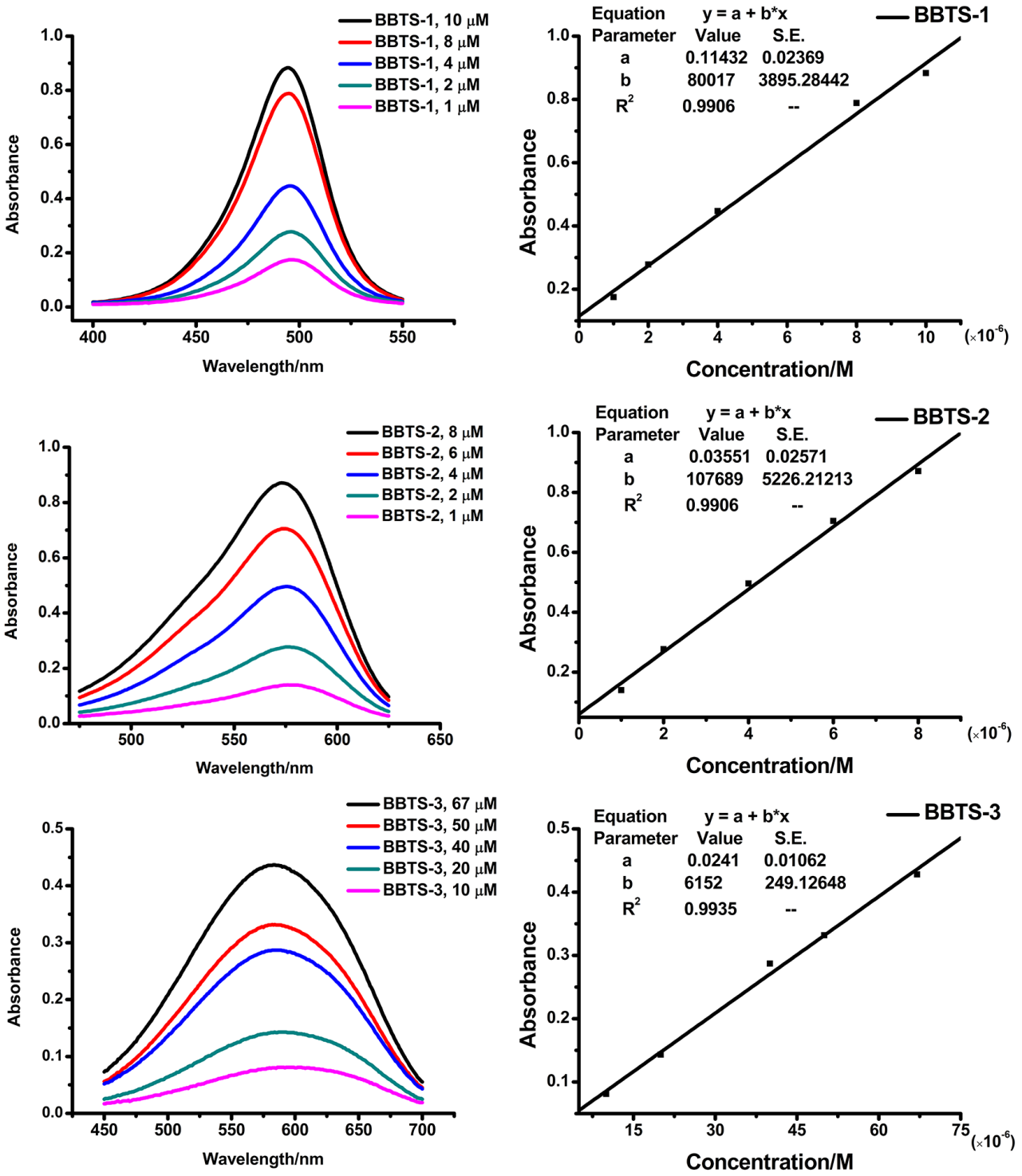


Figure. **S7** Absorption spectra (left panel) and linear fitting curve between absorbance and

concentration (right panel) of the BBTS1-3 in CH_2Cl_2, molar absorption coefficients (ε) equals to b.

Table S1 The spectroscopic properties (absorption, excitation and emission wavelength, molar absorption coefficient, and fluorescence quantum yield) of BBTOM 1-5.

Probe	Solvent	Fluorescence spectrum				UV-vis spectrum	
		λ_{em} (nm)	λ_{ex} (nm)	Stokes shift (nm)	Φ^a %	λ_{abs} (nm)	ε L mol^{-1}cm^{-1}
BBTOM-1	CH_2Cl_2	522	468	54	0.08	461.5	329936
	DMSO	537	476	61			
	MeOH	546	505	41			
	THF	512	467	45			
	PBS	550	466	84			
BBTOM-2	CH_2Cl_2	598	549	49	4.64	530	83346
	DMSO	623	567	56			
	MeOH	613	568	45			
	THF	596	534	62			
	PBS	630	593	37			
	With Aβ	606	574	32			
BBTOM-3	CH_2Cl_2	691	593	98	7.81	548.5	36372
	DMSO	727	621	106			
	MeOH	718	619	99			
	THF	680	585	95			
	PBS	729	650	79			
	With Aβ	690	626	64			
BBTOM-4	CH_2Cl_2	788	625	163	1.64	564	141686
	DMSO	820	635	185			
	MeOH	816	629	187			
	THF	773	620	153			
	PBS	823	642	181			
	With Aβ	793	632	161			
BBTOM-5	CH_2Cl_2	822	636	186	0.26	574.5	87774
	DMSO	826	639	187			
	MeOH	827	634	193			
	THF	817	628	189			
	PBS	829	647	182			
	With Aβ	822	632	190			

[a] Measured in CH_2Cl_2.

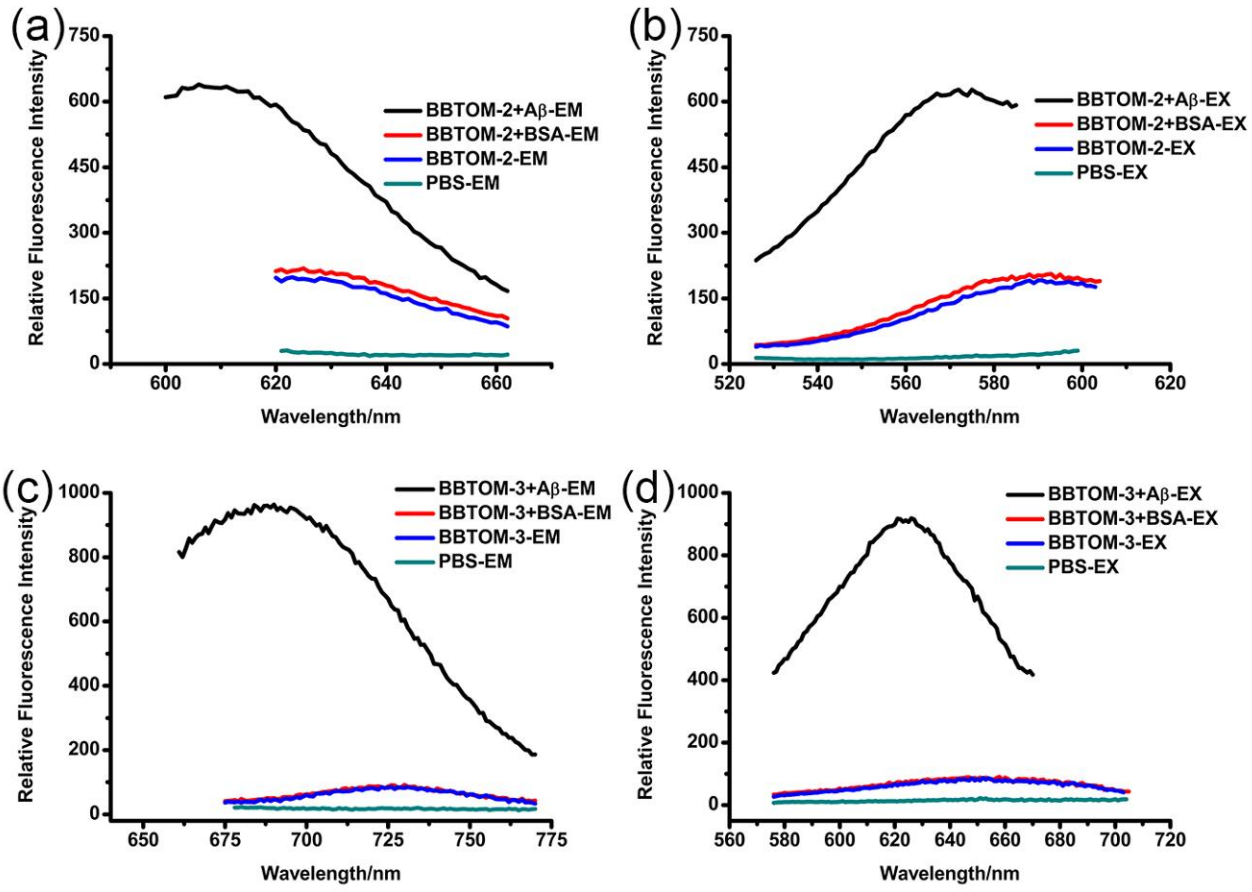
Table S2 The spectroscopic properties (absorption, excitation and emission wavelength, molar absorption coefficient, and fluorescence quantum yield) of BBTO 1-3 and BBTS 1-3.

Probe	Solvent	Fluorescence spectrum				UV-vis spectrum	
		λ_{em} (nm)	λ_{ex} (nm)	Stokes shift (nm)	Φ^a %	λ_{abs} (nm)	ε $L\ mol^{-1}cm^{-1}$
BBTO-1	CH_2Cl_2	518	469	49	0.05	459.5	1146
	DMSO	528	468	60			
	MeOH	514	467	47			
	THF	509	464	45			
	PBS	502	426	76			
BBTO-2	CH_2Cl_2	602	557	45	5.71	537	61602
	DMSO	620	559	61			
	MeOH	611	566	45			
	THF	588	522	66			
	PBS	627	589	38			
BBTO-3	CH_2Cl_2	696	619	77	8.16	536	25342
	DMSO	722	617	105			
	MeOH	712	620	92			
	THF	674	584	90			
	PBS	720	646	74			
	With Aβ	693	630	63			
BBTS-1	CH_2Cl_2	546	500	46	0.79	496	80017
	DMSO	554	504	50			
	MeOH	549	494	55			
	THF	535	489	46			
	PBS	502	426	76			
BBTS-2	CH_2Cl_2	632	595	37	5.93	576.5	107689
	DMSO	651	601	50			
	MeOH	641	599	42			
	THF	617	571	46			
	PBS	649	535	114			
BBTS-3	CH_2Cl_2	727	632	95	6.77	584	6152
	DMSO	734	620	114			
	MeOH	737	628	109			
	THF	714	618	96			
	PBS	727	648	79			
	With Aβ	721	643	78			

[a] Measured in CH_2Cl_2.

Fluorescence Spectral Measurements of the NIR Probes with Aβ Aggregates and BSA.

NIR probes (50 nM in the final test solution) in 2.9 mL PBS were added to a solution of 100 μL of aggregated A$\beta_{1\text{-}42}$ and A$\beta_{1\text{-}40}$ fibrils or A$\beta_{1\text{-}42}$ and A$\beta_{1\text{-}40}$ monomers (30 μg in the final test solution) or BSA (30 μg in the final test solution), then the mixture was incubated at 37 ℃ with constant shaking (120 r/min) for 1 h. The mixture was then transferred to a quartz sampling cell, and the fluorescent properties (fluorescence excitation/emission wavelength and intensity) were measured on a SHIMADZU RF-5301PC spectrofluorophotometer (Japan). The fluorescence properties of the solutions of the NIRFs in PBS (50 nM) were also measured in the same manner as a blank control.



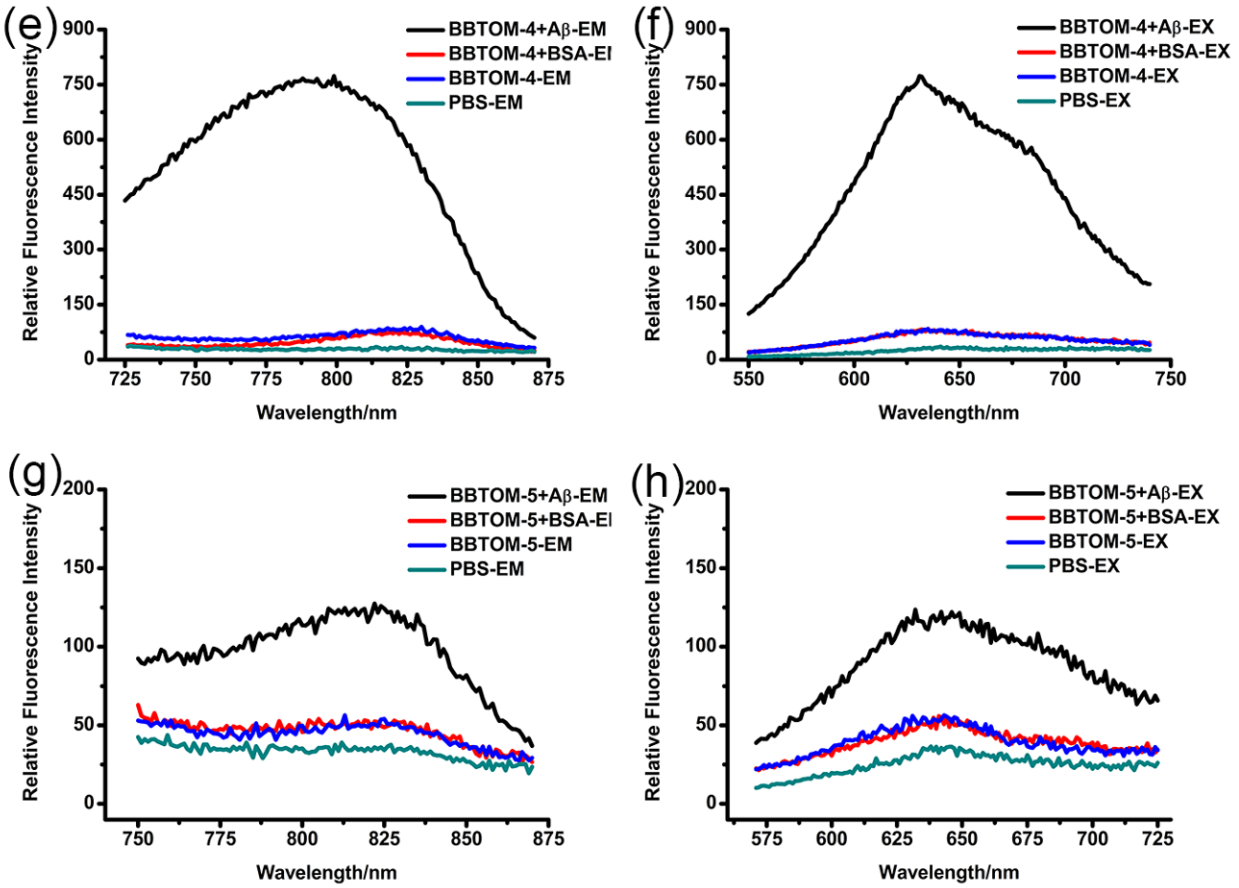
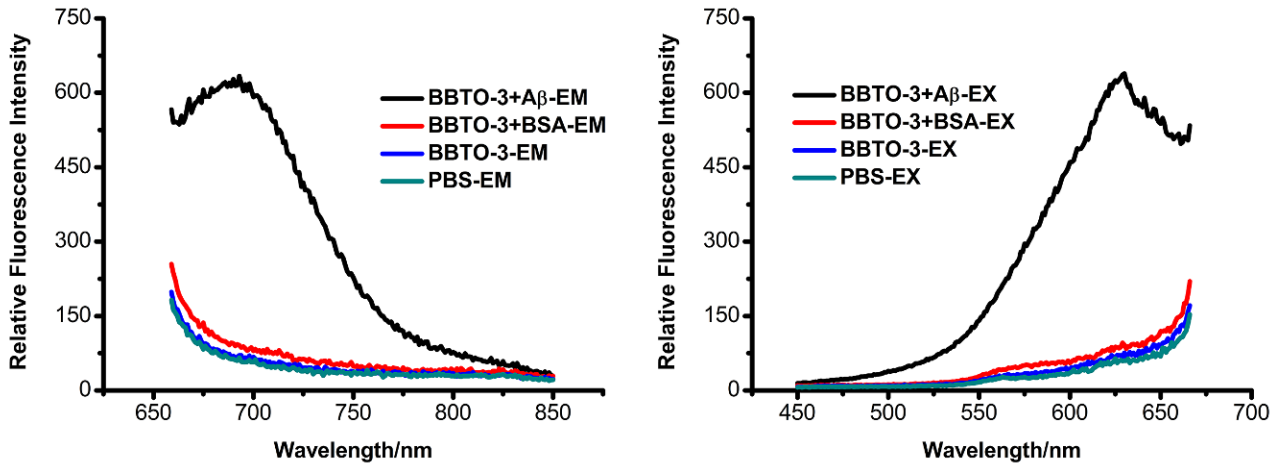


Figure. **S8** The excitation (right panel) and emission (left panel) spectra of BBTOM2-5 upon interaction with Aβ_{1-42} aggregates and BSA. The spectra of BBTOM2-5 solutions in PBS and PBS alone were also measured in the same condition.



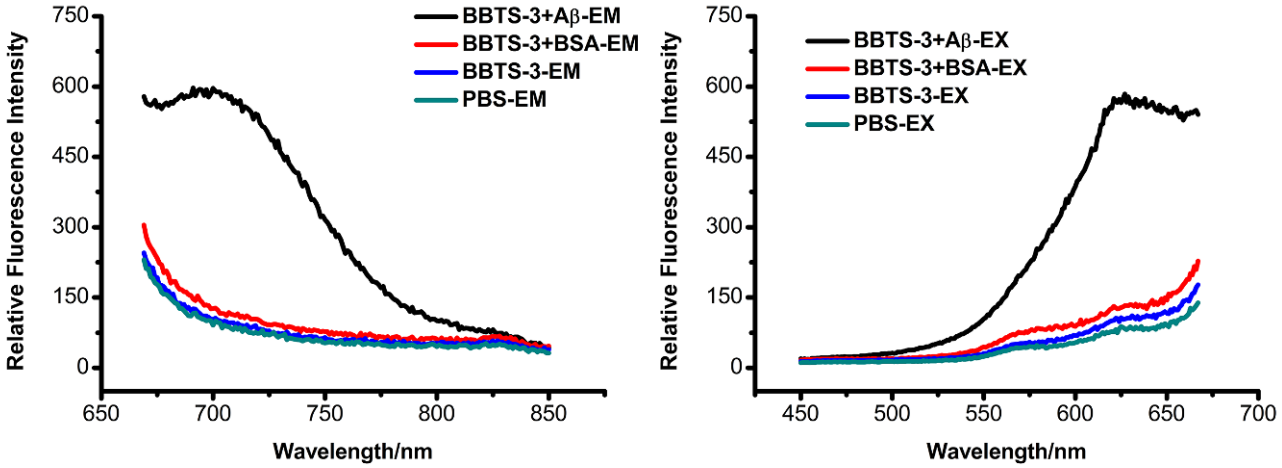


Figure. **S9** The excitation (right panel) and emission (left panel) spectra of BBTO-3 and BBTS-3 upon interaction with Aβ_{1-42} aggregates and BSA. The spectra of BBTO-3 and BBTS-3 solutions in PBS and PBS alone were also measured in the same condition.

***In Vitro* Fluorescent Staining.**

Paraffin-embedded brain sections from Tg mice (C57BL6, APPswe/PSEN1, 12-month old, male), wild-type mice (C57BL6, 12-month old, male), AD human (91-year old, male; 64-year old, female, temporal lobe) and healthy human (72-year old, male, temporal lobe) were used for the neuropathological staining. The brain slices were deparaffinized with 10 min washes in xylene, 5 min washes in 100% ethanol, and running double distilled water for 5 min. Next, the brain sections were incubated with aqueous solutions of NIR probes (1.0 μM, 5% DMSO, 10% ethanol) for 5 min at room temperature. The localization of plaques was confirmed by staining the adjacent sections with Th-S (0.125% in water). Fluorescent observation was carried out on an Axio Oberver Z1 (Zeiss, Germany) equipped with DAPI, AF488, AF546, and Cy5 filter sets.

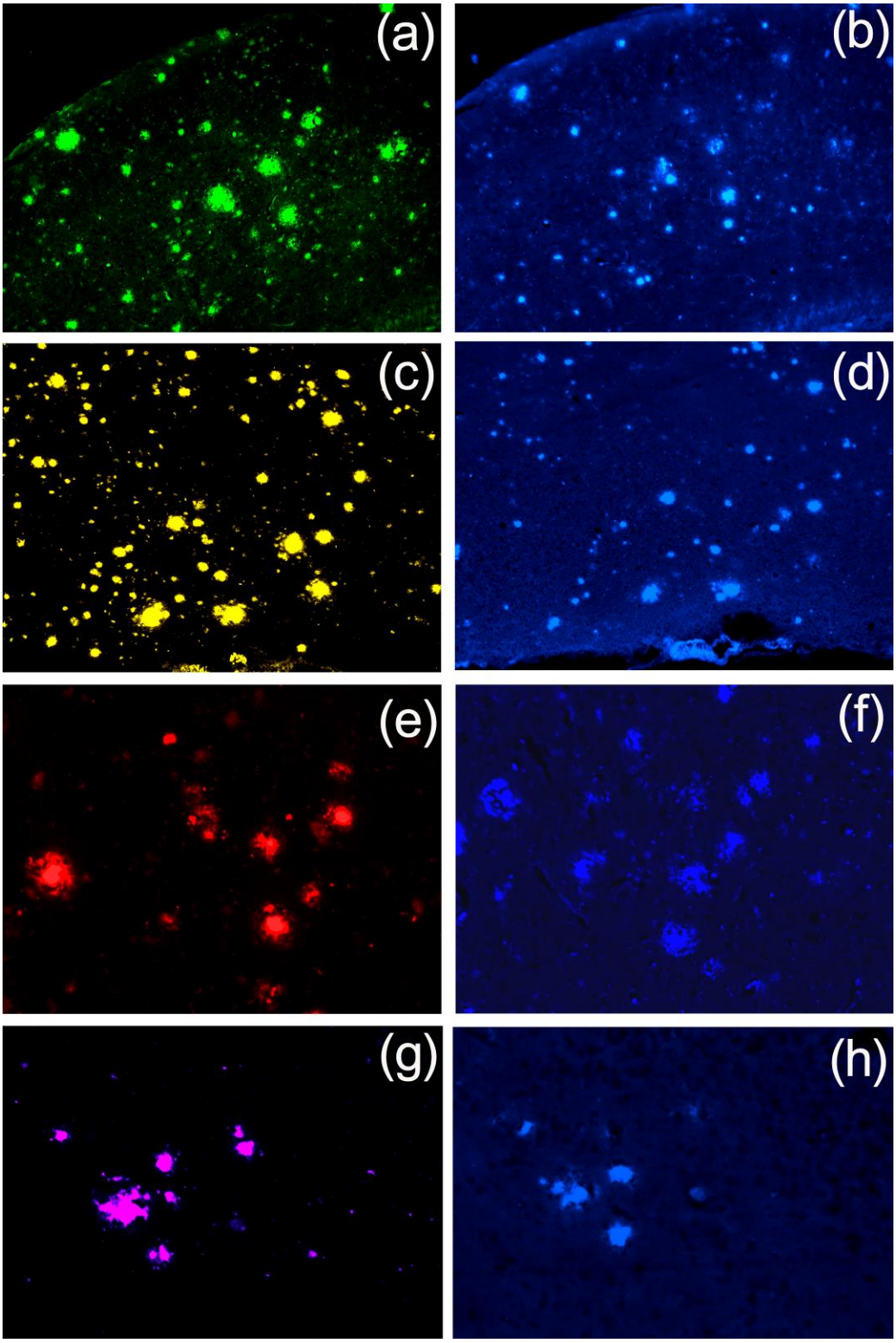


Figure. **S10** *In vitro* fluorescent staining of Aβ plaques on brain sections of Tg mice by BBTOMs. (a, 10×), (c, 10×), (e, 10×) and (g, 20×) were stained by BBTOM-1, -2, -4, and BBTOM-5, respectively. The presence and distribution of plaques on the sections were confirmed by fluorescence staining

using Th-S on the adjacent section (b, 10×), (d, 10×), (f, 10×) and (h, 20×).

