Electronic Supplementary Material (ESI) for ChemComm.


# Electronic Supplementary Information for:

# Photo-oxygenation inhibited tau amyloid formation

Takanobu Suzuki[a#], Yukiko Hori[a#], Taka Sawazaki[b#], Yusuke Shimizu[b], Yu Nemoto[a], Atsuhiko Taniguchi[b,c], Shuta Ozawa[a], Youhei Sohma[b*], Motomu Kanai[b*], Taisuke Tomita[a*]

a. Laboratory of Neuropathology and Neuroscience, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: taisuke@mol.f.u-tokyo.ac.jp
b. Laboratory of Synthetic Organic Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mails: ysohma@mol.f.u-tokyo.ac.jp; kanai@mol.f.u-tokyo.ac.jp
c. Present address: Department of Medicinal Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences
#T.S., Y.H. and T.S. contributed equally to the present work

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## Experimental Procedures

### *General*

All solvents and chemicals were purchased from commercial suppliers, Kanto Chemical Co., Inc., Sigma–Aldrich, Inc., Tokyo Chemical Industry Co., Ltd., Wako Pure Chemical Co., Inc., and Watanabe Chemical Industries, Ltd., and were used without further purification. Nuclear magnetic resonance (NMR) spectra were recorded on JEOL ECS400 (400 MHz for $^{1}$H NMR, 100 MHz for $^{13}$C{$^{1}$H} NMR, 370 MHz for $^{19}$F NMR, 125.7 MHz for $^{11}$B NMR) spectrometer. Chemical shifts are reported relative to the solvent used as an internal reference for $^{1}$H NMR and $^{13}$C{$^{1}$H} NMR, $C_6F_6$ (–164.90 ppm) as an external reference for $^{19}$F NMR, and $BF_3$ (0.00 ppm) as an external reference for $^{11}$B NMR. Electrospray ionization mass spectra (ESI–MS) were measured on a JEOL JMS–T100LC AccuTOF spectrometer (for HRMS). MALDI-TOF MS spectra were recorded on Shimadzu Biotech Axima-TOF2 using α-cyano-4-hydroxy cinnamic acid as a matrix. An aliquot of the reaction solution was de-salted with ZipTip U-C18 (Millipore Corporation) prior to the MS analysis. Absorbance measurement was performed using Shimadzu UV-1800 spectrometer with a rectangular quartz cell (5 mm pathlength). Fluorescence measurement was performed using Shimadzu RF-5300PC spectrometer with a rectangular quartz cell (3 mm pathlength). Amino acid analysis was performed by Peptide Institute, Inc. Analytical HPLC charts were obtained by using a JASCO HPLC system equipped with a UV-2075 spectrometer, PU-2080 pumps, a DG-2080-54 degasser, and an MX-2080-32 mixer, with a YMC Triart C18 column (150 × 4.6 mm) using a linear gradient of 0–100% acetonitrile in 0.1% aqueous trifluoroacetic acid over 40 min with a flow rate of 0.9 mL min$^{-1}$ at 40 $^{o}$C, detected by absorbance at 215 nm.

### *Synthesis of di-iodo B-CF$_3$-boron-tetramethyldipyromethene (di-iodo B-CF$_3$-BODIPY)*

To $CH_2Cl_2$ (5 mL) containing B-$CF_3$-BODIPY [1] (28.6 mg, 0.096 mmol) was added *N*–iodosuccinimide (86.3 mg, 0.38 mmol). The reaction mixture was stirred at room temperature for 1 hr, and then washed with saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (hexane/DCM =19:1) to afford the title compound as red solid (26.9 mg, 51% yield); $^{1}$H NMR ($CDCl_3$) δ 7.18 (s, 1H), 2.60 (s, 6H), 2.26 (s, 6H); $^{13}$C{$^{1}$H} NMR ($CDCl_3$) δ 159.0, 145.2, 132.7, 121.0, 83.2, 16.3 (m), 14.1; $^{19}$F NMR ($CDCl_3$) δ –73.3 (3F), –181.0; $^{11}$B NMR ($CDCl_3$) δ –1.83. HRMS (ESI$^{+}$): *m/z* calcd for $C_{14}H_{13}BF_4I_2N_2Na^{+}$ [M+Na]$^{+}$: 572.9095. Found: 572.9098.

### *Synthesis of catalyst 3, new photo-oxygenation catalyst*

To a solution of dry toluene (2 mL) containing di-iodo B-$CF_3$-BODIPY (27.5 mg, 0.050 mmol) and 6-(6-formyl-3,4-dihydroquinolin-1(2*H*)-yl)hexanoic acid (16.5 mg, 0.060 mmol) was added piperidine (49.4 μL, 0.50 mmol). The reaction mixture was heated at reflux for 2 hrs under Ar. Then, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane/acetone = 4:1 with 1% AcOH, then $Et_2O$/hexane/MeOH =5:4:1 with 1% AcOH) to afford the title compound as dark blue solid (10.2 mg, 30%). $^{1}$H NMR (acetone–$d_6$) δ 10.5 (br, 1H), 7.57 (dd, *J* =2.2, 16.1 Hz, 1H), 7.50 (s, 1H), 7.38 (d, *J* =16.1 Hz, 1H), 7.33 (dd, *J* =2.2, 8.5 Hz, 1H), 7.24 (s, 1H), 7.06 (s, 1H), 6.70 (d, *J* =8.5 Hz, 1H), 3.38–3.46 (m, 4H), 2.78 (t, J =6.3 Hz, 2H), 2.56 (s, 3H), 2.36 (s, 3H), 2.32 (t, J =7.4 Hz, 2H), 2.25 (s, 3H), 1.89–1.97 (m, 2H), 1.63–1.71 (m, 4H), 1.39–1.47 (m, 2H); $^{13}$C{$^{1}$H} NMR (acetone–$d_6$) δ 174.5, 161.0, 151.7, 148.6, 145.1, 142.8, 139.6,

137.5, 132.3, 129.7, 129.6, 124.2, 123.4, 118.7, 118.6, 113.2, 113.1, 111.4, 80.0, 51.7, 50.3, 34.1, 28.7, 27.2, 26.8, 25.5, 16.0 (m), 13.6, 11.4; $^{19}$F NMR (acetone–$d_6$) δ –73.1 (3F), –178.3; $^{11}$B NMR (acetone–$d_6$) δ –1.22. HRMS (ESI$^+$): *m/z* calcd for $C_{30}H_{33}BF_4IN_3O_2Na^+$ [M+Na]$^+$: 704.1544. Found: 704.1545. Note that one iodine atom was removed during the reaction, and the residual iodine atom in **3** connected at C2 position (Fig. S1).

***Recombinant tau RDWT/RD (P301S) preparation***

The human tau plasmid (2N4R full-length tau cloned into pRK172 vector) was gifted from Dr. Hasegawa.[2] Tau Repeat Domain (RD; aa 244-332 of the 441 amino acids of full-length tau 2N4R) was constructed by deletion from full-length (FL) tau sequence. In addition, the P301S mutation (RD P301S) was generated, and all sequences were confirmed.

Recombinant tau preparation was according to previous report with some modification.[3] Briefly, wild-type or P301S mutant repeat domain of tau protein (RDWT or RD (P301S)) was expressed in Rosetta (DE3) (Novagen) and purified using the cation exchange chromatography column with cellulose phosphate as previously reported. After the elution, the eluates were applied to reverse-phase chromatography to increasing the purity. After the evaporating of elution buffer from reverse-phase chromatography, the pellets were dissolved with phosphate buffered saline (PBS), measured the concentration of tau by BCA assay kit (Takara), and stocked at -80°C until use.

***Photo-oxygenation***

To a solution of tau RDWT (40 μM) in sodium acetate buffer (pH 7.0) was added 1,4-dithiothreitol (DTT: 2 μM) and Heparin-sodium (40 μM). The mixture was incubated at 37°C for 3 hrs, and then added a stock solution of **3** (final concentration: 4 μM). The mixture was irradiated with 660 nm LED at 37°C and analyzed using MALDI-TOF-MS.

***Enzymatic digestion***

After the photo-oxygenation for 60 min, trypsin aqueous solution (1/50 of protein by weight) was added to the reaction mixture, and incubated at 37°C overnight. Then, the mixture was analyzed using MALDI-TOF-MS.

***Tau aggregation assay***

4 μM of catalyst **3** or dimethyl sulfoxide (DMSO) was added to PBS (pH 7.4) containing recombinant tau RDWT (40 μM), Heparin-sodium (0.48 mg/ml) and DTT (2 μM). Samples were shaken 1,000 rpm at 37°C and irradiated with light at 660 nm. To evaluate the amount of tau fibrils, samples were mixed with 3 μM of Thioflavin T solution (ThT) and measured fluorescence intensity (Excitation: 437 nm, Emission: 505 nm).

***Negative stain electron microscopy***

To stain the fibrils, samples were incubated with 2% sodium phosphotungstate for 1 min on the membrane (NISSHIN EM Co., Ltd. TOKYO). Tau fibrils were observed using the electron microscopy (JEM-1200EX).

