Supplemental Data

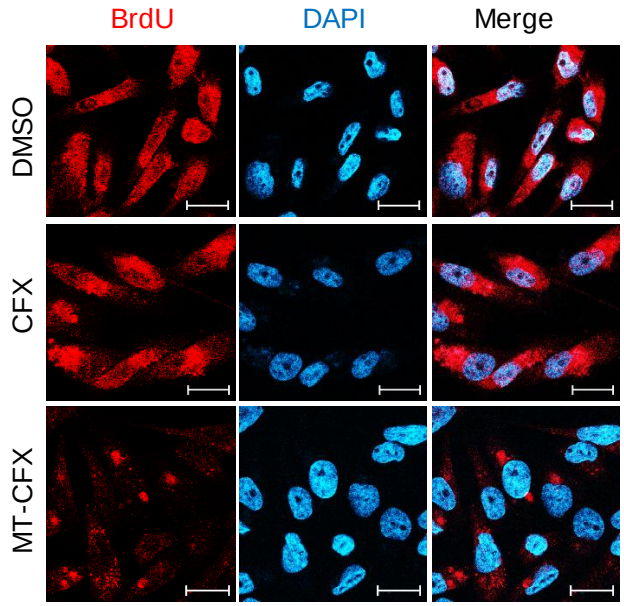


Figure S1. BrdU confocal fluorescence assay, Related to Figure 1.
BrdU incorporation assay in MDA-MB-231 cells incubated with 50 µM **CFX**, **Mt-CFX**, or a control for 24 hours, followed by incubation with BrdU 20 µM for 4 hours. Fixed cells were stained with DAPI (blue channel) and BrdU was visualized using immunolabeling (red channel). Scale bar 20 µm.

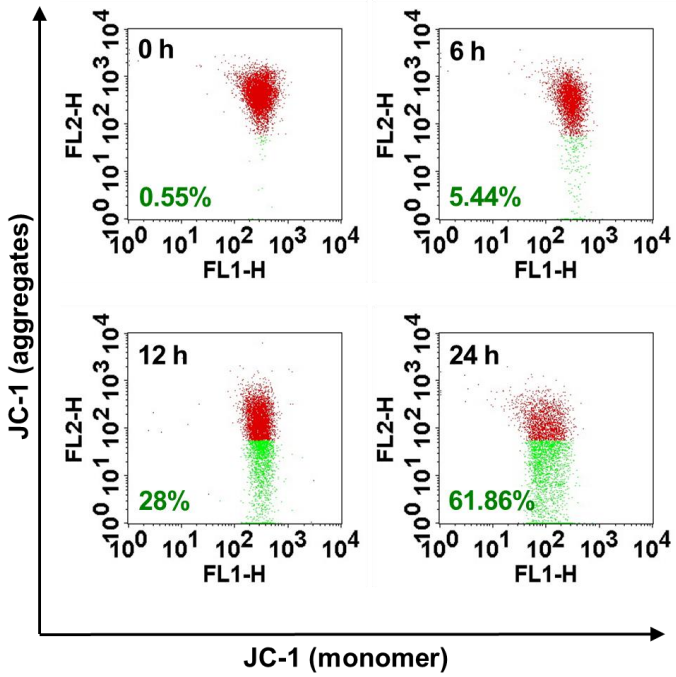


Figure S2. Time-dependent JC-1 flow cytometry, Related to Figure 1.
Proportion of JC-1 monomer fluorescence in a flow cytometry assay of MDA-MB-231 pretreated with 30 µM **Mt-CFX** with incubation times as indicated in the figure. These results are reported in Figure 1D of the main text.

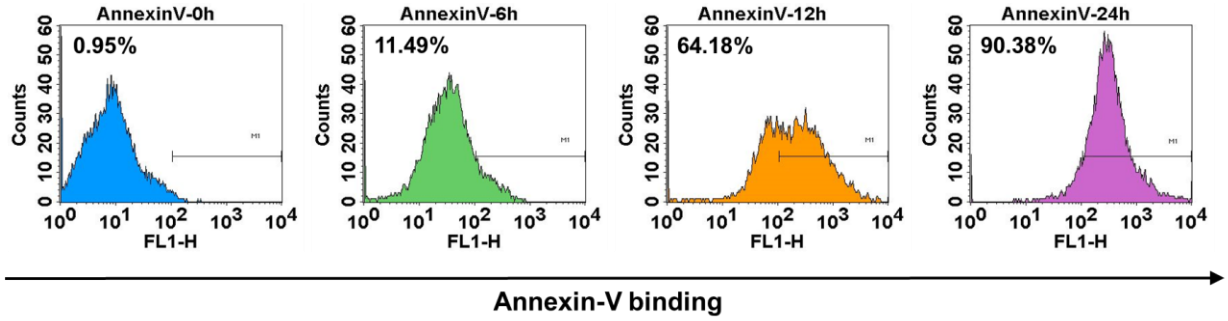


Figure S3. Time-dependent Annexin V flow cytometry, Related to Figure 1.
Individual time depended Annexin V-FITC flow cytometry analysis results of MDA-MB-231 cells pretreated with 30 μM **Mt-CFX** with incubation times as indicated in the figure. The merged data are reported in Figure 1E of the main text.

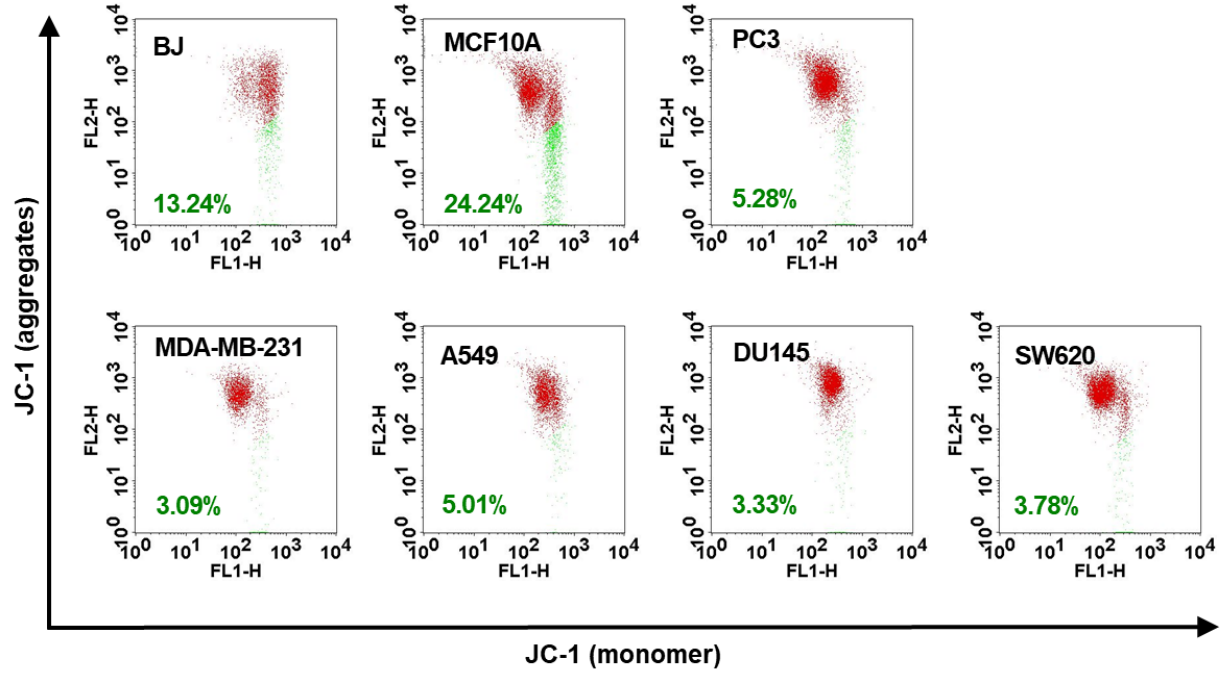


Figure S4. Cell line-dependent JC-1 flow cytometry, Related to Figure 1.
Proportion of JC-1 monomer fluorescence in a flow cytometry assay of various cell lines pretreated. These results are reported in Figure 1G of the main text.

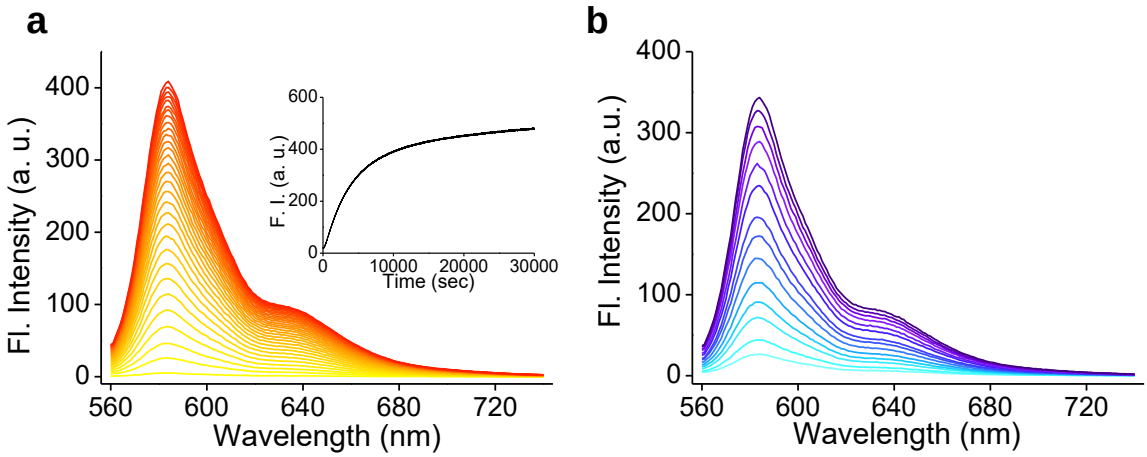


Figure S5. Time and concentration-dependent release of resorufin from Bo-Mt-CFX, Related to Figure 2.
(a) Time-dependent fluorescence spectral evolution of **Bo-Mt-CFX** (5 μM) with H_2O_2 (250 μM) in PBS (pH = 7.8), excitation at 550 nm. (b) Dose dependent fluorescence increase of **Bo-Mt-CFX** (5 μM) upon treatment of H_2O_2 (0 to 1,500 μM) in PBS (pH = 7.8), excitation at 550 nm.

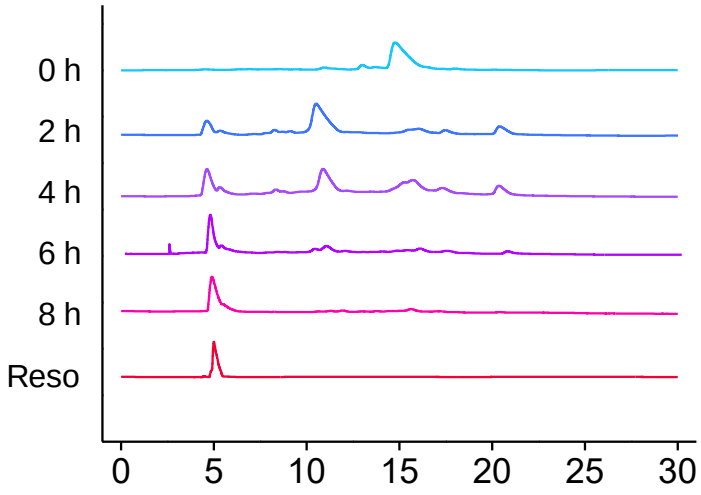


Figure S6. Resorufin time-dependent HPLC assay, Related to Figure 2.
HPLC analyses (absorbance at 550 nm) of **Bo-Mt-CFX** in PBS (pH = 7.8), **Bo-Mt-CFX** incubated with 250 μM H_2O_2 at 37 °C for the times indicated in the figure.

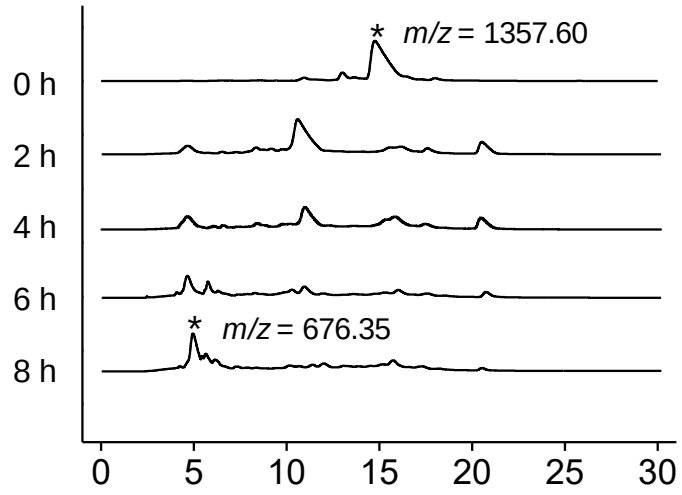


Figure S7. Mt-CFX release HPLC/MS assay, Related to Figure 2.
HPLC analyses (absorbance at 254 nm) of **Bo-Mt-CFX** in PBS (pH = 7.8), **Bo-Mt-CFX** was incubated with 250 µM H_2O_2 at 37 °C for the time incubated in the figure. The mass of the peaks labeled with a star were analyzed and found to correspond to the boronic acids of **Bo-Mt-CFX** (0 h) and **Mt-CFX** (8 h), respectively.

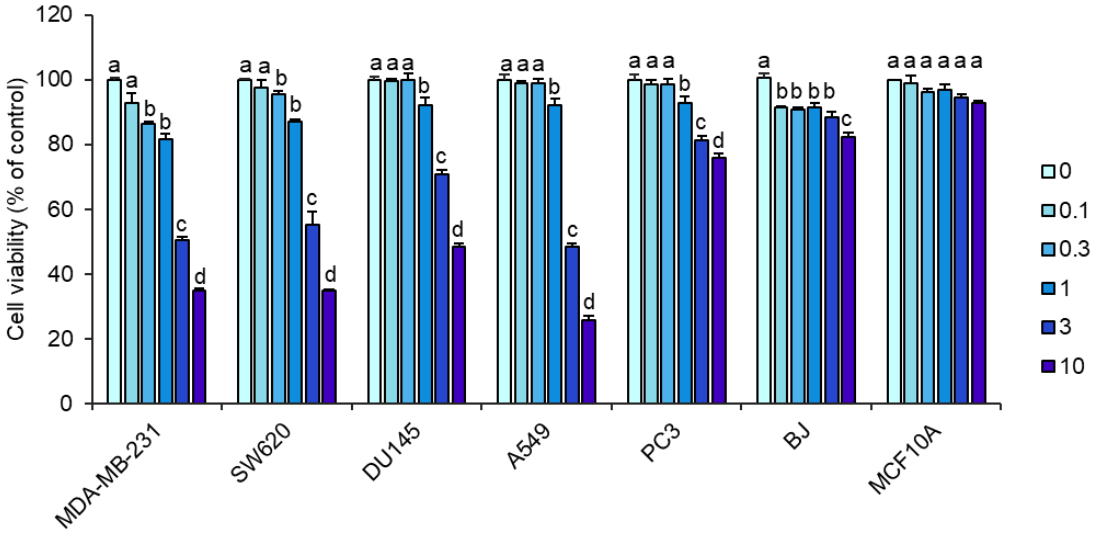


Figure S8. Cell viability of Bo-Mt-CFX treated cells, Related to Figure 2.
Concentration-dependent cell-viability of different cell lines incubated with **Bo-Mt-CFX**, as indicated in the figure, as determined by a CytoTox96 Assay (72h). Cytotoxicity experiments were carried out trice in triplicate wells. Data are represented as mean ± SEM (n = 9). Statistical significance was determined using a one-way ANOVA test with post-hoc Bonferroni test. Different letters signify data that are statistically different ($p < 0.05$).
Note the similarity between the cell type selectivity for **Bo-Mt-CFX** and **Mt-CFX** (Main text, Figure 1F), while **Bo-Mt-CFX** exhibits an increased cytotoxicity *vs* **Mt-CFX** as the combined result of ROS induction and GSH depletion (*vide infra*).