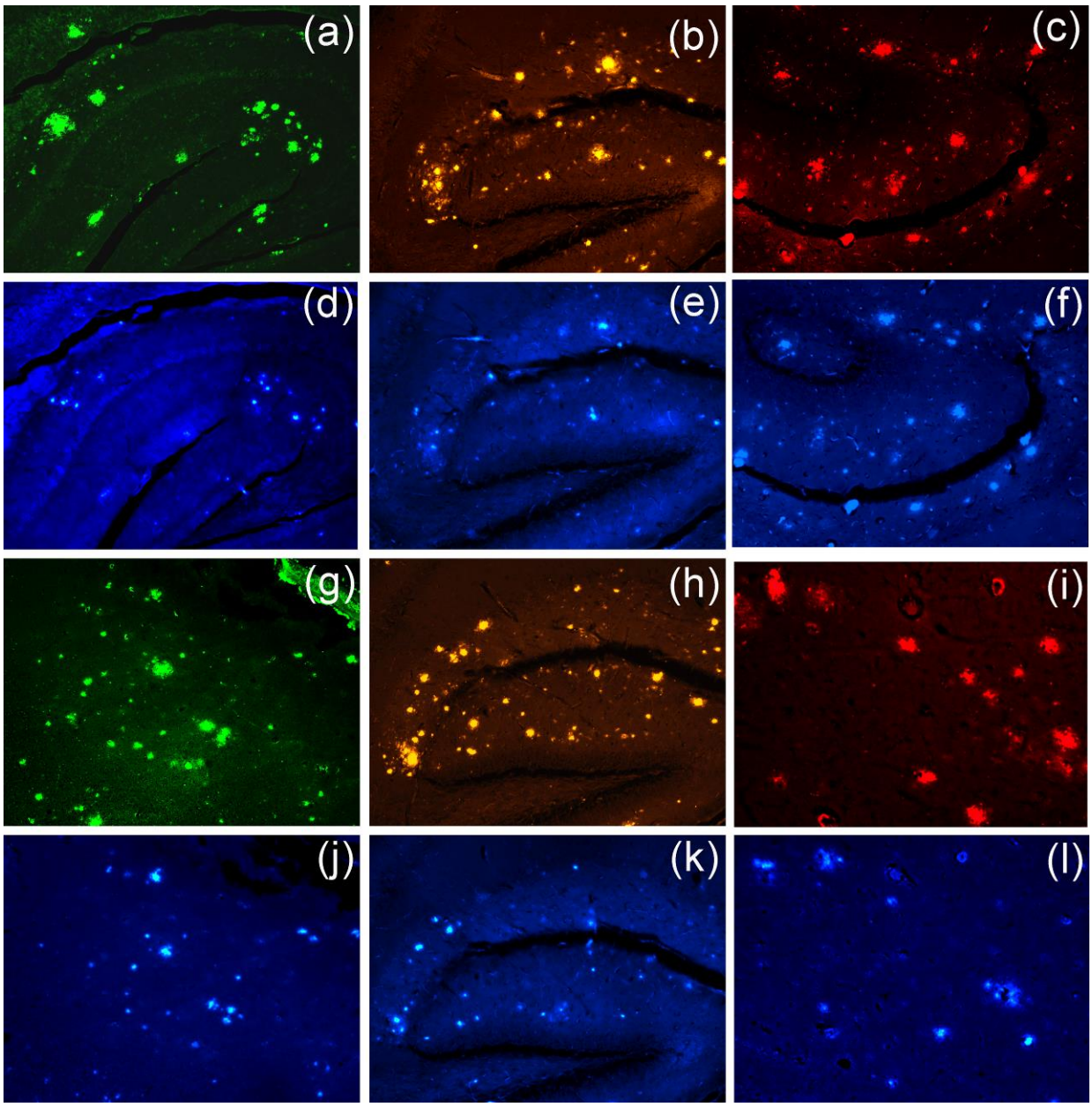


Figure. **S11** *In vitro* fluorescent staining of Aβ plaques on brain sections of Tg mice by BBTO1-3 and BBTS1-3. (a, 10×), (b, 10×), (c, 10×), (g, 10×), (h, 10×) and (i, 20×) were stained by BBTO-1, -2, -3, and BBTS-1, -2, -3, respectively. The presence and distribution of plaques on the sections were confirmed by fluorescence staining using Th-S on the adjacent section (d, 10×), (e, 10×), (f, 10×), (j, 10×), (k, 10×) and (l, 20×).

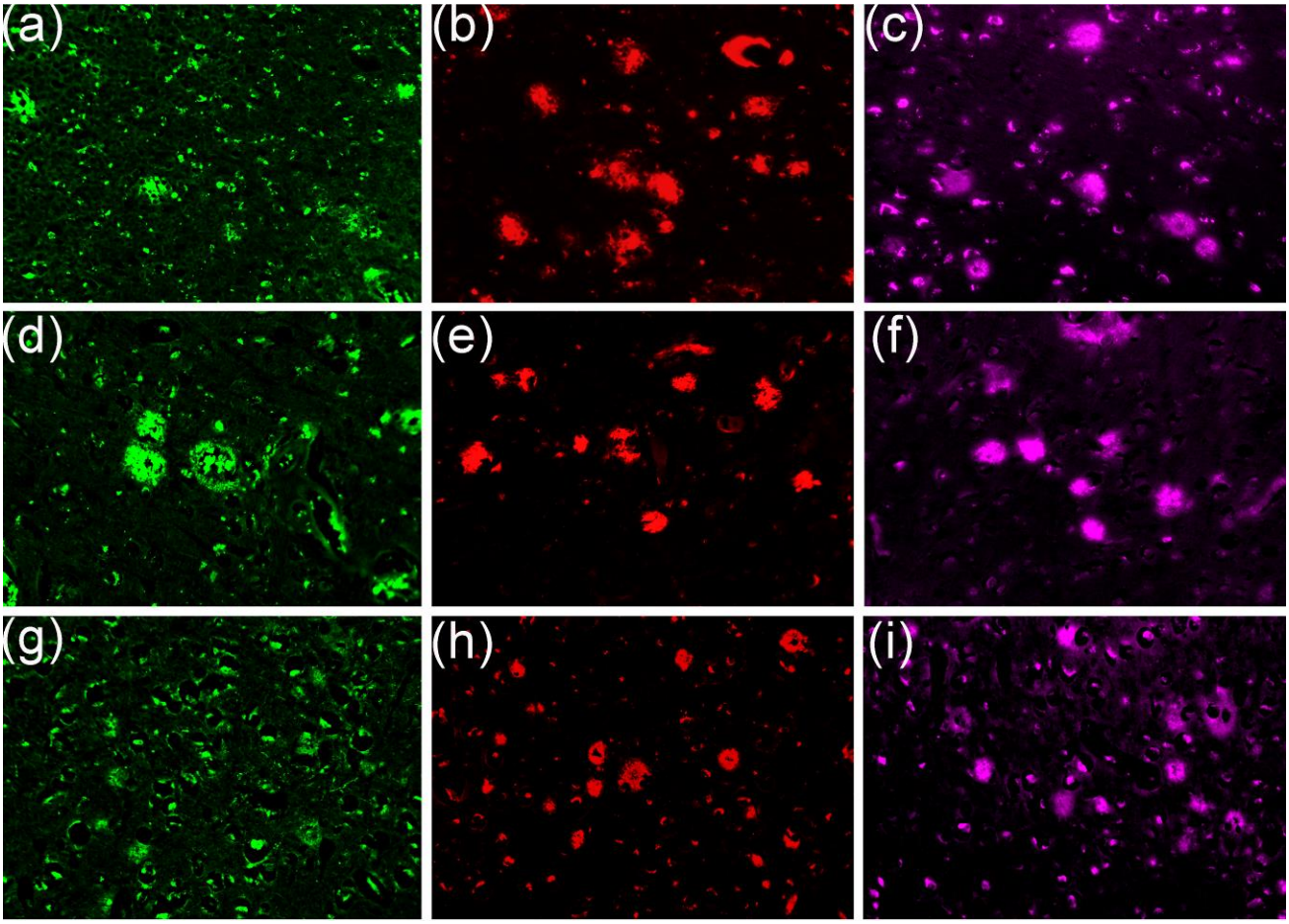


Figure. **S12** *In vitro* fluorescent staining of Aβ plaques on brain sections of AD patients. (a), (b) and (c) were stained by BBTOM-1, -2, -4, respectively; (d), (e) and (f) were stained by BBTO-1, -2, -3, respectively. (g), (h) and (i) were stained by BBTS-1, -2, -3, respectively. Magnification: 20×

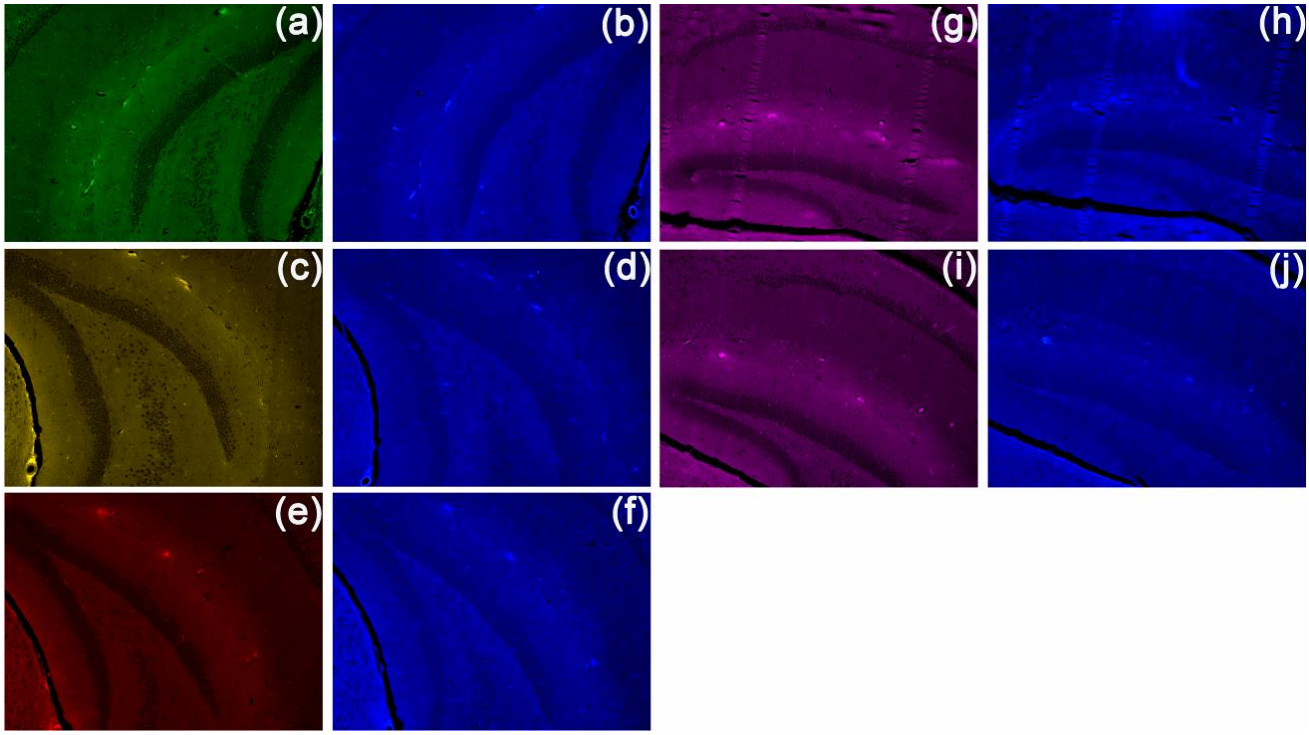


Figure. **S13** *In vitro* fluorescent staining on brain sections from wild-type mouse. (a), (c), (e), (g) and (i) were stained by BBTOM-1, -2, -3, -4, and -5, respectively. The adjacent brain sections were stained with Th-S (b), (d), (f), (h) and (j). Magnification: 10×

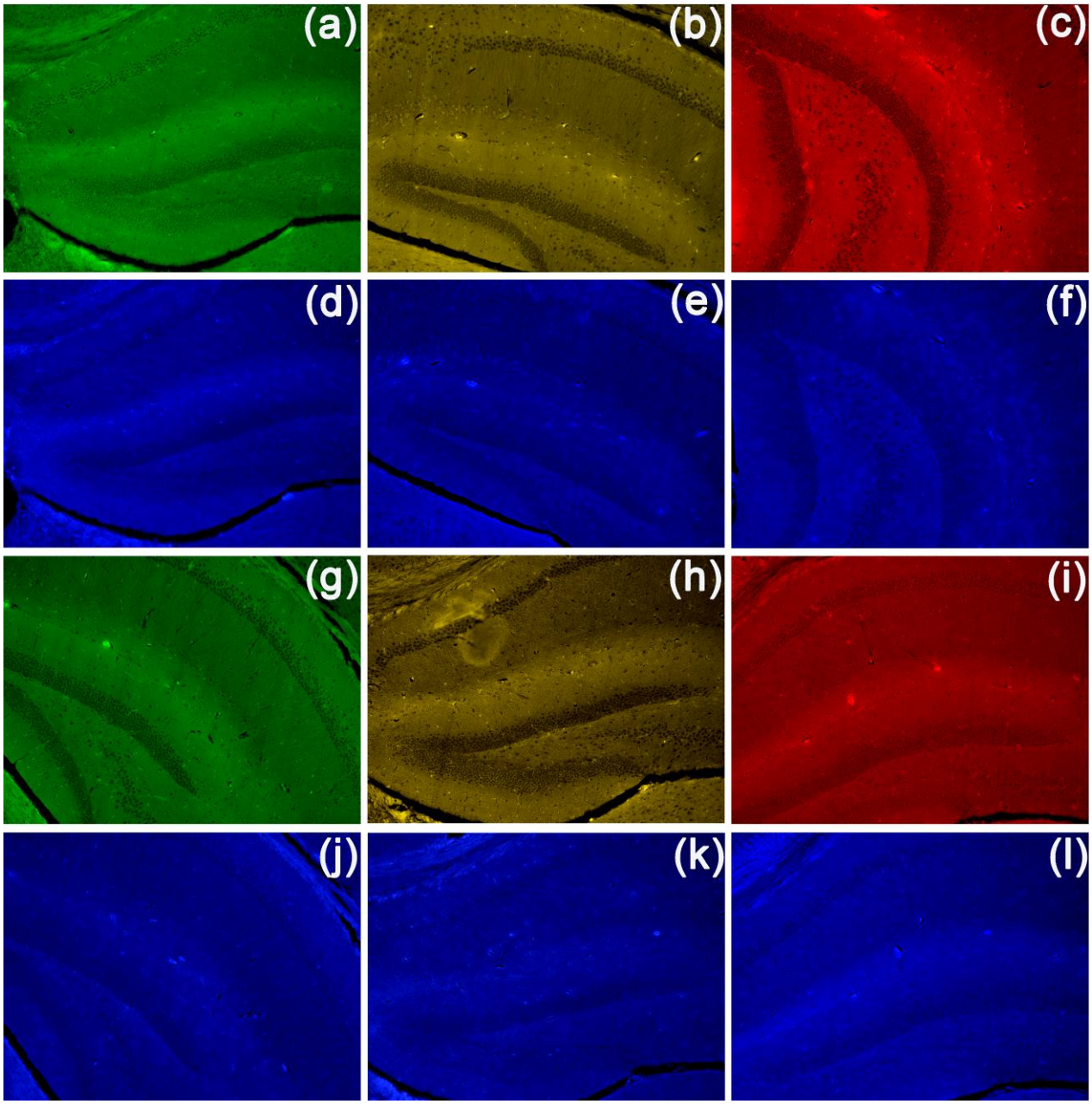


Figure. **S14** *In vitro* fluorescent staining on brain sections from wild-type mouse. (a), (b) and (c) were stained by BBTO-1, -2 and -3, respectively. (g), (h) and (i) were stained by BBTS-1, -2, -3, respectively. The adjacent brain sections were stained with Th-S (d), (e), (f), (j), (k) and (l). Magnification: 10×.

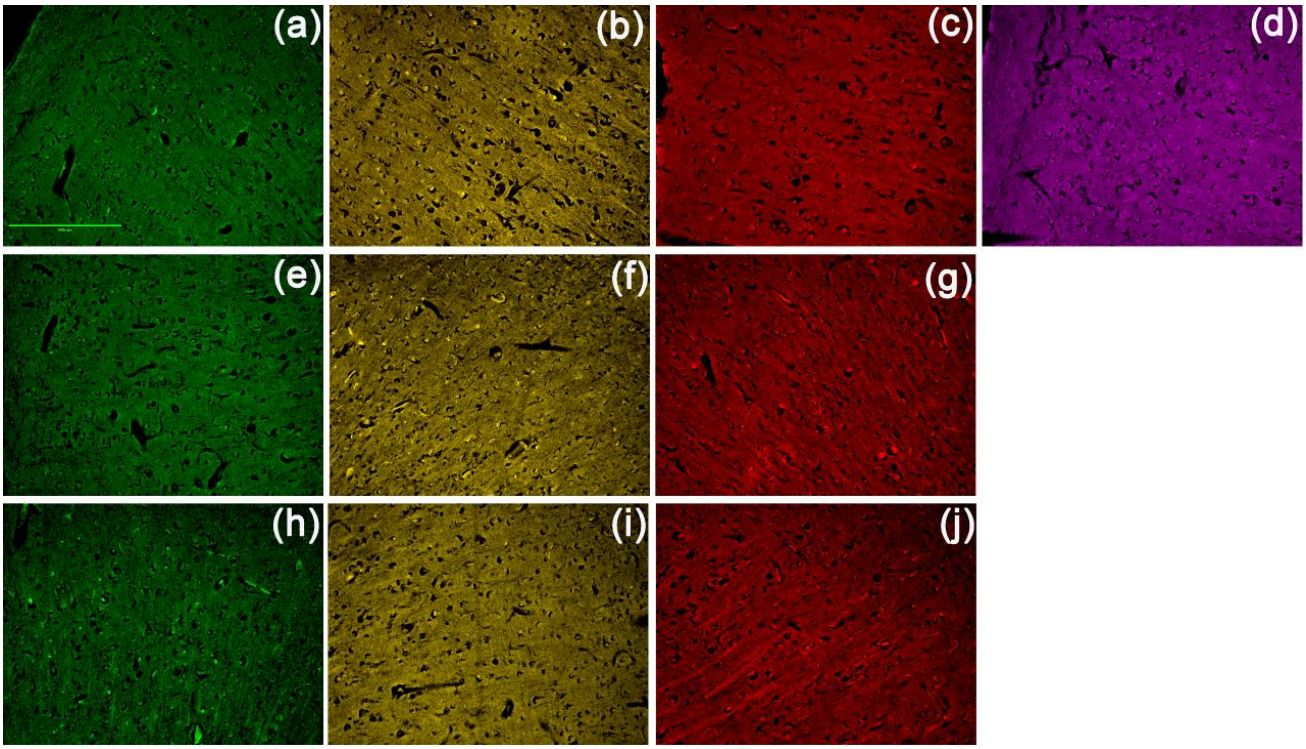


Figure. **S15** *In vitro* fluorescent staining on brain sections from healthy human (72-year old, male, temporal lobe). (a), (b), (c) and (d) were stained by BBTOM-1, -2, -3 and -4, respectively. (e), (f) and (g) were stained by BBTO-1, -2, -3, respectively. (h), (i) and (j) were stained by BBTS-1, -2, -3, respectively. Magnification: 10×.

***In vitro* Inhibition Binding Assays Using A$\beta_{1\text{-}42}$ Aggregates.**

[^{125}I]IMPY was employed as radioligand to determine the binding affinities of the NIR probes in *in vitro* competitive binding assays. [^{125}I]IMPY and A$\beta_{1\text{-}42}$ aggregates were prepared according to procedures described previously.[4] Inhibition experiments were manipulated in 12 × 75 mm borosilicate glass tubes, 100 μL of aggregated Aβ fibrils (60 nM in the final assay mixture) was added to a mixture containing 100 μL of [^{125}I]IMPY at appropriate concentration, 100 μL of probes (10^{-5} to 10^{-10} M in ethanol), and 700 μL of PBS (0.2 M, pH = 7.4, containing 0.1 % BSA) in a final volume of 1 mL. The mixture was incubated at 37 ℃ for 2 h, then it was transferred to borosilicate

glass microfiber filters (Whatman, GF/B) using a Brandel Mp-48T cell harvester. After the bound and free [^{125}I]IMPY were separated, filters containing the bound [^{125}I]IMPY were measured by gamma counter (WALLAC/Wizard 1470, USA) with 70% counting efficiency. The half maximal inhibitory concentration (IC_{50}) was determined from the displacement curves of three independent assays using GraphPad Prism 4.0 and the inhibition constant (K_i) was calculated using the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + [L]/K_d)$.[5]

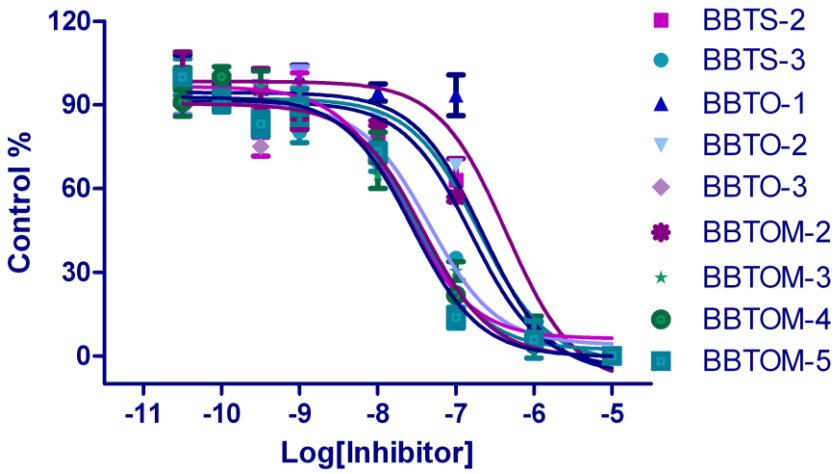


Figure. **S16** Inhibition curves for the binding of [^{125}I]IMPY to A$\beta_{1\text{-}42}$ aggregates.