### *In vitro Tau seeding activity assay*

PBS (pH 7.4) containing recombinant tau RDWT (40 μM), Heparin-sodium (0.48 mg/ml) and DTT (2 μM) was shaken 1,000 rpm for more than 12 hrs at 37°C. Then, samples were centrifuged at 113,000 x g for 20 min and the pellet was resuspended with PBS (pH 7.4). After sonication of the resuspended tau solution at output 5 for 300 sec using SONIFIER 250 (BRANSON), the tau concentration was calculated by BCA protein assay kit (Takara) and sample was irradiated at 660 nm for 3 hrs with catalyst **3** (0.3 μM) or DMSO. This photo-oxygenated tau was added to recombinant tau RDWT monomer solution with 1 mol% ratio as seed. To evaluate the amount of tau fibrils, ThT fluorescence was measured as above.

### *Generation of FScV cell line*

The human 2N4R FL tau plasmid cloned into Puro4 vector was gifted from Dr. Murata. The 1N4R tau plasmid was generated by deletion of exon 3 in 2N4R tau sequence. P301S mutation was added and all sequences were confirmed.

The HEK293A cells were transfected with plasmid encoding human 1N4R FL tau harboring P301S mutation tagged with Venus at the C-terminal using polyethylenimine (Polyscience). The monoclonal cell line (FScV cell) was generated from these stably-expressing tauFL(P301S)-cVenus cells. Cells were kept to culture in Dulbecco's Modified Eagle Medium supplemented with 10% defined fetal bovine serum (Cell Culture Technologies) and 1% penicillin and streptomycin (Thermo Fisher Scientific).

### *Tau seed transfection into cells*

A previously reported fibril-transduction protocol was used with minor modifications.[4] Briefly, FScV cells were plated on coverslips at 50,000 cells/well in 12-well plates. The photo-oxygenated tau seeds of recombinant RD (P301S) fibrils were combined with OptiMEM (Gibco) to a final volume of 100 μL. 96 μL OptiMEM and 4 μL lipofectamine-2000 (Invitrogen) were then added to the OptiMEM mixture to a final volume of 200 μL. After the incubation for 20 min, liposome preparations were added to cells. 18 hrs later, cells were washed, fixed with 4% paraformaldehyde, and observed using confocal microscopy (Leica MICROSYSTEMS, TCS-SP5 Ver2.0).

### *Statistical analysis*

In the experiments, no randomization was performed. Data were presented as mean ± SEM. The samples were compared by the two-tailed Student *t*-test. $p<0.05$ was considered as statistically significant difference between two groups. R version 3.4.3 was used to perform the statistical analysis.

**Determination of the position of the iodine atom in 3**

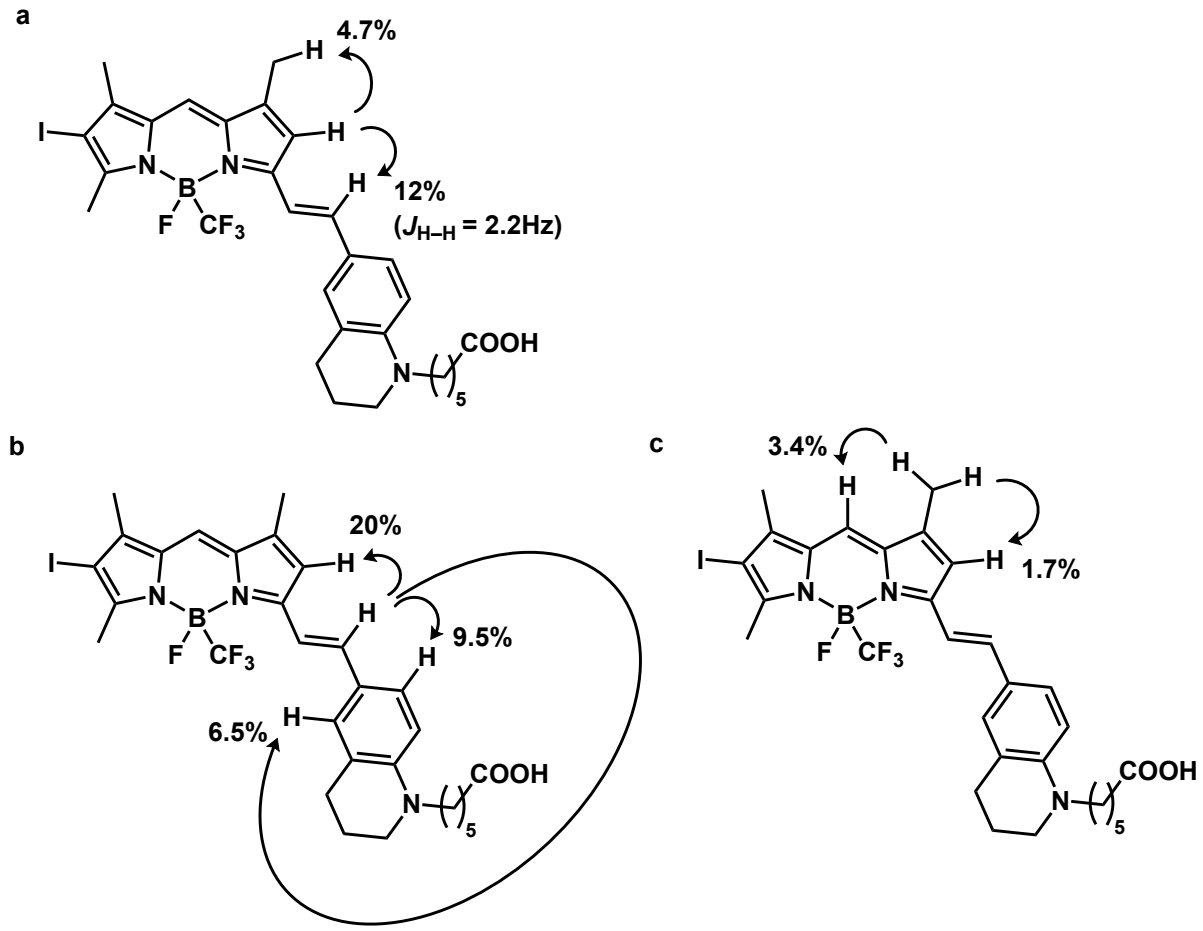


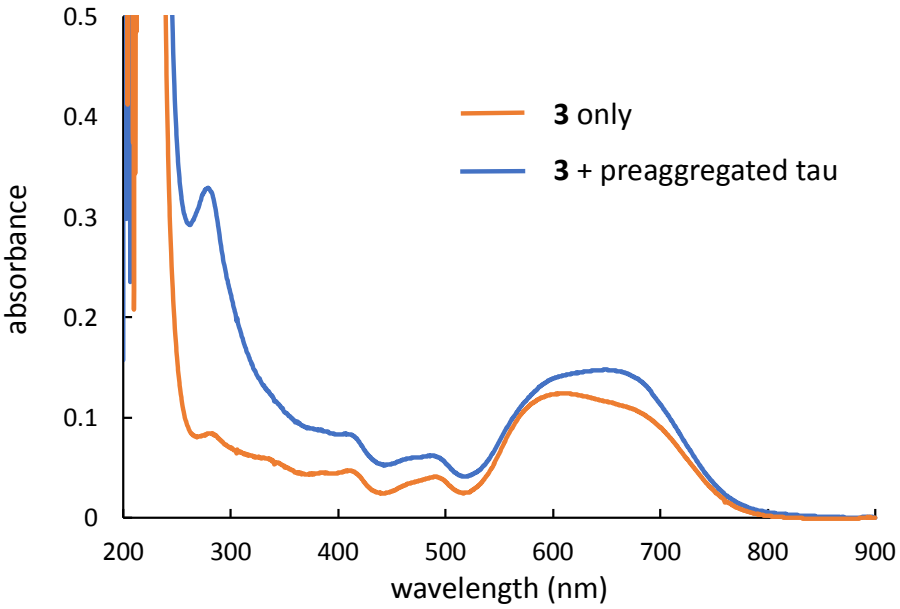

Figure S1 NOE correlations in **3**. (a) Irradiation at δ7.06; (b) irradiation at δ7.57; (c) irradiation at δ2.36.

**Photophysical properties**

Table S1

| compound | $\lambda_{abs}$max (nm) | $\lambda_{flu}$max | ϕ |
|---|---|---|---|
| B-$CF_3$-BODIPY[a,b] | 502 | 509 | 0.83 |
| C2,C6-$I_2$-B-$CF_3$-BODIPY[a] | 532 | 546 | 0.014[c] |
| BAP-1[d,e] | 604 | 648 | 0.46 |
| **3**[d] | 652 | – | N.D.[f] |

[a]Data measured in MeOH; [b]from ref 5; [c]calculated using fluorescein (0.86 in 0.1 N NaOH solution); [d]Data measured in $CHCl_3$; [e]from ref 6; [f]not detected.