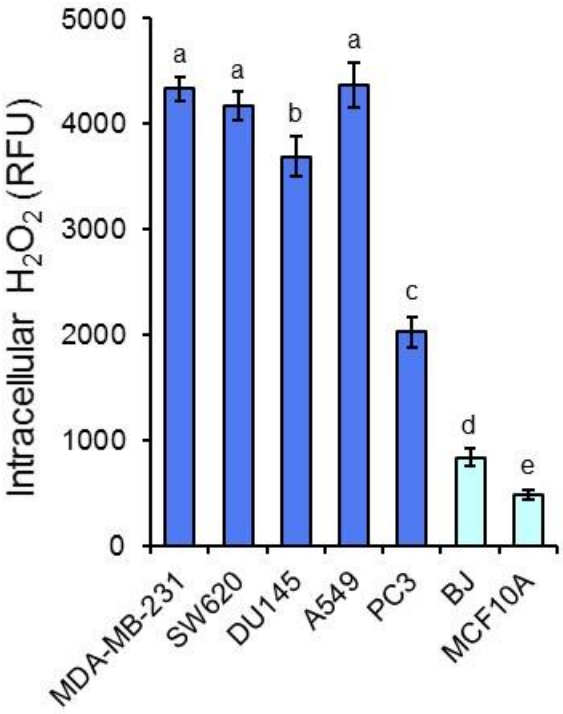


Figure S9. Endogenous H_2O_2 levels in various cell lines, Related to Figure 2.
Endogenous H_2O_2 levels in various cell lines were determined using the Intracellular H_2O_2 Assay Kit. Data are represented as mean ± SEM (*n* = 3).
Statistical significance was determined using a one-way ANOVA test with post-hoc Bonferroni test. Different letters (e.g., a–d) signify data which are statistically different ($p < 0.05$). Note the correlation between the endogenous H_2O_2 levels and the susceptibility to **Bo-Mt-CFX** (Figure S8).

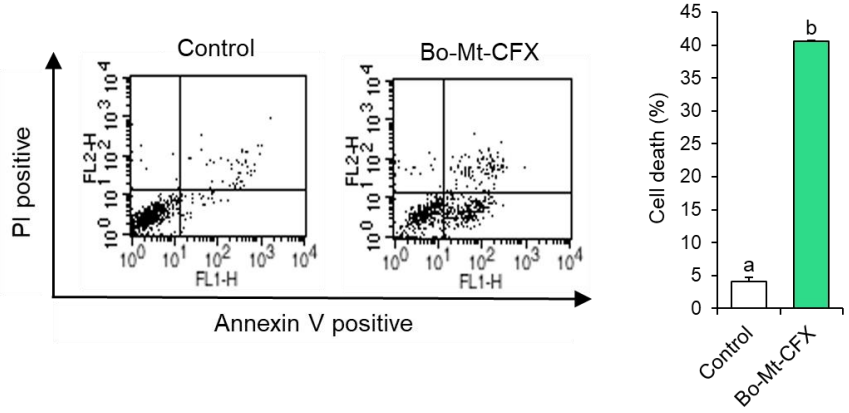


Figure S10. Annexin V-FITC/PI flow cytometry, Related to Figure 2.
(a) Flow cytometry plots of MDA-MB-231 treated with 3 µM **Bo-Mt-CFX** for 48 hours. (b) Proportion of annexin V positive cells. Data are represented as mean ± SEM (*n* = 3). Statistical significance was determined using a one-way ANOVA test with post-hoc Bonferroni test. Different letters signify data that are statistically distinct ($p < 0.05$).
The assay provides support for the contention that **Bo-Mt-CFX** induces apoptotic cell death in MDA-MB-231 cells.

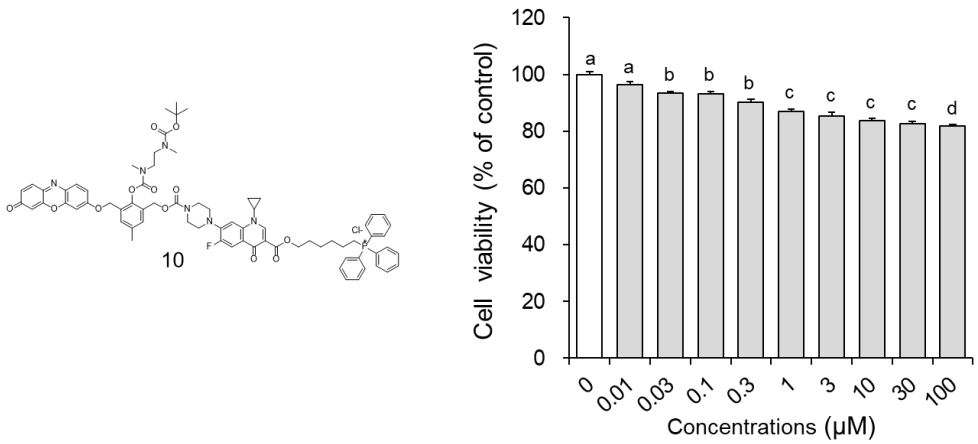


Figure S11. Cell viability of 10-treated cells, Related to Figure 2.

Concentration-dependent cell-viability of MDA-MB-231 incubated with **10**, as determined by a CytoTox96 Assay (72 h). Cytotoxicity experiments were carried out trice in triplicate wells. Data are represented as mean ± SEM (n = 9). Statistical significance was determined using a one-way ANOVA test with post-hoc Bonferroni test. Different letters signify data that are statistically distinct ($p < 0.05$).

Compound **10**, missing a H_2O_2 labile triggering group, does not cause a strong reduction in cytotoxicity in MDA-MB-231 cells, lending support to the mode of action for **Bo-Mt-CFX** proposed in Figure 2A of the main text.

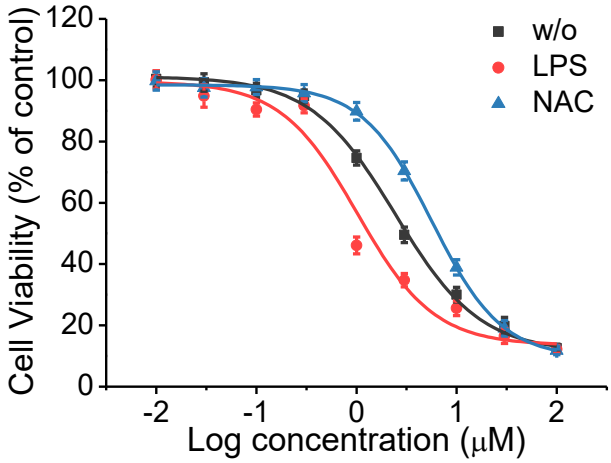


Figure S12. Cell viability of Bo-Mt-CFX -treated cells as a function of cellular ROS concentration, Related to Figure 2.

Cell-viability of MDA-MB-231 incubated with **Bo-Mt-CFX**, in the presence of a ROS inducer (LPS) or quencher (NAC), as determined by a CytoTox96 Assay (72 h). Data are represented as mean ± SEM (n = 9).

The correlation between higher ROS levels and increased cytotoxicity, as also observed in Figure S9, is taken as evidence that **Bo-Mt-CFX** is activated by cellular ROS.

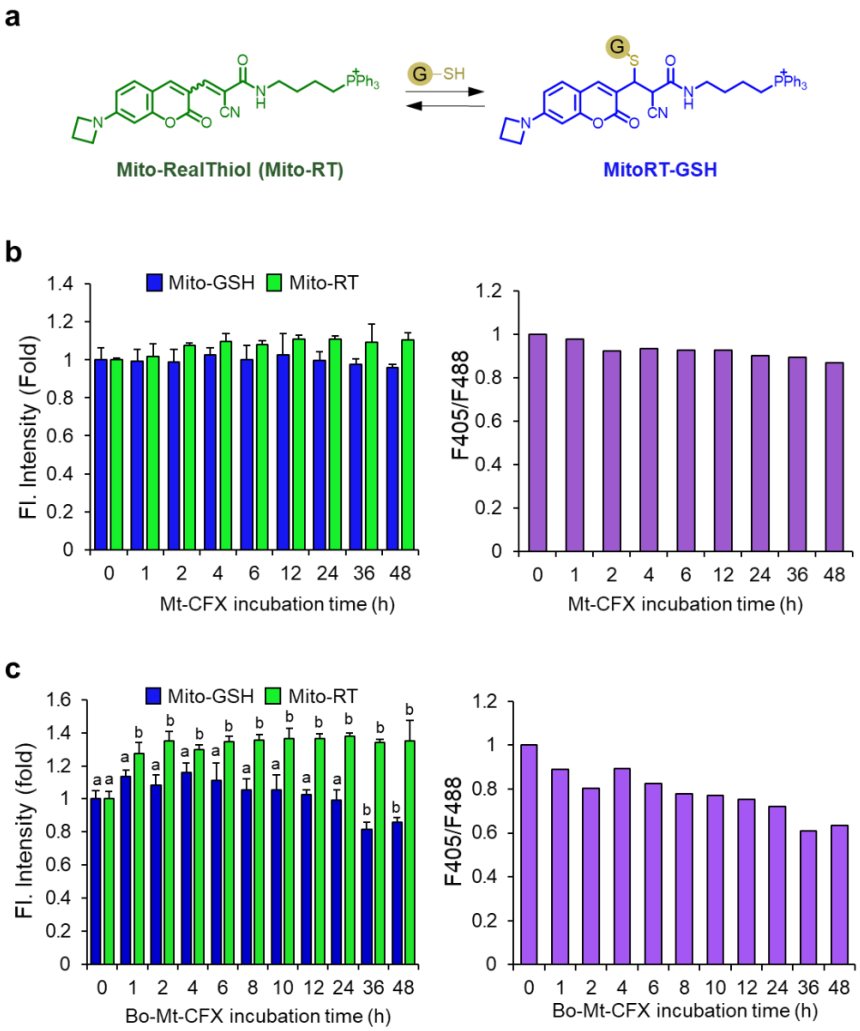


Figure S13. Mito-RealThiol fluorescence assessment for Mt-CFX and Bo-Mt-CFX, Related to Figure 2.
(a) GSH sensing mechanism of the Mito-RealThiol, the unreacted probe exhibits fluorescence at 488 nm, while the GSH adduct is fluorescent at 405 nm. (b) Fluorescence of MDA-MB-231 incubated with **Mt-CFX** as indicated in the figure and the corresponding fluorescence ratios. (c) Fluorescence of MDA-MB-231 incubated with **Bo-Mt-CFX** as indicated in the figure and the corresponding fluorescence ratios. Mito-RealThiol (100 nM) was added to the cells 30 minutes prior to fluorescence intensity determination. Data are represented as mean ± SEM (*n* = 6). Statistical significance was determined using a one-way ANOVA test with post-hoc Bonferroni test. Different letters signify data that are statistically distinct ($p < 0.05$).
Using a mitochondria-selective fluorescent GSH sensor,[S1] we determined that the total GSH levels in the mitochondria are depleted to a greater extent in **Bo-Mt-CFX**-loaded cells than in cells incubated with **Mt-CFX**. This observation could be a result of quinone methide generation,[S2] which is thought to occur upon activation of **Bo-Mt-CFX** (main text, Figure 2A). The greater depletion of the GSH levels could also serve to rationalize the stronger decrease in cell viability seen for this DDS conjugate relative to free **Mt-CFX**.

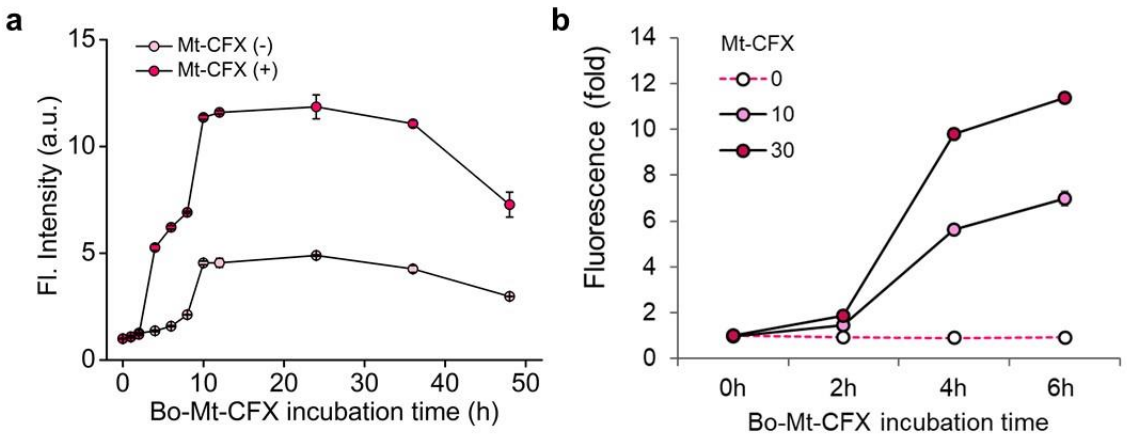


Figure S14. Concentration- and time-dependent self-amplification effect, Related to Figure 2.
(a) Fluorescence intensity as determined by a multi-detection microplate reader (n = 3) incubated with 10 µM **Bo-Mt-CFX** alone or 10 µM **Bo-Mt-CFX** and 10 µM **Mt-CFX**, fluorescence normalized at t = 0 hour. (b) Resorufin fluorescence obtained using a Multi-detection Microplate Reader System from MDA-MB-231 cells incubated with 10 µM **Bo-Mt-CFX** and 0, 10 or 30 µM **Mt-CFX**. (*n* = 2). Data are represented as mean ± SEM.
Because the fragmentation of **Bo-Mt-CFX** and drug activation is both dependent on, and resulting in, intracellular ROS production, this probe benefits from a self-amplification effect. After a lag time of around 8 hours, a sigmoidal time-dependent fluorescence enhancement can be observed in the case of MDA-MB-231 cells incubated with 10 µM **Bo-Mt-CFX** (Figure S13A). Addition of 10 µM **Mt-CFX** to 10 µM solutions of **Bo-Mt-CFX** shortened the lag time. As expected, the extent of the effect is concentration dependent (Figure S13B).