Blood-Brain Barrier (BBB) Penetrating studies of BBTOM-3.

A solution of BBTOM-3 (20% DMSO and 80% propylene glycol, 50 μL, 2 mg/Kg) was injected via tail vein of ICR mice (20-22 g, male). Next, the mice were sacrificed at proper time points of 2, 10, 30 and 60 min (n = 3 for each time point), and the brain samples were dissected out to homogenize with 1 mL acetonitrile for twice. Then, the extracts were filtered by flashing nylon membrane (0.22 μm) and dried over anhydrous sodium sulfate and washed by 1 mL acetonitril. After that, 100 μL of the combined acetonitrile were analyzed by Agilent 1260 Infinity Quaternary LC (Agilent

Technologies) system, HPLC conditions: Venusil MP C18 column (Agela Technologies, 5 μm, 4.6 mm × 250 mm), CH_3CN/H_2O = 80%/20%, 1 mL/min. The blank control was obtained by analyzing the solution of the probe (10 μL diluted to 3.0 mL acetonitrile).

***In Vivo* Near-Infrared Imaging.**

Double Tg mice (n = 3, C57BL6, APPsw/PSEN1, 14 month-old, male) and an age-matched control mice (n = 3, C57BL6, 14-month-old, male) were shaved before background imaging and were intravenous injected with BBTOM-3 (0.5 mg/kg, 15% DMSO, 15% tween-80, 70% saline, 50 μL). The fluorescence signals of the brain are recorded at different time points after intravenous injected with BBTOM-3 on an IVIS Lumina III system. A filter set (ex. at 560 nm and em. at 710 nm) was used for the measurement. The mice were kept on the imaging stage under anesthesia with 2.5% isoflurane gas in an oxygen flow (0.8 L/min) during the imaging process. Living Image Software was employed to analyze the imaging date, and an ROI was drawn around the brain section. Intensity of brain fluorescence was calculated from the radiant efficiency. The date were analyzed according to the procedures described previously.[6]

***Ex Vivo* Fluorescent Staining of BBTOM-3 to A*β* Plaques in the Brain of Transgenic Mouse.**

BBTOM-3 (1 mg/kg, 20% DMSO, 80% propylene glycol, 50 μL) was intravenous injected to a double Tg mouse (C57BL6, APPsw/PSEN1, 14-month-old, male) and an age-matched control mouse (C57BL6, 14-month-old, male). Next, they were sacrificed at 20 min after injection. The brains were

excised, embedded in optimum cutting temperature compound (OCT). Frozen sections of 20 μm were cut, and fluorescent observation was performed by Axio Oberver Z1 (Zeiss, Germany) equipped with an AF488 filter sets. Furthermore, the Aβ plaques were further confirmed by the staining of the same section with Th-S (0.125%) using a DAPI filter set.

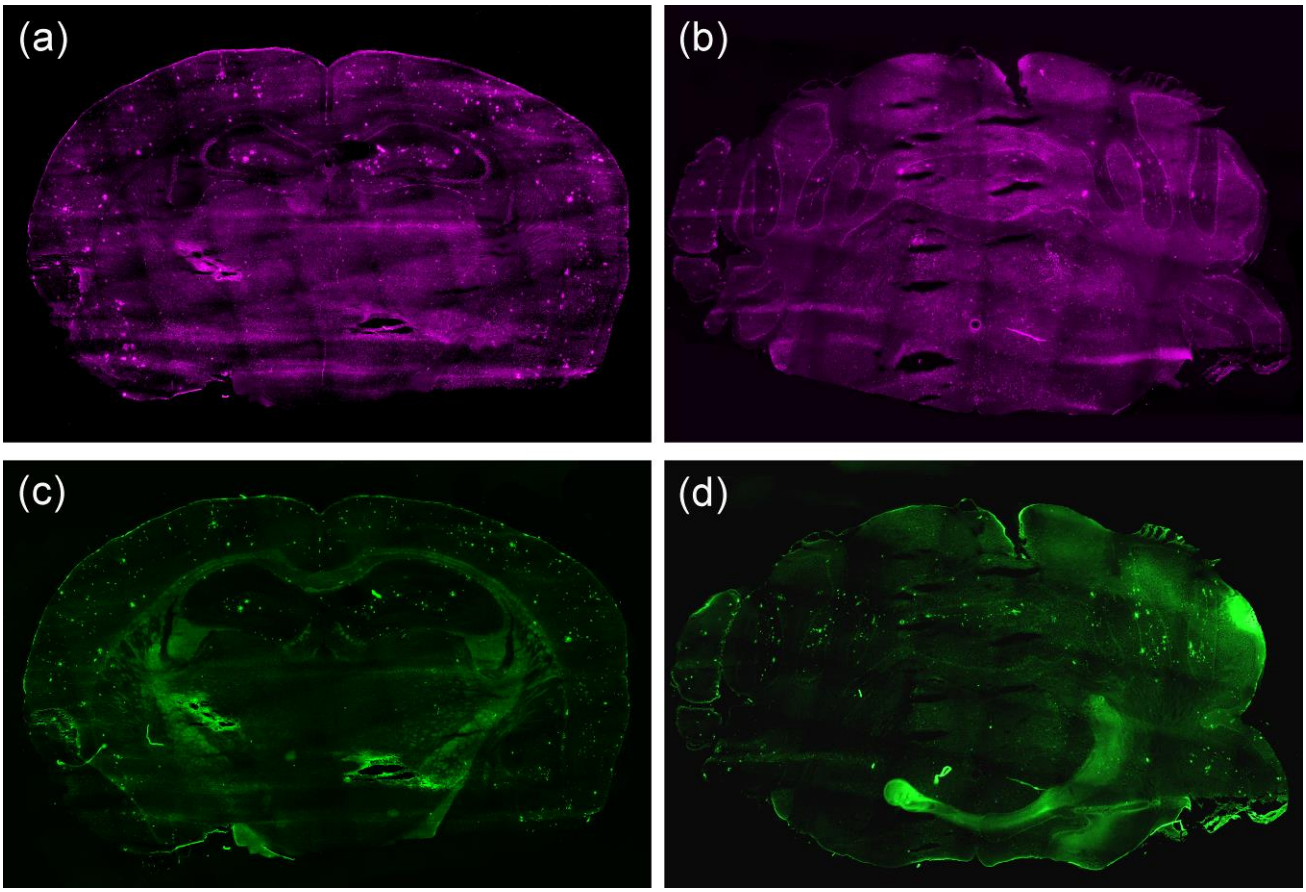


Figure. S17 *Ex vivo* fluorescence observation of coronal brain slices from a Tg mouse (a: cerebrum region; b: cerebellum region) after injection of BBTOM-3 (1 mg/kg). The Aβ plaques were further confirmed by staining the same sections with Th-S (c and d).

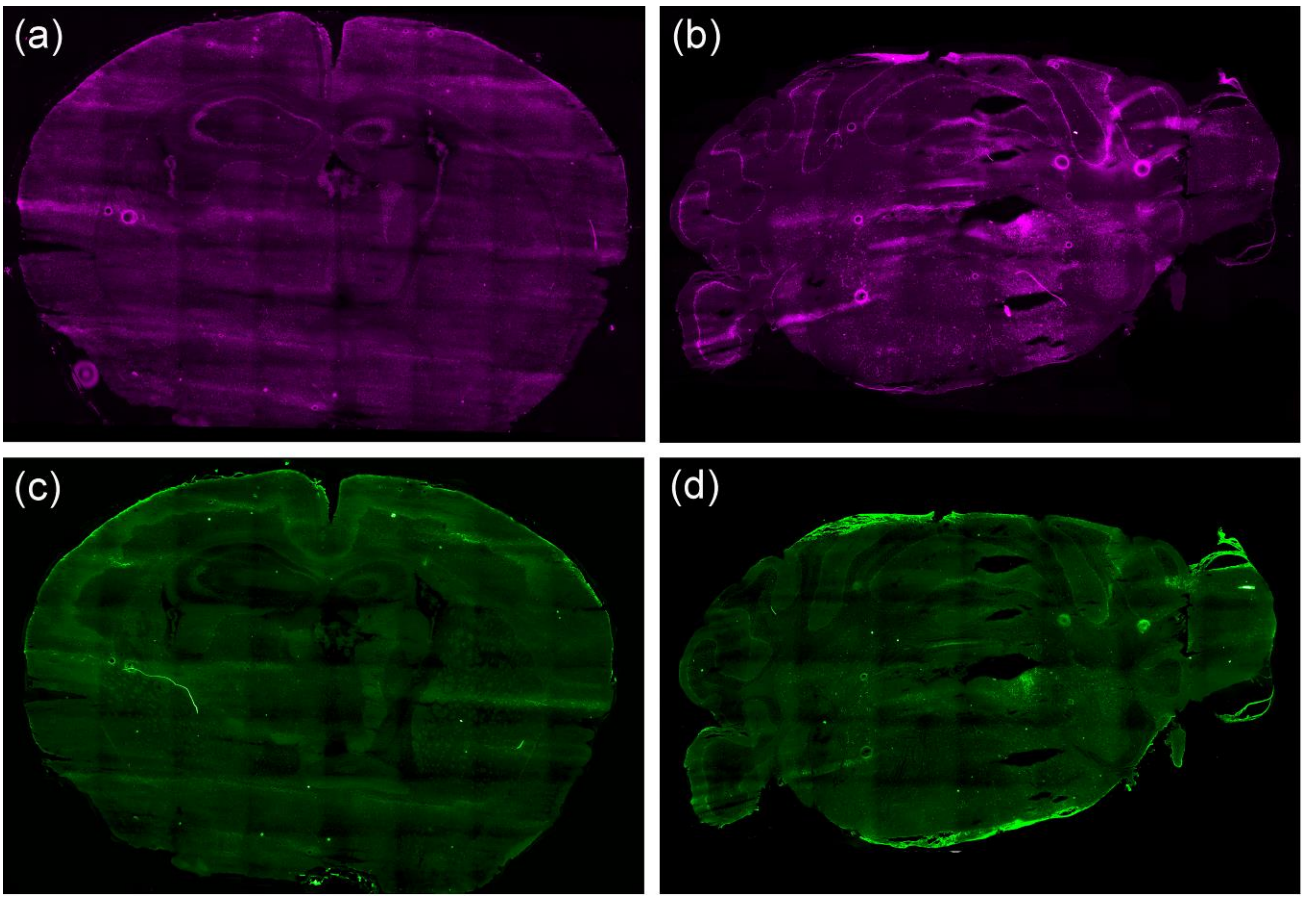


Figure. S18 *Ex vivo* fluorescence observation of coronal brain slices from a wild-type mouse (a: cerebrum region; b: cerebellum region) after injection of BBTOM-3 (1 mg/kg). The Aβ plaques were further confirmed by staining the same sections with Th-S (c and d).

Photo-isomerization.

BBTOM-3 was dissolved in acetonitrile and was irradiated under UV light (365 nm) for 1 hour, then the samples were analyzed by HPLC to verify the retention time and purity with or without irradiation. As shown in Fig S19, there was no retention time and purity change of BBTOM-3 after UV irradiation, which indicated that BBTOM-3 was stable towards photochemical isomerization.

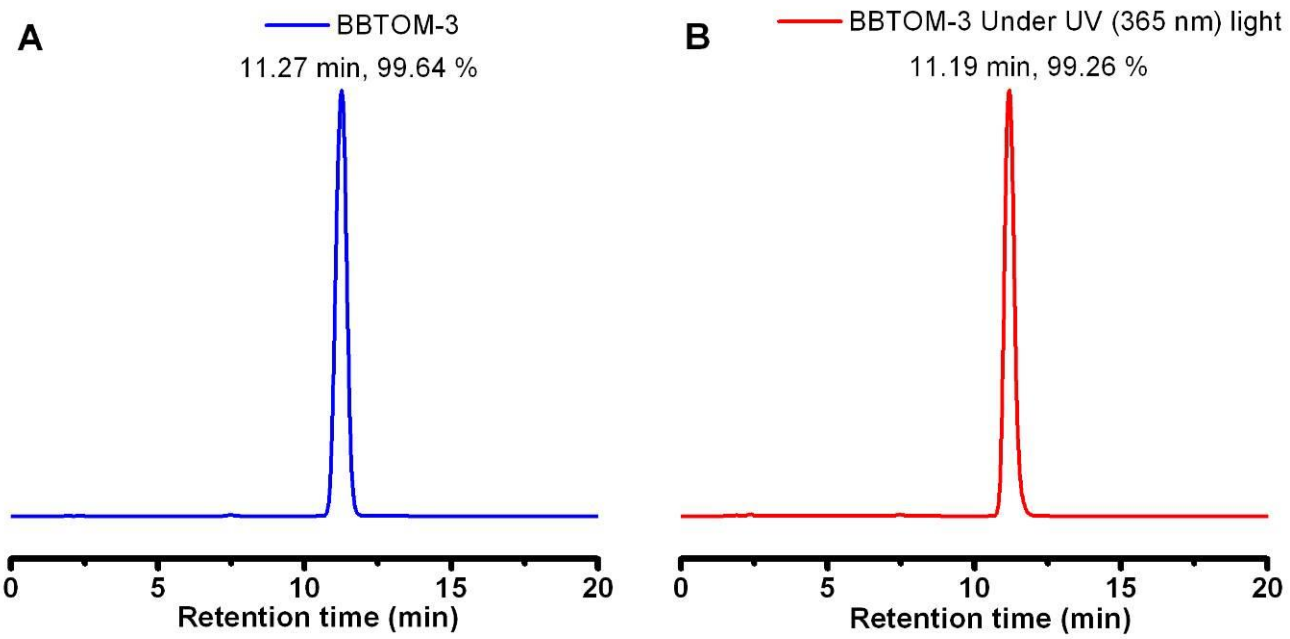
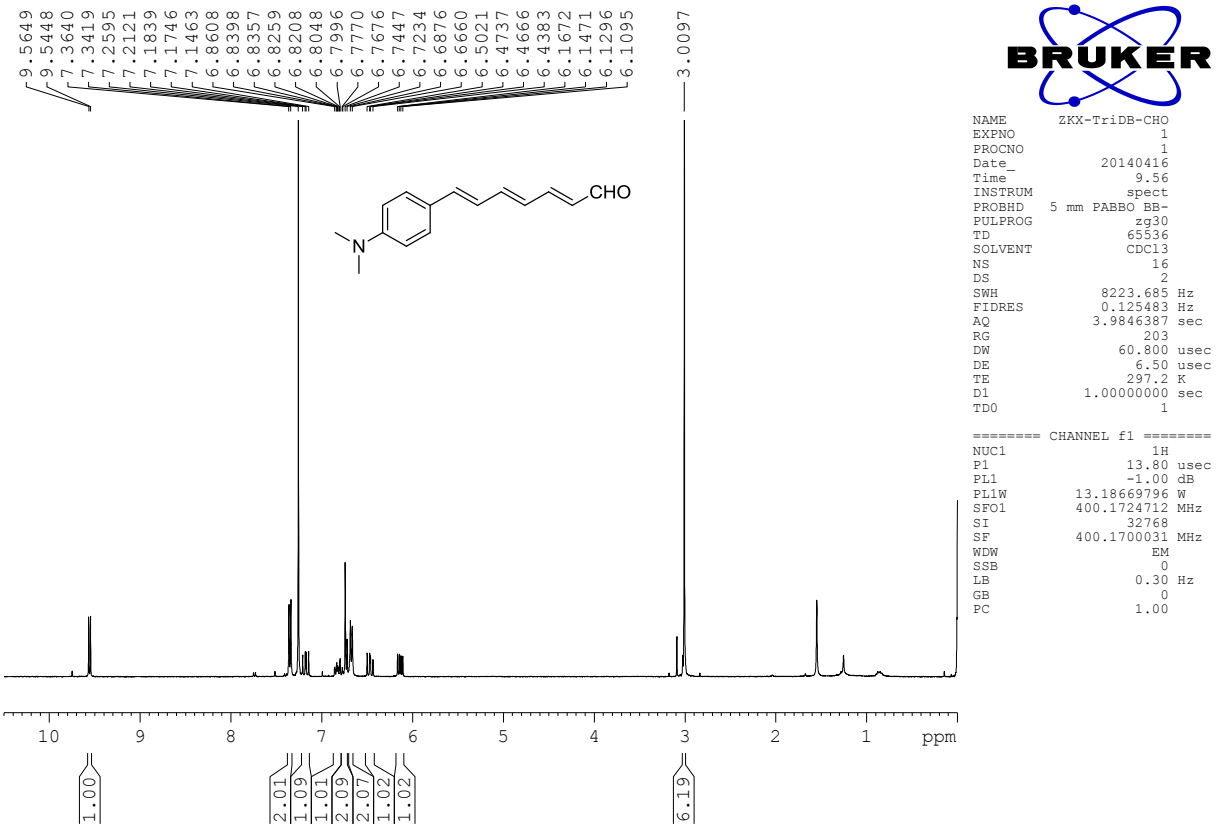
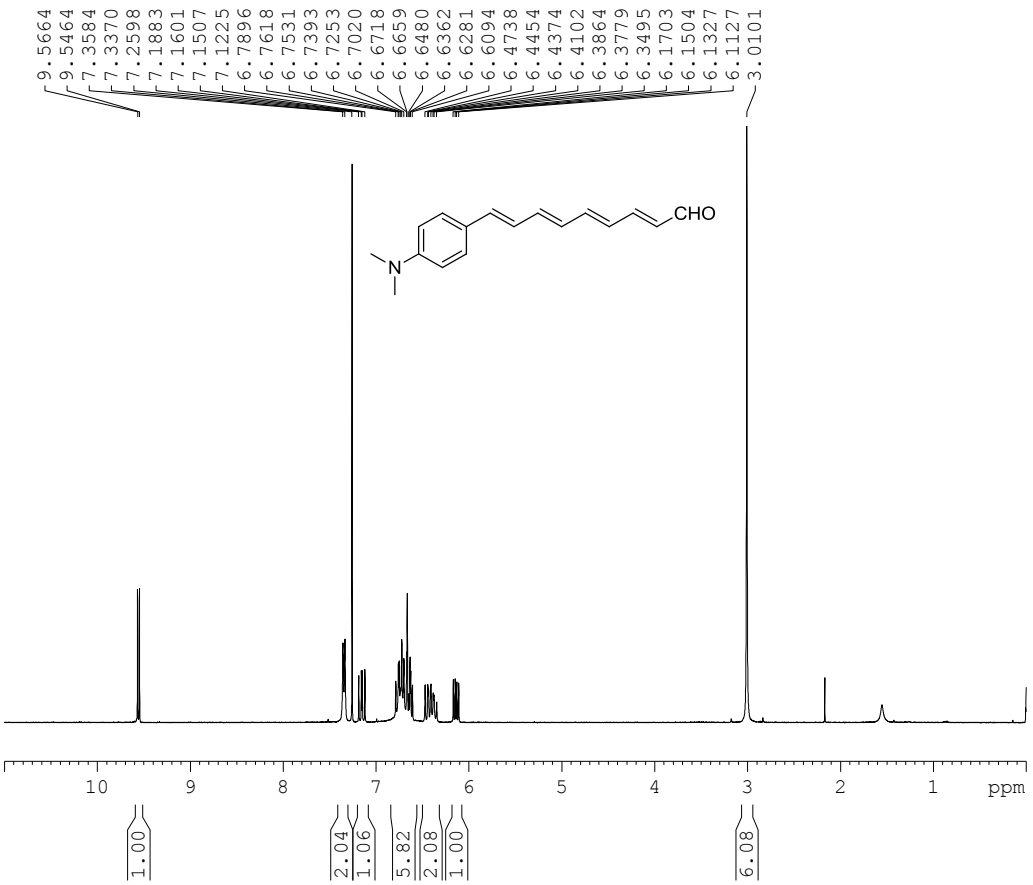


Figure. S19 HPLC profiles of BBTOM-3 (A) and BBTOM-3 under 1 hour of UV light (365 nm) irradiation (B).

NMR and MS Spectra of All Compounds.