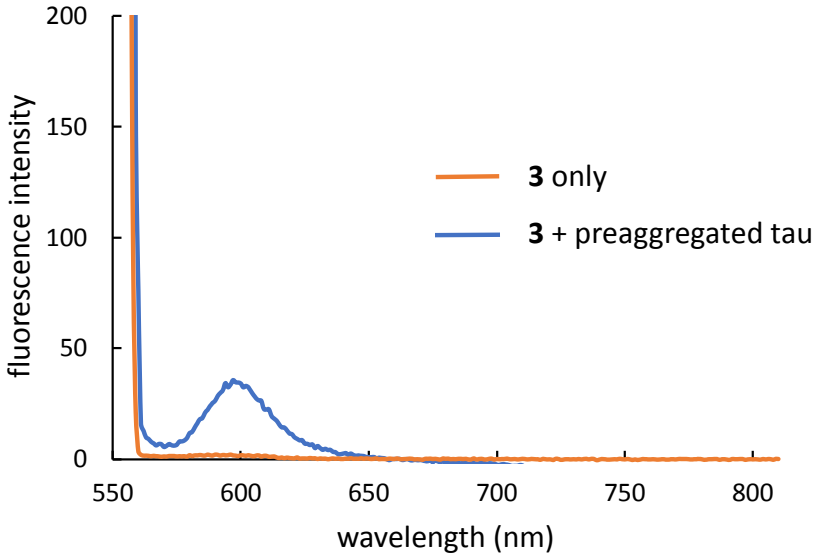




Figure S2 (upper part) Absorbance spectra of **3**. A sodium acetate buffer (pH 7.0) containing **3** (4 μM) in the presence or absence of preaggregated tau RDWT (40 μM) was measured. (lower part) Fluorescence spectra of **3**. A sodium acetate buffer (pH 7.0) containing **3** (4 μM) in the presence or absence of preaggregated tau RDWT (40 μM) was measured. The fluorescence intensity of **3** was slightly increased by the presence of preaggregated tau, suggesting that **3** binds with tau aggregates.

**Photo-oxygenation reaction of tau RDWT**

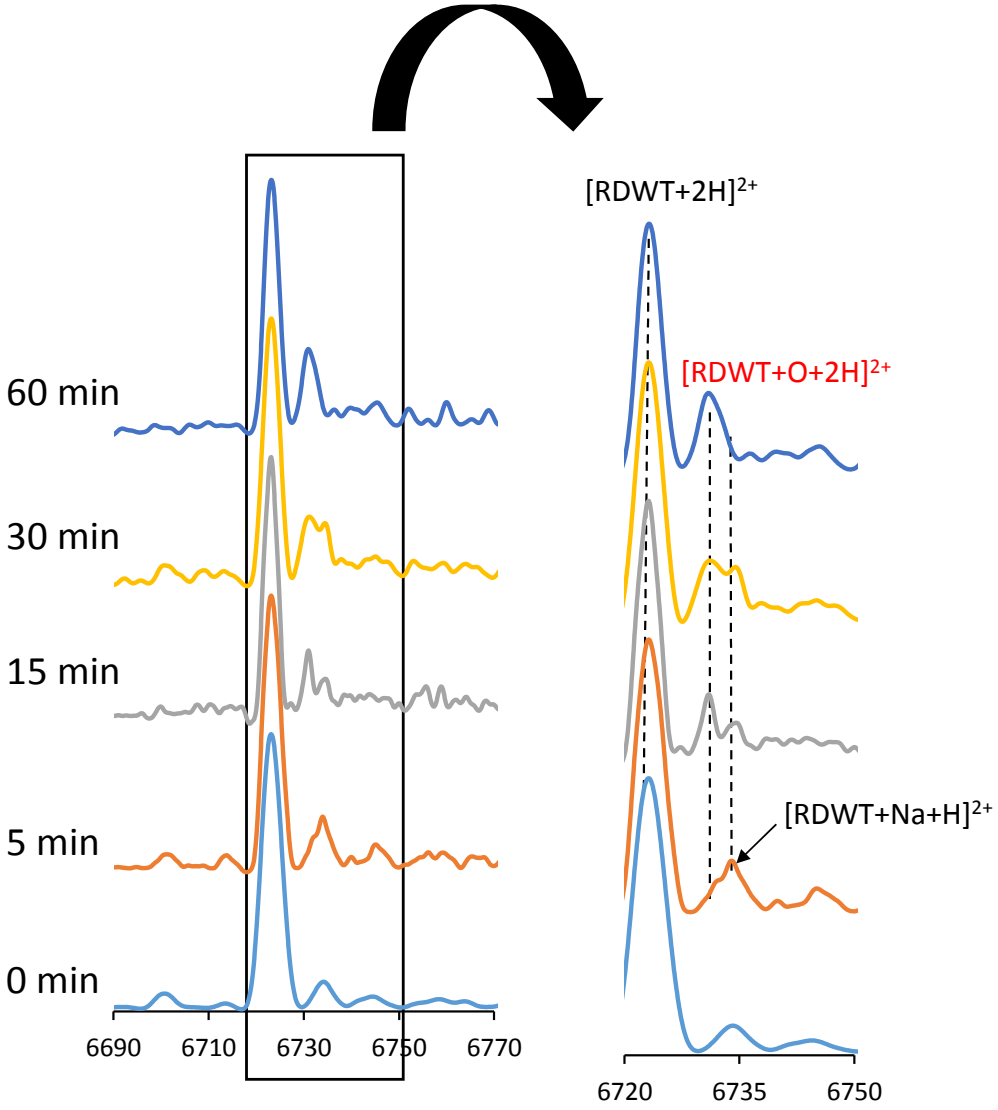



Figure S3 MALDI-TOF MS spectra of the oxygenation reactions of aggregated tau RDWT using **3**. A sodium acetate buffer solution (pH 7.0) containing tau RDWT (40 μM) and **3** (4 μM) was photoirradiated (λ = 660 nm) at 37°C, and the reaction mixture was analyzed at arbitrary time points (t). Horizontal axis denotes the values of *m/z*.

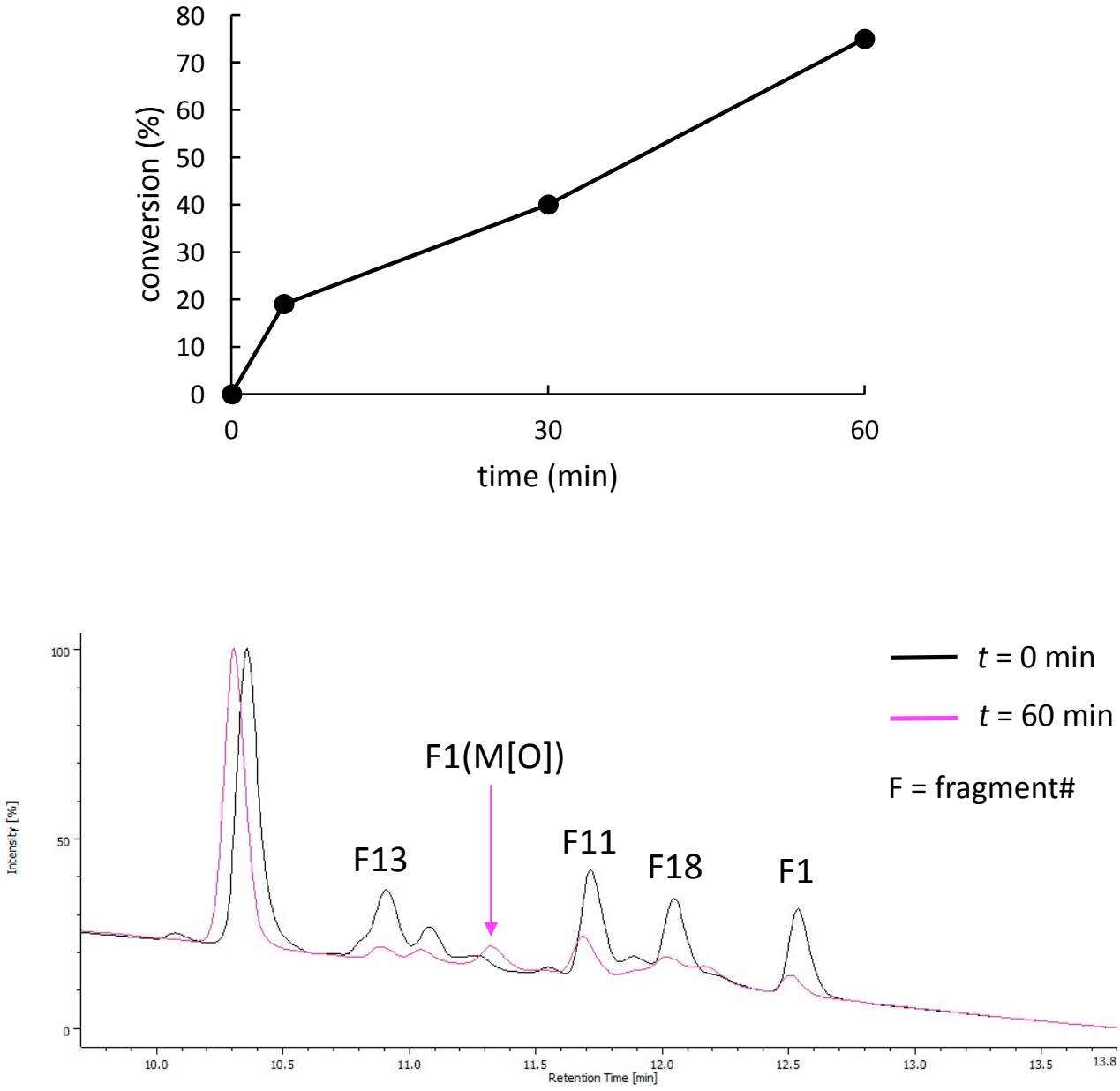



Figure S4 Photo-oxygenation of the preaggregated tau RDWT using **3**, verified using HPLC after tryptic digestion of the reaction mixture. A sodium acetate buffer solution (pH 7.0) containing the preaggregated tau RDWT (40 μM, preaggregated tau RDWT was prepared via preincubation in the presence of dithiothreitol (DTT) and heparin for 3 hrs) and **3** (4 μM) was photoirradiated ($\lambda$ = 660 nm) at 37°C for arbitrary time periods (*t*), the reaction mixture was treated with trypsin, and analyzed using HPLC (lower part: HPLC trance at *t* = 60 min). Upper part: conversion (%) = 100 × ([sum of peak areas of peptide fragments at *t* = 0 min] – [sum of peak areas of remaining peptide fragments at *t*])/ [sum of peak areas of peptide fragments at *t* = 0 min].