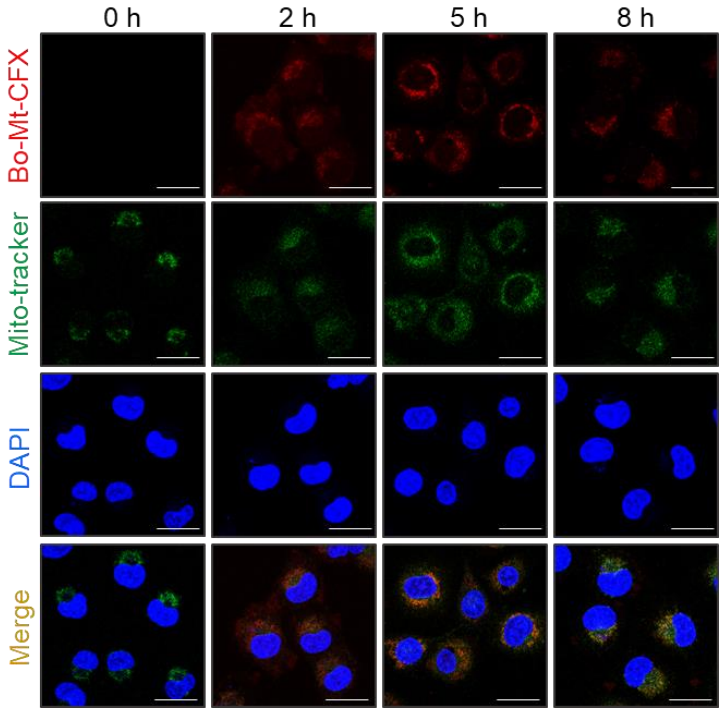


Figure S15. Time-dependent self-immolative activation of Bo-Mt-CFX, Related to Figure 2.
Resorufin fluorescence (Red channel) in MDA-MB-231 cells pretreated with 3 µM **Bo-Mt-CFX** for the time indicated in the figure. Cells were stained 30 min prior to imaging with 500 nM Mitotracker green (green channel) and 100 nM DAPI (blue channel). Scale bar 20 µm.

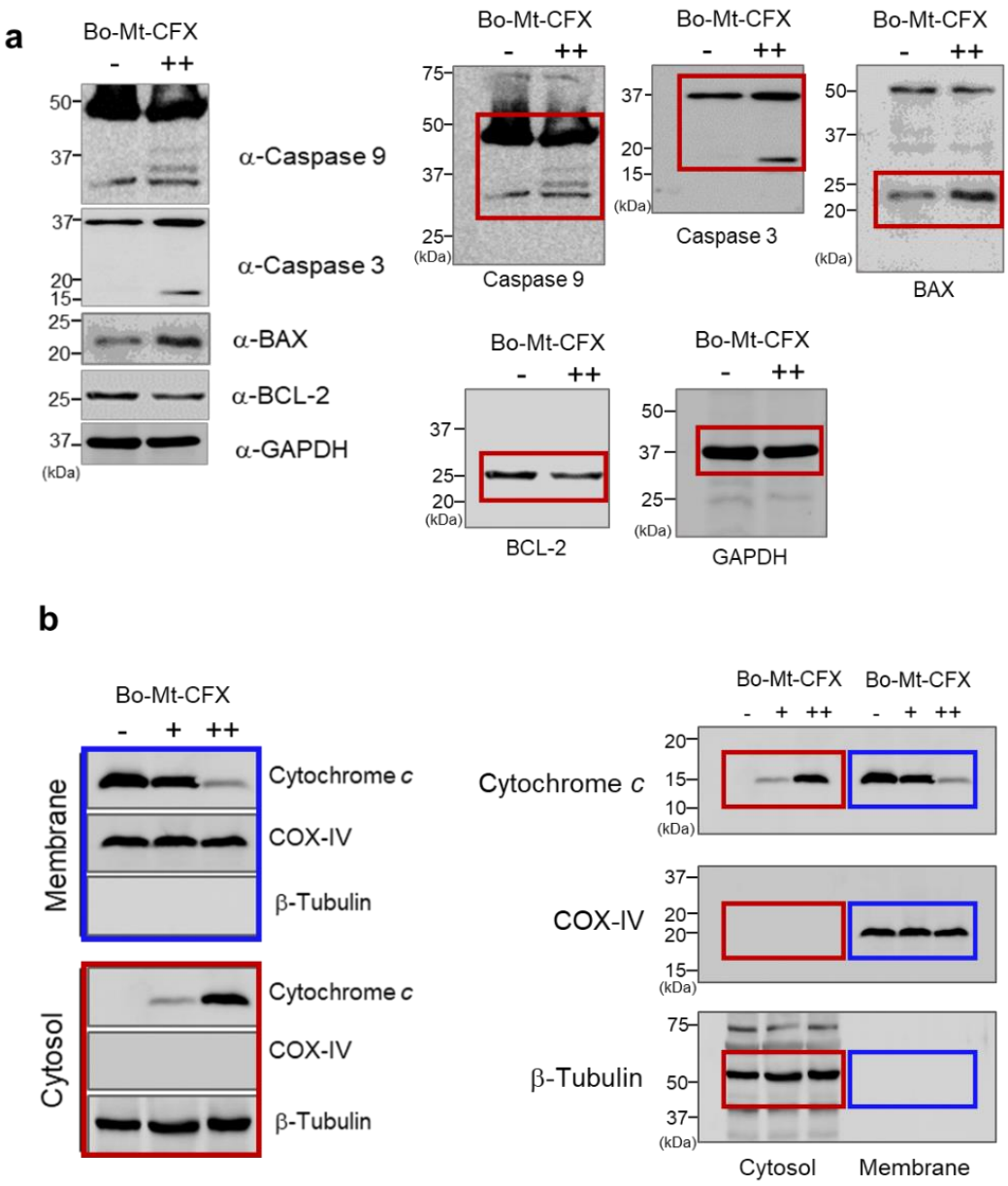


Figure S16. Full-length western blots corresponding to Figures 2C and 2D in the main text.
a: Figure 2C. **b**: Figure 2D.

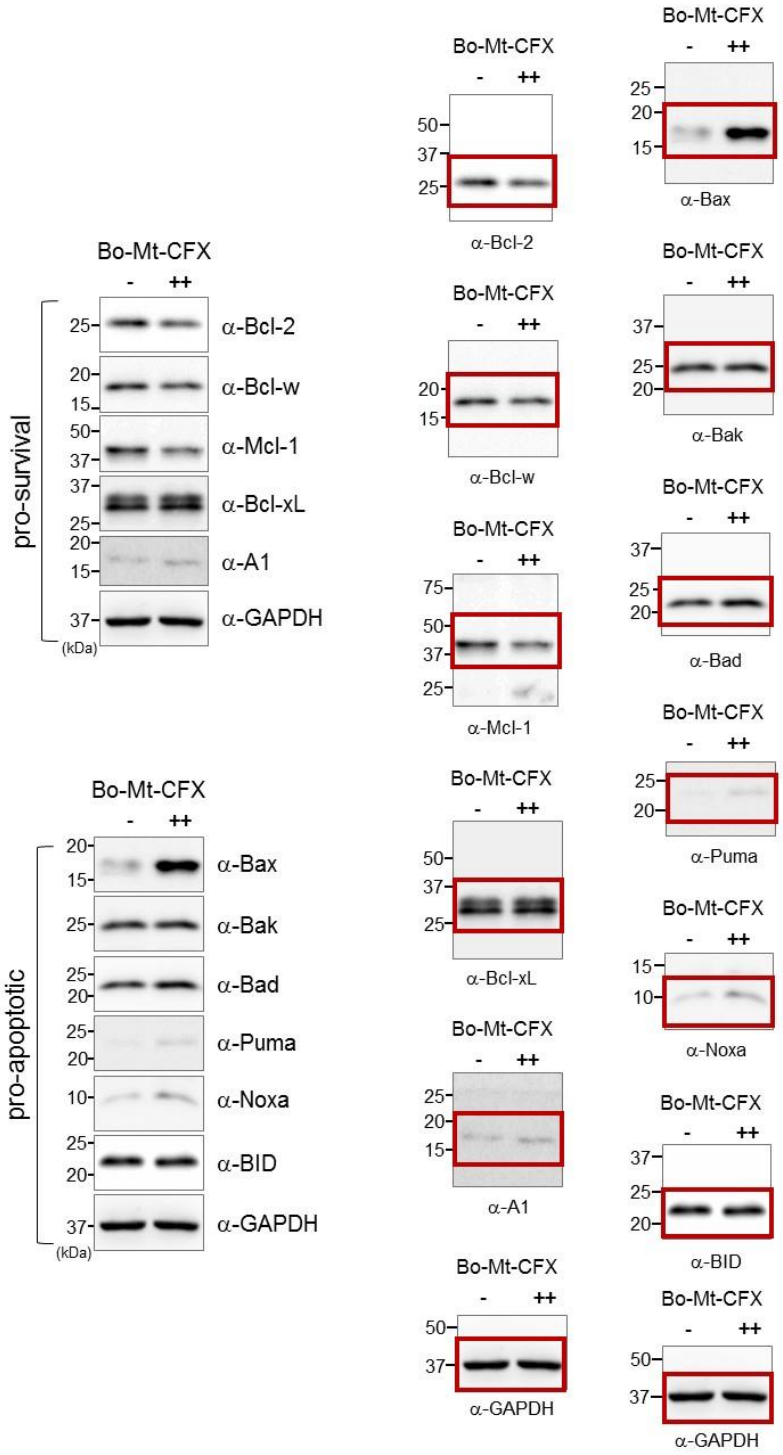


Figure S17. Western blot analysis of the Bcl-2 protein family in MDA-MB-231 cells, grouped by their pro-apoptotic or anti-apoptotic function, Related to Figure 2.

Cells were treated with **Bo-Mt-CFX** (10 µM) or DMSO (1%). Sections of the blots used to construct the composite images are indicated.

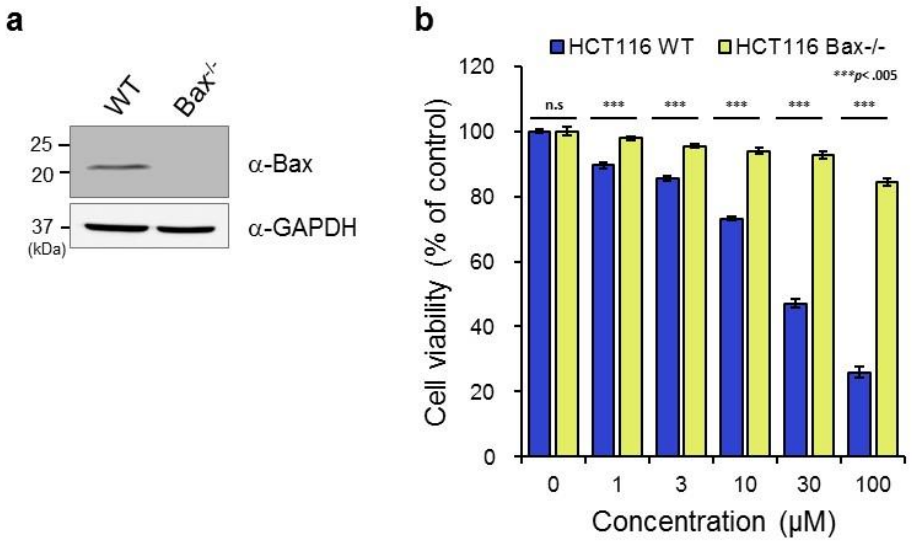


Figure S18 Influence of BAX on Mt-CFX induced apoptosis, Related to Figure 2.
(a) Western blot analysis showing expression of Bax in HCT116 WT and HCT116 $Bax^{-/-}$ cells. (b) Concentration dependent cell viability of HCT116 WT and HCT116 $Bax^{-/-}$ cells incubated with indicated concentrations (0–100 μM) of **Mt-CFX**. Cell viability was determined by means of a cyto-Tox96 assay (72 h). Data are represented as mean ± SEM (*n* = 9). Statistical significance was determined using a Student t-test ***P<0.005.

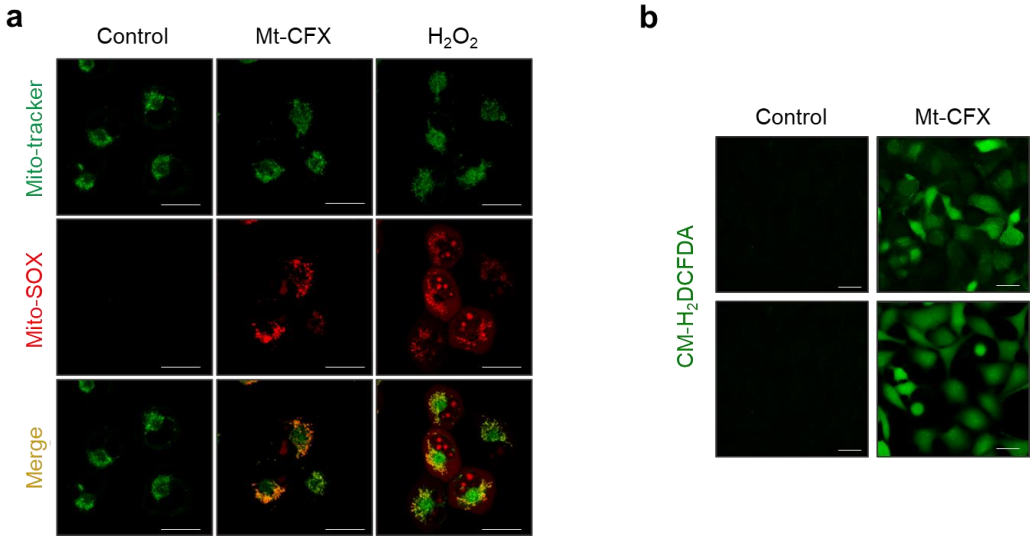


Figure S19. Confocal imaging of ROS generation, Related to Figure 3.
(a) Mito-SOX fluorescence (Red channel) in MDA-MB-231 cells was recorded 15 minutes after the addition of the dye to cells pretreated with 30 μM **Mt-CFX**, 100 μM H_2O_2 or a control and Mitochondrial localization was demonstrated using a MitoTracker™ Green FM pretreatment for 30 minutes prior to imaging (green channel). Note that while Mito-SOX fluorescence in **Mt-CFX** treated cells was largely confined to the mitochondria, H_2O_2 induced fluorescence throughout the cell, including the nuclear compartment. (b) CM-H_2DCFDA fluorescence (green channel) recorded 30 minutes after the addition of the dye in **Mt-CFX** treated MDA-MB-231 cells versus a control. Scale bar 20 μm.

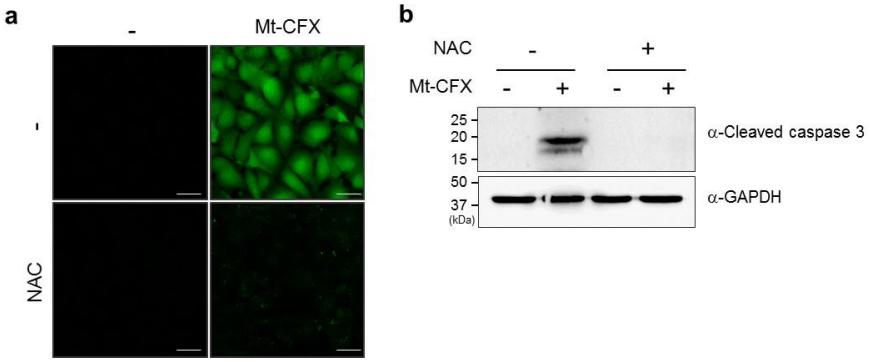


Figure S20. The effect of NAC on the ROS burden and caspase activation in Mt-CFX-challenged cells, Related to Figure 3.