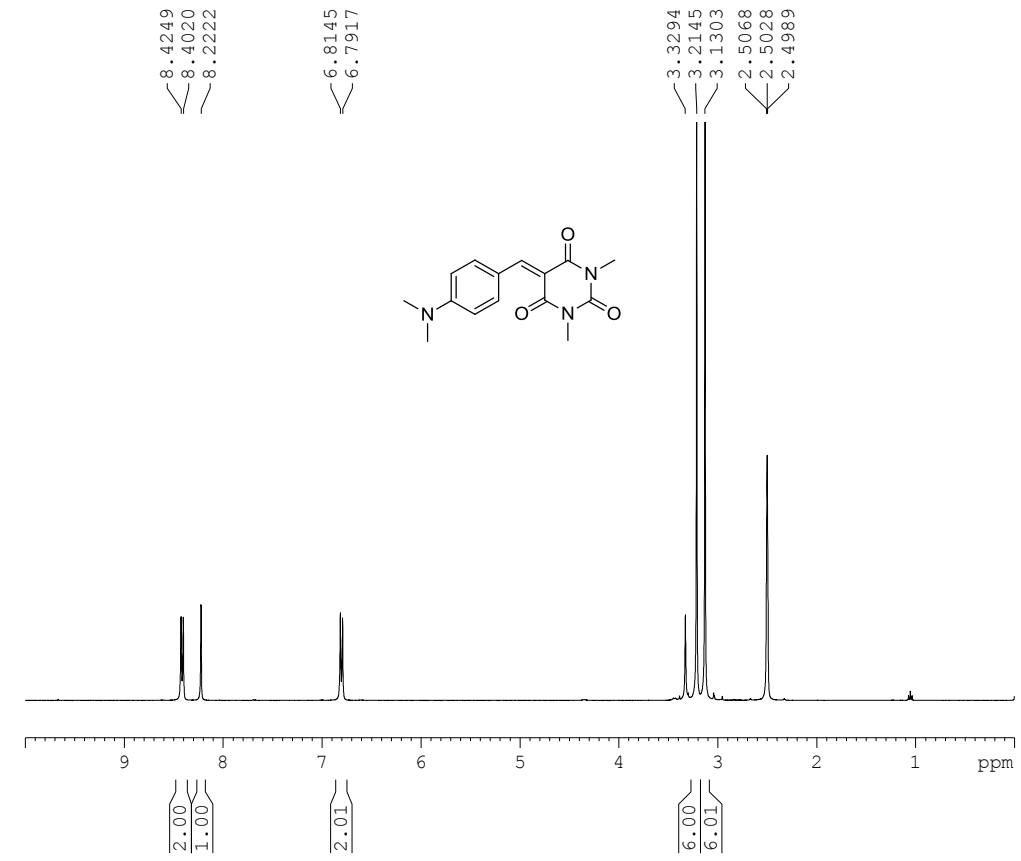


^{1}H NMR ($CDCl_3$) spectrum of compound **2**



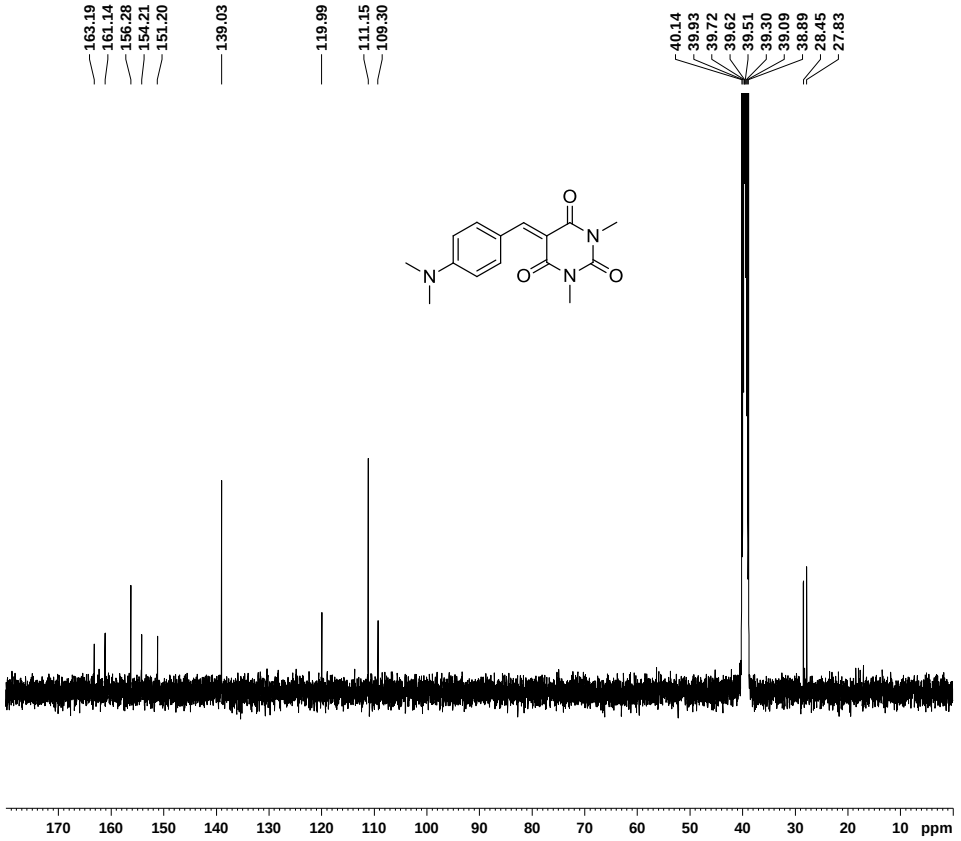
```
NAME          ZKX-PH-4-CHO-20150527
EXPNO                            1
PROCNO                           1
Date_                     20150527
Time                         21.13
INSTRUM                      spect
PROBHD     5 mm PABBO BB-
PULPROG                       zg30
TD                           65536
SOLVENT                      CDCl3
NS                              16
DS                               2
SWH                       8223.685 Hz
FIDRES                    0.125483 Hz
AQ                        3.9846387 sec
RG                             203
DW                          60.800 usec
DE                           6.50 usec
TE                          297.2 K
D1                    1.00000000 sec
TD0                              1

======== CHANNEL f1 ========
NUC1                            1H
P1                          13.80 usec
PL1                         -1.00 dB
PL1W                 13.18669796 W
SFO1                400.1724712 MHz
SI                           32768
SF                  400.1700034 MHz
WDW                             EM
SSB                              0
LB                            0.30 Hz
GB                               0
PC                            1.00
```

^{1}H NMR ($CDCl_3$) spectrum of compound **3**



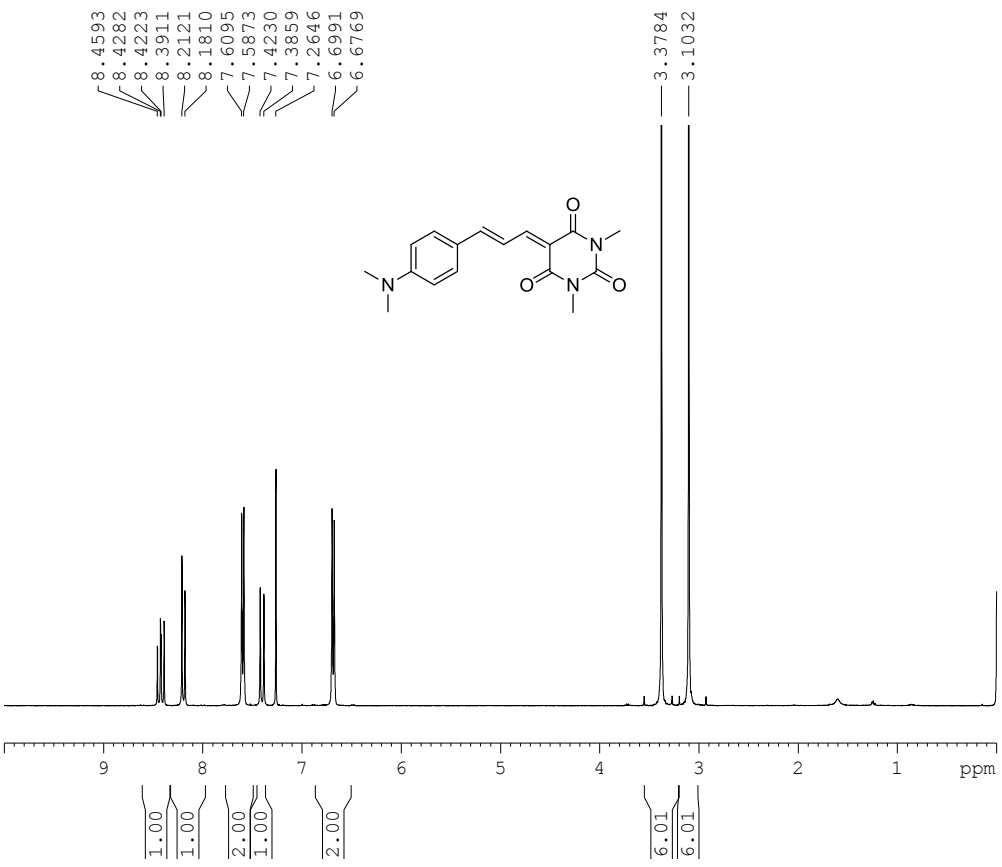
```
NAME              BBATO-M-1
EXPNO                     1
PROCNO                    1
Date_              20111025
Time                   9.33
INSTRUM               spect
PROBHD     5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       16
DS                        2
SWH                8223.685 Hz
FIDRES             0.125483 Hz
AQ                 3.9846387 sec
RG                      203
DW                   60.800 usec
DE                    6.50 usec
TE                   295.4 K
D1             1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                   13.80 usec
PL1                  -1.00 dB
PL1W          13.18669796 W
SFO1         400.1724712 MHz
SI                    32768
SF           400.1699942 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```

^{1}H NMR (DMSO-d_6) spectrum of BBTOM-1



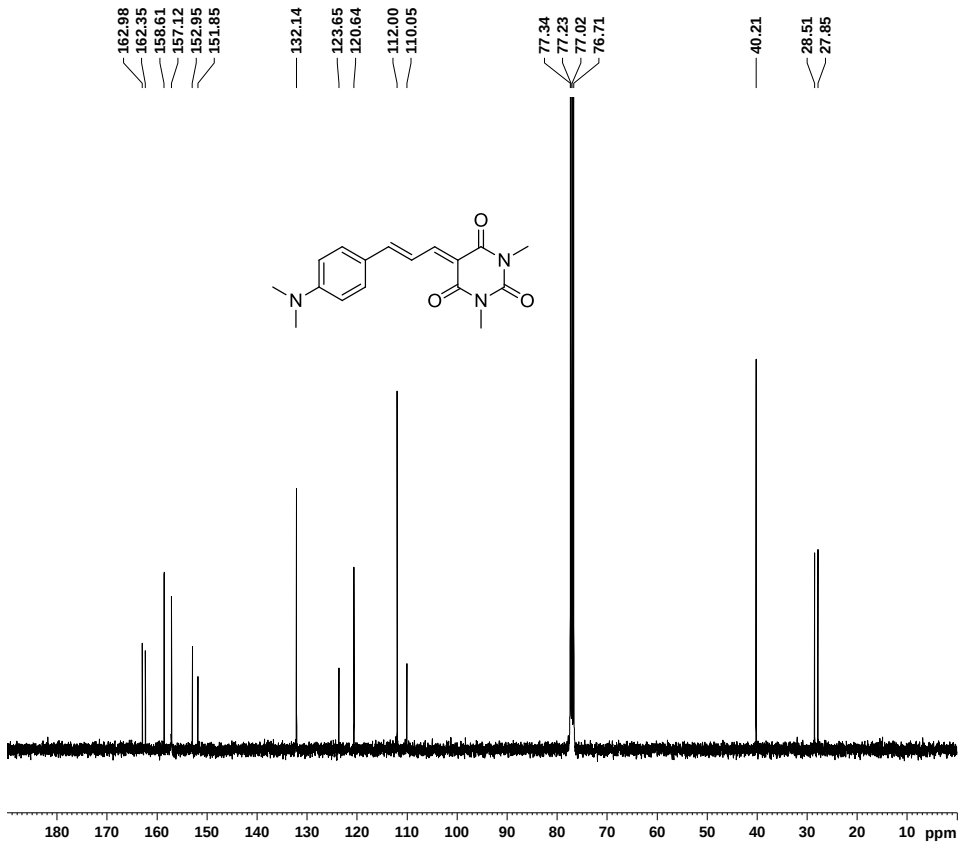

```
NAME            BBATO-M-1-C13
EXPNO                       1
PROCNO                      1
Date_            20111026
Time                14.03
INSTRUM             spect
PROBHD    5 mm PABBO BB-
PULPROG            zgpg30
TD                  65536
SOLVENT               DMSO
NS                    3080
DS                       4
SWH             24038.461 Hz
FIDRES           0.366798 Hz
AQ               1.3631988 sec
RG                     203
DW                  20.800 usec
DE                   6.50 usec
TE                  298.7 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                    8.50 usec
PL1                  -2.00 dB
PL1W          57.32743073 W
SFO1         100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2                80.00 usec
PL2                  -1.00 dB
PL12                 14.26 dB
PL13                 14.46 dB
PL2W          13.18669796 W
PL12W          0.39276794 W
PL13W          0.37509048 W
SFO2         400.1716007 MHz
SI                   32768
SF           100.6228773 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

^{13}C NMR (DMSO-d_6) spectrum of BBTOM-1



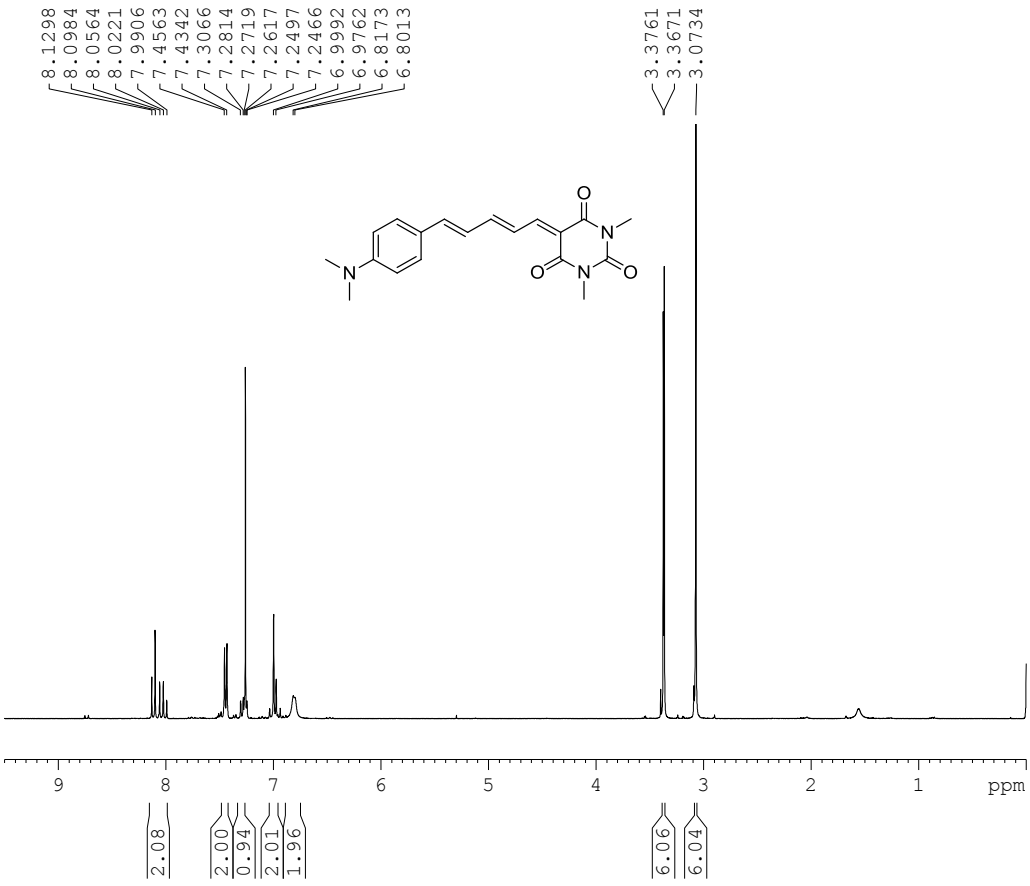

```
NAME              BBATO-M-2
EXPNO                     1
PROCNO                    1
Date_          20111025
Time               9.29
INSTRUM           spect
PROBHD    5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ              3.9846387 sec
RG                   203
DW                60.800 usec
DE                 6.50 usec
TE                295.5 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 13.80 usec
PL1                -1.00 dB
PL1W        13.18669796 W
SFO1       400.1724712 MHz
SI                 32768
SF         400.1700017 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

^{1}H NMR ($CDCl_3$) spectrum of BBTOM-2



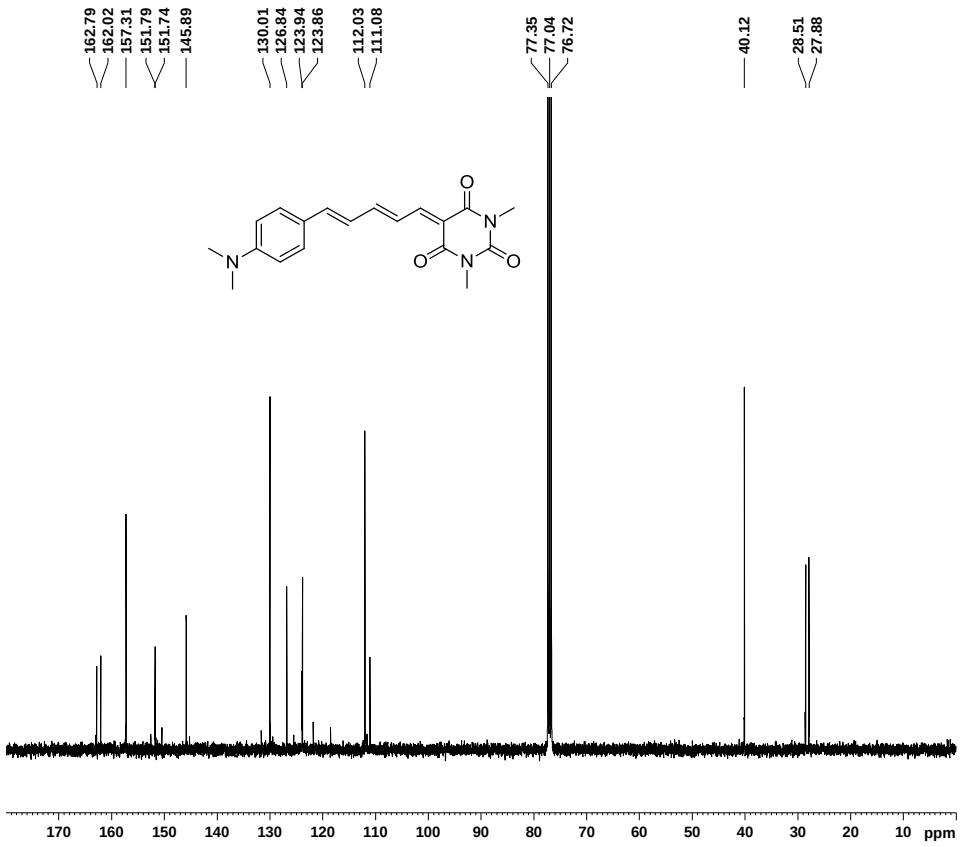

```
NAME          BBATO-M-2-C13
EXPNO                    1
PROCNO                   1
Date_             20111025
Time                 22.27
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  2000
DS                     4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631988 sec
RG                   203
DW                20.800 usec
DE                 6.50 usec
TE                300.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.50 usec
PL1                -2.00 dB
PL1W        57.32743073 W
SFO1       100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -1.00 dB
PL12               14.26 dB
PL13               14.46 dB
PL2W        13.18669796 W
PL12W        0.39276794 W
PL13W        0.37509048 W
SFO2       400.1716007 MHz
SI                 32768
SF         100.6228252 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

^{13}C NMR ($CDCl_3$) spectrum of BBTOM-2



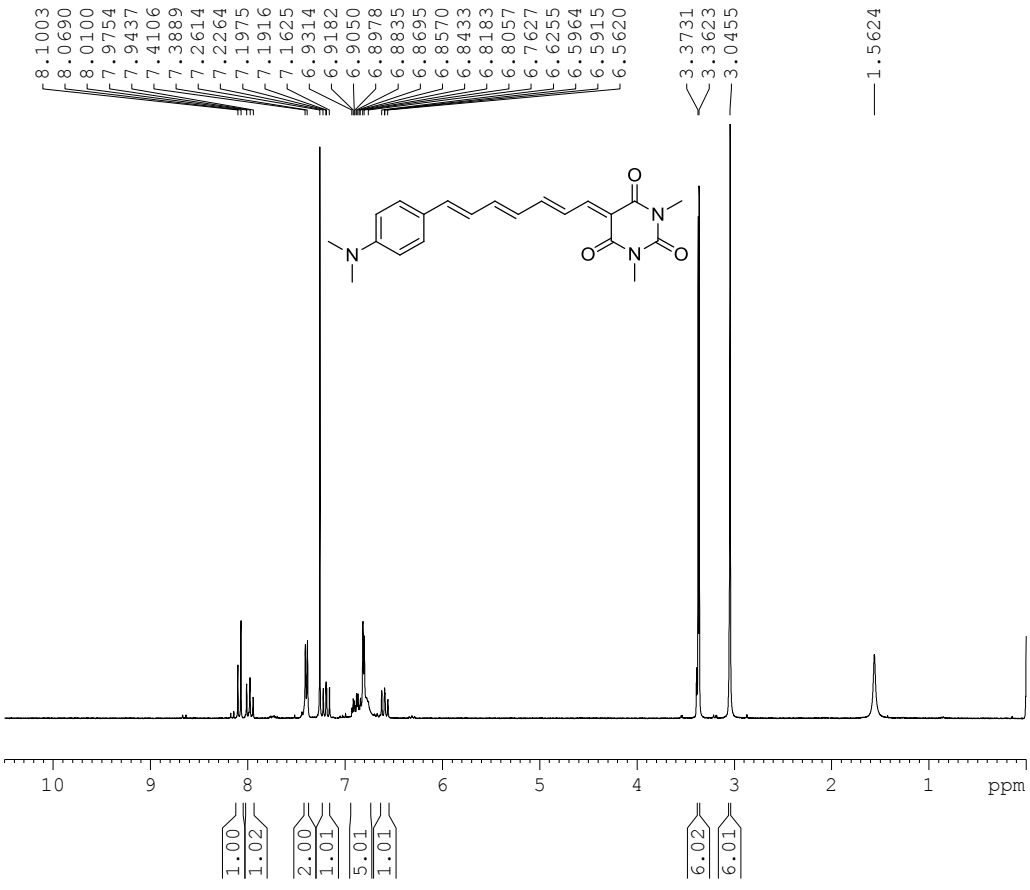

```
NAME      ZKX-BBTOM-3-20150527
EXPNO                    1
PROCNO                   1
Date_             20150527
Time                 9.37
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG              zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH            8223.685 Hz
FIDRES         0.125483 Hz
AQ             3.9846387 sec
RG                   203
DW                60.800 usec
DE                 6.50 usec
TE                297.2 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 1H
P1                 13.80 usec
PL1                -1.00 dB
PL1W        13.18669796 W
SFO1       400.1724712 MHz
SI                 32768
SF         400.1700026 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

^{1}H NMR ($CDCl_3$) spectrum of BBTOM-3



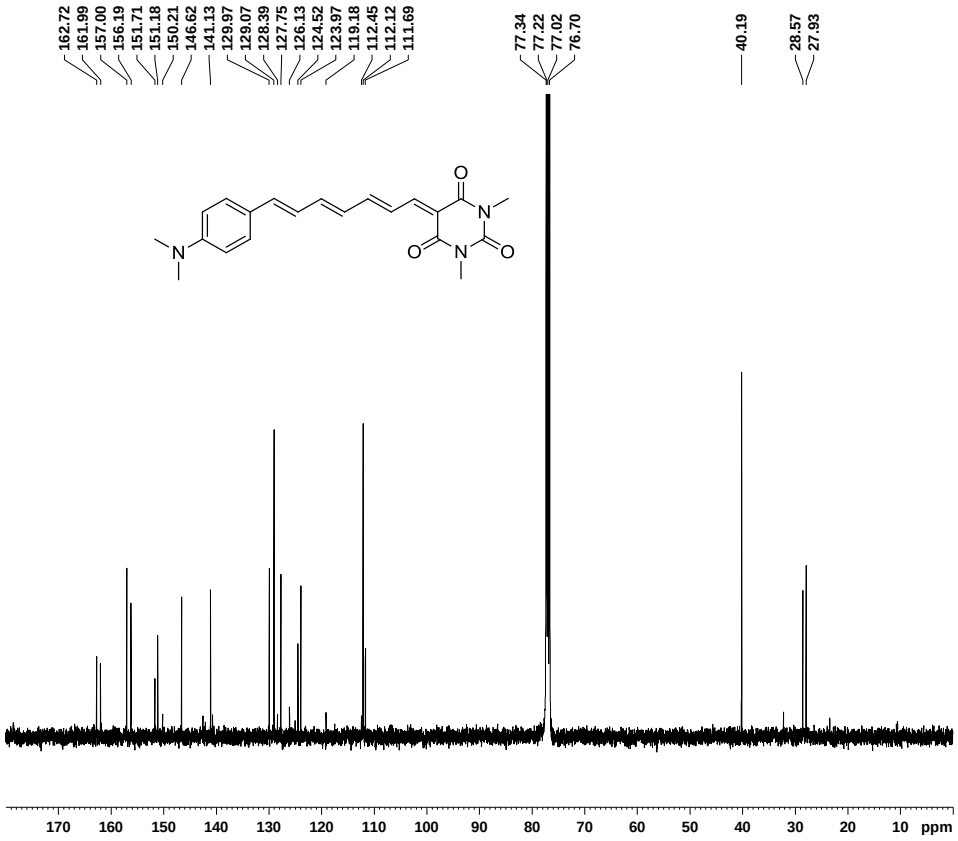

