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Supplementary Information

Selective and Efficient Mitochondrial Staining with Designed 2,1,3-Benzothiadiazole Derivatives as Live Cell Fluorescence Imaging Probes

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Experimental

General

Chemicals and solvents were purchased from commercial sources (Acros or Aldrich) and used without further purification.

X-ray diffraction analysis

The molecular structures of BTDPyMe was determined by X-ray diffractometry. The X-ray measurements were carried out in a Bruker SMART CCD APEX II area-detector diffractometer with graphite-monochromated Mo K_α radiation ($\lambda = 0.71073$ Å) at room temperature ($T = 293$ K).

Mass spectrometry analyses

ESI-MS (electrospray ionization mass spectrometry) and ESI-MS/MS (electrospray ionization tandem mass spectrometry) measurements were performed in the positive ion mode (*m/z* 50-2000 range) on a Waters Synapt HDMS (high definition mass spectrometer, Manchester, U. K.) instrument. This instrument has a hybrid quadrupole/ion mobility/orthogonal acceleration time-of-flight (oa-TOF) geometry and was used in the TOF mode, with the mobility cell switched off and working only as an ion guide. All samples were dissolved in methanol to form 50 mmol L^{-1} solutions (with 0.1% HCOOH) and were infused directly into the ESI source in a flow rate of 5 μL min^{-1}. ESI source conditions were as follows: capillary voltage 3.0 kV, sample cone 30 V and extraction cone 3 V.

NMR analyses

NMR (nuclear magnetic resonance) spectra were recorded on a Varian Mercury Plus spectrometer 7.05 T (300 MHz for proton) at room temperature, using a 5-mm internal diameter probe. DMSO-d_6 (deuterated dimethyl sulfoxide) was used as internal standard. Temperature dependent NMR spectra were recorded on a Varian Inova 300 MHz spectrometer.

Infrared analyses

FTIR-ATR (attenuated total reflection using infrared Fourier transform) spectra were obtained on an Equinox 55 Fourier transform instrument from Bruker. The FTIR-ATR spectra were recorded using a horizontal ATR cell, 7 cm long (10 reflections), from Pike Technologies, covering the 650-4000 cm^{-1} spectral range, using a DTGS detector. Each FTIR-ATR spectrum is the average of 32 scans, using air as reference, at 4 cm^{-1} nominal spectral resolution. All spectra were collected at 21 °C.

Melting point analysis

Melting points were measured on an Electrothermal IA9000 Melting Point apparatus.

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Theoretical calculations

All theoretical calculations were carried out using Gaussian 09[1] suite of programs. Optimizations for ground state (S_0) were performed using the *ab initio* Hartree-Fock (HF) and density functional theory (DFT) methods, while the configuration interaction with single excitation (CIS) method was employed to optimize the geometries for first excited state (S_1). According to Brillouin's theorem, CIS method is the HF equivalent for the excited electronic states. The transition states were located using the synchronous transit-guided quasi-Newton QST2 method. Harmonic frequency calculations were performed for every structure to confirm it as a local minimum or transition structures. It is well known that the CIS method could produce reliable geometry but it predicts too high excitation energy. To introduce the dynamic electron correlation, single point energy calculation for the HF and CIS optimized geometries in S_0 e S_1 states has been done at Kohn-Sham DFT and its time-dependent (TD-DFT) formalism, respectively, with Beck three-parameters hybrid exchange-correlation functional, known as B3LYP. The hybrid method such as DFT//HF or TDDFT//CIS (denoted as single-point calculation//optimization method) has been proved to be an efficient approach in the predicting energy parameters or optical properties for LED materials. All calculations were performed using the 6-311+G(2d,p)/LANL2DZ basis set (*i.e.*, LANL2DZ pseudo potential for bromine and the 6-311+G(2d,p) split-valence basis set for all other atoms). Absorption spectra in close agreement with experiments have been obtained using the TD-B3LYP/6-311+G(2d,p) level. It was calculated partial charges of each atom using CHELPG (charges from electrostatic potentials using a grid based method).