(a) Confocal microscopy imaging of MDA-MB-231 with abCM-H2DCFDA ROS marker. (b) Western blot analysis. MDA-MB-231 cells were pretreated with or without NAC (100 µM) and then treated with **Mt-CFX** (30 µM) or DMSO (1%).

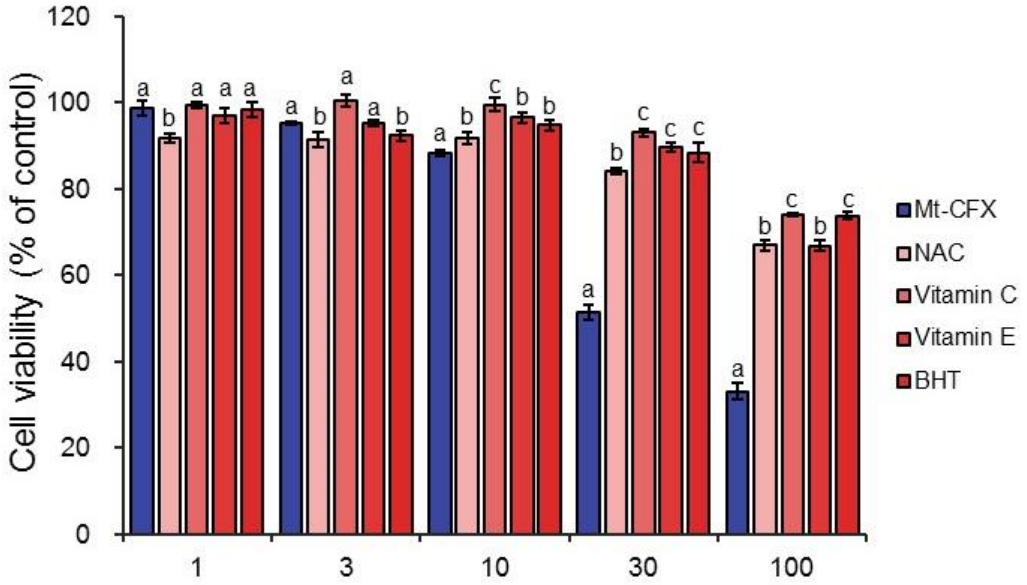


Figure S21. Concentration dependent cell viability of MDA-MB-231 cells pretreated with various antioxidants, Related to Figure 3.

Cells were pretreated with (NAC, Vitamin C (ascorbic acid), Vitamin E (α-tocopherol) and BHT) and then incubated with **Mt-CFX** or DMSO (1%). Cell viability was determined by a cyto-Tox96 assay (72h). Data are represented as mean ± SEM (*n* = 6). Statistical significance was determined using a one-way ANOVA test with post-hoc Bonferroni test within each concentration group. Different letters (e.g. a–d) signify data which are statistically different ($p < 0.05$).

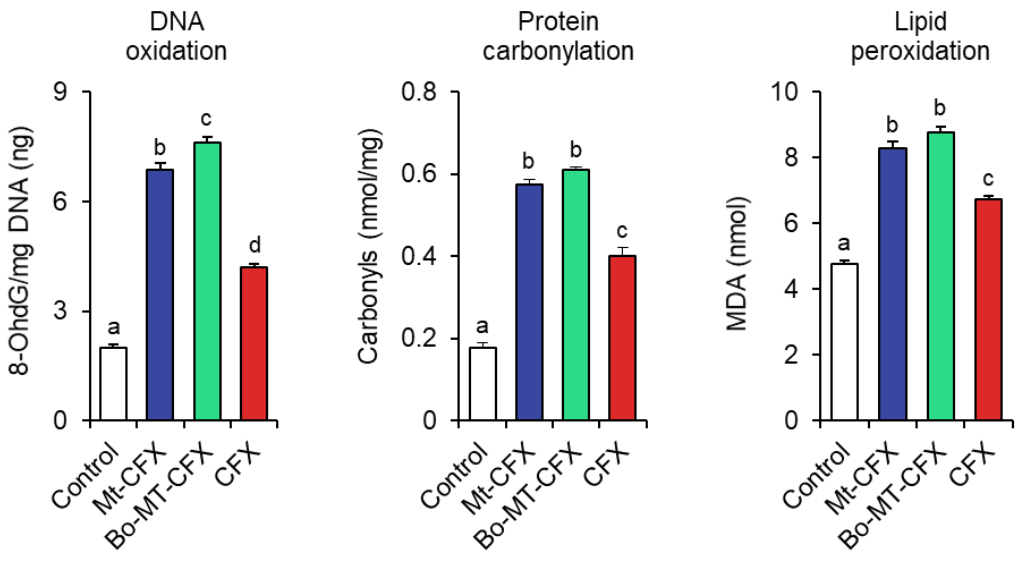


Figure S22. Treatment-induced oxidative damage to DNA, proteins and lipids, Related to Figure 3.
ELISA DNA oxidation and protein carbonylation assay results of MDA-MB-231 cells treated with 30 µM **Mt-CFX**, 30 µM **CFX** or 3 µM **Bo-Mt-CFX** as well as control samples and colorimetric malondialdehyde (MDA) assay results, as a measure of lipid peroxidation, of similarly treated MDA-MB-231 cells. Data are represented as mean ± SEM (*n* = 6). Statistical significance was determined using a one-way ANOVA test with post-hoc Bonferroni test. Different letters signify data that are statistically distinct ($p < 0.05$).

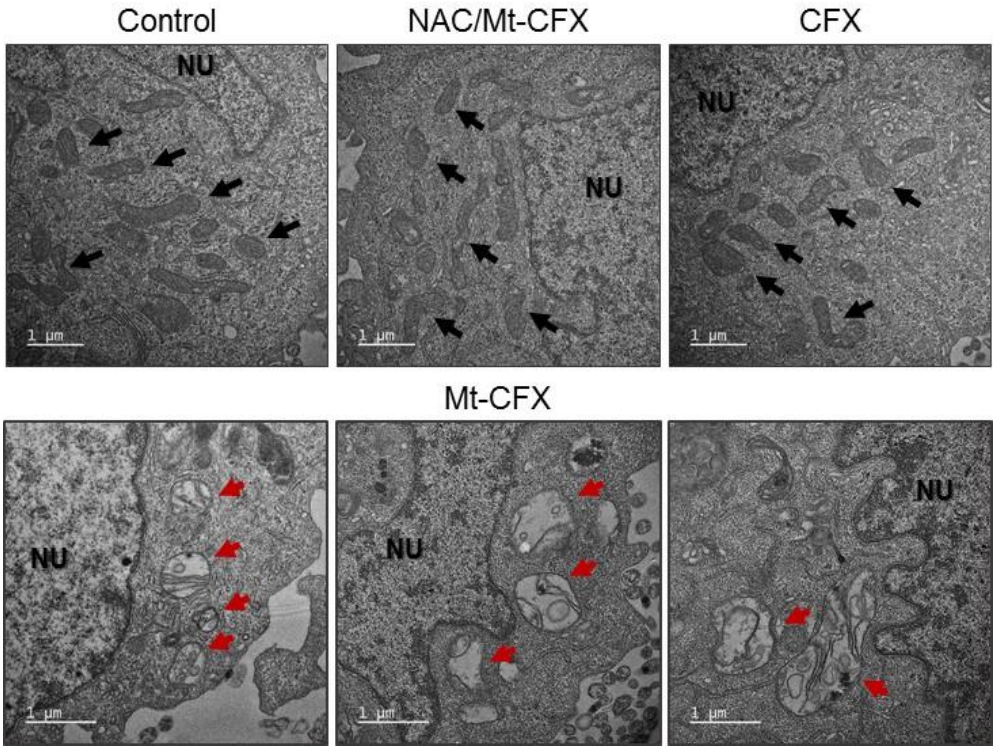


Figure S23. Bio-TEM imaging of MDA-MB-231 cells, Related to Figure 3.
Cells were treated with the indicated agents **Mt-CFX** (30 µM), **CFX** (30 µM), with or without pretreatment with NAC (100 µM), and DMSO (1%). Black arrows indicate normal mitochondria while red arrows indicate abnormally deformed and swollen mitochondria. Nu: Nucleus.

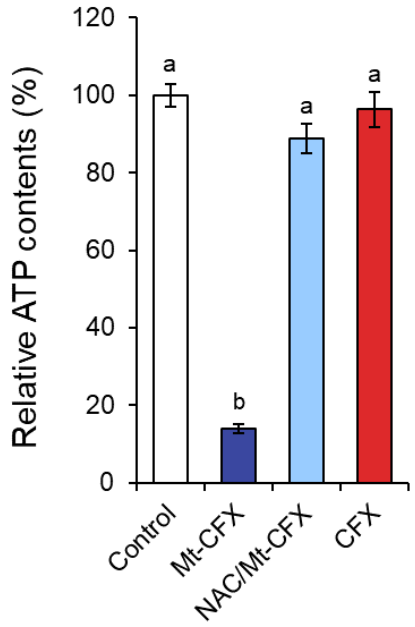


Figure S24. Cellular ATP content, Related to Figure 3.
MDA-MB-231 cells were pretreated with the chemicals indicated in the figure **Mt-CFX** (30 µM), **CFX** (30 µM), NAC (100 µM) or DMSO (1%). ATP levels were measured using an ATP Bioluminescence Assay Kit HS II. Data are represented as mean ± SEM (*n* = 3). Statistical significance was determined using a one-way ANOVA test with post-hoc Bonferroni test. Different letters (e.g. a–d) signify data which are statistically different ($p < 0.05$).

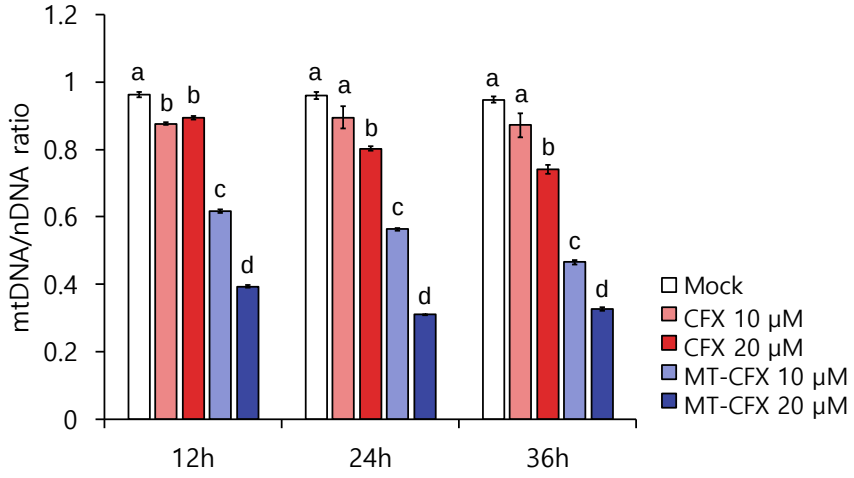


Figure S25. Time and dose-responsive effect of CFX and Mt-CFX on the MtDNA/nDNA ratios, Related to Figure 3.
Time-dependent expression levels (PCR) of ND1 and GAPDH as mitochondrial and nuclear housekeeping proteins, respectively. Data are represented as mean ± SEM (*n* = 3). Statistical significance was determined using a one-way ANOVA test with post-hoc Bonferroni test. Different letters signify data that are statistically distinct ($p < 0.05$).

Table S1. Mitochondrial damage related genes primers

Genes	5' sequences	3' sequences
Nu-16sRNA	GGG ATA ACA GCG CAA TCC TA	CCT GGA TTA CTC CGG TCT GA
Mt-ND1	ATA CCC CCG ATT CCG CTA CGA C	GTT TGA GGG GGA ATG CTG GAG A
ERCC1	CTA CGC CGA ATA TGC CAT CTC	GTA CGG GAT TGC CCC TCT G
DDB2	ACC TCC GAG ATT GTA TTA CGC C	TCA CAT CTT CTG CTA GGA CCG
POLγ	GCTGCACGAGCAAATCTTCG	GTCCAGGTTGTCCCCGTAGA
Taq1 control	ACA GTT TAT GTA GCT TAC CTC C	TTG CTG CGT GCT TGA TGC TTG
Taq1 1216	AAC TGC TCG CCA GAA CAC TAC	GGG CTA CAC CTT GAC CTA AC
nuclear long PCR	GAG GCA GGA CTC AGG ACA AG	CTC CTC ACA TGC AGC TAC CA
nuclear short PCR	GAG GCA GGA CTC AGG ACA AG	GGA TGC CTC AGG GAC CAG
mito long PCR	CGC CTC ACA CTC ATT CTC AA	AAT GTA TGG GAT GGC GGA TA
mito short PCR	CGC CTC ACA CTC ATT CTC AA	CAA GGA AGG GGT AGG CTA TG

Table S2. Microarray real time PCR primers

Genes	5' sequences	3' sequences
GTP2	GTG ATG GCA CTA TGC ACC TAC	TTC ACG GAT GCA GTT GAC ACC
ME1	CTG CTG ACA CGG AAC CCT C	GAT CTC CTG ACT GTT GAA GGA AG
ALDH2	ATG GCA AGC CCT ATG TCA TCT	CCG TGG TAC TTA TCA GCC CA
PCK2	GCC ATC ATG CCG TAG CAT C	AGC CTC AGT TCC ATC ACA GAT
ALDH1B1	CCC ATT CTG AAC CCA GAC ATC	AAT GAC CTC CCC GGT GGT A
SLC25A10	AGG ATG CAG AAC GAC GTG AAG	GGT TGC ACC CGA GAA CAG TC
DDIT4	TGA GGA TGA ACA CTT GTG TGC	CCA ACT GGC TAG GCA TCA GC
SESN2	TCT TAC CTG GTA GGC TCC CAC	AGC AAC TTG TTG ATC TCG CTG
MAP1LC3B	AAG GCG CTT ACA GCT CAA TG	CTG GGA GGC ATA GAC CAT GT
MTHFD2	AGG ACG AAT GTG TTT GGA TCA G	GGA ATG CCA GTT CGC TTG ATT A
NLRP1	GCA GTG CTA ATG CCC TGG AT	GAG CTT GGT AGA GGA GTG AGG
CCL2	CAG CCA GAT GCA ATC AAT GCC	TGG AAT CCT GAA CCC ACT TCT
CCL8	TGG AGA GCT ACA CAA GAA TCA CC	TGG TCC AGA TGC TTC ATG GAA
TNFSF21	ATT GGC ACA TAC CGC CAT GTT	GGC TTG TGT TGG TAC AAT GCT C
GADD45A	GAG AGC AGA AGA CCG AAA GGA	CAC AAC ACC ACG TTA TCG GG

Supplemental Experimental Procedures

Uv/Vis and Fluorescence Spectroscopy

A stock solution (1.0 mM) of **Bo-Mt-CFX** was prepared in DMSO and a 100 mM stock solution of hydrogen peroxide was prepared in triple-distilled water. Quartz cuvettes with a 1 cm light path were used for all experiments. For all fluorescence emission spectral studies, samples were excited at 550 nm with a slit width 5/5 nm. For time course spectra, **Bo-Mt-CFX** (5 μM) was incubated with hydrogen peroxide (250 μM) and it was recorded at λ_{max} = 583 nm. For the concentration dependent spectral analyses, **Bo-Mt-CFX** (5 μM) was treated with increasing concentrations of hydrogen peroxide (0, 1, 2.5, 10, 25, 50, 75, 100, 150, 250, 400, 500, 750, 1000 μM).