```
NAME          ZKX-BBATO-M-3-C
EXPNO                       1
PROCNO                      1
Date_              20140429
Time                  20.34
INSTRUM               spect
PROBHD    5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                     618
DS                       4
SWH             24038.461 Hz
FIDRES           0.366798 Hz
AQ               1.3631988 sec
RG                     203
DW                  20.800 usec
DE                   6.50 usec
TE                  301.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                    8.50 usec
PL1                  -2.00 dB
PL1W          57.32743073 W
SFO1          100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2                80.00 usec
PL2                  -1.00 dB
PL12                 14.26 dB
PL13                 14.46 dB
PL2W          13.18669796 W
PL12W          0.39276794 W
PL13W          0.37509048 W
SFO2          400.1716007 MHz
SI                   32768
SF            100.6228244 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

^{13}C NMR ($CDCl_3$) spectrum of BBTOM-3



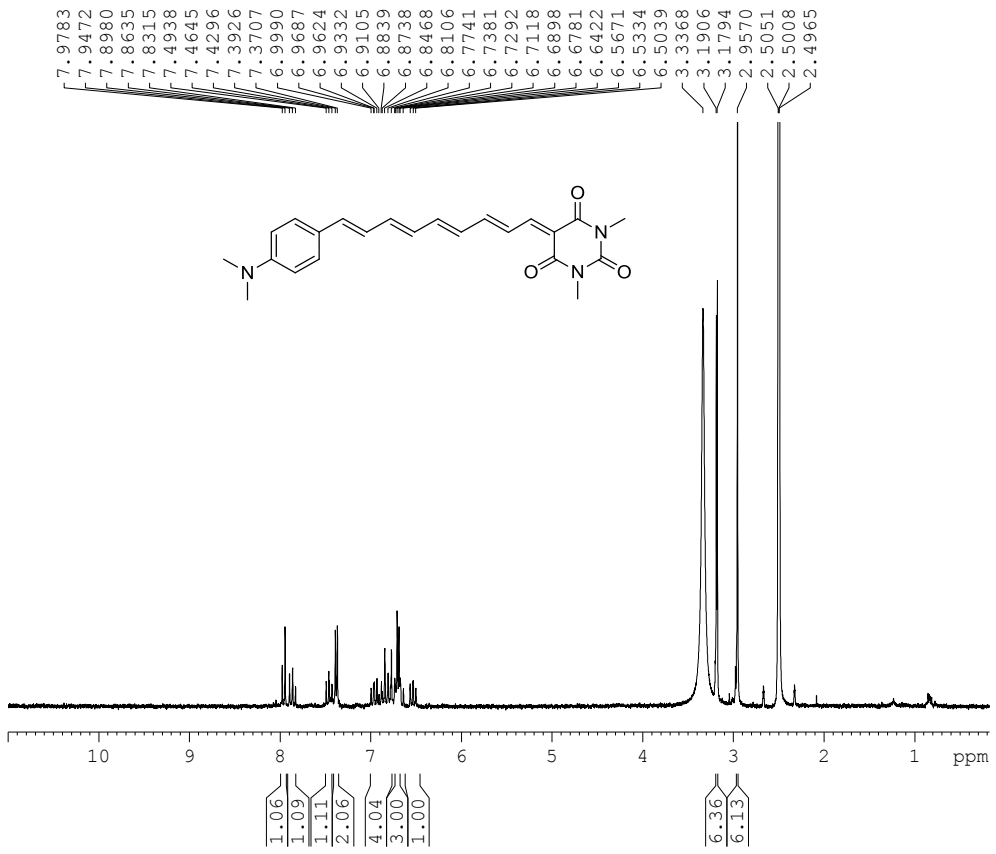

```
NAME          ZKX-BBTOM-4-20150527
EXPNO                       1
PROCNO                      1
Date_              20150527
Time                  9.43
INSTRUM               spect
PROBHD    5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       2
SWH              8223.685 Hz
FIDRES           0.125483 Hz
AQ               3.9846387 sec
RG                     203
DW                  60.800 usec
DE                   6.50 usec
TE                  297.2 K
D1              1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   13.80 usec
PL1                  -1.00 dB
PL1W          13.18669796 W
SFO1          400.1724712 MHz
SI                   32768
SF            400.1700027 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

^{1}H NMR ($CDCl_3$) spectrum of BBTOM-4



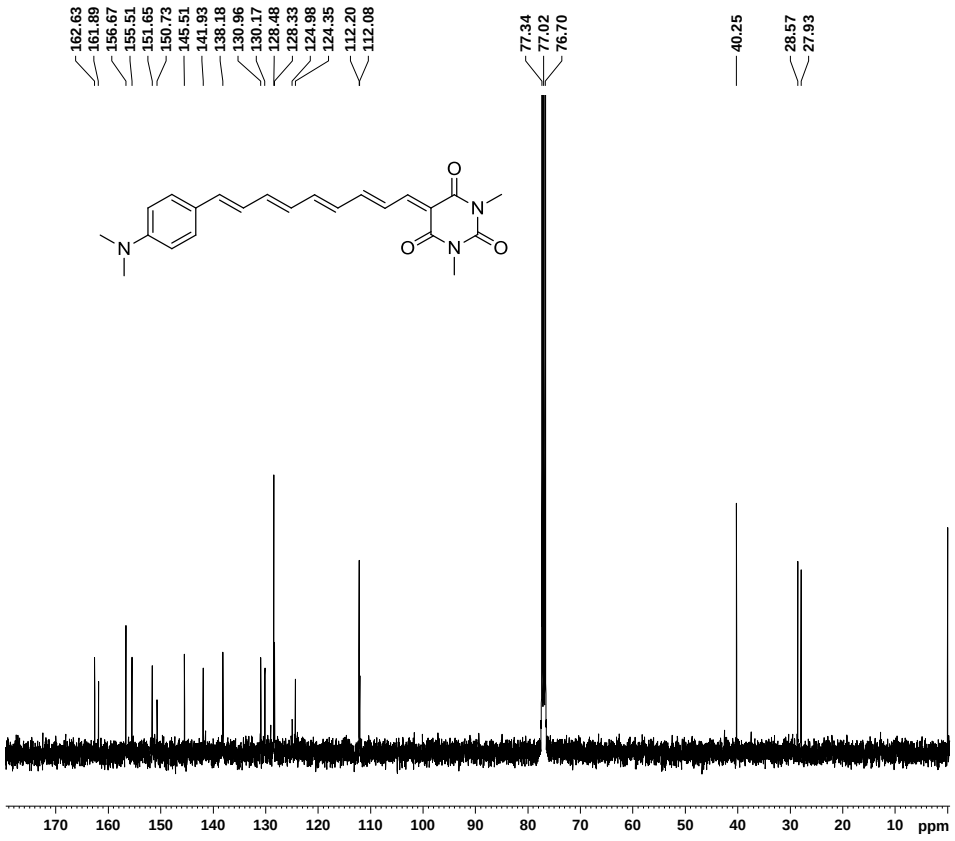

```
NAME        ZKX-M-4-C-20140830
EXPNO                    1
PROCNO                   1
Date_             20140830
Time                21.35
INSTRUM             spect
PROBHD    5 mm PABBO BB-
PULPROG           zgpg30
TD                65536
SOLVENT            CDCl3
NS                12000
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631988 sec
RG                  203
DW                20.800 usec
DE                 6.50 usec
TE                300.6 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                13C
P1                 8.50 usec
PL1               -2.00 dB
PL1W        57.32743073 W
SFO1       100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2              80.00 usec
PL2                -1.00 dB
PL12               14.26 dB
PL13               14.46 dB
PL2W        13.18669796 W
PL12W        0.39276794 W
PL13W        0.37509048 W
SFO2       400.1716007 MHz
SI                32768
SF         100.6228236 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

^{13}C NMR ($CDCl_3$) spectrum of BBTOM-4



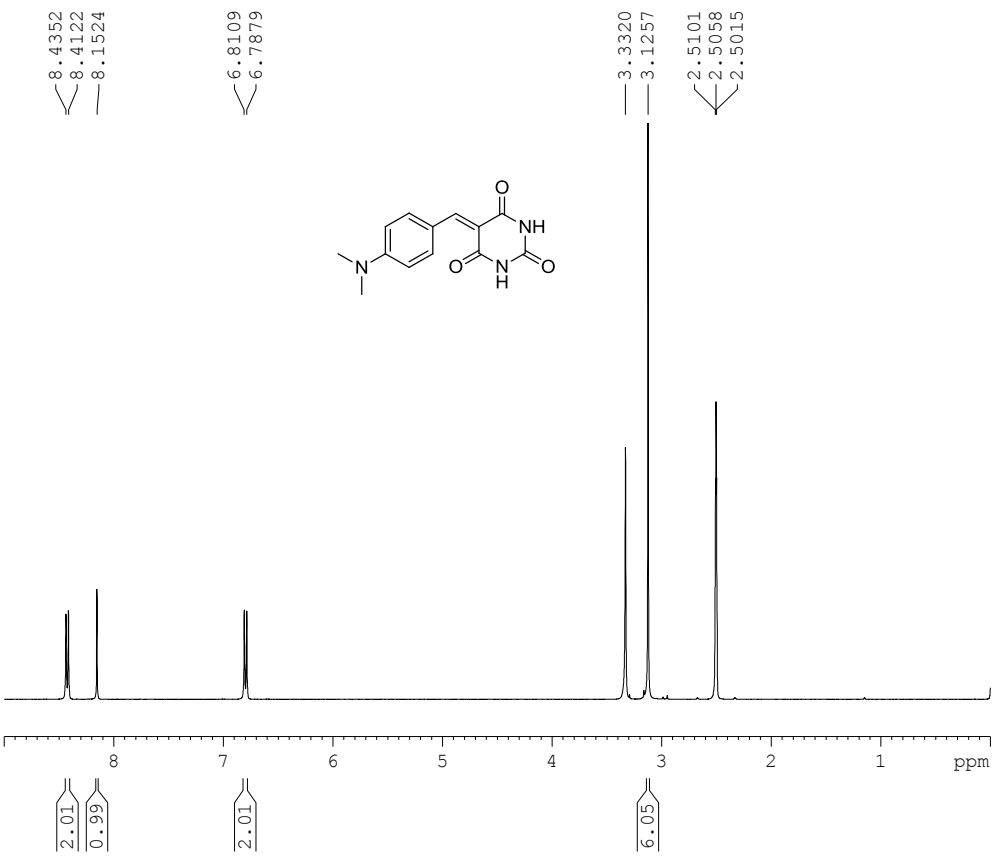

```
NAME      ZKX-BBATO-M--5-2
EXPNO                    1
PROCNO                   1
Date_             20140419
Time                 9.03
INSTRUM             spect
PROBHD    5 mm PABBO BB-
PULPROG              zg30
TD                65536
SOLVENT            DMSO
NS                   16
DS                    2
SWH           8223.685 Hz
FIDRES        0.125483 Hz
AQ            3.9846387 sec
RG                  203
DW                60.800 usec
DE                 6.50 usec
TE                296.0 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                1H
P1                13.80 usec
PL1               -1.00 dB
PL1W        13.18669796 W
SFO1       400.1724712 MHz
SI                32768
SF         400.1699940 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```

^{1}H NMR ($CDCl_3$) spectrum of BBTOM-5



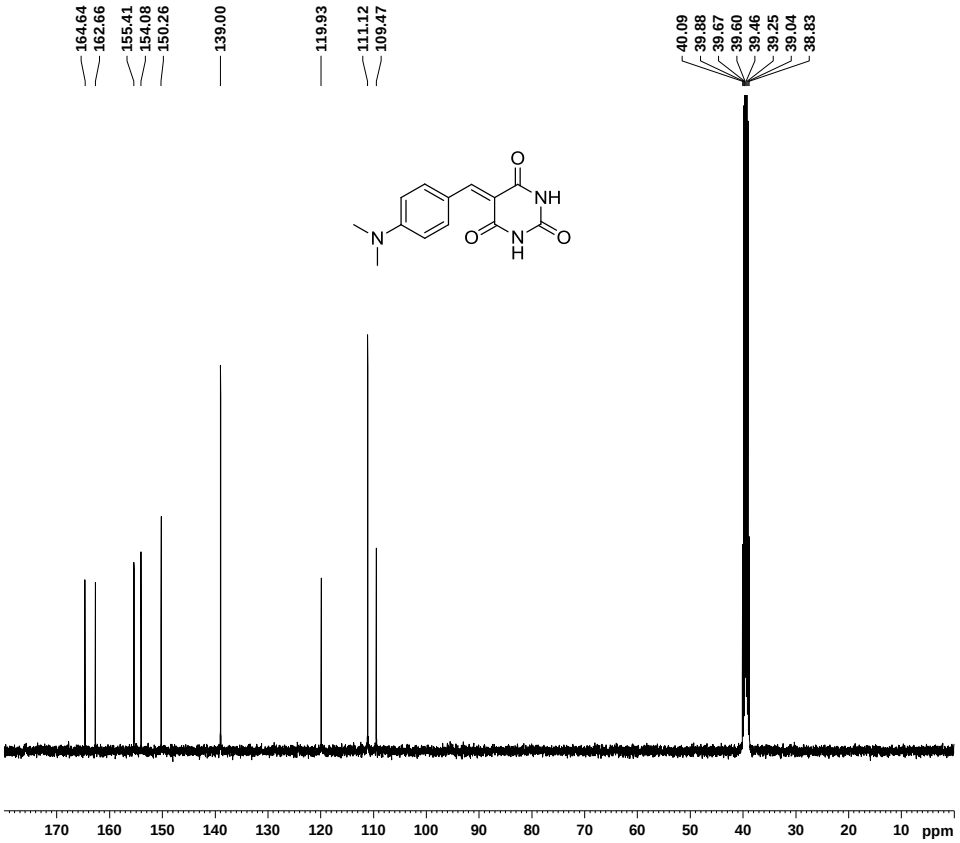

```
NAME          ZKX-BBATO-M-5-C
EXPNO                       1
PROCNO                      1
Date_              20140429
Time                   22.24
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG             zgpg30
TD                      65536
SOLVENT                 CDCl3
NS                       1024
DS                          4
SWH               24038.461 Hz
FIDRES             0.366798 Hz
AQ                 1.3631988 sec
RG                        203
DW                     20.800 usec
DE                      6.50 usec
TE                     301.3 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                      13C
P1                       8.50 usec
PL1                     -2.00 dB
PL1W             57.32743073 W
SFO1            100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                      1H
PCPD2                  80.00 usec
PL2                     -1.00 dB
PL12                    14.26 dB
PL13                    14.46 dB
PL2W             13.18669796 W
PL12W             0.39276794 W
PL13W             0.37509048 W
SFO2            400.1716007 MHz
SI                       32768
SF              100.6228238 MHz
WDW                        EM
SSB                         0
LB                       1.00 Hz
GB                          0
PC                       1.40
```

^{13}C NMR ($CDCl_3$) spectrum of BBTOM-5



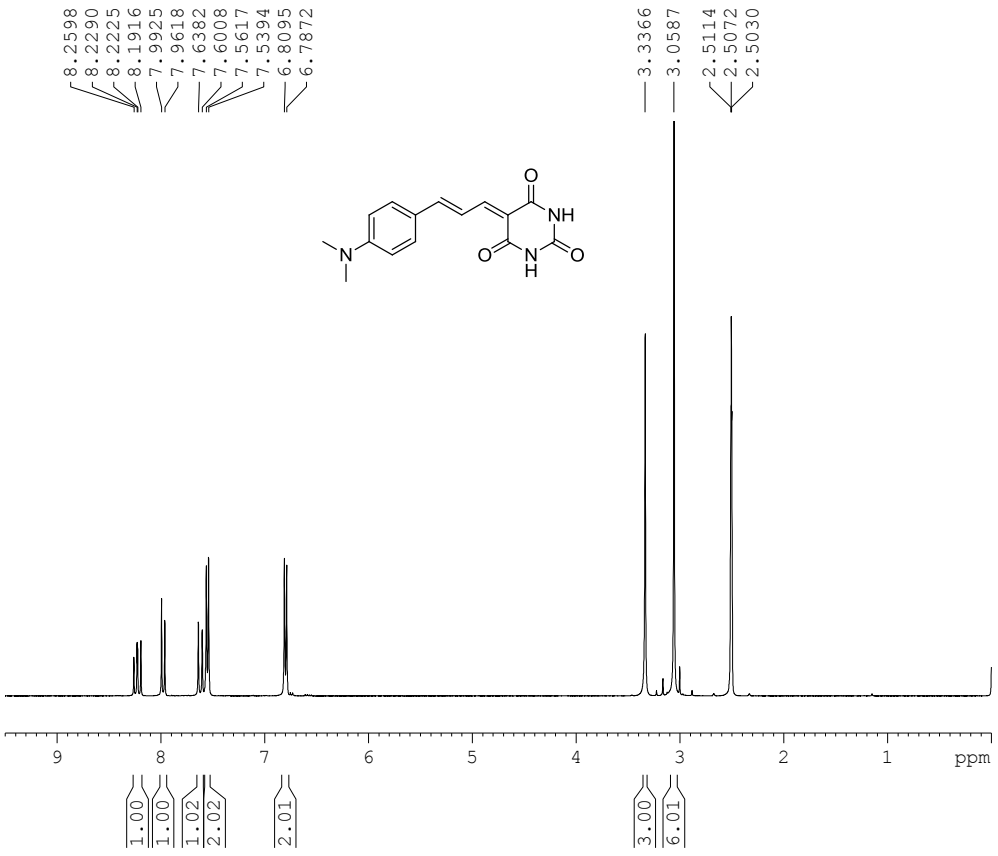

```
NAME          BBAT-1-NMe2
EXPNO                       1
PROCNO                      1
Date_              20110615
Time                   17.03
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG                 zg30
TD                      65536
SOLVENT                  DMSO
NS                         16
DS                          2
SWH                8223.685 Hz
FIDRES             0.125483 Hz
AQ                 3.9846387 sec
RG                        203
DW                     60.800 usec
DE                      6.50 usec
TE                     299.4 K
D1                1.00000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                       1H
P1                      14.20 usec
PL1                     -1.00 dB
PL1W             13.18669796 W
SFO1            400.1724712 MHz
SI                       32768
SF              400.1699932 MHz
WDW                        EM
SSB                         0
LB                       0.30 Hz
GB                          0
PC                       1.00
```

^{1}H NMR (DMSO-d_6) spectrum of BBTO-1



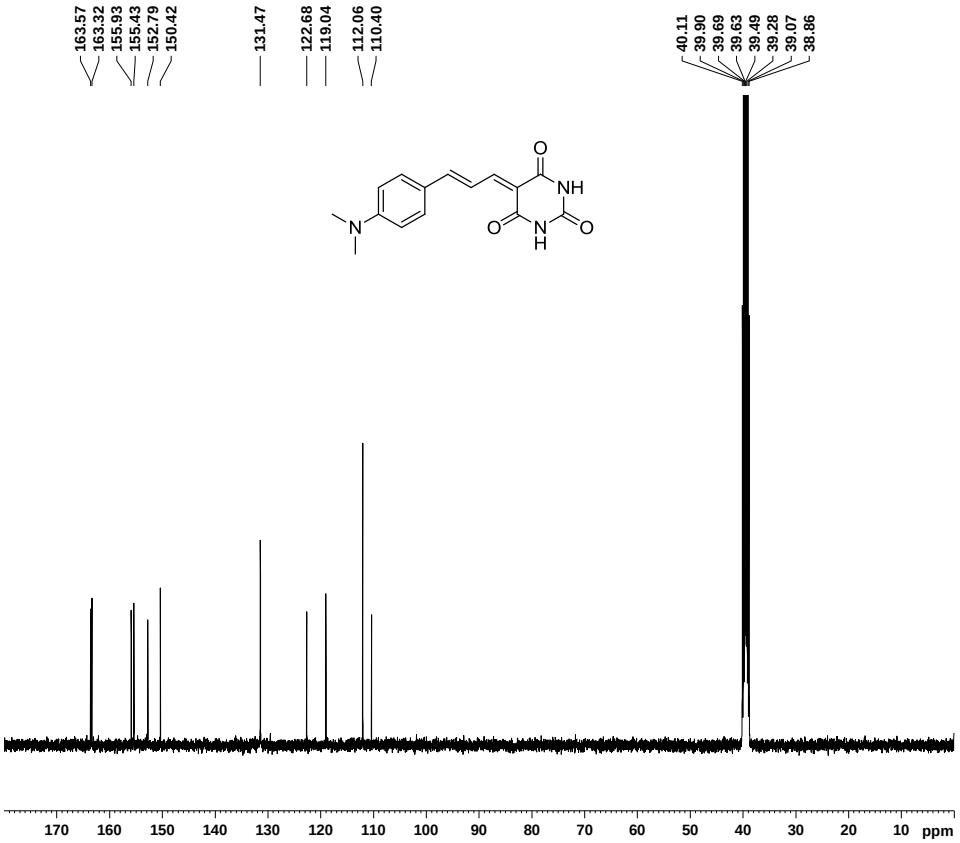

```
NAME          ZKX-BBTO-1-C13
EXPNO                      1
PROCNO                     1
Date_               20150313
Time                   23.03
INSTRUM                spect
PROBHD   5 mm PABBO BB-
PULPROG                zgpg30
TD                      65536
SOLVENT                 DMSO
NS                        500
DS                          4
SWH                24038.461 Hz
FIDRES              0.366798 Hz
AQ                  1.3631988 sec
RG                        203
DW                     20.800 usec
DE                      6.50 usec
TE                     296.7 K
D1                 2.00000000 sec
D11                0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                      13C
P1                      8.50 usec
PL1                    -2.00 dB
PL1W              57.32743073 W
SFO1             100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                      1H
PCPD2                  80.00 usec
PL2                    -1.00 dB
PL12                   14.26 dB
PL13                   14.46 dB
PL2W              13.18669796 W
PL12W              0.39276794 W
PL13W              0.37509048 W
SFO2             400.1716007 MHz
SI                      32768
SF               100.6228773 MHz
WDW                        EM
SSB                         0
LB                      1.00 Hz
GB                          0
PC                      1.40
```

^{13}C NMR (DMSO-d_6) spectrum of BBTO-1