Amino acid analysis of native (without light irradiation) and oxygenated tau RDWT using **2** indicated that the amount of His residue was not changed in both samples. The result suggests that His did not oxygenated (Fig. S5b). Consistently, MALDI-TOF-MS analysis of the digested sample (using trypsin) after the oxygenation with **2** revealed that the mass peak corresponding to the oxygen atom-adduct was below detectable limit on the peptide fragments containing His residues (Fig. S5c, lower part), instead a peak derived from the oxygenated product was observed on the peptide containing Met residue (Fig. S5c, upper part).

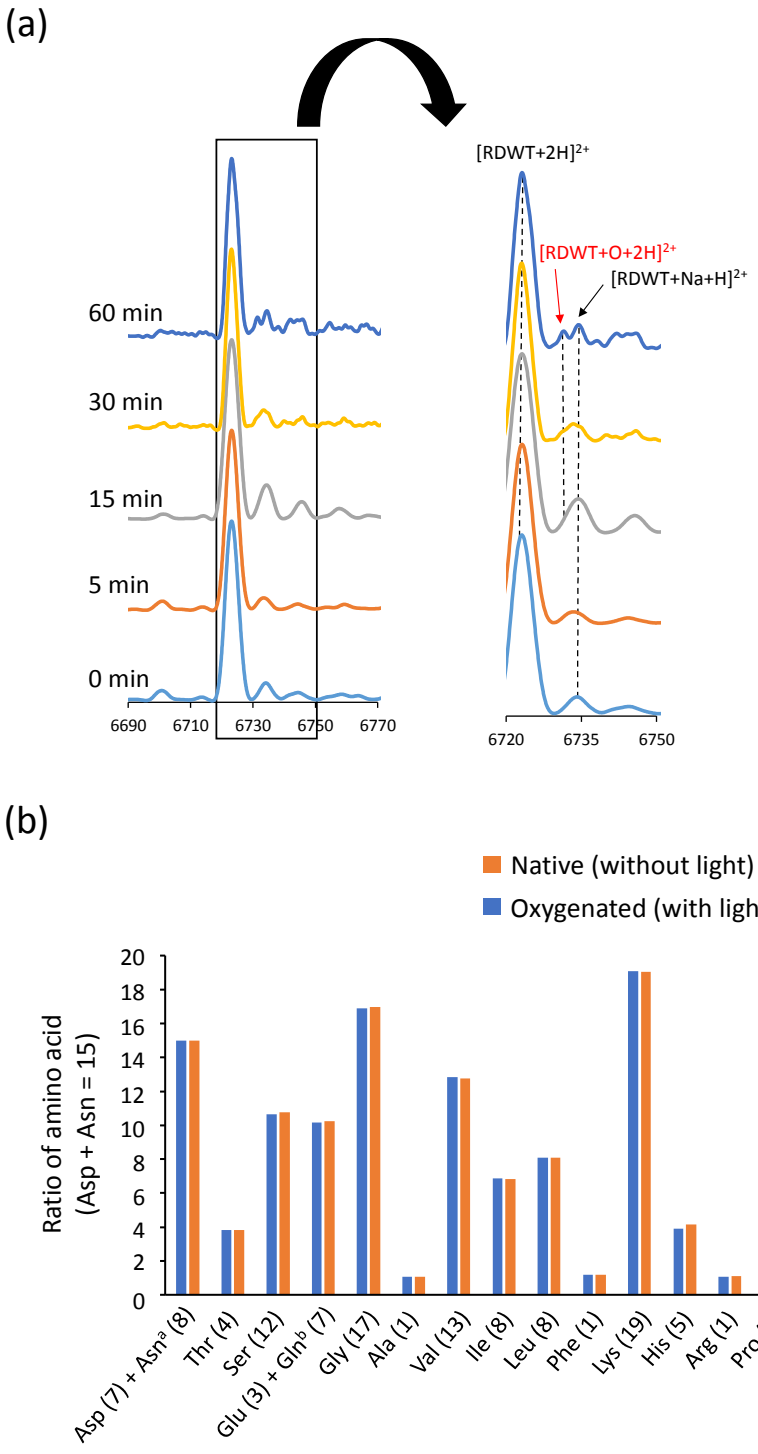



Figure S5 (to be continued)

(c)

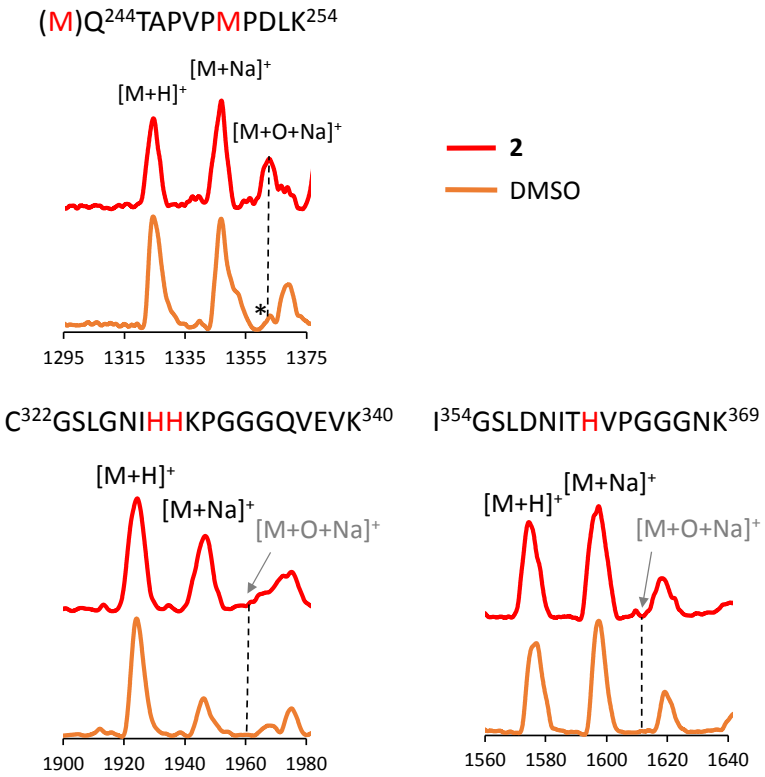



Figure S5 (a) MALDI-TOF MS spectra of the oxygenation reactions of aggregated tau RDWT using **2**. A sodium acetate buffer solution (pH 7.0) containing tau RDWT (40 μM) and **2** (4 μM) was photoirradiated (λ = 660 nm) at 37°C, and the reaction mixture was analyzed at arbitrary time points (*t*). Horizontal axis denotes the values of *m/z*. (b) Amino acid analysis of native (without light irradiation) and oxygenated tau RDWT (*t* = 60 min) (hydrolyzed with 6 M HCl at 110°C for 24 hrs). The number in parenthesis following each amino acid represents a theoretical ratio in the native tau RDWT. [a]detected as Asp; [b]detected as Glu. (c) MALDI-TOF MS spectra after digestion (using trypsin) of the photo-oxygenation reaction mixture.

(M)Q$^{244}$TAPVPMPDLK$^{254}$

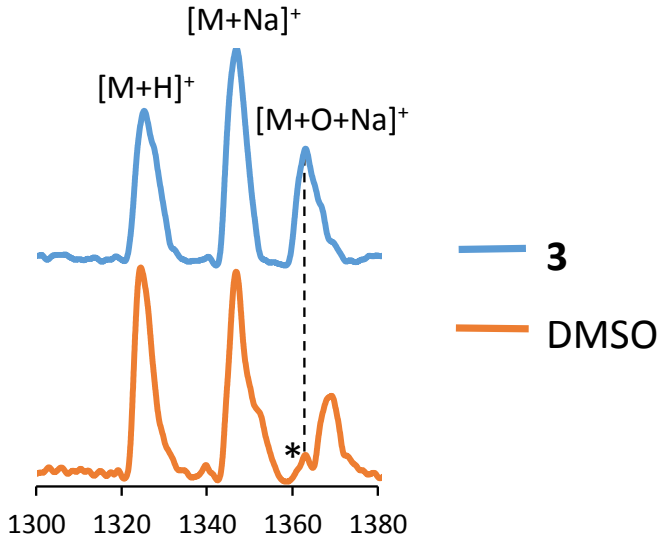



Figure S6 MALDI-TOF MS spectra of digested mixture (using trypsin) of the reaction mixture. A sodium acetate buffer solution (pH 7.0) containing preaggregated tau RDWT (40 μM) and DMSO or **3** (4 μM) was photoirradiated ($\lambda$ = 660 nm) at 37°C for 60 min, and the reaction mixture was treated with trypsin. *Met(O) was included in the original protein sample.