Cell-imaging experiments

MCF-7 cells (breast cancer cells) were plated on 13 mm round glass coverslips on the bottom of 24 well plates and allowed to adhere for 30 min at 37 ºC in 5% CO_2 atmosphere. Subsequently, non-adherent cells were removed by washing with serum free medium. MCF-7 cells were cultivated in D-MEM medium plus 10% fetal bovine serum at 37 ºC in 5% CO_2 atmosphere. After confluence was reached, the culture were washed three times in PBS (phosphate-buffered saline) pH 7.4 and fixed for 15 min in 3.7% formaldehyde solution in PBS at room temperature. The cells were washed three times with PBS and were incubated with markers BTDPy, BTDPyMe or BTDShiny diluted 1:1 in DMSO during 30 min at room temperature. As negative control, the cells were incubated at same conditions in PBS. After the incubation time, the cells were washed three times with PBS and incubated with DAPI (Invitrogen, Oregon, USA) according to manufacture recommendations. The coverslips were mounted by using ProLong Gold Antifade (Invitrogen, Oregon, USA) according manufacture recommendations. The samples were analyzed in a Leica Confocal Microscopy TCS SP5. The assays were performed by experimental triplicate in three independent series.

Synthesis of BTDPyMe

4-Pyridyl-7-bromo-2,1,3-benzothiadiazole (BTDPy, 1.00 mmol) was added to sealed Schlenk tube. Iodomethane (3 mL) was then added. The Schlenk is sealed and the reaction temperature set to 80 °C (overnight). After that, the excess of iodomethane is removed and the product crystallized in benzene:acetone mixtures, affording BTDPyMe in 18%.

IR (KBr) ν/cm^{-1} 3056, 2961, 2911, 2921, 2851, 1621, 1439, 1115, 737; ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm 9.33-9.29 (m, 1H), 8.83-8.75 (m, 1H), 8.38-8.27 (m, 2H), 8.06-8.02 (m, 1H), 7.82-7.75 (m, 1H), 4.15 (s, 3H); ^{13}C NMR-APT (75 MHz, DMSO- d_6) δ / ppm 153.2, 152.0, 150.9, 133.8, 133.7, 133.2, 132.6, 130.7 130.5, 124.0, 119.8, 117.8.

Synthesis of BTDShiny

4-Pyridyl-7-bromo-2,1,3-benzothiadiazole (BTDPy, 1.00 mmol) was added to sealed Schlenk tube. Methanol (3 mL), K_3PO_4 (3.00 mmol) and 4-MeO-Ph-B(OH)$_2$ was added with the palladium catalyst (1 mol%)[2] The reaction mixture was heated at 110 °C overnight. The crude mixture was filtered with celite and separated in a silica column eluted with mixtures of hexane/ethyl acetate affording the desired compound in 99% yield.

IR (KBr) ν/cm^{-1} 3015, 2955, 2847, 1643, 1248, 1183, 1017, 823; ^{1}H NMR (300 MHz, DMSO-d_6) δ / ppm 8.02-7.98 (m, 2H), 7.63-7.51 (m, 8H), 7.14-7.11 (m, 2H), 7.04-7.96 (m, 8H), 3.84 (s, 3H), 3.83 (s, 3H); ^{13}C NMR-APT (75 MHz, DMSO-d_6) δ / ppm 158.3, 158.2, 132.3, 131.2, 130.4, 129.3, 128.9, 127.7, 127.5, 127.2, 127.1, 126.7, 126.2, 125.8, 114.4, 114.3, 114.0, 55.3, 55.1.

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Table S1. Selected bond lengths (Å, left) and bond angles (degree, right) for BTDPyMe

Br(7)-C(7)	1.883(4)	C(6)-C(7)-Br(7)	124.0(4)
N(11)-C(12)	1.333(5)	C(8)-C(7)-Br(7)	117.4(3)
S(2)-N(1)	1.613(4)	C(12)-N(11)-C(16)	117.3(4)
S(2)-N(3)	1.609(3)	C(10)-N(11)-C(16)	120.7(4)
N(11)-C(16)	1.481(5)	N(11)-C(10)-C(4)	120.0(3)
N(1)-C(8)	1.346(5)	N(3)-C(9)-C(4)	126.1(4)
N(3)-C(9)	1.351(5)	C(9)-N(3)-S(2)	106.4(3)
C(4)-C(9)	1.481(5)	C(8)-N(1)-S(2)	106.1(3)
C(4)-C(10)	1.464(6)	N(3)-S(2)-N(1	101.06(2)
C(8)-C(9)	1.425(5)	C(15)-C(10)-C(4)	122.9(4)