```
NAME          BBAT-2-NMe2
EXPNO                      1
PROCNO                     1
Date_               20110615
Time                   17.07
INSTRUM                spect
PROBHD   5 mm PABBO BB-
PULPROG                zg30
TD                      65536
SOLVENT                 DMSO
NS                         16
DS                          2
SWH                 8223.685 Hz
FIDRES              0.125483 Hz
AQ                  3.9846387 sec
RG                        203
DW                     60.800 usec
DE                      6.50 usec
TE                     299.3 K
D1                 1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                       1H
P1                     14.20 usec
PL1                    -1.00 dB
PL1W              13.18669796 W
SFO1             400.1724712 MHz
SI                      32768
SF               400.1699925 MHz
WDW                        EM
SSB                         0
LB                      0.30 Hz
GB                          0
PC                      1.00
```

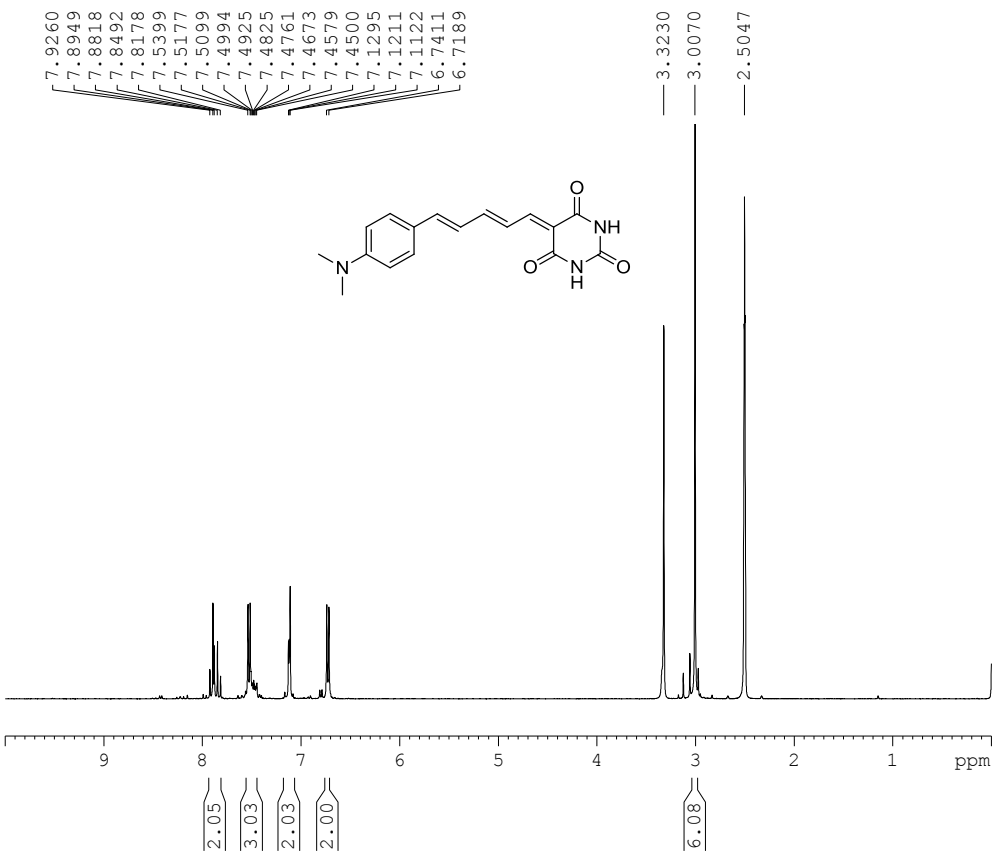
^{1}H NMR (DMSO-d_6) spectrum of BBTO-2



BRUKER

```
NAME            ZKX-BBTO-2-C13
EXPNO                        1
PROCNO                       1
Date_                 20150311
Time                     14.36
INSTRUM                  spect
PROBHD    5 mm PABBO BB-
PULPROG                 zgpg30
TD                       65536
SOLVENT                   DMSO
NS                        1746
DS                           4
SWH                 24038.461 Hz
FIDRES               0.366798 Hz
AQ                   1.3631988 sec
RG                         203
DW                      20.800 usec
DE                       6.50 usec
TE                      298.6 K
D1                   2.00000000 sec
D11                  0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                       13C
P1                       8.50 usec
PL1                     -2.00 dB
PL1W               57.32743073 W
SFO1              100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                        1H
PCPD2                   80.00 usec
PL2                     -1.00 dB
PL12                    14.26 dB
PL13                    14.46 dB
PL2W               13.18669796 W
PL12W               0.39276794 W
PL13W               0.37509048 W
SFO2              400.1716007 MHz
SI                       32768
SF                100.6228773 MHz
WDW                         EM
SSB                          0
LB                        1.00 Hz
GB                           0
PC                        1.40
```

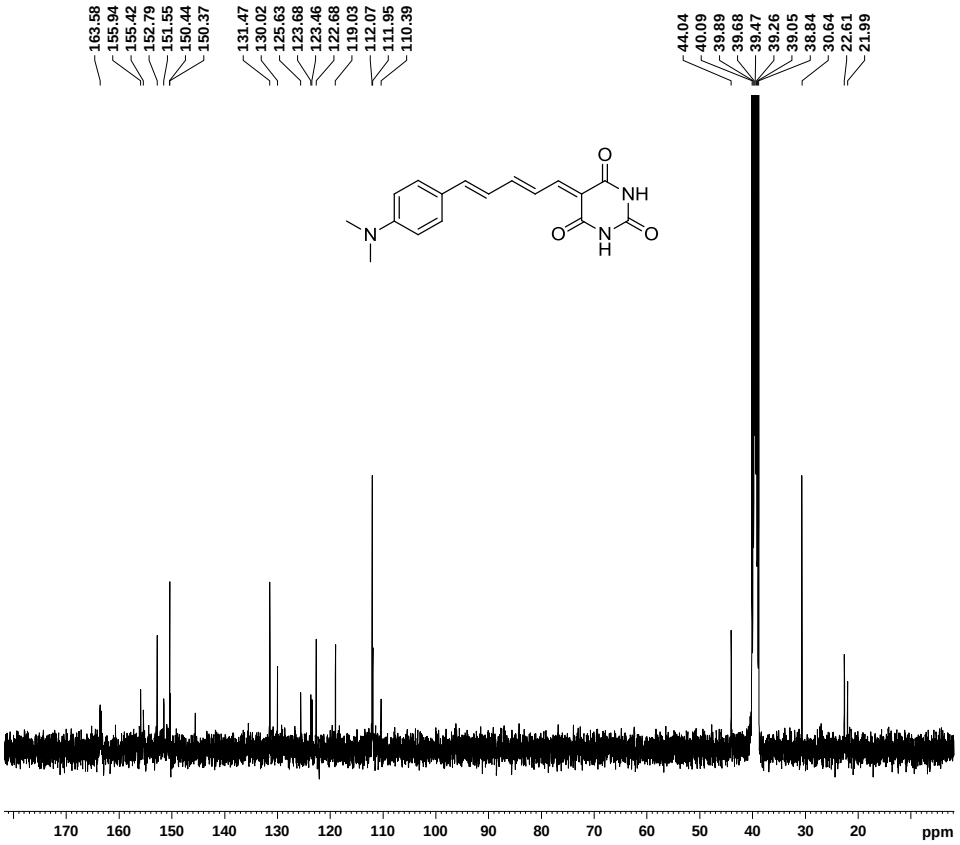
^{13}C NMR (DMSO-d_6) spectrum of BBTO-2



BRUKER

```
NAME            BBAT-3-NMe2
EXPNO                        1
PROCNO                       1
Date_                 20110617
Time                     11.19
INSTRUM                  spect
PROBHD    5 mm PABBO BB-
PULPROG                  zg30
TD                       65536
SOLVENT                   DMSO
NS                          16
DS                           2
SWH                  8223.685 Hz
FIDRES               0.125483 Hz
AQ                   3.9846387 sec
RG                         203
DW                      60.800 usec
DE                       6.50 usec
TE                      300.0 K
D1                   1.00000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                        1H
P1                      14.20 usec
PL1                     -1.00 dB
PL1W               13.18669796 W
SFO1              400.1724712 MHz
SI                       32768
SF                400.1699938 MHz
WDW                         EM
SSB                          0
LB                        0.30 Hz
GB                           0
PC                        1.00
```

^{1}H NMR (DMSO-d_6) spectrum of BBTO-3

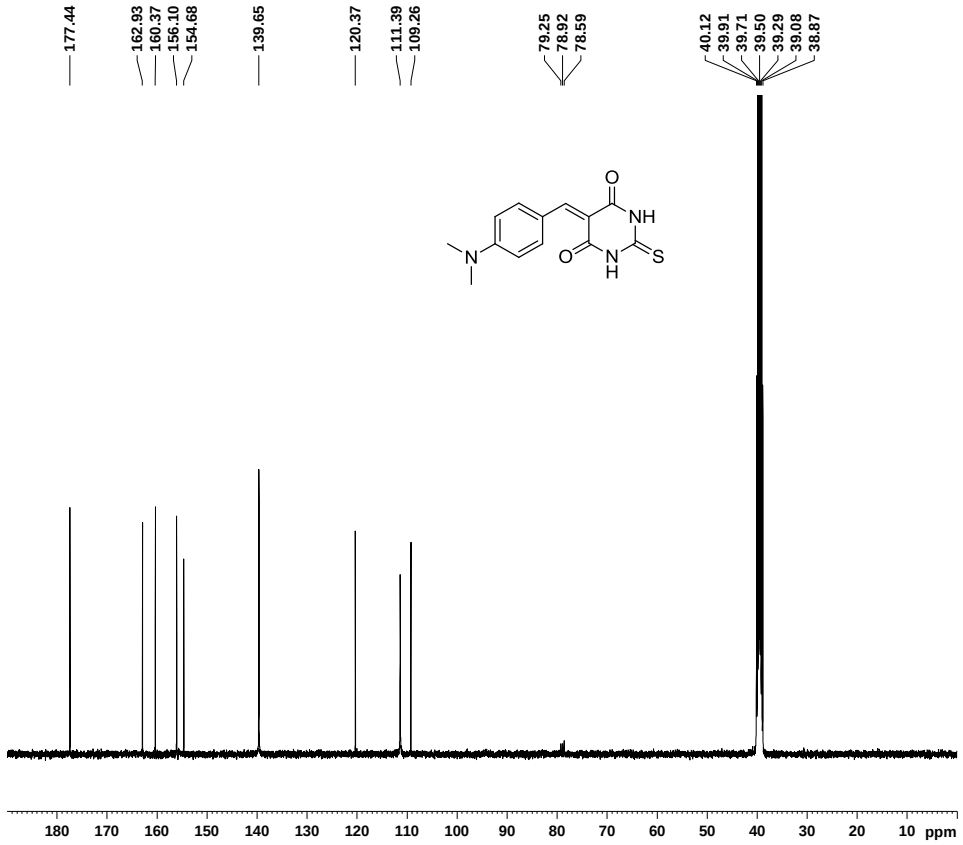



```
NAME            ZKX-BBTO-3-C13
EXPNO                        1
PROCNO                       1
Date_                 20150314
Time                      1.29
INSTRUM                  spect
PROBHD    5 mm PABBO BB-
PULPROG                 zgpg30
TD                       65536
SOLVENT                   DMSO
NS                        2500
DS                           4
SWH                  24038.461 Hz
FIDRES                0.366798 Hz
AQ                    1.3631988 sec
RG                         203
DW                      20.800 usec
DE                       6.50 usec
TE                      296.3 K
D1                 2.00000000 sec
D11                0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                       13C
P1                        8.50 usec
PL1                      -2.00 dB
PL1W               57.32743073 W
SFO1              100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                        1H
PCPD2                    80.00 usec
PL2                      -1.00 dB
PL12                     14.26 dB
PL13                     14.46 dB
PL2W               13.18669796 W
PL12W               0.39276794 W
PL13W               0.37509048 W
SFO2              400.1716007 MHz
SI                       32768
SF                100.6228773 MHz
WDW                         EM
SSB                          0
LB                        1.00 Hz
GB                           0
PC                        1.40
```

^{13}C NMR (DMSO-d_6) spectrum of BBTO-3

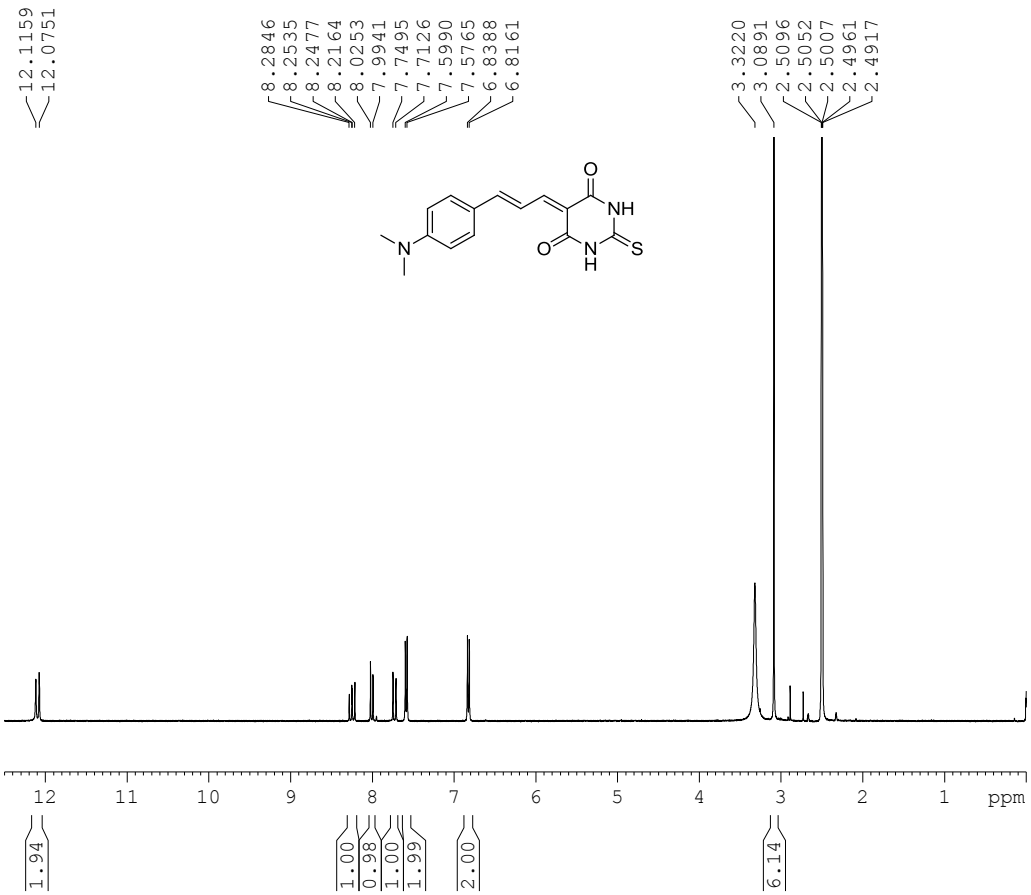

```
NAME                BBATS-1
EXPNO                     1
PROCNO                    1
Date_              20110818
Time                  15.49
INSTRUM               spect
PROBHD    5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       16
DS                        2
SWH                8223.685 Hz
FIDRES            0.125483 Hz
AQ                 3.9846387 sec
RG                      203
DW                   60.800 usec
DE                    6.50 usec
TE                   300.1 K
D1              1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                      1H
P1                    13.80 usec
PL1                   -1.00 dB
PL1W           13.18669796 W
SFO1          400.1724712 MHz
SI                    32768
SF            400.1699937 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```

^{1}H NMR (DMSO-d_6) spectrum of BBTS-1



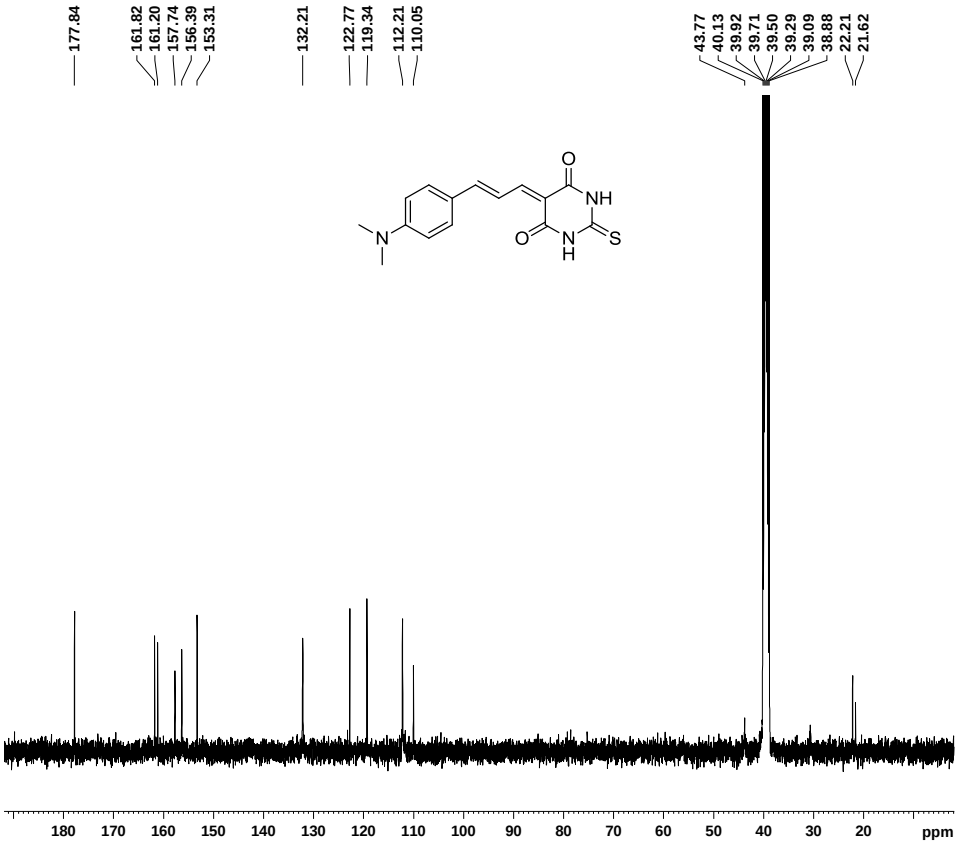

```
NAME            BBATS-1-C13
EXPNO                     1
PROCNO                    1
Date_              20110901
Time                 14.06
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT                DMSO
NS                    3639
DS                       4
SWH             24038.461 Hz
FIDRES           0.366798 Hz
AQ               1.3631988 sec
RG                     203
DW                  20.800 usec
DE                   6.50 usec
TE                  299.8 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                    8.50 usec
PL1                  -2.00 dB
PL1W          57.32743073 W
SFO1         100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2                80.00 usec
PL2                  -1.00 dB
PL12                 14.26 dB
PL13                 14.46 dB
PL2W          13.18669796 W
PL12W          0.39276794 W
PL13W          0.37509048 W
SFO2         400.1716007 MHz
SI                   32768
SF           100.6228773 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

^{13}C NMR (DMSO-d_6) spectrum of BBTS-1



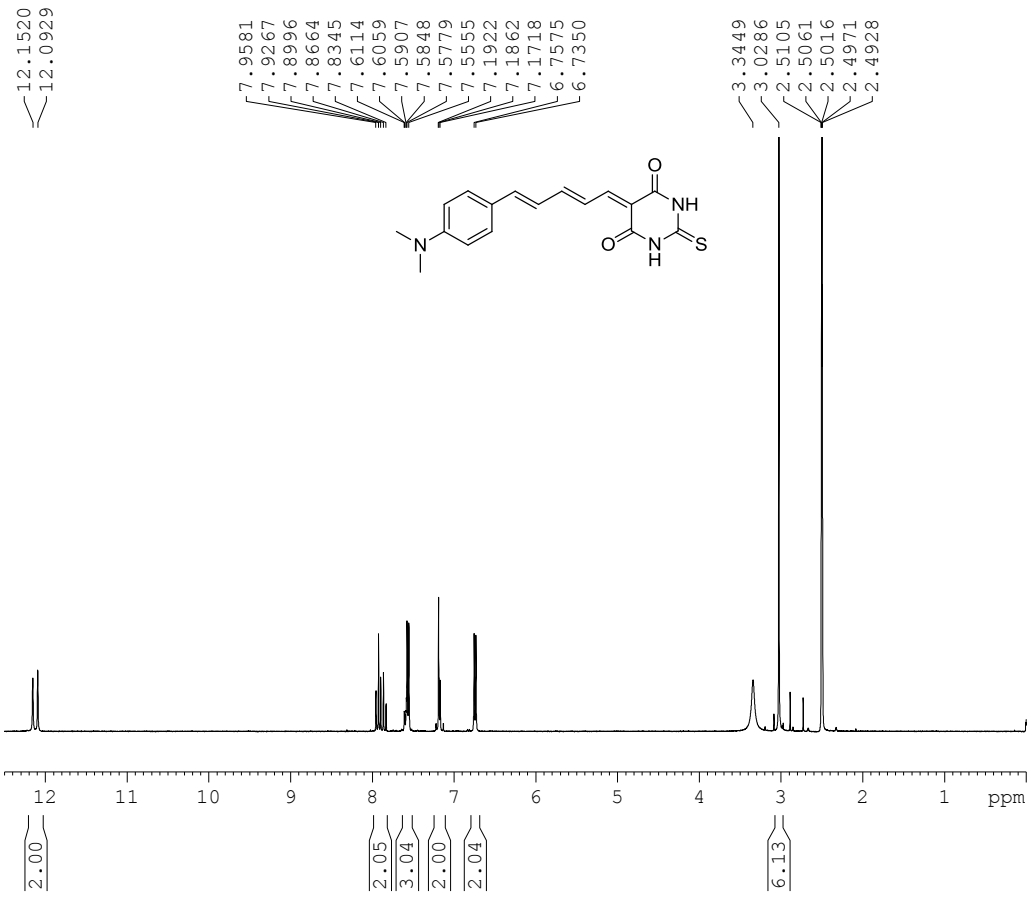

```
NAME      ZKX-BBTS-2-20150518
EXPNO                     1
PROCNO                    1
Date_              20150518
Time                 19.04
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT                DMSO
NS                      16
DS                       2
SWH              8223.685 Hz
FIDRES           0.125483 Hz
AQ               3.9846387 sec
RG                     203
DW                  60.800 usec
DE                   6.50 usec
TE                  298.1 K
D1              1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   1H
P1                   13.80 usec
PL1                  -1.00 dB
PL1W          13.18669796 W
SFO1         400.1724712 MHz
SI                   32768
SF           400.1699949 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