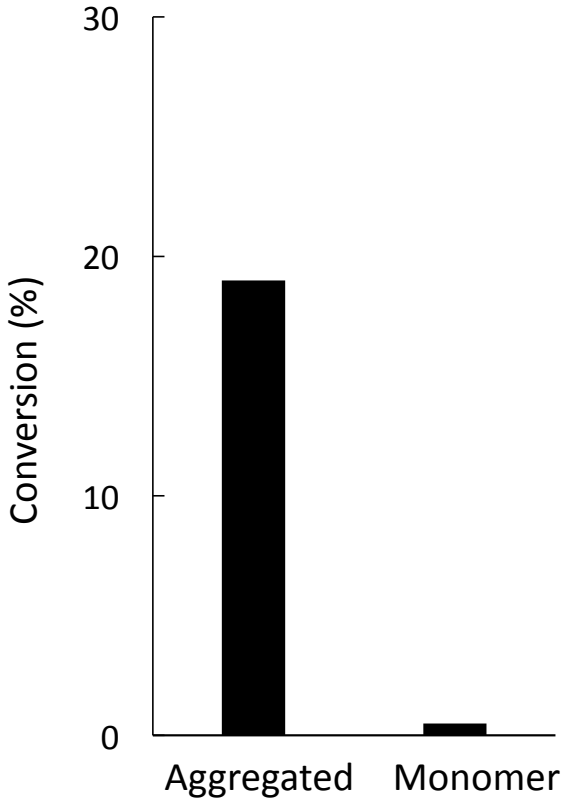



Figure S7 Photo-oxygenation of preaggregated and monomeric tau RDWT using **3**. A sodium acetate buffer solution (pH 7.0) containing the tau RDWT (40 μM, preaggregated and monomeric tau were prepared with/without pre-incubation in the presence of DTT and heparin) and **3** (4 μM) was photoirradiated ($\lambda$ = 660 nm) at 37°C for 5 min, the reaction mixture was treated with trypsin, and analyzed using HPLC. Conversion (%) = 100 × ([sum of peak areas of peptide fragments at *t* = 0 min (preaggregated and monomeric tau, respectively)] – [sum of peak areas of remaining peptide fragments at *t* = 5 min])/ [sum of peak areas of peptide fragments at *t* = 0 min (preaggregated and monomeric tau, respectively)].

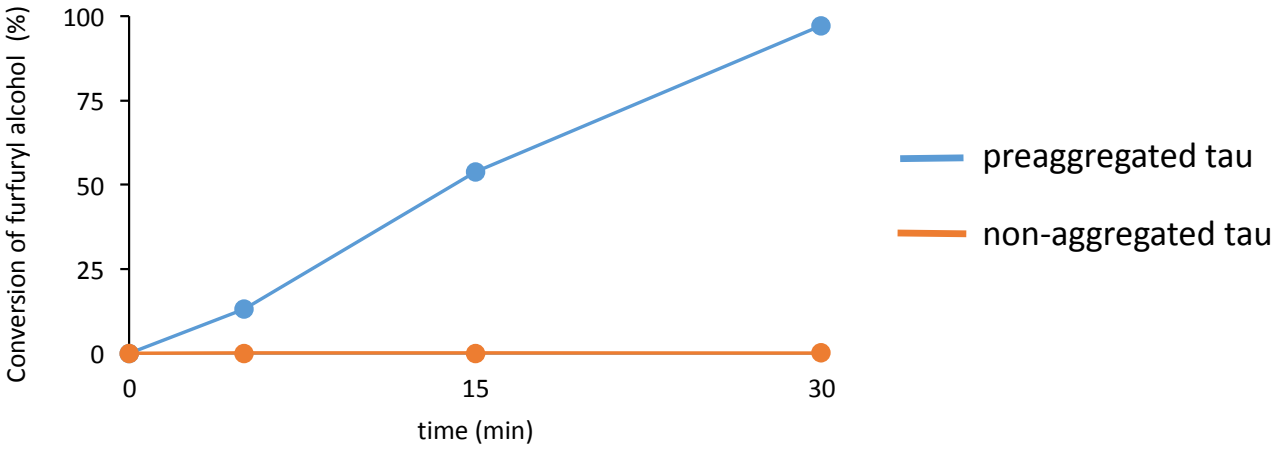



Figure S8 Assessment of the production of $^1O_2$. A sodium acetate buffer solution (pH 7.0) containing the preaggregated or non-aggregated tau RDWT (40 μM), furfuryl alcohol (4 mM), and **3** (4 μM) was photoirradiated ($\lambda$ = 660 nm) at 37°C for arbitrary time periods, and the concentrations of furfuryl alcohol were quantified by UV absorbance using HPLC.

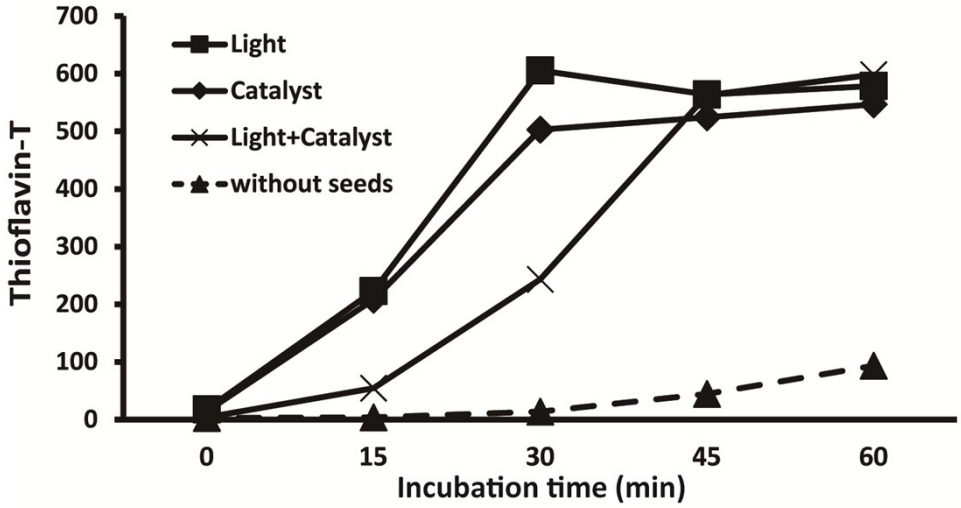



Figure S9 Time-dependent profiles of ThT fluorescences after the addition of each type of seeds. PBS (pH 7.4) containing recombinant tau RDWT (40 μM) were preaggregated in presence of heparin and DTT, and centrifuged. The pellet was resuspended with PBS (pH 7.4), sonicated and used as seeds. These tau seeds were photo-oxygenated in each conditions (with/without photoirradiated ($\lambda$ = 660 nm) for 3 hrs, with/without catalyst **3** (0.3 μM)). These tau seeds with/without photo-oxygenation were added to recombinant tau RDWT monomer solution (40 μM) with 1 mol% ratio as seed (Light, Catalyst, Light+Catalyst in this figure) and incubated for indicated times. As control, recombinant tau RDWT monomer solution without seeds were also incubated (without seeds in this figure).To evaluate the amount of tau fibrils at various times, ThT fluorescence was measured.

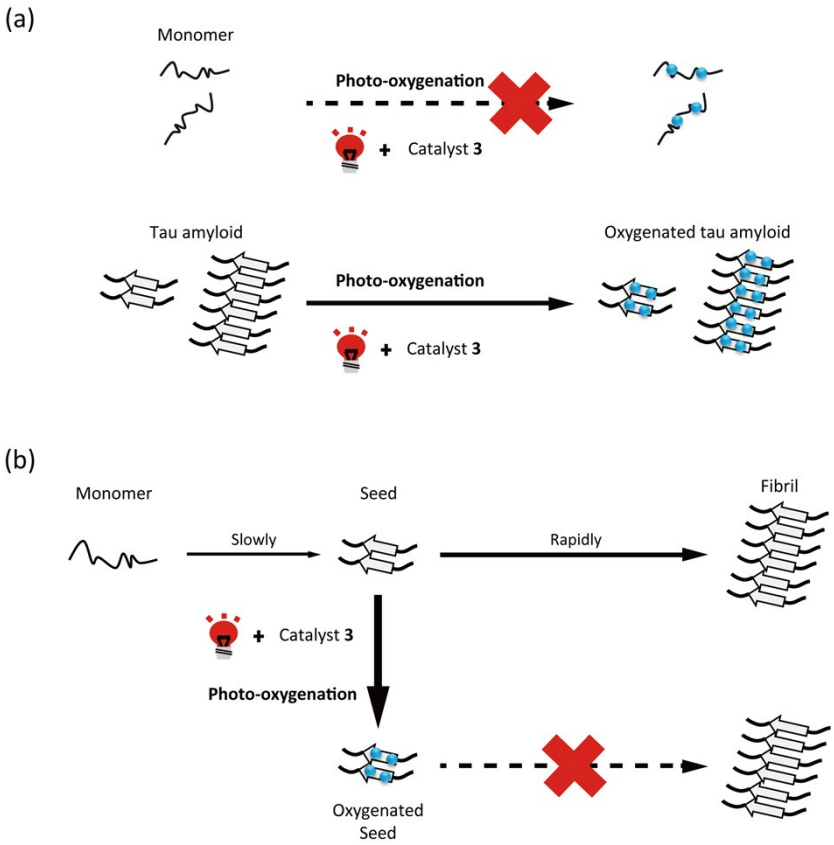



Figure S10. (a) Cartoon of amyloid-selective photo-oxygenation. The photo-oxygenation occur for amyloid, not monomer. (b) Cartoon of inhibition of tau fibril formation by photo-oxygenation. The oxygenated tau seed has lower seeding activity, resulting in the inhibition of tau fibril formation.