Table S2. NMR data of temperature dependent experiments

Temperature / °C	Atropoisomer	Area / a.u.	Frequency / Hz
20	*syn*	20.86466	1150.794
	anti	79.13534	1153.147
30	*syn*	21.73127	1151.915
	anti	78.26873	1154.273
40	*syn*	26.48731	1152.996
	anti	73.51269	1155.315
50	*syn*	31.32456	1154.163
	anti	68.67544	1156.263
60	*syn*	38.66242	1156.403
	anti	61.33758	1157.303
70	*syn*	100.00000	1158.259
	anti		

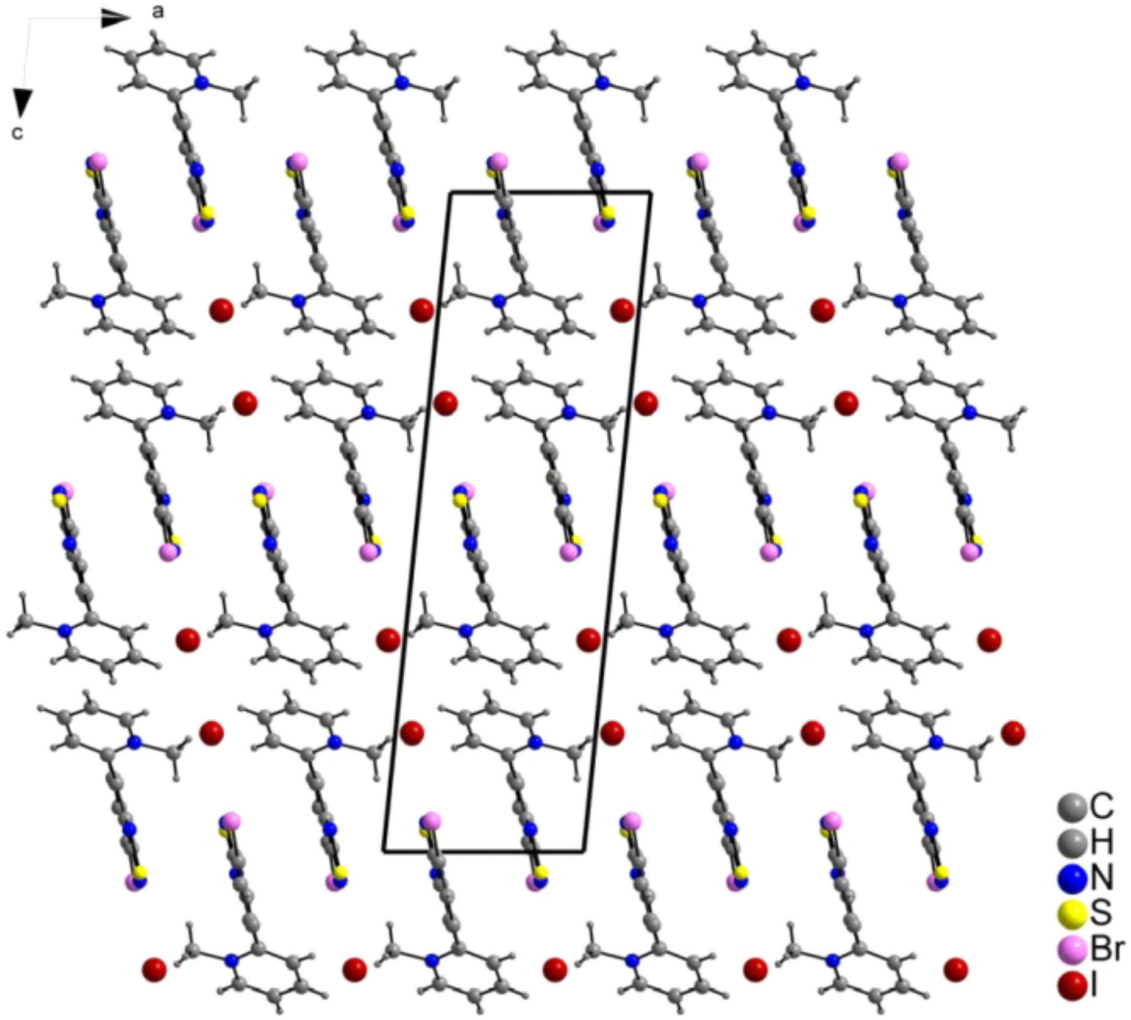

Figure S1. Unit cell of BTDPyMe showing the packing of molecules in *ac* plane.