^{1}H NMR (DMSO-d_6) spectrum of BBTS-2



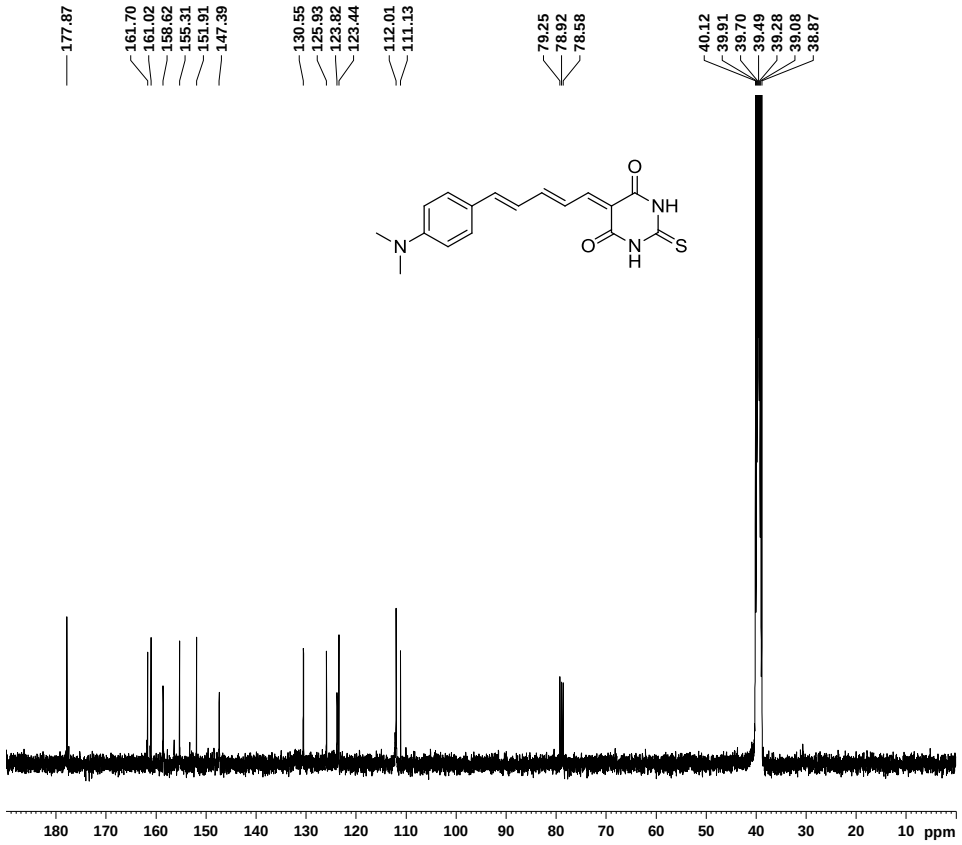

```
NAME            BBATS-2-C13
EXPNO                     1
PROCNO                    1
Date_              20110831
Time                 21.50
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT                DMSO
NS                    6000
DS                       4
SWH             24038.461 Hz
FIDRES           0.366798 Hz
AQ               1.3631988 sec
RG                     203
DW                  20.800 usec
DE                   6.50 usec
TE                  301.3 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                    8.50 usec
PL1                  -2.00 dB
PL1W          57.32743073 W
SFO1         100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2                80.00 usec
PL2                  -1.00 dB
PL12                 14.26 dB
PL13                 14.46 dB
PL2W          13.18669796 W
PL12W          0.39276794 W
PL13W          0.37509048 W
SFO2         400.1716007 MHz
SI                   32768
SF           100.6228773 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

^{13}C NMR (DMSO-d_6) spectrum of BBTS-2



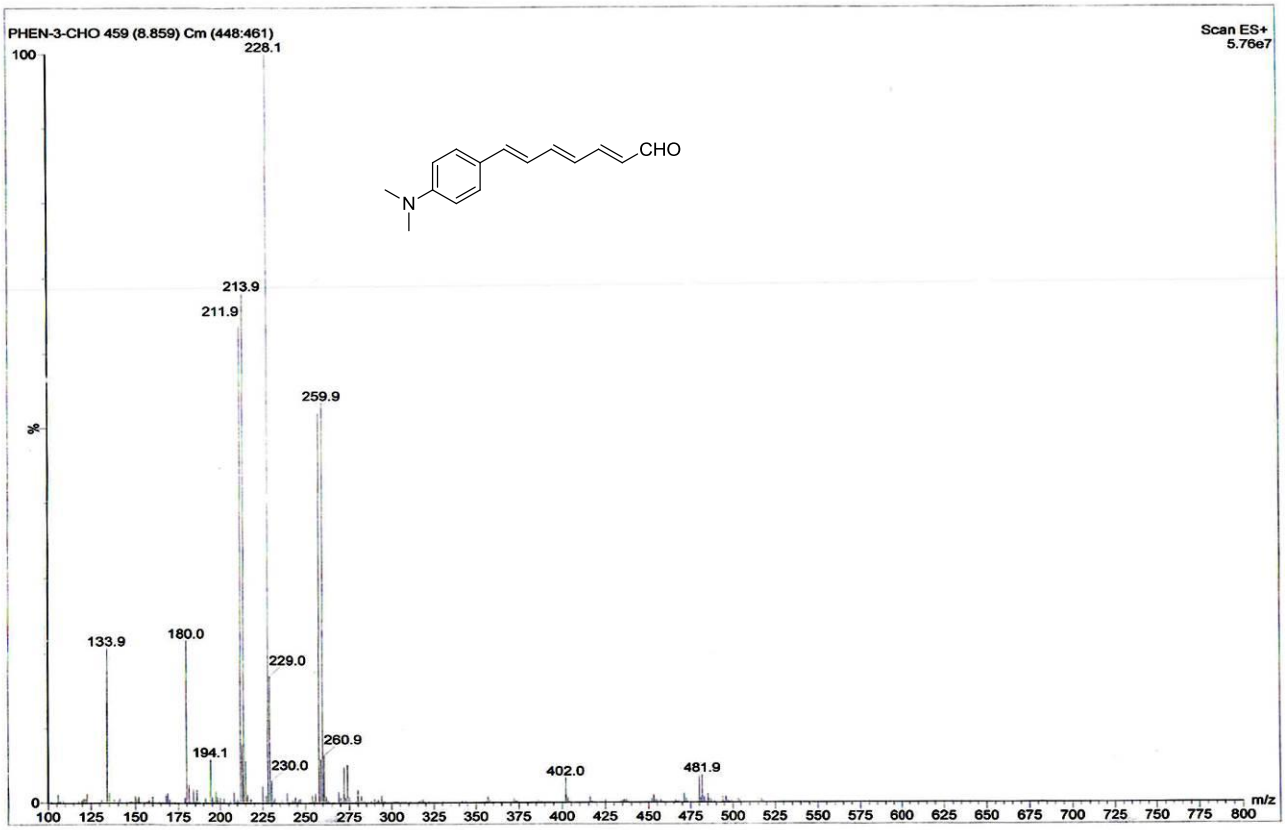

```
NAME      ZKX-BBTS-3-20150518
EXPNO                       1
PROCNO                      1
Date_              20150518
Time                 19.08
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT                DMSO
NS                       16
DS                        2
SWH              8223.685 Hz
FIDRES           0.125483 Hz
AQ               3.9846387 sec
RG                     203
DW                  60.800 usec
DE                   6.50 usec
TE                  297.8 K
D1              1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   1H
P1                   13.80 usec
PL1                  -1.00 dB
PL1W          13.18669796 W
SFO1         400.1724712 MHz
SI                   32768
SF           400.1699945 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

^{1}H NMR (DMSO-d_6) spectrum of BBTS-3

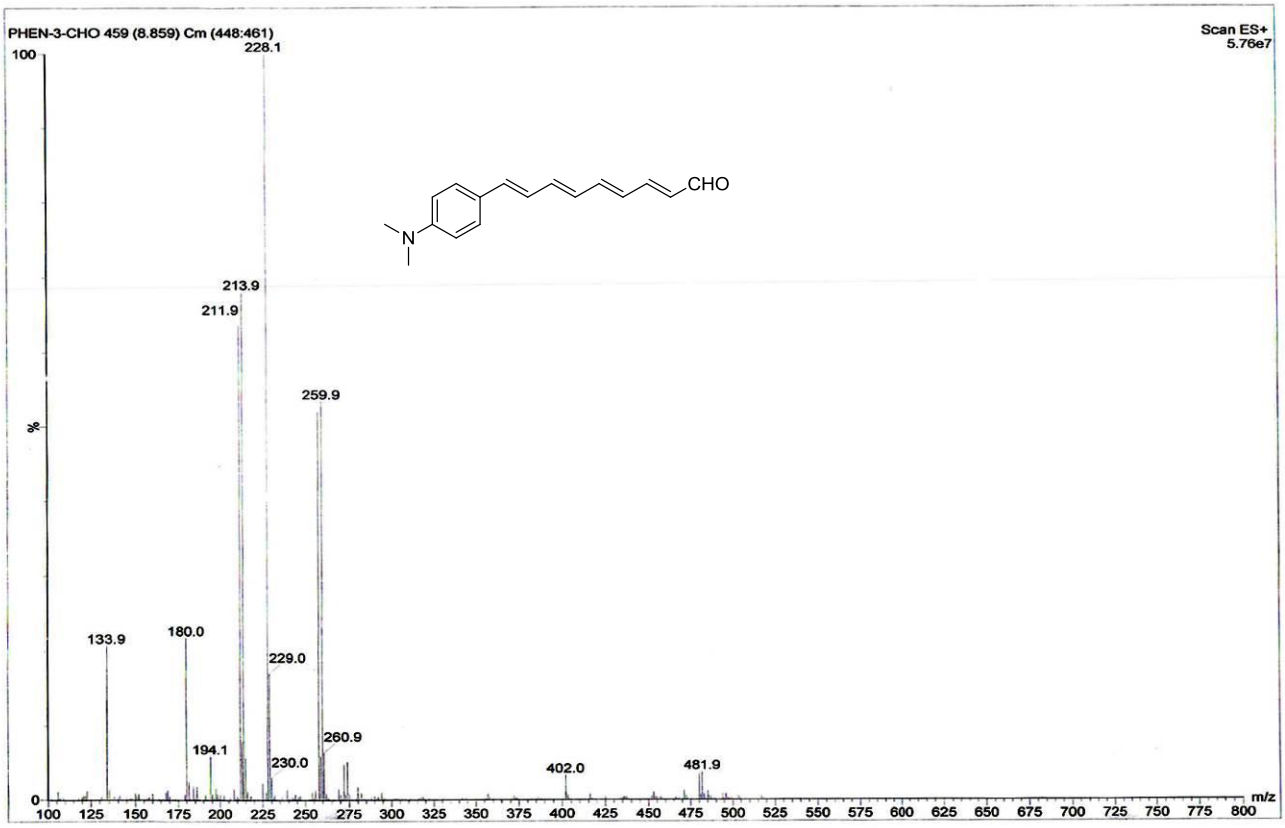



```
NAME              BBATS-3-C13
EXPNO                       1
PROCNO                      1
Date_              20110830
Time                    6.40
INSTRUM                spect
PROBHD    5 mm PABBO BB-
PULPROG                zgpg30
TD                      65536
SOLVENT                   DMSO
NS                        8000
DS                           4
SWH               24038.461 Hz
FIDRES             0.366798 Hz
AQ                 1.3631988 sec
RG                         203
DW                    20.800 usec
DE                     6.50 usec
TE                     297.0 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                      13C
P1                     8.50 usec
PL1                   -2.00 dB
PL1W             57.32743073 W
SFO1            100.6328888 MHz

======== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                      1H
PCPD2                 80.00 usec
PL2                   -1.00 dB
PL12                  14.26 dB
PL13                  14.46 dB
PL2W             13.18669796 W
PL12W             0.39276794 W
PL13W             0.37509048 W
SFO2            400.1716007 MHz
SI                        32768
SF              100.6228773 MHz
WDW                         EM
SSB                          0
LB                        1.00 Hz
GB                           0
PC                        1.40
```

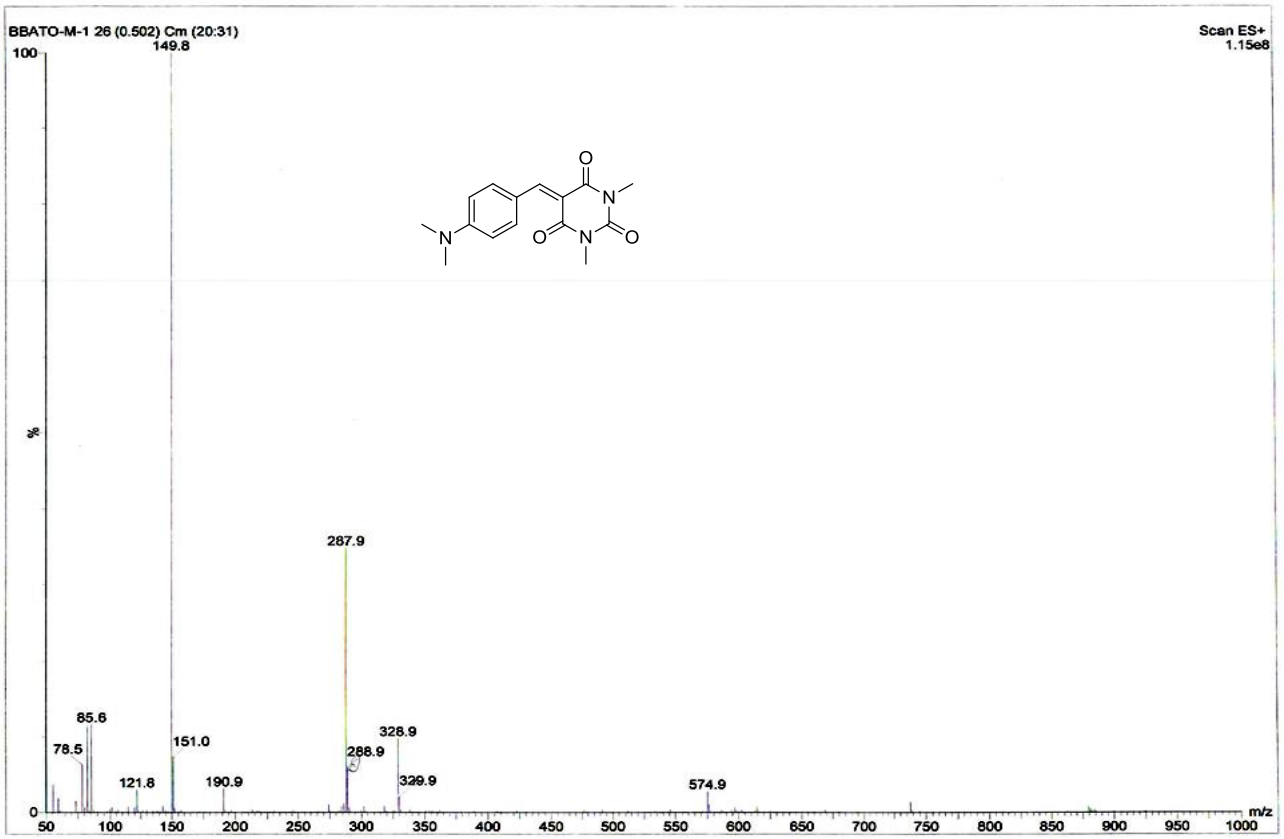
^{13}C NMR (DMSO-d_6) spectrum of BBTS-3



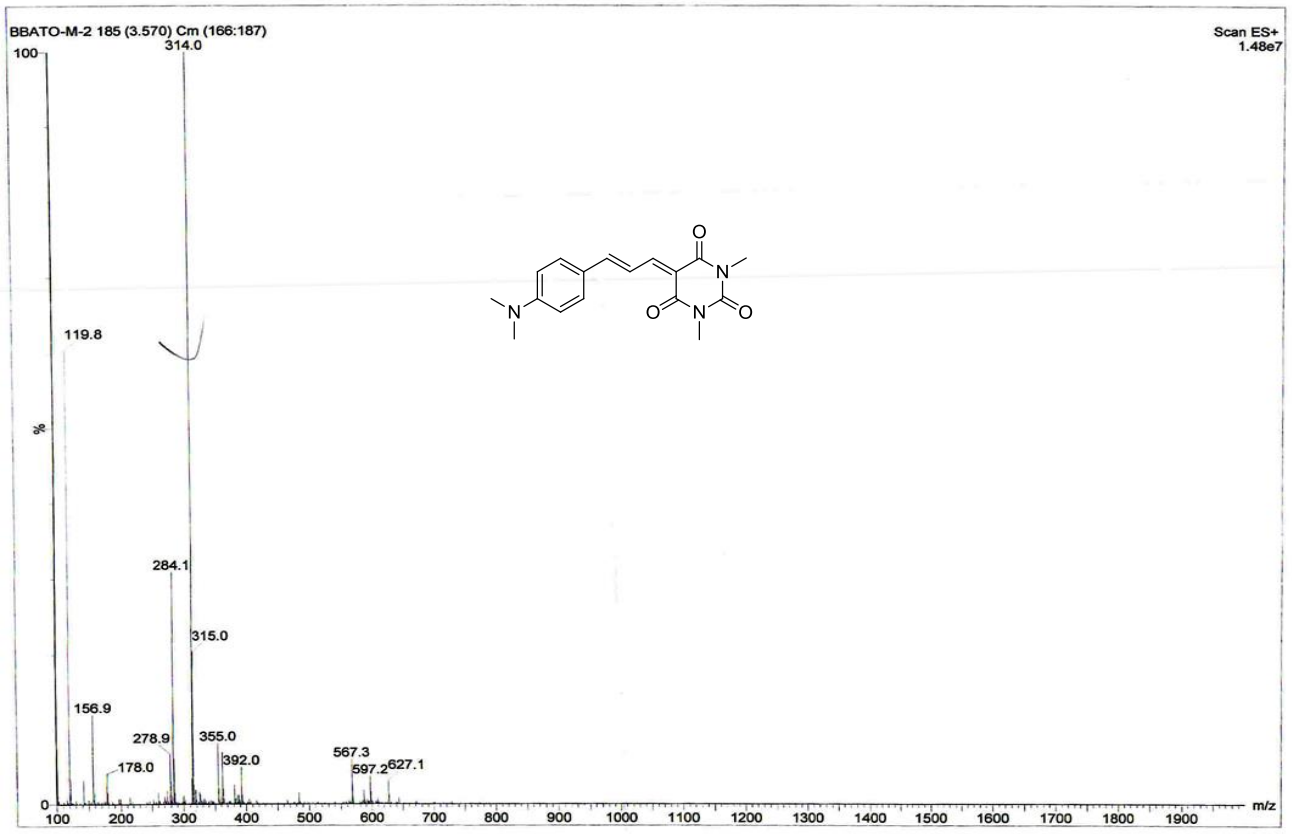
MS spectrum of compound **2**



MS spectrum of compound **3**

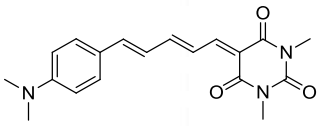


MS spectrum of BBTOM-1



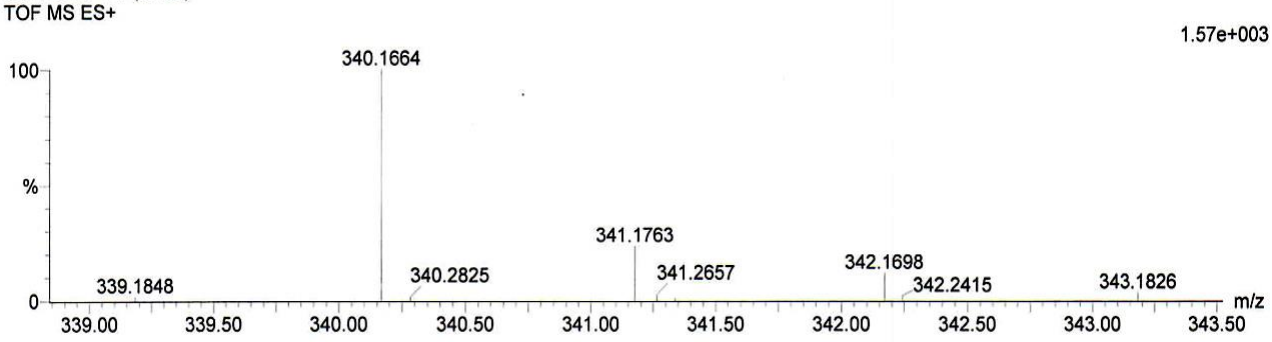
MS spectrum of BBTOM-2

Elemental Composition Report **Page 1**



Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

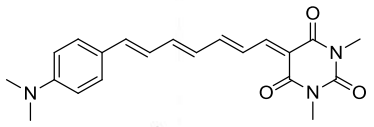
Monoisotopic Mass, Even Electron Ions
205 formula(e) evaluated with 1 results within limits (up to 100 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-30 N: 0-10 O: 0-6
BBATO-M -3 6 (0.102)
TOF MS ES+



Minimum:				-1.5		
Maximum:		5.0	3.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
340.1664	340.1661	0.3	0.9	10.5	0.6	C19 H22 N3 O3

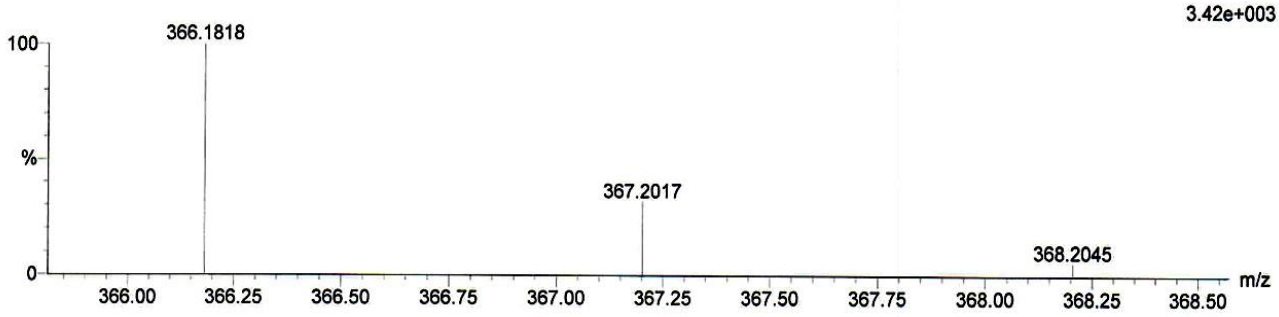
HRMS spectrum of BBTOM-3

Elemental Composition Report **Page 1**



Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

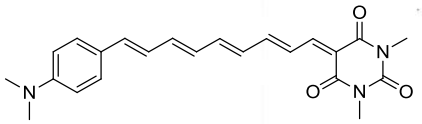
Monoisotopic Mass, Even Electron Ions
206 formula(e) evaluated with 1 results within limits (up to 100 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-30 N: 0-10 O: 0-6
BBATO-M -4 2 (0.034)
TOF MS ES+



Minimum:				-1.5		
Maximum:		5.0	3.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
366.1818	366.1818	0.0	0.0	11.5	25.7	C21 H24 N3 O3