**Identification of the oxygenated amino acids**

Table S2[a]

| Fragment | Tau | peptide | $[M+H]^+$ calcd. |
|---|---|---|---|
| 1 | (Met)-244-254 | (M)QTAPVPMPDLK | 1328.6 |
| 2 | 255-257 | NVK | 360.4 |
| 3 | 258-259 | SK | 234.3 |
| 4 | 260-267 | IGSTENLK | 862.0 |
| 5 | 268-274 | HQPGGGK | 680.7 |
| 6 | 275-280 | VQIINK | 714.9 |
| 7 | 281 | K | 147.2 |
| 8 | 282-290 | LDLSNVQSK | 1004.1 |
| 9 | 291-294 | CGSK | 394.5 |
| 10 | 295-298 | DNIK | 489.5 |
| 11 | 299-317 | HVPGGGSVQIVYKPVDLSK | 1981.3 |
| 12 | 318-321 | VTSK | 434.5 |
| 13 | 322-340 | CGSLGNIHHKPGGGQVEVK | 1918.2 |
| 14 | 341-343 | SEK | 363.4 |
| 15 | 344-347 | LDFK | 522.6 |
| 16 | 348-349 | DR | 290.3 |
| 17 | 350-353 | VQSK | 461.5 |
| 18 | 354-369 | IGSLDNITHVPGGGNK | 1579.8 |

[a]Identified oxygenated amino acid residues are shown in red; either or both of two His residues written in orange was/were oxygenated; unidentified residues are shown in green. Note that signals derived from $H^{268}QPGGGK^{274}$ and $H^{299}VPGGGSVQIVYKPVDLSK^{317}$ could not be detected due to the poor ionization potency. Met residue written in parenthesis is the initiating amino acid, not residue in tau.

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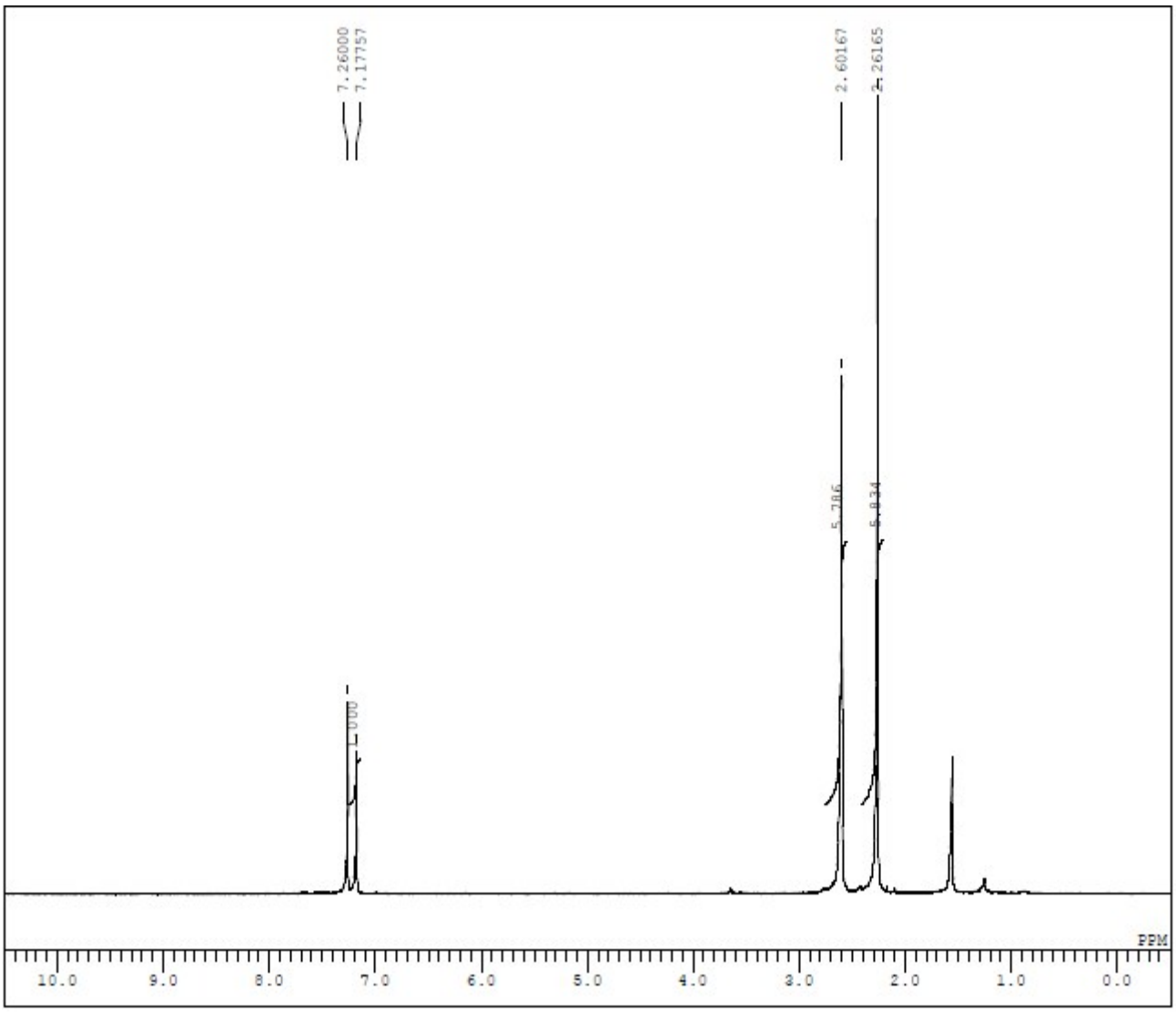
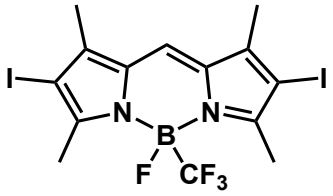
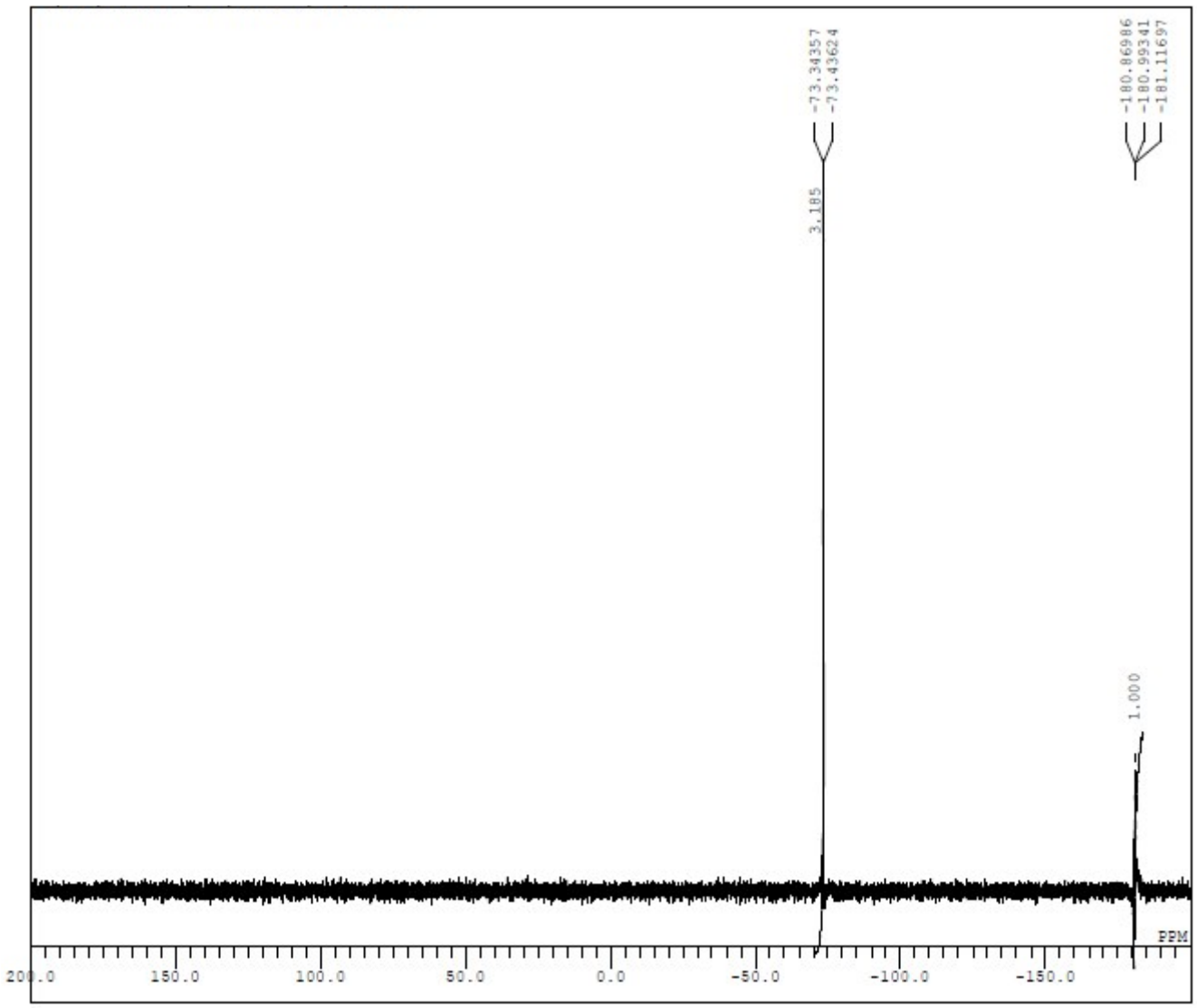
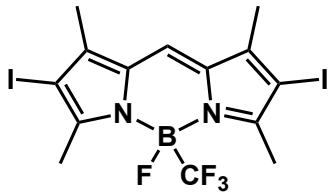