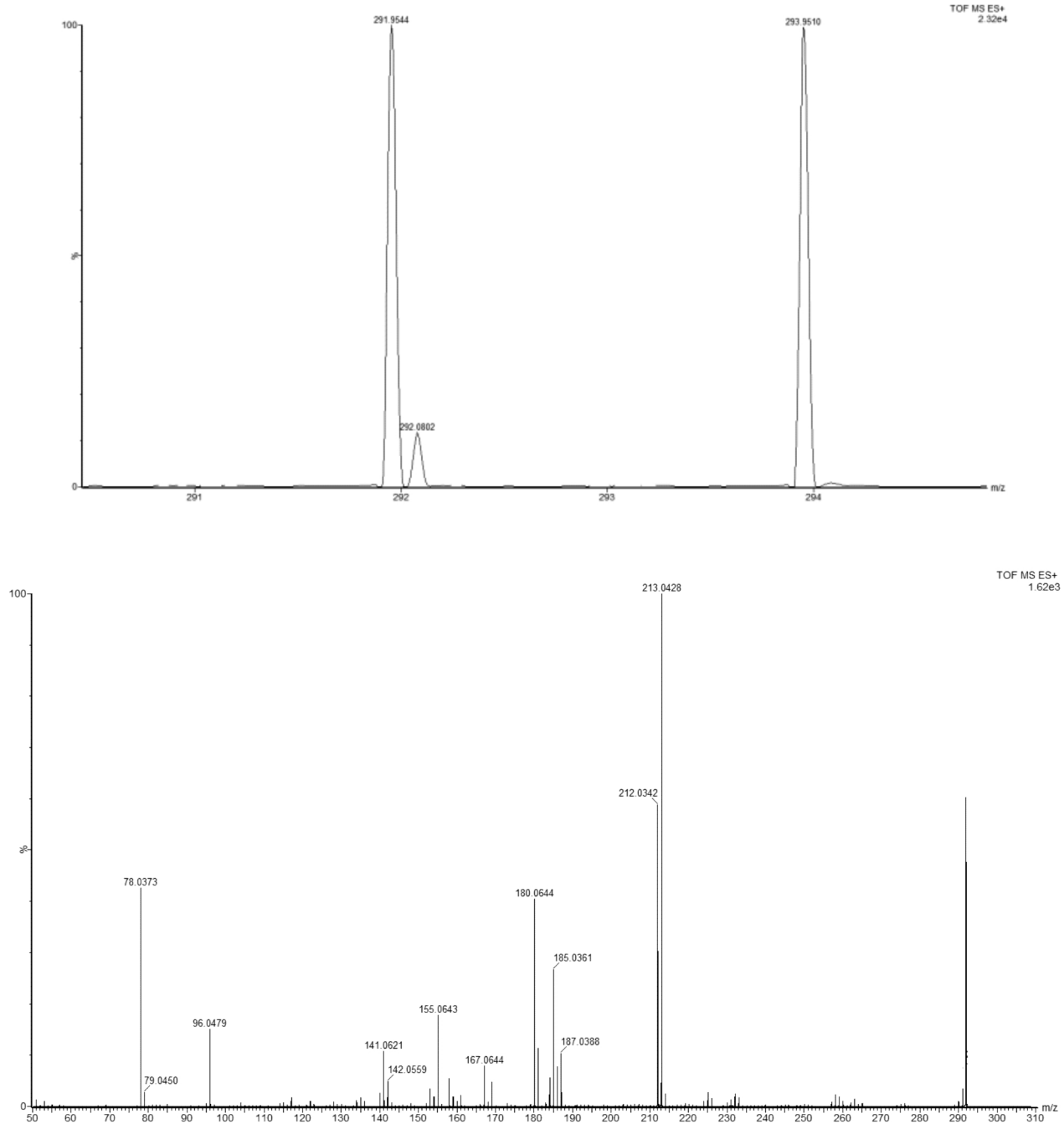

Figure S2. ESI(+)-QTOF (top) and ESI(+)-QTOF product ion spectrum (bottom) of protonated BTDPy.