Cell lines and Reagents

Human cancer cell lines MDA-MB-231, SW620, DU145, A549, PC3 and HCT116 and the non-cancerous BJ and MCF10A cell lines were purchased from Korea Cell Line Bank (Seoul, Korea). MDA-MB-231, SW620, DU145 and PC3 cells were cultured in RPMI 1640 media (HyClone, Chicago, IL, USA). A549 cells were cultured in Modified Eagle's media (Hyclone). BJ cells were cultured in Dulbecco's modified Eagle's media (Hyclone). MCF 10A cells were cultured Mammary Epithelial Cell Growth basal medium (Lonza, Alpharetta, GA, USA) supplemented with Endothelial Cell Growth Medium BulletKit (Lonza). HCT116 and HCT Bax$^{-/-}$ cells were cultured in McCoy's 5A media (Hyclone). All media were supplemented with 10% FBS (GIBCO, Grand Island, NY, USA) and 1% penicillin /streptomycin (GIBCO) and cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell viability assay

MDA-MB-231, A549, SW620, DU145, PC3, HCT116, HCT Bax$^{-/-}$, BJ and MCF10A cells were seeded in 96 well plates at 4×10^3 cells per well and were allowed to adhere for at least 24 hours. To assess the effect of antioxidants on **Mt-CFX**-induced cell death, MDA-MB-231cells were pretreated with 100 μM NAC (N-acetyl-L-cysteine, Sigma Aldrich), 100 μM Vitamin C (ascorbic acid, Sigma Aldrich), 10 μM Vitamin E (ᾳ-tocopherol, Sigma Aldrich), or 50 μM BHT (butylated hydroxytoluene, Sigma Aldrich) for 12 hours. Subsequent to incubation, the cells were treated with **CFX**, **Mt-CFX**, **Bo-Mt-CFX** or 1% DMSO as a control for 72 hours. To determine the cytotoxicity, a CytoTox96® Non-Radioactive Cytotoxicity Assay Kit (Promega, Madison, WI, USA) was used according to the manufacturer's instructions in the presence of the three test agents under study. The absorbance of the wells was detected at 490 nm by a SpectraMax Gemini EM microplate reader (Molecular Devices, San Jose, CA, USA). Cell viability assays were performed in triplicate and the cytotoxicity was recorded as a percentage calculated for the treated cells relative to the control group.

Assessment of Mitochondrial Membrane Potential

To assess the mitochondrial membrane potential, MDA-MB-231 cells (1.5×10^6 cells) were seeded in 60 mm dishes and were allowed to adhere for at least 24 h. The cells were treated with 30 μM **Mt-CFX** for 0, 6, 12 and 24 h. After incubation, the cells were stained with 2 mL PBS solution containing 5 μM JC-1 (Thermo Fisher Scientific, Waltham, MA, USA) for 15 min at 37 °C. The cells were then washed with phosphate-buffered saline (PBS). The proportion of monomer to aggregate of JC-1 was quantified using a FACS Calibur™ flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) with 488 nm excitation and 530 nm and 585 nm band pass emission filters. The results were analyzed using the CELLQUEST™ software (BD Biosciences).

Annexin V assay

To detect apoptotic cells, MDA-MB-231 cells (1.5×10^6 cells) were seeded in 60 mm dishes and were allowed to adhere for at least 24 h. The cells treated with 30 μM **Mt-CFX** and 0, 6, 12 and 24 h. The Annexin V-FITC Early Apoptosis Detection Kit (BD Bioscience) was used and apoptotic cells were quantified on a FACS Calibur™ flow cytometer and analyzed using the CELLQUEST™ software (BD Biosciences).

BrdU assay

MDA-MB-231 cells (1.0×10^4 cells) were seeded on Lab-Tek Chamber Slides (Merck Inc., NJ, USA) in DMEM containing 10% FBS. After 48 hours, the media was changed to DMEM containing 50 μM of **CFX** or **MT-CFX**. After 24 hours of incubation, cells were labeled with 20 μM of BrdU for 4 hours. Then, the cells were washed with PBS, and immediately fixed with 4% formaldehyde. Cells were incubated with anti-BrdU antibody (1:100; sc-32323, Santa Cruz

Biotechnology, Dallas, TX, USA) and followed with 1:200 dilution of Alexa Fluor-conjugated donkey anti-mouse antibody (Thermo Fisher Scientific, Waltham, MA, USA) for monitoring BrdU incorporation. Nuclei were stained with DAPI. Confocal microscopy was carried out using an LSM 700 confocal microscope (Carl Zeiss, Oberkochen, Barden-Württemberg, Germany).

ROS and mitochondrial superoxide imaging assay

To detect mitochondrial superoxide, MDA-MB-231 cells (1×10^5 cells) were seeded on glass bottom dishes and were allowed to adhere for at least 24 h followed by **Mt-CFX** (30 μM) treatment for 48 h. Cells were washed twice with PBS and then incubated with 5 μM MitoSOX™, a red mitochondrial superoxide indicator (Molecular Probes™, USA) at 37°C for 15 min in the dark. To detect general levels of ROS, cells were seeded on glass bottom dishes followed by 100 μM NAC or no NAC pretreatment for 12 h and then **Mt-CFX** (30 μM) treatment for 48 h. Cells were washed twice with PBS and then incubated with 10 μM CM-H_2DCFDA (Invitrogen, Carlsbad, CA, USA) at 37°C for 30 min. After incubation, cells were washed twice with PBS. Fluorescent images were obtained using an LSM 700 confocal laser scanning microscope (Carl Zeiss). To detect hydrogen peroxide, MDA-MB-231 cells (1×10^4 cells) were seeded on 96 well plates and were allowed to adhere for at least 24h followed by **Mt-CFX** (30 μM) treatment for 48 h. The treated cells were washed twice with PBS and 50 μL of Amplex Red® reagent (400 μM) and 50 μL PBS phosphate buffer (50 mM, pH 7.4) were added. 100 μl of hydrogen peroxide standards, ranging from 0 to 20 μM, and a control buffer, at the same concentrations as the treatment buffers, were added separately to the wells in triplicates. Horseradish peroxidase (1 U/mL) was added to each well and incubated for 30 min at 37°C. The fluorescence intensities were then recorded using a Multi-Detection Microplate Reader system (HIDEX, Turku, Finland). The fluorescence channel was excited at 530 nm and the emission was recorded using a 590 nm band-pass filter.

Intracellular hydrogen peroxide assay

To detect endogenous intracellular hydrogen peroxide levels, MDA-MB-231, SW620, DU145, A549, PC3, BJ, and MCF10A cells (1×10^4 cells) were seeded on 96 well plates and were allowed to adhere for at least 24 h. To determine the hydrogen peroxide levels, an Intracellular Hydrogen Peroxide Assay Kit (MAK164, Sigma Aldrich) was used according to the manufacturer's instructions. The fluorescence at 520 nm following excitation at 490 nm was recorded using a Multi-detection Microplate Detection System (HIDEX).

Protein, lipid and DNA oxidation damage assay

To detect oxidative damage, MDA-MB-231 cells (4×10^3 cells) were seeded on 96 well plates and were allowed to adhere for at least 24 h followed by **Mt-CFX** (30 μM) or **Bo-Mt-CFX** (3 μM) treatment for 48 h. Protein carbonylation was measured with a Protein Carbonyl ELISA Kit (Enzo Life Science, Farmingdale, NY, USA). Lipid peroxidation was measured with the Lipid Peroxidation MDA Assay Kit (Abcam, Cambridge, UK), while 8-OHdG levels were quantified using an OxiselectTM Oxidative DNA damage ELISA Kit (Cell BioLabs, Inc., San Diego, CA, USA). All assays were performed according to the manufacturer's instructions.

Subcellular localization analyses.

To detect mitochondrial superoxide, MDA-MB-231 cells (1×10^5 cells) were seeded on glass bottom dishes and were allowed to adhere for at least 24 h followed by **Mt-CFX** (30 μM) or **Bo-Mt-CFX** (3 μM) treatment for 48 h. The cells were washed twice with PBS and the mitochondria, lysosome, and ER (Endoplasmic reticulum) were stained for 30 min with 500 nM MitoTracker™ Green FM, 500 nM LysoTracker® Green DND-26, and 1 μM ER-Tracker® Green (BODIPY™ FL Glibenclamide) (Invitrogen), respectively. After incubation, the cells were washed with PBS × 3. Fluorescent images were obtained using a FLUOVIEW FV3000 confocal laser scanning microscope (Olympus, Shinjuku, Tokyo, Japan).

Immunoblotting analyses

Immunoblot analyses were performed using antibodies specific for Caspase-9 (9508), Caspase-3 (9662), Cleaved Caspase-3 (9661), Bak (12105), Bad (9239), Puma (12450), Noxa (14766), BID (2002), Bcl-w (2724), Mcl-1 (94296), Bcl-xL (2764), A1(14093) and Bcl-2 (sc-65392), Bax (sc-7480), GAPDH (sc-47724), β-Tubulin (sc-166729), cytochrome c (sc-13156) and COX IV (A21347), purchased from Cell Signaling Technology (Danvers, MA, USA), Santa Cruz Biotechnology, or Invitrogen. Briefly, attached cells were washed with ice-cold PBS three times and scraped to collect cell pellets. After the removal of PBS, a radioimmunoprecipitation assay (RIPA) lysis buffer containing 50 mM Tris/HCl

(pH 7.5), 150 mM NaCl, 0.1 % sodium dodecyl sulfate (SDS), 1% Triton X-100, 1% sodium deoxycholate, and a protease inhibitor mixture, as provided by the manufacturer (Biosesang, Seongnam, Gyeonggi-do, Korea) was added into the cell pellets to obtain protein lysates. A BCA assay (Bicinchoninic acid assay) was conducted to measure the protein concentration of each samples and then proteins (30 µg/lane) from each cell line were loaded onto a sodium dodecyl sulfate poly-acrylamide gel electrophoresis gel (SDS-PAGE) to separate the proteins into bands. The separated protein bands were transferred to polyvinylidene difluoride (PVDF) membranes (Merk Millipore, Burlington, MA, USA). The membranes were incubated with antibodies overnight at 4 °C. The resulting membranes were washed with Tris-buffered saline 0.1% tween-20 (TBS-T) and then incubated with anti-mouse horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology; sc-2357) and anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies (GTX213110-01; GeneTex, Irvine, CA, USA) for 2 h at room temperature. To detect immunoreactive protein bands, an enhanced chemiluminescence reagent (Luminate; Merk Millipore) was used according to the manufacturer's instructions.

Separation of cytosolic and mitochondrial fractions

Separation of cytosolic and mitochondrial fractions was performed using the Mitochondria Isolation Kit (Thermo Fisher Scientific). Briefly, the cells under study were washed with cold PBS and lysed by a 5-cycle freeze-thawing process in Mitochondria Isolation Reagent A buffer, containing 10 mM phenylmethylsulfonyl fluoride (PMSF). After incubation on ice for 10 min, and the addition of Mitochondria Isolation Reagent B buffer, the lysates were centrifuged at 700× g for 10 min, following inverting by a treatment with Mitochondria Isolation Reagent C buffer. The supernatant was transferred to a new tube, centrifuged at 12 000 × g for 15 min at 4 °C, and collected as the cytosolic fraction. After adding 500 mL of Mitochondria Isolation Reagent C, the pellet was centrifuged at 12,000× g for 5 min and collected as the mitochondrial fraction.

Assay for ATP content

To detect ATP levels, MDA-MB-231 cells (4×10^3 cells) were seeded on 96 well plates and were allowed to adhere for at least 24 h followed by pretreated with 100 µM NAC or without NAC for 12 h and thentreated with **Mt-CFX** (30 µM) for 48 h. To determine the APT level, an ATP Bioluminescence Assay Kit HS II (11699709001, Roche, Mannheim, Germany) was used according to the manufacturer's instructions. Briefly, cells were lysed by boiling with the lysis reagent and the supernatant was collected. Fifty microliters of the diluted sample were mixed with 50 µL of luciferin/luciferase reagents. The luminescence was measured using a Multi-detection Microplate Detection System (HIDEX).

Transmission electron microscopy (TEM) imaging

MDA-MB-231 cells (2×10^7 cells) were seeded on 100 mm dishes and were allowed to adhere for at least 24 h followed by pretreatment with 100 µM NAC or without NAC for 12 h and then treated with **Mt-CFX** (30 µM) or **CFX** (30 µM) for 48 h. To allow for the TEM analyses, cells were pre-fixed with a mixed buffer solution (0.2 M PBS, pH7.2) of glutaraldehyde 2% and paraformaldehyde 2% for 24 h. Then, a buffer (0.2 M PBS, pH7.2) containing osmium tetroxide 2% was used to post-fix the cells for 40 minutes. Dehydration was effected for 20 minutes with 50, 60, 70, 80, 90 and 100% ethanol, respectively. The cells were added to a solution of ethanol and propylene oxide at a ratio of 2:1, 1:1, or 1:2 and propylene oxide 100% for 20 minutes, respectively. The embedding process was conducted in a solution of propylene oxide and Epon 812 resin at a ratio of 2:1, 1:1, 1:2 and Epon 812 100% for 20 minutes, respectively. The samples were polymerized in an oven at 60 °C for 48 hours. Ultra-thin sectioning was carried out using a 80 nm thickness microtome (Leica Microsystems AG, Wetzlar, Hessne, Germany) and stained with 2% uranyl acetate for 30 min followed by lead citrate for 10 min at room temperature. The sections were examined using a transmission electron microscope JEM-2100F (JEOL Ltd., Akishima-shi, Tokyo, Japan).

HPLC assays

Reverse-phase high pressure liquid chromatography (RP-HPLC) was performed on a YL9101S (YL-Clarity) instrument equipped with a reverse-phase column VDSpher 100 C18-E (5 µm, 250 × 4.6 mm). HPLC-grade acetonitrile was purchased from J. T. Baker and deionized H_2O, containing 0.1% TFA, was used during separations. For analysis, a water-acetonitrile gradient (0-30 min; acetonitrile from 50% to 100%) with a 5.0 mL min^{-1} flow rate was used. The UV-Vis detector was set at 254 nm and 550 nm. Prior to HPLC analyses, **Bo-Mt-CFX** was incubated in a 1:1 mixture of deionized H_2O, containing 0.1% TFA, and acetonitrile for 10 hours to avoid artefacts associated with the hydrolysis

of the pinacol ester. All HPLC analyses were carried out on the corresponding boronic acid and the identity was confirmed with ESI-MS prior to HPLC assays.