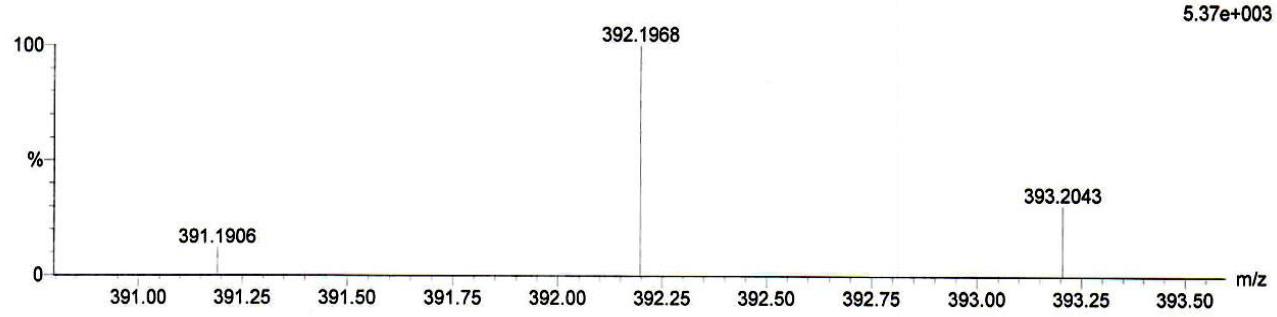
HRMS spectrum of BBTOM-4

Elemental Composition Report **Page 1**



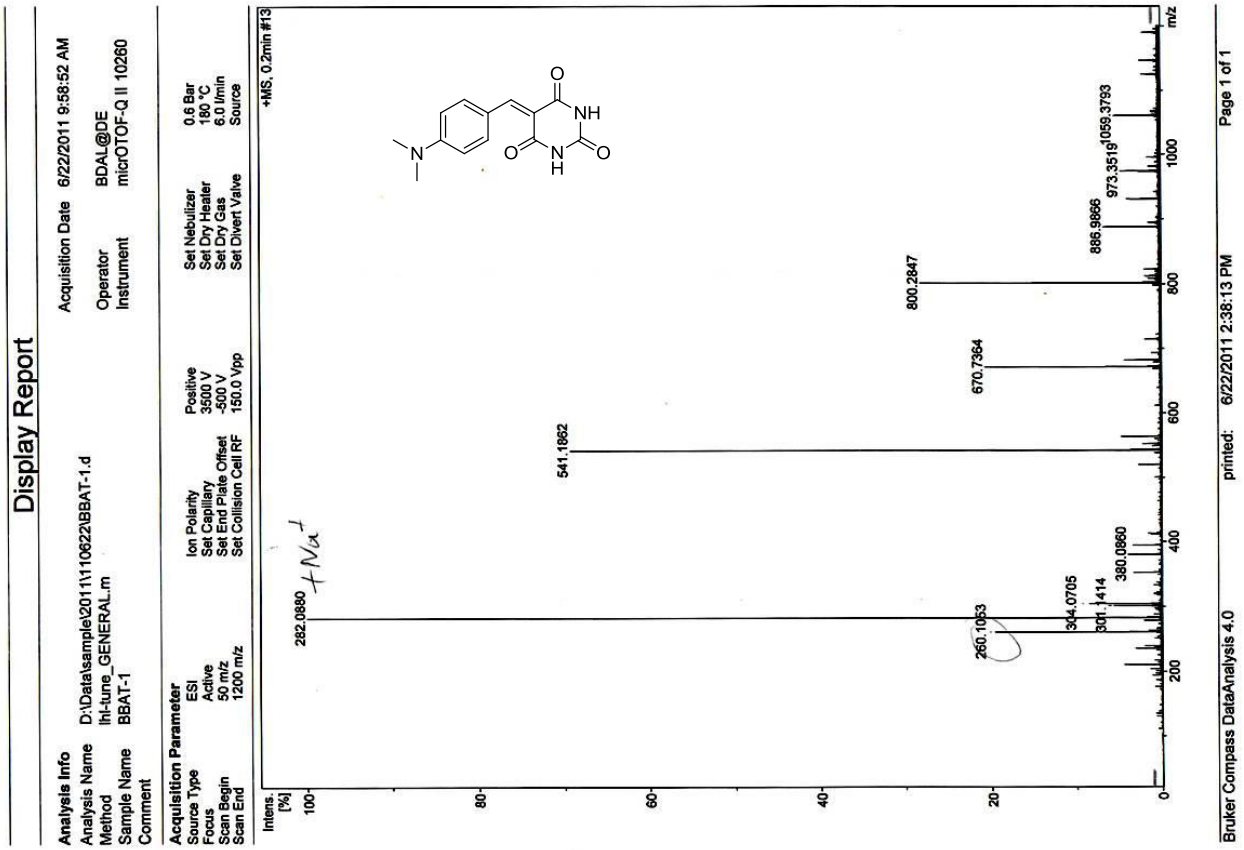
Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
202 formula(e) evaluated with 1 results within limits (up to 100 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-30 N: 0-10 O: 0-6
BBATO-M -5 10 (0.170)
TOF MS ES+

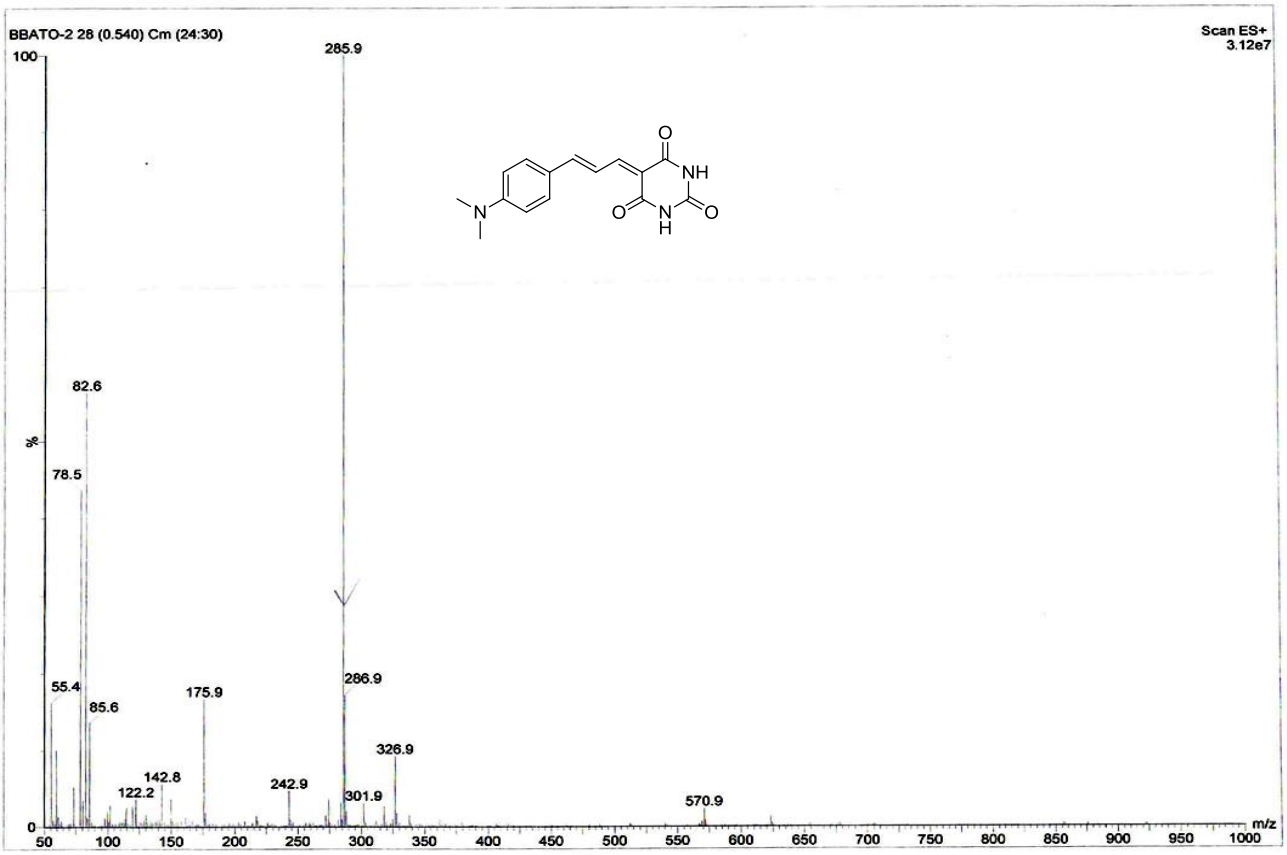


Minimum:				-1.5		
Maximum:		5.0	3.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
392.1968	392.1974	-0.6	-1.5	12.5	8.4	C23 H26 N3 O3

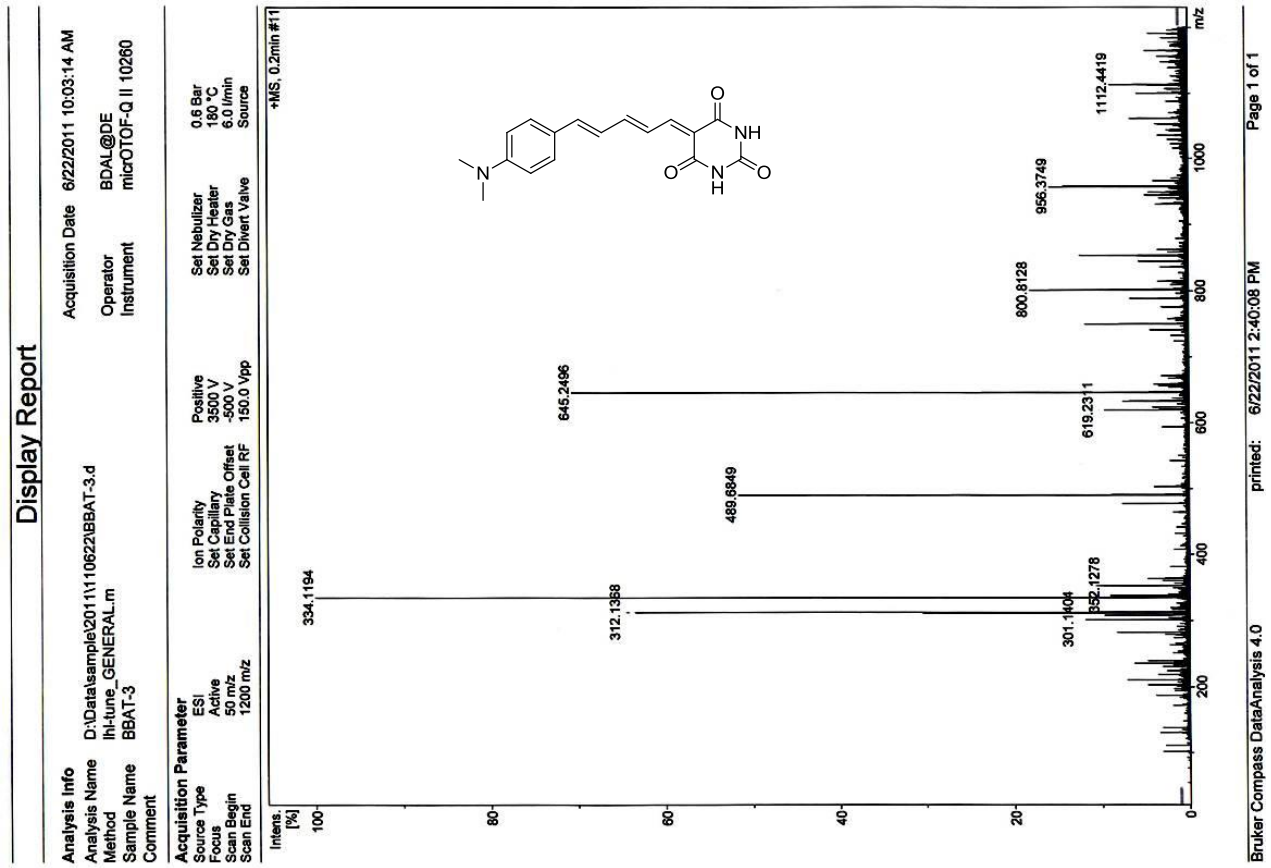
HRMS spectrum of BBTOM-5



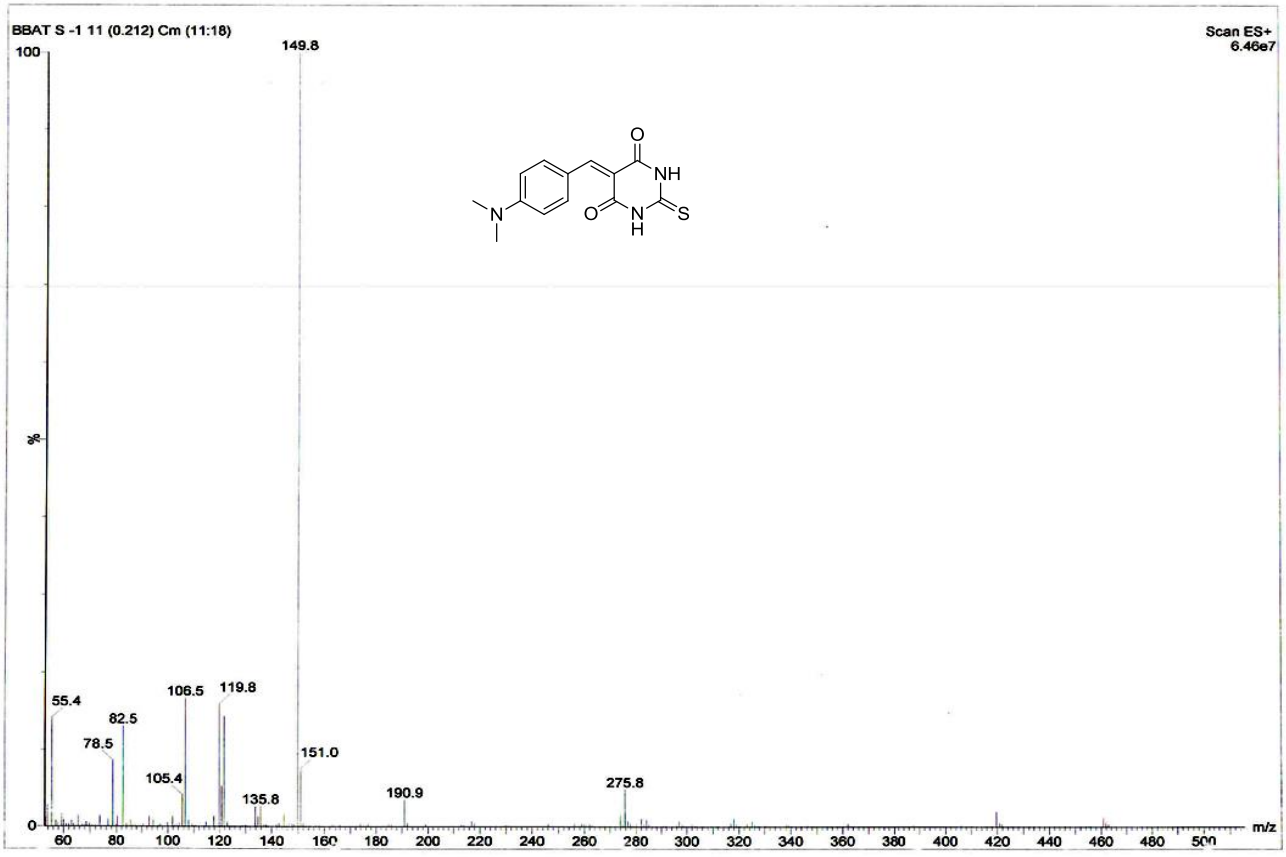
MS spectrum of BBTO-1



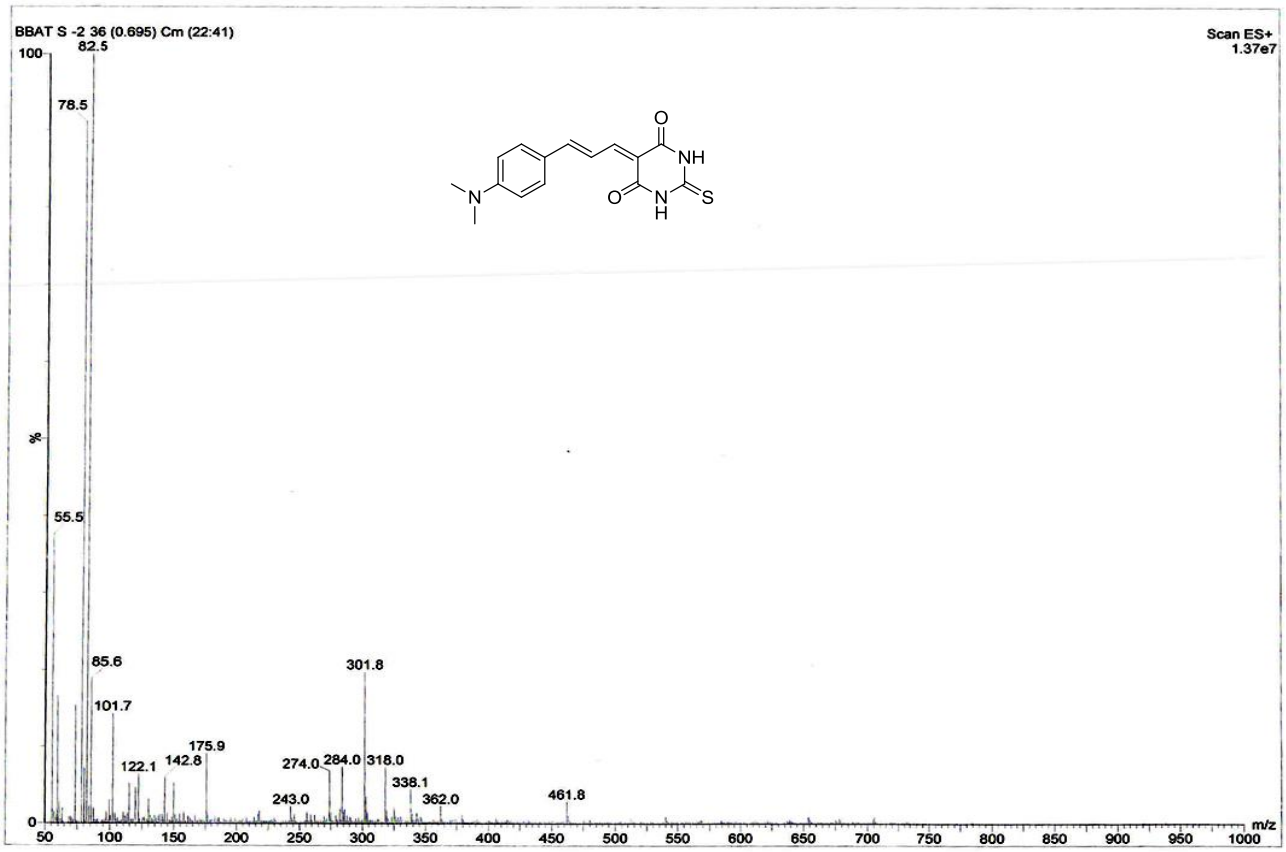
MS spectrum of BBTO-2



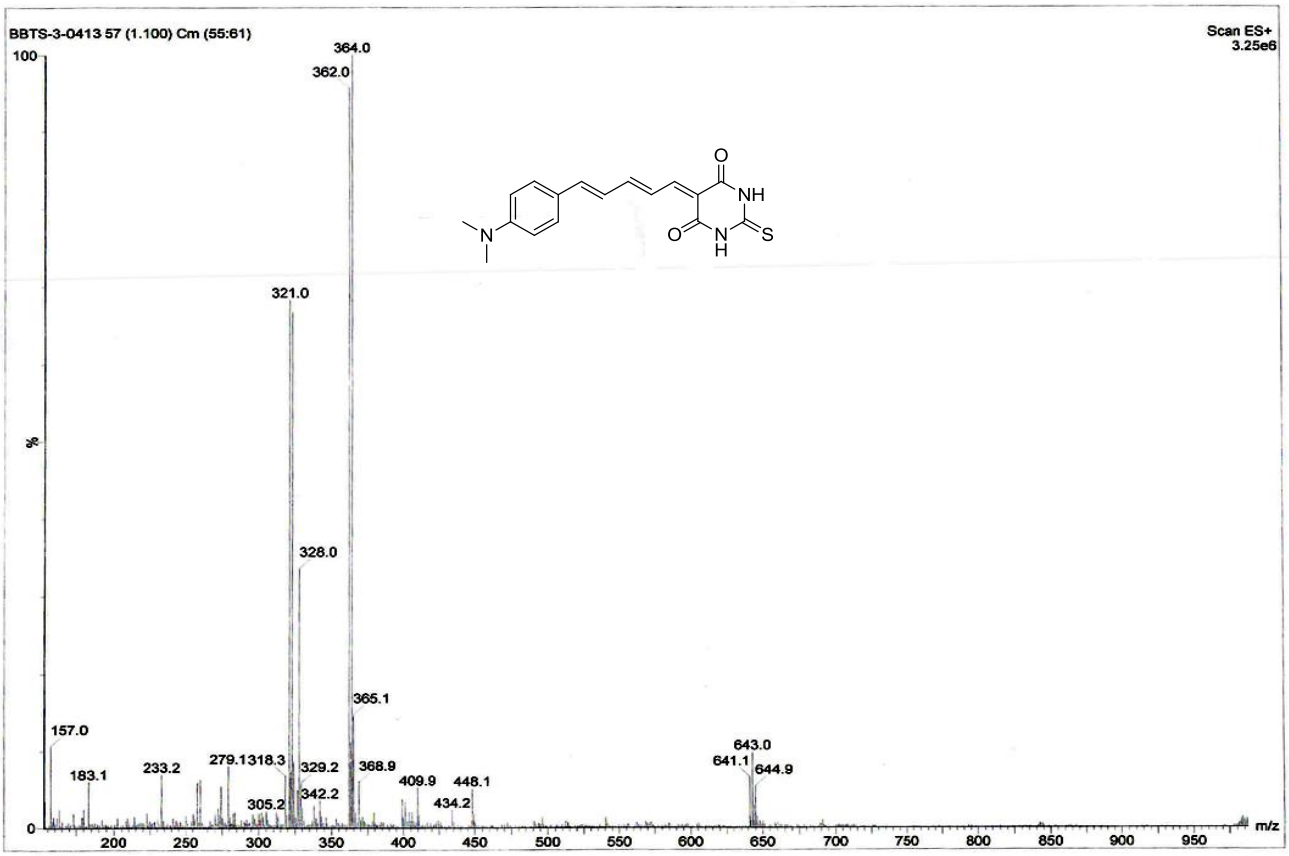
MS spectrum of BBTO-3



MS spectrum of BBTS-1



MS spectrum of BBTS-2



MS spectrum of BBTS-3

References.

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2. J. Olmsted, *Journal of Physical Chemistry*, 1979, **83**, 2581-2584.
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