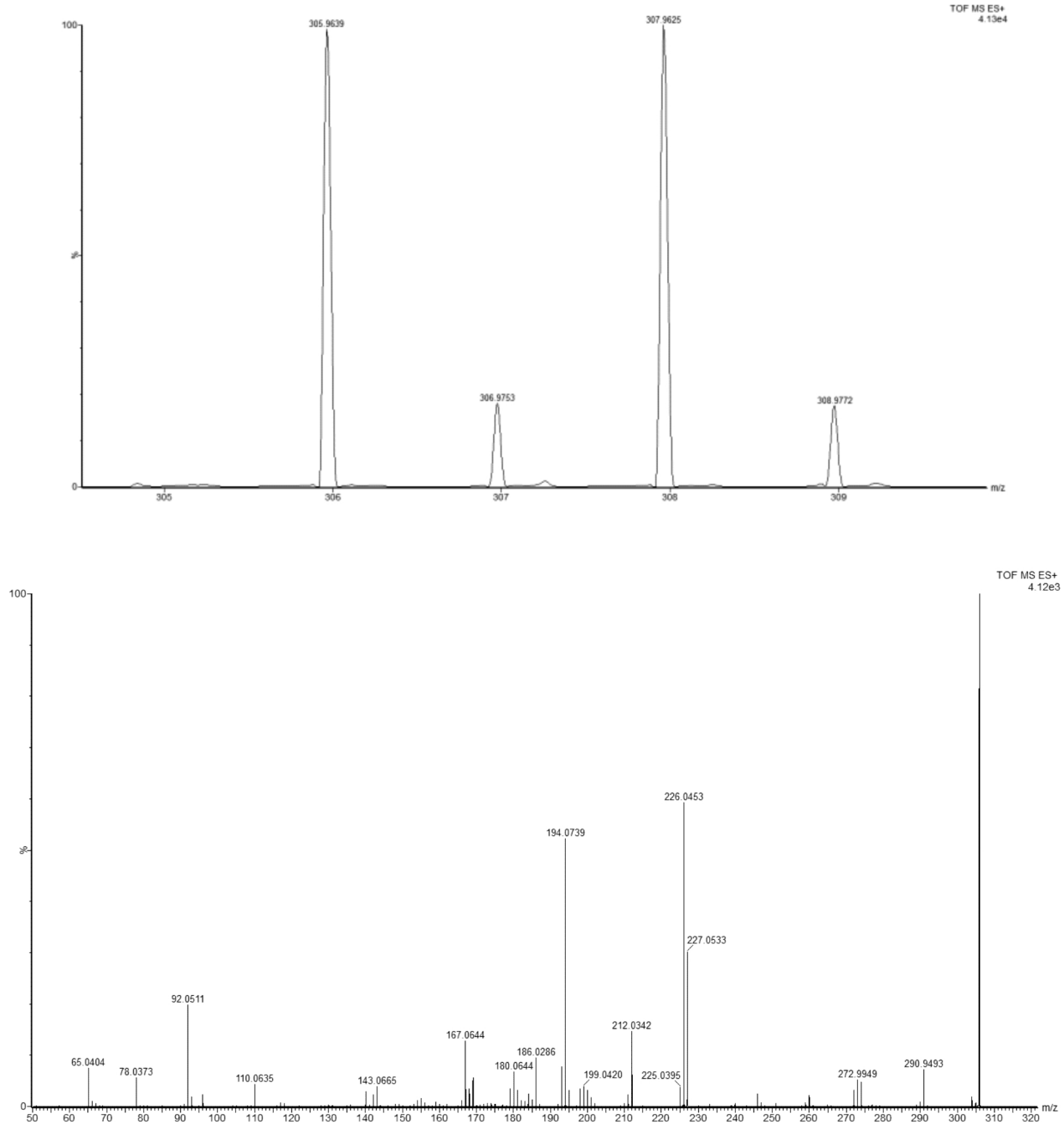

Figure S3. ESI(+)-QTOF (top) and ESI(+)-QTOF product ion spectrum (bottom) of protonated BTDPyMe.