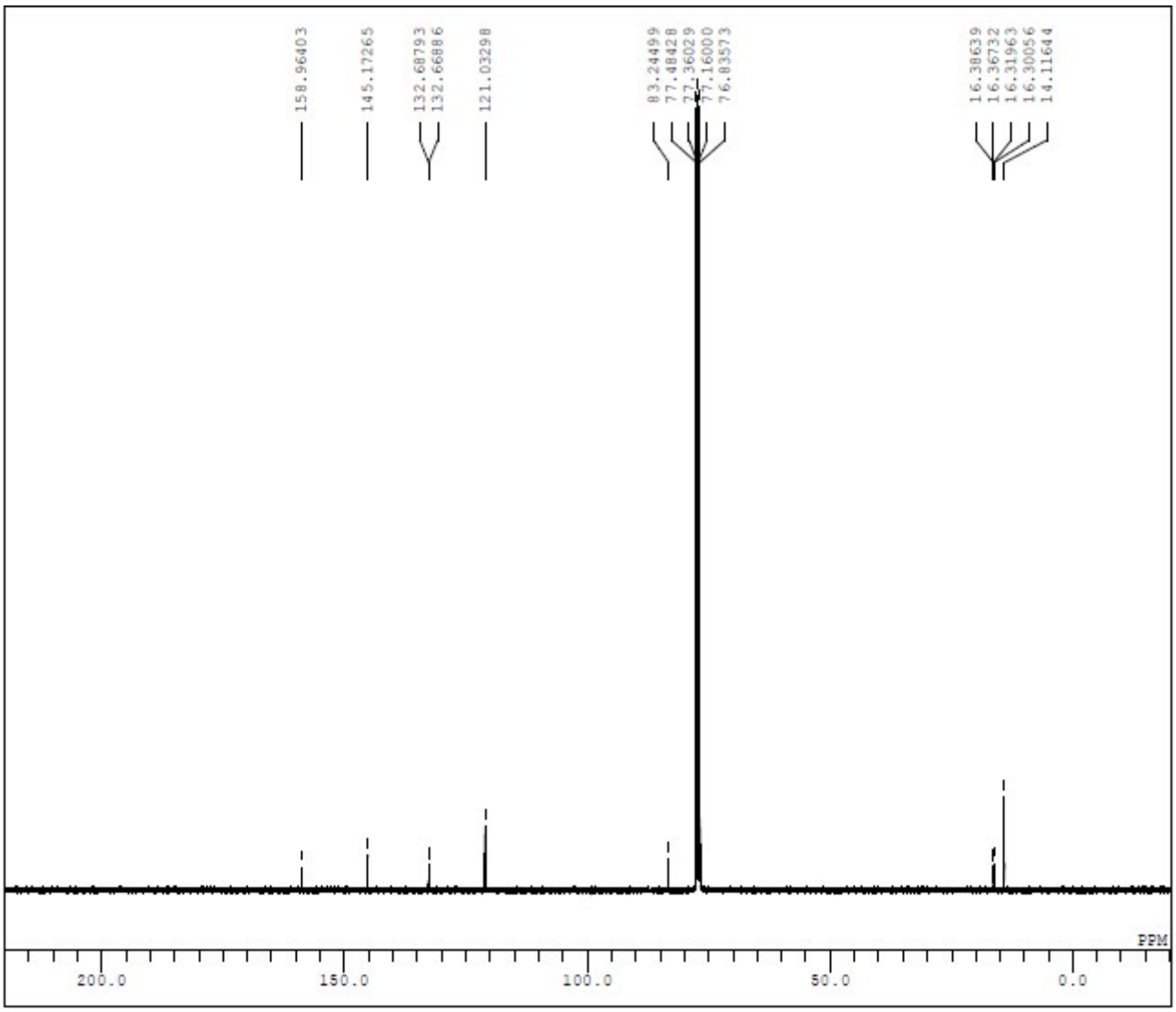



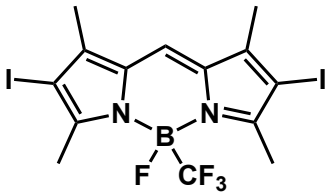

$^{1}$H NMR spectrum



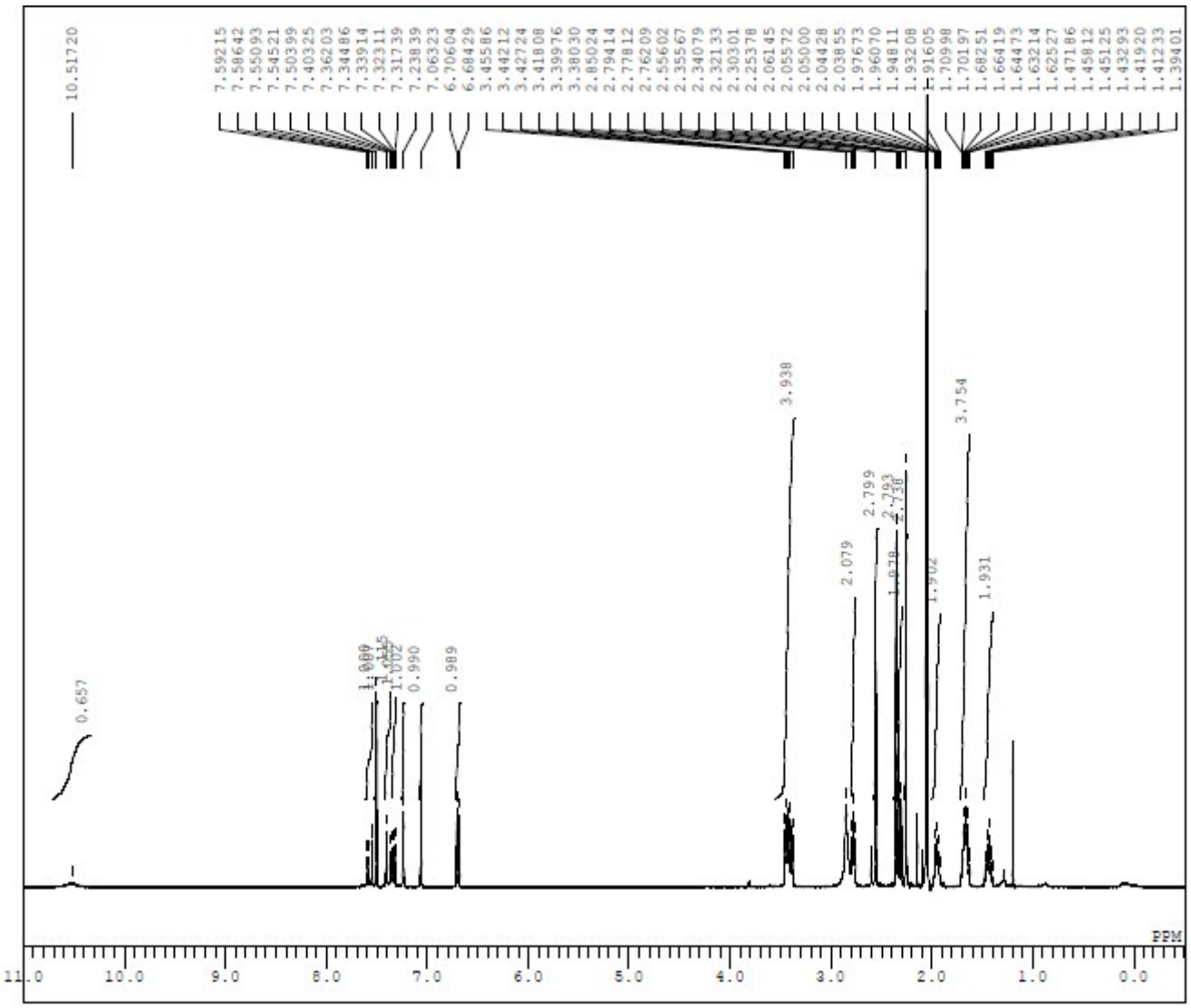



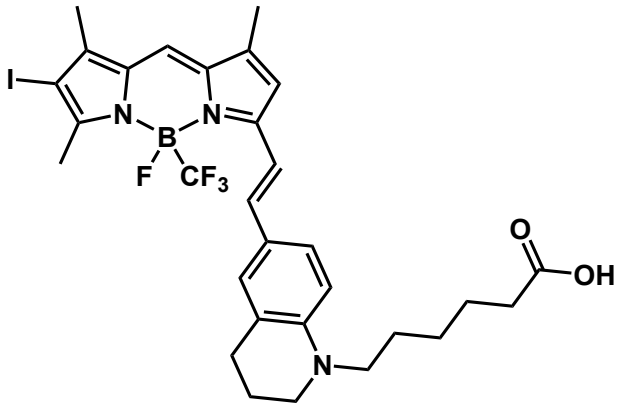

$^{19}$F NMR spectrum



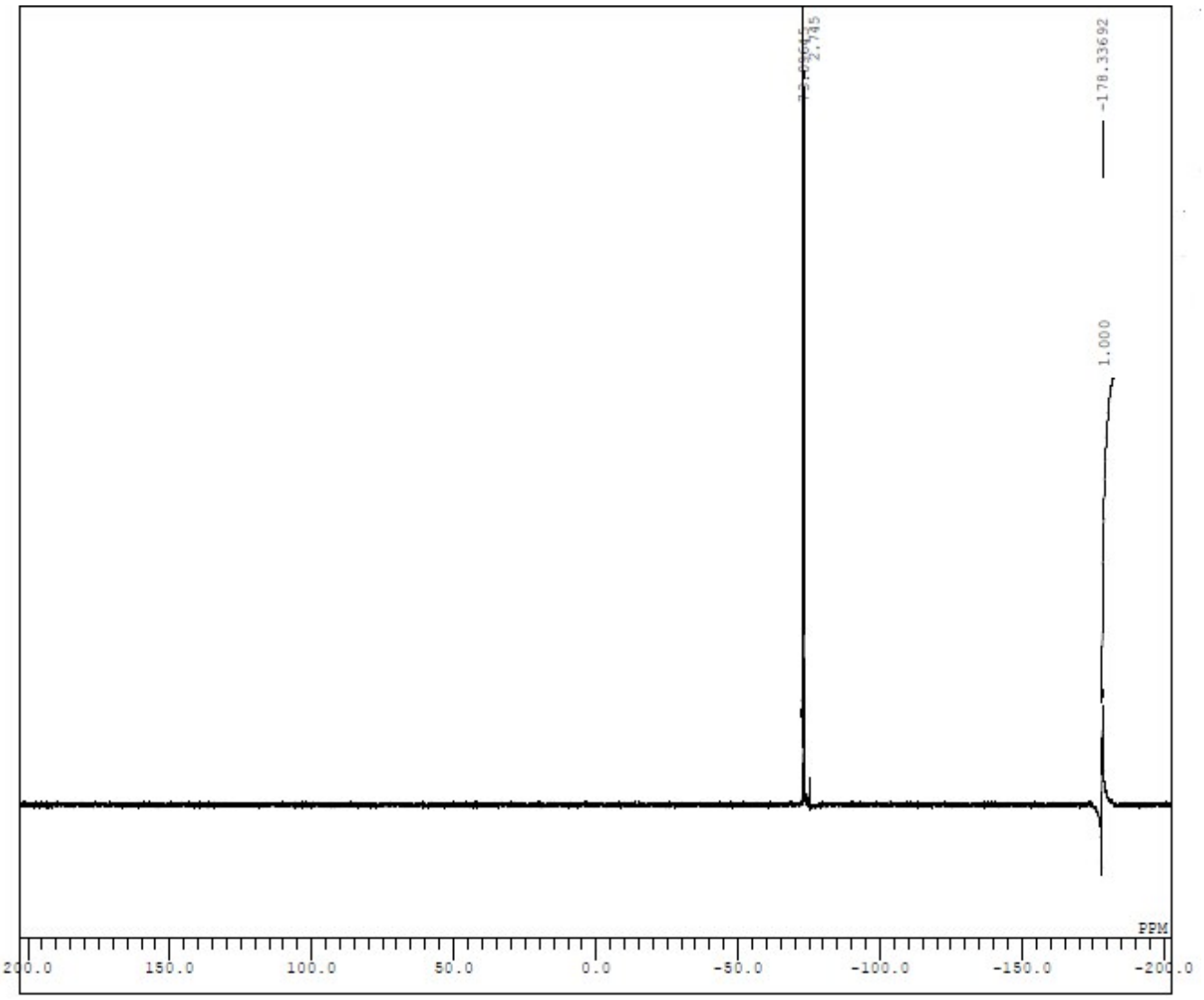