Mitochondrial GSH measuring assay

Mito-RealThiol (MitoRT) for detecting mitochondrial glutathione was purchased from the laboratory of Jin Wang, PhD, Baylor College of Medicine (Kerafast, Inc., Boston, MA, USA). MDA-MB-231 cells (4×10^5 cells) were seeded on 96 well plates and were allowed to adhere for at least 24 h followed by the addition of 30 μM **Mt-CFX** or 3 μM **Bo-Mt-CFX**. After treatment, wells were washed with PBS (× 2), and incubated with 100 nM Mito-RealThiol for 30 min at 37°C. The fluorescence intensities were obtained using a Multi-detection Microplate Detection System (HIDEX) using blue (excitation 405 nm, emission 420-500 nm) and green (excitation 488 nm, emission 500-700) channels. The fluorescence intensity ratio was calculated between 405 and 488 nm excitation, reported as I_{405}/I_{488}.

RNA extraction and Real-time q-PCR analysis

Total RNAs from cell lines were isolated by the PureLink™ total RNA isolation kit (Invitrogen) and TRIzol reagent (Invitrogen), following the manufacturer's instructions. Reverse transcription to cDNA was performed using the iScript™ cDNA synthesis kit (BioRad, Hercules, CA, USA). All cDNAs used in real-time PCR were normalized with GAPDH. Quantitative real-time PCRs were performed using iQ™SYBR Green Supermix (BioRad). Gene expression was quantified by the delta-delta-CT method, and real-time PCRs were performed in a CFX-96 thermal cycler (Applied Biosystems, Foster City, CA, USA) and detection system. The primers for each gene can be found in Tables S1 and S2.

Semi-quantitative DNA-PCR analysis

DNA was isolated using a G-spin Total DNA Extraction Kit (iNtRON Biotechnology, Sungnam. Korea), according to the manufacturer's protocol. The following primers for Human target genes were used for genomic PCR: GAPDH sense 5'-AACCATGAGAAGTATGACAACAGC-3' and antisense 5'-GAGTCCTTCCACGATACCAAA-3'; and ND1 sense 5'-AATTCTCCGATCCGTCCCTA-3' and antisense 5'-GCTTACTGGTTGTCCTCCGAT-3'. PCR was carried out with r-Taq (Takara, Shiga, Japan) at 30 sec at 95 °C, 30 sec at 60 °C, and 30 sec at 72 °C for 20-25 cycles using 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products were resolved using agarose gel electrophoresis and visualized on a GelDoc XR+ imaging system (BioRad, Hercules, CA, USA). The band intensity was measured in a densitometer and was quantified with QuantityOne Software (BioRad, Hercules, CA, USA). The results were expressed as a ratio of ND1 band density to GAPDH band.

Micro array analyses

For microarray analysis, MDA-MB-231 cells (3×10^6 cells) were seeded in 100 mm dishes and were allowed to adhere for at least 24 h. The cells treated with 10 μM **Mt-CFX**, 10 μM **CFX**, and 1% DMSO as a control for 72 h. The cells washed with PBS (× 2) and detached cells were centrifuged at 1000 × g for 3 min at 4°C. After being harvested, the supernatant was removed and the cells were stored at -70°C before being transferred to a Micro array analysis company (Macrogen, Seoul, Korea). Macrogen carried out the micro-array analysis according the following general procedures:

RNA quality control prior to Micro array analysis

RNA purity and integrity were evaluated using an ND-1000 Spectrophotometer (NanoDrop, Wilmington, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Affymetrix whole transcript expression array methods

The Affymetrix Whole Transcript Expression array process was executed according to the manufacturer's protocol (GeneChip Whole Transcript PLUS reagent Kit). cDNA was synthesized using the GeneChip WT (Whole Transcript) Amplification kit as described by the manufacturer. The sense cDNA was then fragmented and biotin-labeled with TdT (terminal deoxynucleotidyl transferase) using the GeneChip WT Terminal labeling kit. Approximately 5.5 μg of labeled DNA target was hybridized to the Affymetrix GeneChip Human 2.0 Array at 45°C for 16 h. Hybridized arrays were washed and stained on a GeneChip Fluidics Station 450 and scanned on a GCS3000 Scanner (Affymetrix). Signal values were computed using the Affymetrix® GeneChip™ Command Console software.

Raw data preparation and statistic analysis for micro arrays

The data were summarized and normalized with the robust multi-average (RMA) method implemented in Affymetrix® Power Tools (APT). The results were exported in the form of gene level RMA analyses and subject to a differentially expressed gene (DEG) analysis. Statistical significance of the expression data was determined as fold

change. For a DEG set, hierarchical cluster analysis was performed using complete linkage and Euclidean distance as a measure of similarity. Gene-Enrichment and Functional Annotation analysis for significant probe list was performed using Gene Ontology (www.geneontology.org/) and KEGG (www.genome.jp/kegg/). All data analysis and visualization of differentially expressed genes was conducted using R 3.3.3 (www.r-project.org).

Animal studies

Five-week-old immunodeficient nude mice (nu/nu) (Orient Bio, Inc., Seongnam, Gyeonggi-do, Korea) were maintained in pressurized ventilated cages under conditions of repeated controlled illumination (12 h dark; 12 h light) with *ad libitum* access to sterilized water and food (Cat no:1314Fort; Altromin Spezialfutter GmbH & Co.Kg, Lage, North Rhine-Westphalia, Germany). All animal studies were performed with the approval of the Korea University Institutional Animal Care and Use Committee (#KUIACUC-2018-57) and in accord with the Korean Animal Protection Act. MDA-MB-231 cells (1×10^6) in 50 µL PBS with 50 µL Matrigel® Basement Membrane Matrix (CORNING, Corning, NY, USA) were injected subcutaneously into both flanks of each nude mouse. When the tumor volumes reached 100 mm^3, the mice were randomized into 4 treatment groups; vehicle control (DMSO), **CFX**, **Mt-CFX**, and **Bo-Mt-CFX** (*n* = 4 mice, *n* = 8 tumors per group). The mice were *i.v.* injected with the test agents (0.5 µmol kg^{-1} $dose^{-1}$) or vehicle (DMSO) in 100 µL PBS using a 0.5 ml syringe (BD Biosciences) once every week for 3 weeks. Tumor growth was monitored periodically, and the tumor volume (V) was calculated by using the modified ellipsoidal formula: $V = 1/2 \times length \times (width)^2$. After 12 weeks of treatment, animals were euthanized by CO_2 asphyxiation. The xenograft tumors were excised and photographed. Each animal was subject to complete post-mortem examination and an effort was made to minimize the number of animals used and their suffering.

***In vivo* and *ex vivo* fluorescent imaging**

To assess tumor target specificity and the organ distribution of **Bo-Mt-CFX** *in vivo* and *ex vivo*, MDA-MB-231 cells were injected subcutaneously into mice, as described above. When tumor growth reached a detectable size, the mice were treated with **CFX** (0.5 µmol kg^{-1} $dose^{-1}$), **Bo-Mt-CFX** (5 mmol kg^{-1} $dose^{-1}$), or vehicle (DMSO) by means of a single *i.v.* injection. After 2 days, the animals were euthanized via CO_2 asphyxiation. The xenograft tumors were excised and photographed. *Ex vivo* fluorescence images of the tumors and organs of the control and treated mice were recorded and quantified using a Maestro2 instrument (excitation and emission wavelengths: 550 and 650 nm, respectively). The fluorescence images and auto-fluorescence were then deconvoluted using the software provided with the instrument (Maestro software ver.2.4; CRi, Woburn, MA, USA) using the multi excitation spectral analysis function.

Hepatotoxicity assay

Blood samples of each mouse were centrifuged at 3,000 rpm for 10 min at 4 °C to produce serum. Then the activities of ALT and AST in serum were analyzed using the AST and ALT Activity Assay Kits (MAK055, MAK052; Sigma Aldrich) according to the manufacturer's instructions.

H&E staining and immunohistochemistry

The tissues were extracted and embedded in frozen section compound (Leica Microsystems AG). Tumor sections (5 µm thickness) were produced using a Leica cryotome CM3050S and fixed onto slides and the slides were stored at -70 °C. For H&E staining, the slides were rehydrated, and counterstained with Gill's Hematoxylin and 0.5% Eosin Y. Immunohistochemistry was performed using anti-cleaved caspase 3 (# 9661; Cell Signaling) and anti-Ki-67 (ab15580; abcam). Immunohistochemistry was performed according to a standard protocol.

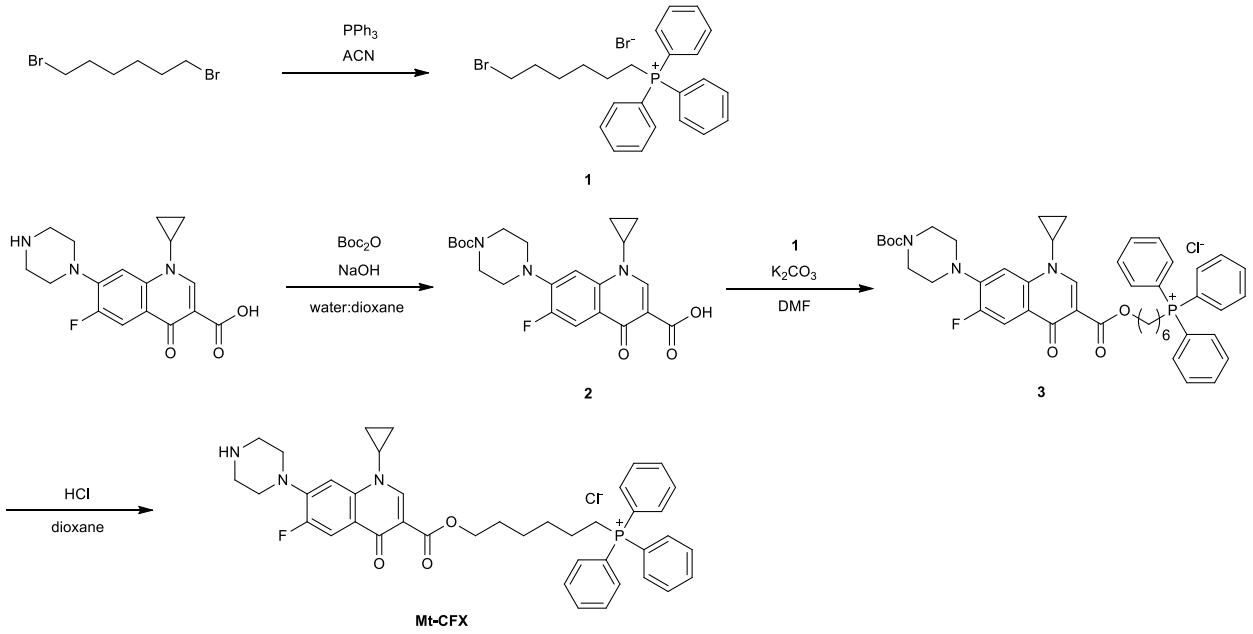
Statistical analyses

All statistical analyses were performed using the SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) software suite, using a one-way ANOVA with post-hoc Bonferroni test, with an alpha level of 0.05. Further details are supplied in the figure captions.

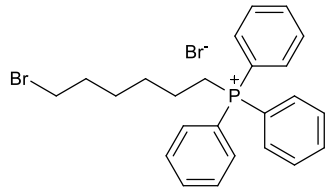
Synthetic Procedures

General Information

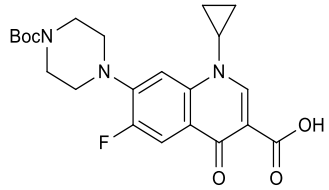
All synthetic steps requiring anhydrous conditions were carried out in oven-dried flasks flushed with argon gas and commercial anhydrous solvents were used. All chemicals were obtained from commercial suppliers and used as received (Alfa Aesar, Sigma Aldrich and TCI Chemicals). Thin layer chromatography was performed on glass plate pre-coated with Merck 60 silica gel F_{254} plates. Column chromatography was performed using Merck 60 (particle size 0.040-0.063 mm) silica gel. NMR spectral analyses were performed with a Bruker NMR instrument (500 MHz for ^{1}H, 125 MHz for ^{13}C) using $CDCl_3$ or DMSO-d_6 as the solvents and TMS as an internal standard. Chemical shifts (δ) are recorded in ppm and coupling constants are given in Hz. Fluorescence and UV-Vis absorption spectra were recorded with Shimadzu RF-5301PC and Agilent 8453 spectrophotometers, respectively. ESI mass spectral analyses were carried out using an LC/MS-2020 Series instrument (Shimadzu). DMSO for molecular biology was obtained from Sigma Aldrich (St. Louis, Mo, USA) and was used for spectral and biological studies. The Dowex® 1X8 chloride form ion exchange resin was prepared using the following procedure: 20 mL of dry Dowex® 1X8 chloride form beads was added to 100 mL MeOH and stirred for 20 min under gentle stirring, after which the solvent was decanted, the procedure was repeated 3 times.



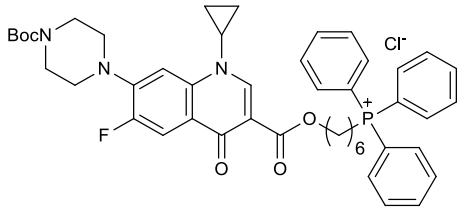
Scheme S1. Synthesis of **Mt-CFX**.



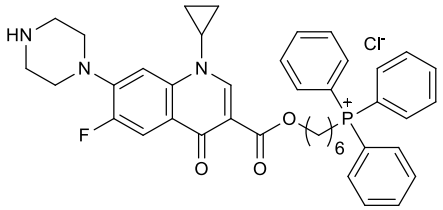
Triphenylphosphonium alkyl bromide (**1**): Compound **1** was synthesized analogously to a literature procedure.[S3] All analytical data matched those reported in the literature.