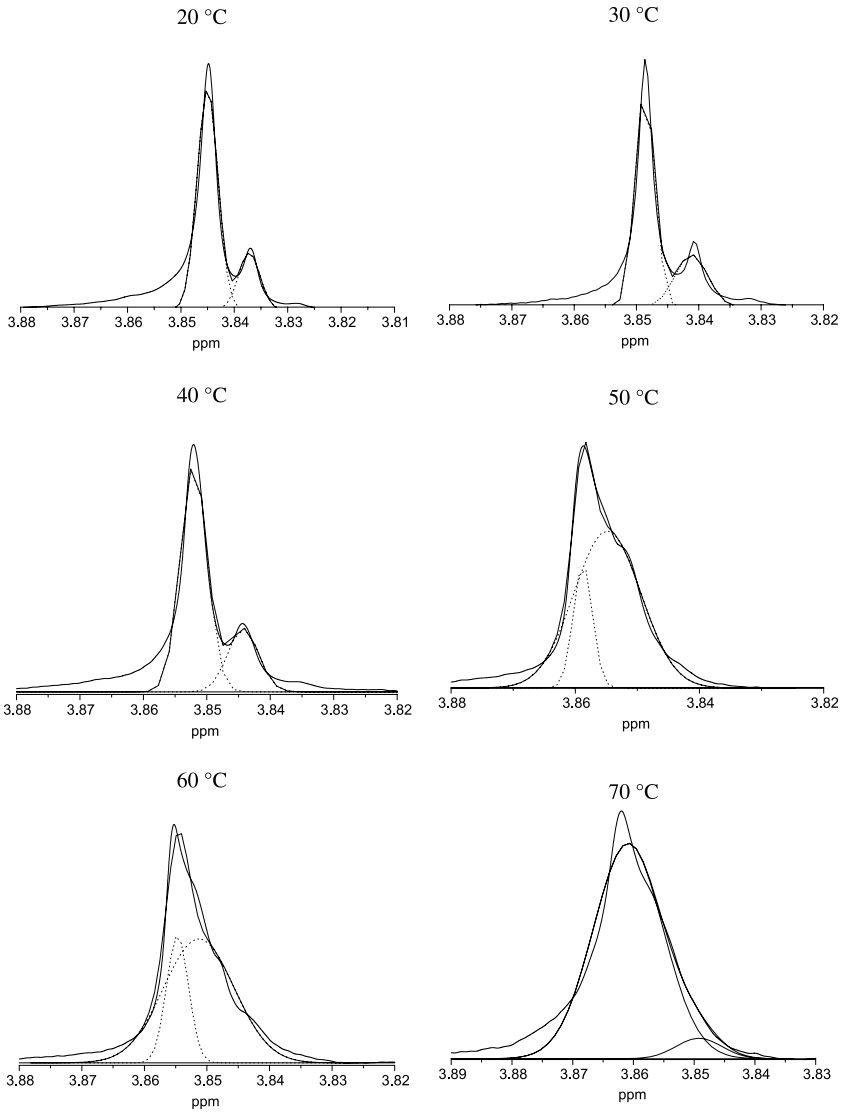

Figure S4. Expansion (solid line) and deconvolution (dotted line) of ^{1}H NMR (300 MHz, DMSO-d_6) of BTDShiny at different temperatures.

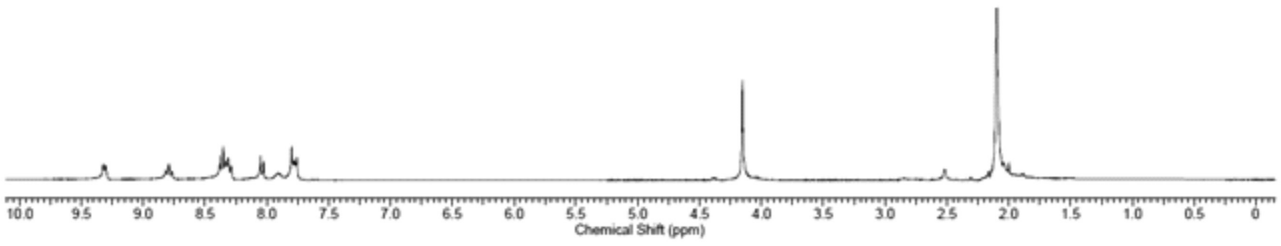

Figure S5. ^{1}H NMR (300 MHz, DMSO-d_6) of BTDPyMe.

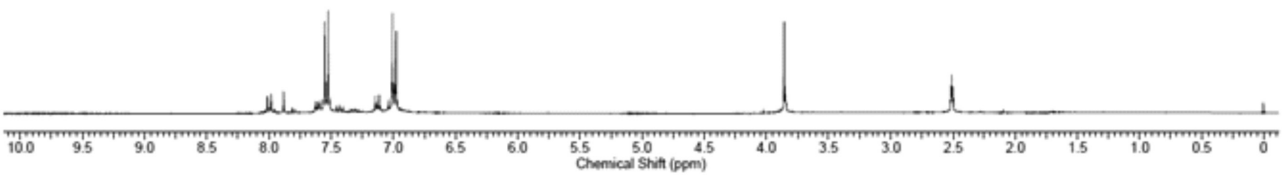

Figure S6. ^{1}H NMR (300 MHz, DMSO-d_6) of BTDShiny.