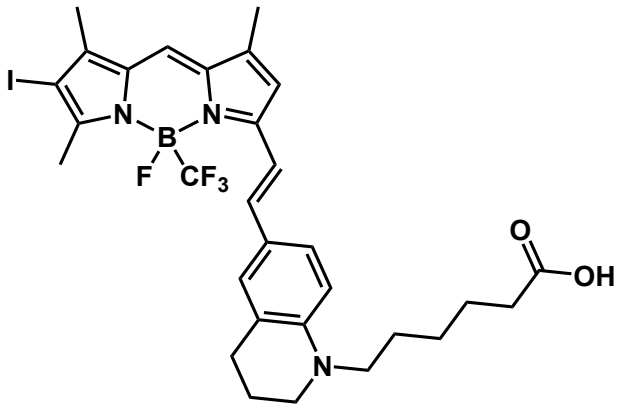

$^{13}$C NMR spectrum



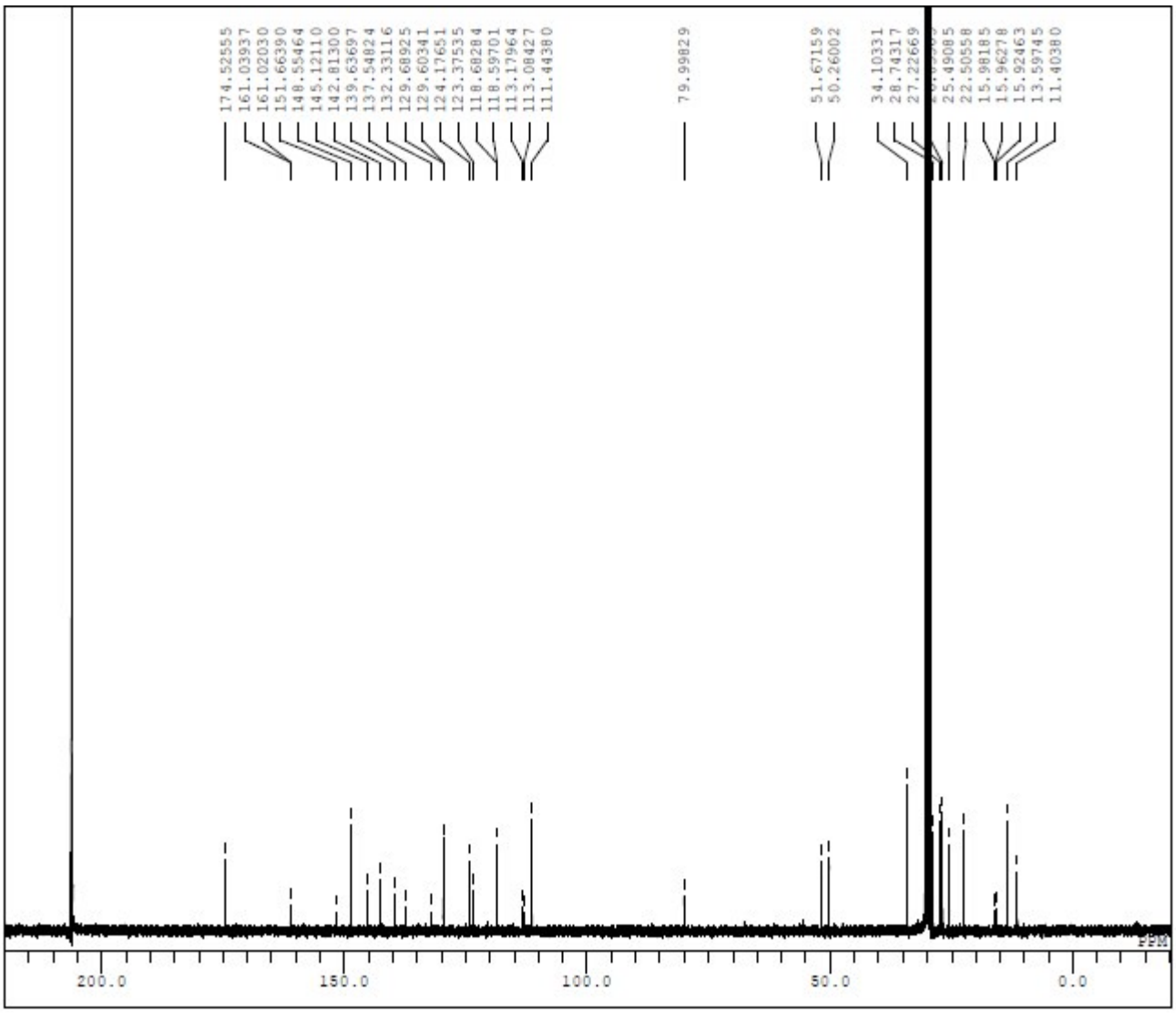



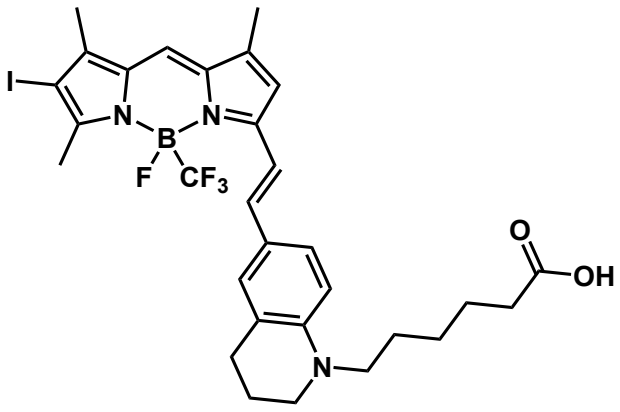

$^{1}$H NMR spectrum




$^{19}$F NMR spectrum




$^{13}C$ NMR spectrum