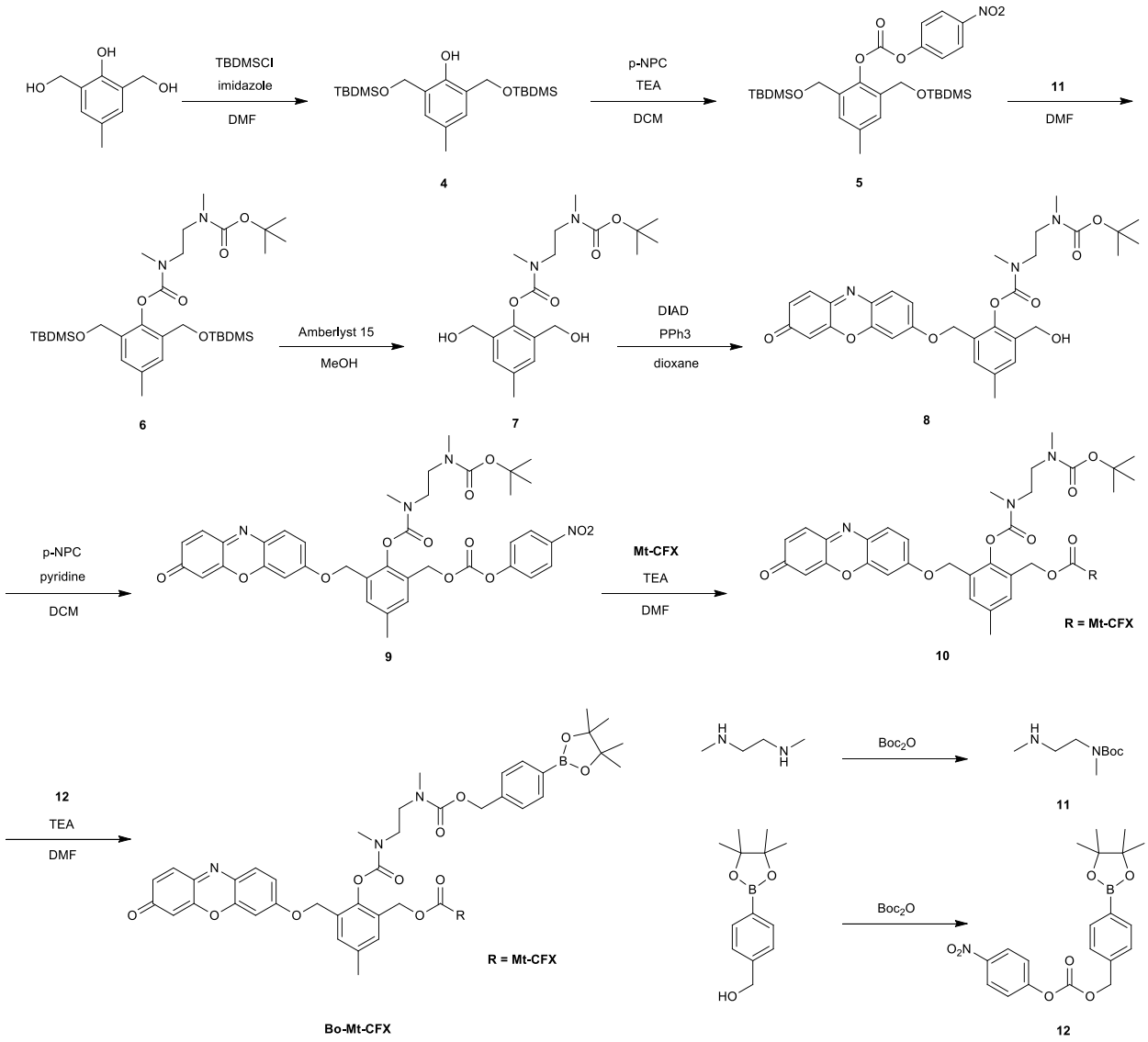
Boc-Ciprofloxacin (**2**): Compound **2** was synthesized analogously to a literature procedure.[S4] All analytical data matched those reported in the literature.



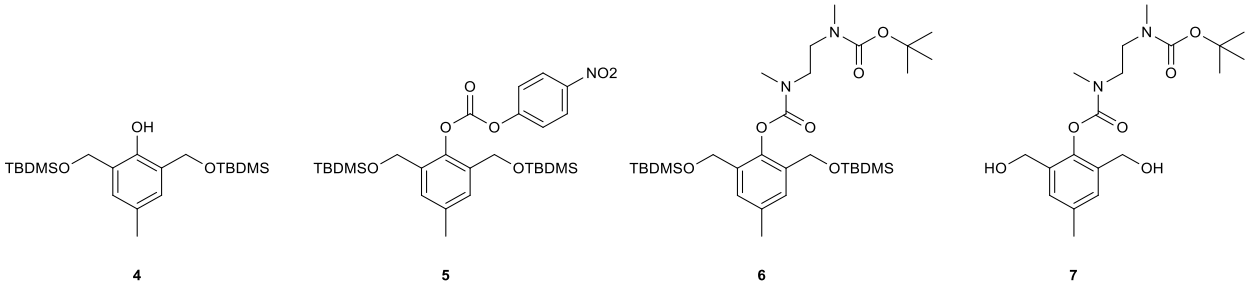
Boc-Mt-CFX (**3**): Compounds **1** (1 g, 1.975 mmol) and **2** (852 mg, 1.975 mmol) were dissolved in DMF (50 mL) and K_2CO_3 (819 mg, 5.925 mmol) was added to the solution. The reaction mixture was heated to 50 °C overnight. After removal of the solvent under reduced pressure, the residue was purified with column chromatography (eluent: DCM:MeOH = 97:3). The product was first dissolved in 30 mL MeOH and 270 mL distilled water was added. To the solution 1 g $NaBF_4$ (9.108 mmol) was added while vigorous stirring 1 hour. The mixture was extracted with DCM (3 × 200 mL), the combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The resulting BF_4 salt was dissolved in MeOH and 20 mL of Dowex® 1X8 chloride form, prepared as described above, was added and the mixture was stirred gently. The ion exchange was monitored with TLC, and after completion the mixture was filtered over a paper filter and the filtrate was evaporated. 1.31 g compound **3** (82%) was obtained; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.50 (s, 1H), 7.82–7.77 (m, 3H), 7.74–7.66 (m, 13H), 7.33 (d, *J* = 7.2 Hz, 1H), 4.25 (t, *J* = 6.0 Hz, 2H), 3.65 (t, *J* = 4.8 Hz, 4H), 3.56–3.50 (m, 1H), 3.39–3.29 (m, 2H), 3.22 (t, *J* = 4.9 Hz, 4H), 1.74–1.63 (m, 6H), 1.58–1.51 (m, 2H), 1.50 (s, 9H), 1.37 (dd, *J* = 14.2 and 6.9 Hz, 2H) and 1.11–1.06 (m, 2H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.0, 165.5, 154.6, 153.2 (d, *J* = 247.7 Hz), 148.2, 144.4 (d, *J* = 10.4 Hz), 138.1, 135.1, 133.6 (d, *J* = 10.0 Hz), 130.5 (d, *J* = 12.7 Hz), 122.9 (d, *J* = 6.6 Hz), 118.1 (d, *J* = 85.4 Hz), 112.7 (d, *J* = 22.9 Hz), 110.0, 105.5, 80.1, 64.3, 53.5, 49.9 (d, *J* = 4.2 Hz), 34.9, 29.5 (d, *J* = 16.0 Hz), 28.4, 28.0, 25.0, 22.3 (d, *J* = 50.3 Hz), 22.1 (d, *J* = 4.3 Hz) and 8.2 ppm; ESI-MS (*m*/*z*): $[M]^+$ calcd for $C_{46}H_{52}FN_3O_5P$: 776.36; found: 776.35.



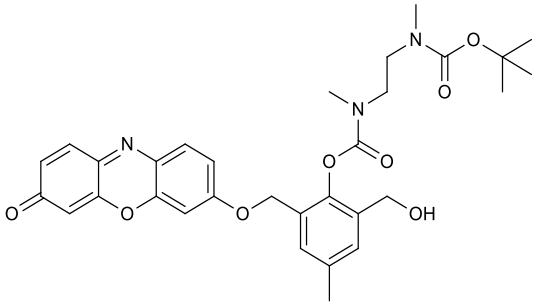
Mt-CFX: Compound **3** (1 g, 1.231 mmol) was dissolved in 30 mL of solvent (1,4-Dioxane:DCM 4:1) and 10 mL of anhydrous 4N HCl in Dioxane was added dropwise in an ice bath, to obtain a final HCl concentration of 1N. The solution was stirred overnight at room temperature and the solvent was evaporated. **Mt-CFX** was obtained as light yellow powder: yield 90% (790 mg, 1.11 mmol). For biological assays, it was further purified by passage through a flash column (eluent 10% MeOH in DCM to 12% MeOH in DCM). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.51 (s, 1H), 7.85–7.66 (m, 16H), 7.33 (d, *J* = 7.1 Hz, 1H), 4.27 (t, *J* = 5.8 Hz, 2H), 3.73–3.61 (m, 2H), 3.55–3.50 (m, 1H), 3.43–3.37 (m, 4H), 3.23 - 3.19 (m, 4H), 1.75–1.67 (m, 6H), 1.61–1.52 (m, 2H), 1.41–1.34 (m, 2H), 1.15–1.08 (m, 2H) ppm.; ^{13}C NMR (DMSO-d_6,125 MHz): δ 171.8, 164.8, 152.6 (d, *J* = 246.8 Hz), 148.2, 144.1 (d, *J* = 10.1 Hz), 138.1, 134.9 (d, *J* = 2.7), 133.6 (d, *J* = 10.1 Hz), 130.3 (d, *J* = 12.6 Hz), 121.7 (d, *J* = 6.8 Hz), 118.6 (d, *J* = 86.2 Hz), 111.5 (d, *J* = 22.8 Hz), 109.1, 106.1 (d, *J* = 2.8 Hz), 63.7, 49.7 (d, *J* = 4.1 Hz), 44.7, 34.9, 29.2 (d, *J* = 16.9 Hz), 27.8, 24.5, 21.6 (d, *J* = 4.3 Hz), 20.1 (d, *J* = 49.9 Hz) and 7.6 ppm; ESI-MS (*m*/*z*): $[M]^+$ calcd for $C_{41}H_{44}FN_3O_3P$: 676.31; found: 676.35.



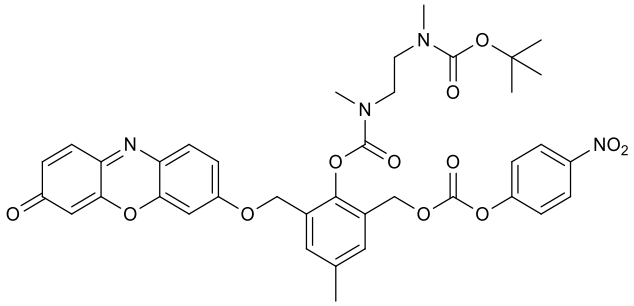
Scheme S2. Synthesis of **Bo-Mt-CFX.**



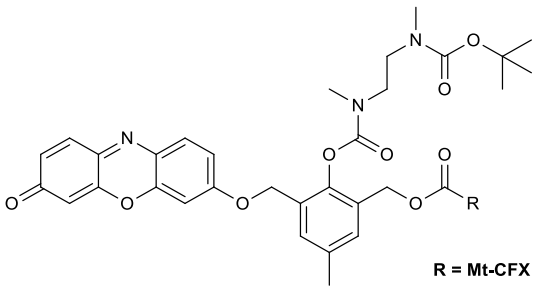
Compounds 4, 5, **6**, and **7** were synthesized as described previously.[S5] All analytical data matched those reported in the literature.



Compound 8: Compound **7** (300 mg, 0. 78 mmol), resorufin (167 mg, 0.78 mmol) and DIAD (206 mg, 1.02 mmol) were dissolved in anhydrous dioxane (20 mL). PPh_3 (267 mg, 1.02 mmol), dissolved in anhydrous dioxane (10 mL), was added dropwise to the solution at 10 °C with vigorous stirring. The mixture was stirred overnight and the solvent was evaporated. The residue was redissolved in DCM (100 mL) and washed with brine (3 × 100 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The resultant residue was purified with column chromatography (eluent: DCM:acetone 95:5) to yield a red viscous product (117 mg, 26%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.70 (d, *J* = 8.9 Hz, 1H), 7.41 (dd, *J* = 9.8 and 1.1 Hz, 1H), 7.32–7.28 (m, 1H), 7.23–7.18 (m, 1H), 7.04–6.96 (m, 1H), 6.95–6.87 (m, 1H), 6.84–6.80 (m, 1H), 6.32–6.27 (m, 1H), 5.21–5.07 (m, 2H), 4.57–4.49 (m, 2H), 3.56–3.38 (m, 4H), 3.12–3.02 (m, 3H), 2.94–2.87 (m, 3H), 2.36–2.30 (m, 3H) and 1.49–1.42 (m, 9H) ppm.; ^{13}C NMR (CDCl3 , 125 MHz): δ 186.3, 162.6, 156.3–155.8 (m), 155.7–155.3 (m), 154.8, 154.6, 149.8, 145.6, 145.3–144.8 (m), 136.2 (br), 134.7, 134.1, 131.6, 129.2 (br), 128.9 (br), 128.6, 128.5, 114.2, 106.7, 101.1, 80.0 (m), 66.9–66.2 (m), 60.6–59.8 (m), 47.3, 46.1, 34.6, 30.9, 28.4 and 21.0–20.8 (m) ppm; ESI-MS (*m*/*z*): $[M+H]^+$ calcd for $C_{31}H_{36}N_3O_8$: 578.25; found: 578.35.
[Note: The presence of rotamers results in excessive splitting of the ^{13}C signals.]

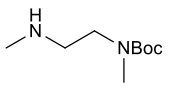


Compound 9: A solution of compound **8** (250 mg, 0.43 mmol) and pyridine (0.12 mL, 1.08 mmol) in anhydrous DCM (50 mL) was cooled in an ice bath. To the solution, 4-nitrophenyl chloroformate (175 mg, 0.87 mmol) in anhydrous DCM (10 mL) was added dropwise with vigorous stirring. The reaction was monitored with TLC and after 6 hours the reaction mixture was washed with brine (3 × 100 mL) and 1N aqueous HCl (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The resultant crude product was further purified with column chromatography (eluent: DCM:acetone 92:8), to obtain 249 mg **9** (78%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.27 (d, *J* = 8.9 Hz, 2H), 7.72 (d, *J* = 9.0 Hz, 1H), 7.42 (dd, *J* = 9.8 and 1.8 Hz, 1H), 7.40–7.35 (m, 2H), 7.35–7.29 (m, 2H), 7.08–6.99 (m, 1H), 6.98–6.90 (m, 1H), 6.84 (dt, *J* = 9.8 and 1.9 Hz, 1H), 6.32–6.30 (m, 1H), 5.30-5.07 (m, 4H), 3.66–3.32 (m, 4H), 3.17–3.01 (m, 3H), 2.96–2.84 (m, 3H), 2.40–2.34 (m, 3H), 1.48–1.38 (m, 9H) ppm.
[Note: Compound **9** exhibits a poor bench stability and was therefore only characterized via ^{1}H NMR spectroscopy before being used directly for the next step.]

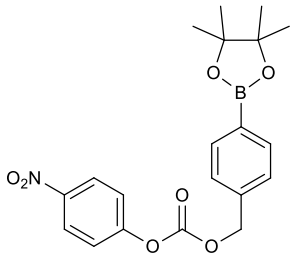


Compound 10: To a solution of compound **9** (200 mg, 0.27 mmol) and TEA (137 mg, 1.35 mmol) in anhydrous DMF (10 mL), cooled in an ice bath, a solution of **Mt-CFX** (192 mg, 0.27 mmol) in anhydrous DMF (2 mL) was added dropwise and stirred overnight at room temperature. After the reaction was completed, the solvent was removed *in vacuo*. The residue was redissolved in DCM (100 mL) and washed with brine (3 × 100 mL). The organic layer was dried over Na_2SO_4 and the residue was further purified with flash column chromatography (eluent: DCM:MeOH 92:8), to obtain 280 mg **10** (81%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.52 (s, 1H), 7.85–7.79 (m, 3H), 7.75–7.66 (m, 14H), 7.43 (dd, *J* = 9.9 and 2.0 Hz, 1H), 7.36–7.23 (m, 3H), 7.09–7.02 (m, 1H), 7.00–6.92 (m, 1H), 6.83 (d, *J* = 10.1 Hz, 1H), 6.31 (s, 1H), 5.24–5.08 (m, 4H), 4.28 (t, *J* = 5.8 Hz, 2H), 3.78–3.63 (m, 4H), 3.56–3.47 (m, 3H) 3.46–3.34 (m, 4H), 3.28–3.18 (m, 4H), 3.18–3.03 (m, 3H), 2.95–2.88 (m, 3H), 2.39–2.34(m, 3H), 1.73–1.61 (m, 6H), 1.59–1.52 (m, 2H), 1.47–1.39 (m, 9H), 1.37–1.32 (m, 2H), 1.13–1.07 (m, 2H) ppm.; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 185.6, 172.5, 164.9, 162.1, 155.5–155.1 (m), 155.1–154.7 (m), 154.4, 153.3, 152.6 (d, *J* = 248.0 Hz), 149.2, 147.8, 145.0, 144.9–144.4 (2 C), 143.7 (d, *J* = 10.4 Hz), 137.6, 135.5, 134.6 (d, *J* = 2.8 Hz), 134.2, 133.4, 133.0 (d, *J* = 10.0 Hz), 131.1, 130.0 (d, *J* = 12.5 Hz), 129.0, 128.9, 128.6, 128.0, 122.4 (d, *J* = 6.6 Hz), 117.7 (d, *J* = 85.3 Hz), 113.8, 112.1 (d, *J* = 21.5 Hz), 109.4, 105.9, 105.3, 100.8, 79.4–78.9 (m), 66.1–65.6 (m), 63.8, 62.2–61.8, 49.3, 46.8, 45.6, 43.2, 34.4, 34.2, 30.4, 29.0 (d, *J* = 16.2 Hz), 27.9, 27.5, 24.5, 21.6 (d, *J* = 4.0 Hz), 21.4 (d, *J* = 50.9 Hz), 20.5 and 7.7 ppm.; ESI-MS (*m/z*): [M]+ calcd for $C_{73}H_{77}FN_6O_{12}P$: 1279.53; found: 1279.50.

[Note: The presence of rotamers results in excessive splitting of the ^{13}C signals.]

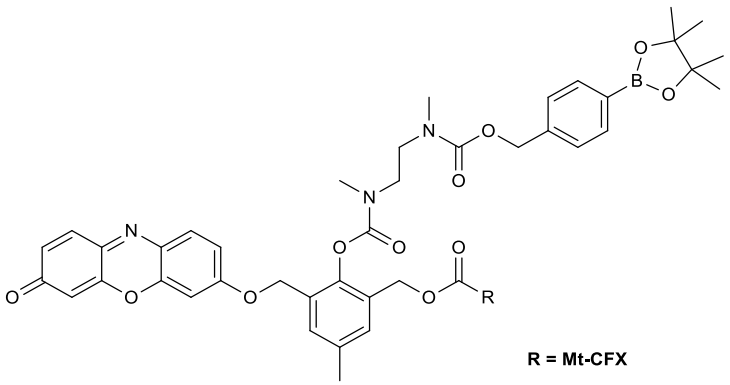


11

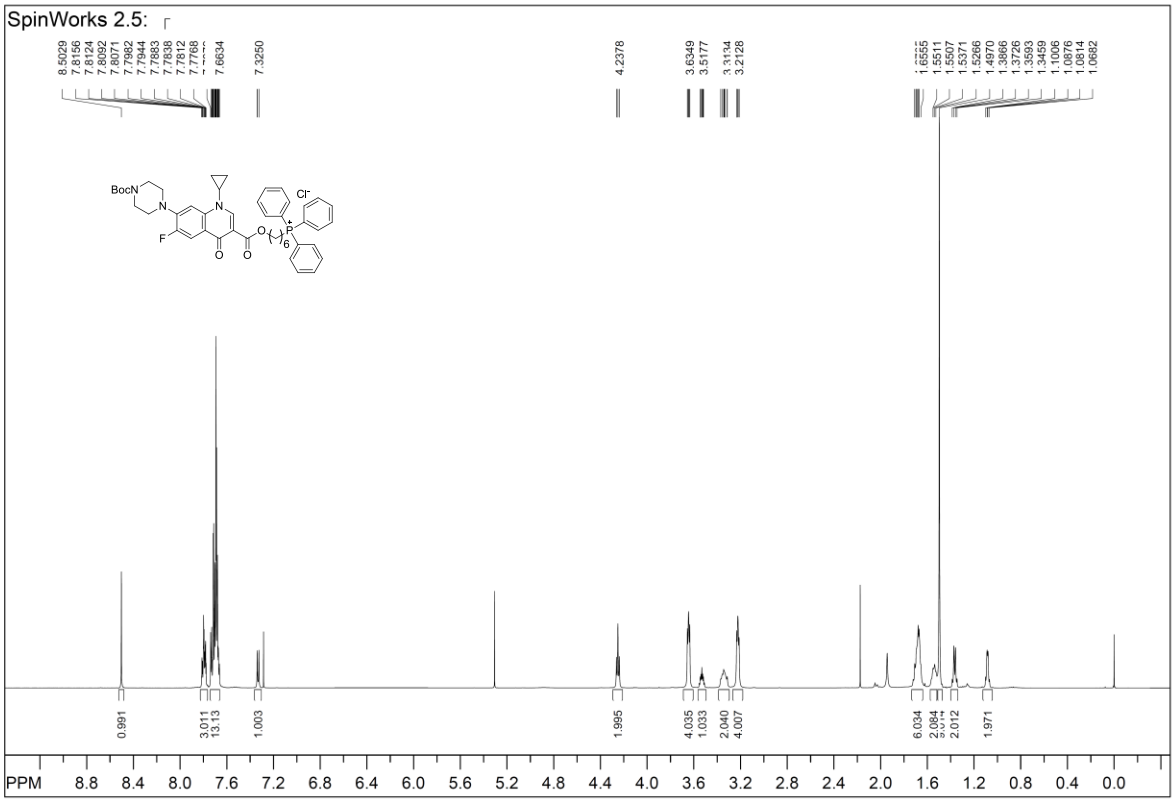


12

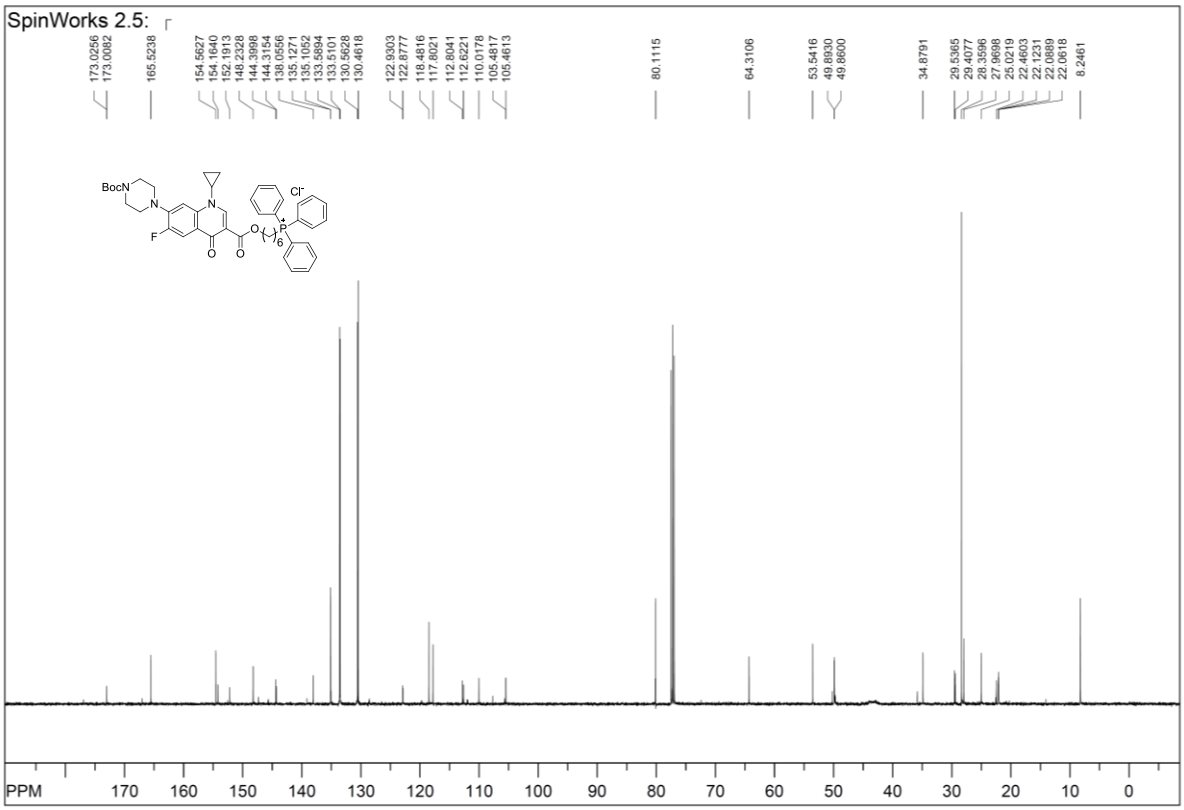
Compounds 11[S6] **and 12**[S7] were synthesized as described previously. All analytical data matched those reported in the literature.



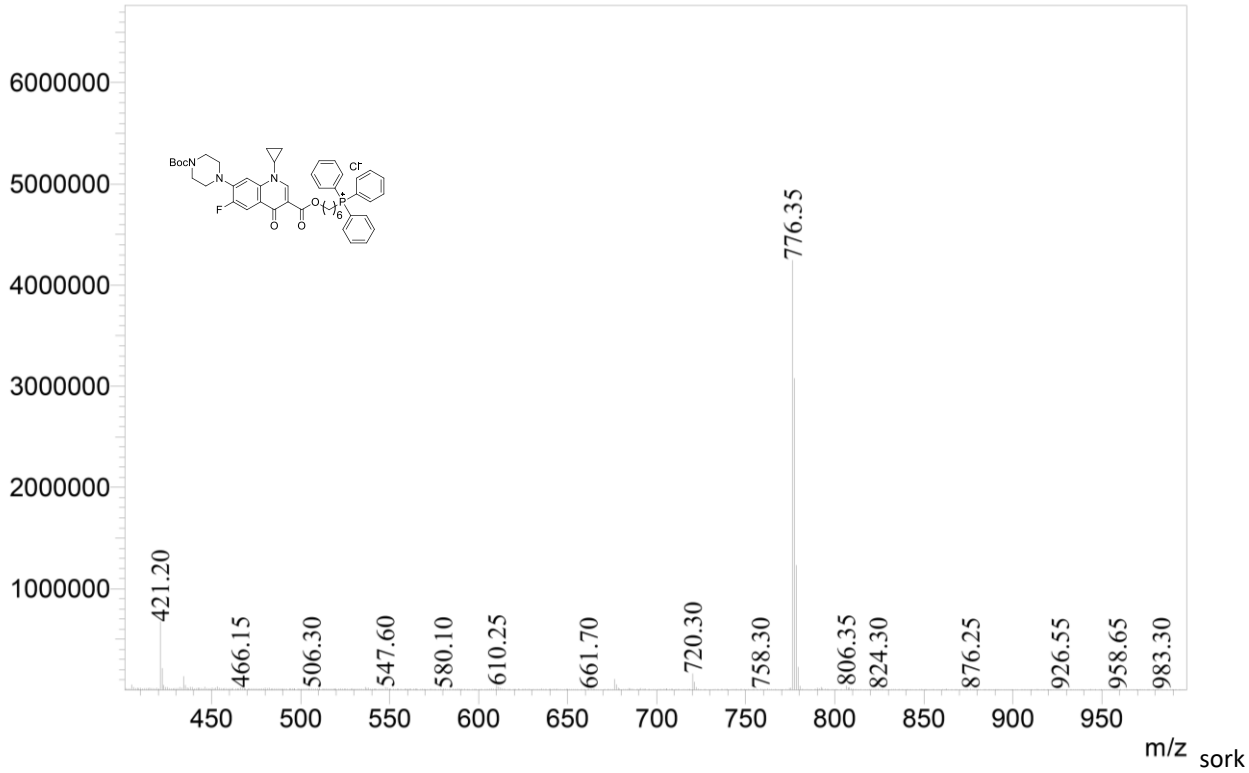
Bo-Mt-CFX: Compound **10** (200 mg, 0.156 mmol) was dissolved in 5 mL anhydrous solvent (EA:DCM 5:1) and the temperature was maintained at 0 °C. A solution of anhydrous 1N HCl in EA (5 mL) was added dropwise and the solution was stirred for 3 hours at 0 °C. The solvent was removed in vacuo, while the temperature bath was maintained at a temperature below 35 °C. The residue was redissolved in anhydrous DMF (1 mL) and added dropwise to a solution of compound **11** (93 mg, 0.234 mmol) and TEA (0.1 mL) in anhydrous DMF (10 mL) in an ice bath. The reaction was monitored with TLC and after completion, the solvent was evaporated under reduced pressure. The resultant residue was dissolved in DCM (30 mL) and washed with brine (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and the mixture was further purified with flash column chromatography (eluent: DCM:MeOH 92:8 to 88:12), to obtain 124 mg **Bo-Mt-CFX** (55%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.53 (s, 1H), 7.86–7.69 (m, 19H), 7.45–7.23 (m, 6H), 7.08–6.81 (m, 3H), 6.35–6.29 (m, 1H), 5.21–5.00 (m, 6H), 4.28 (t, *J* = 6.19 Hz, 1H), 3.82–3.75 (m, 2H), 3.75–3.47 (m, 8H), 3.28–3.20 (m, 4H), 3.20–3.02 (m, 3H), 3.02–2.89 (m, 3H), 2.41-2.35 (m, 3H), 1.76-1.65 (m, 6H), 1.58-1.50 (m, 2H), 1.48-1.40 (m, 2H), 1.34 (s, 12H) and 1.13 (br, 2H) ppm.; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.1, 172.9, 165.6, 162.6–162.3 (m), 156.4, 156.3–155.8 (m), 154.8, 154.7, 154.0, 153.7, 153.1 (d, *J* = 249 Hz), 149.7, 148.2, 145.5, 145.2, 145.0–144.8 (m), 144.1 (d, *J* = 10.8 Hz), 138.0, 136.2–136.0 (m), 135.0 (d, *J* = 2.9 Hz), 134.8, 134.6, 134.0, 133.5 (d, *J* = 9.9 Hz), 131.5, 130.4 (d, *J* = 12.8 Hz), 127.0, 126.9, 126.8, 126.6, 123.1 (d, *J* = 6.6 Hz), 118.2 (d, *J* = 86.2 Hz), 114.3–114.0 (m), 112.7 (d, *J* = 23.5 Hz), 110.1, 106.5, 105.5, 101.2, 83.7, 79.7, 66.2, 64.3, 62.7–62.3 (m), 49.8, 47.0, 46.4, 44.0–43.3 (m), 34.7, 29.6, 29.4 (d, *J* = 15.9 Hz), 27.9, 25.0, 24.8, 22.0, 22.0 (d, *J* = 50.9 Hz), 21.0, 8.2 ppm.; ESI-MS (*m*/*z*): $[M]^+$ calcd for $C_{82}H_{86}BFN_6O_{14}P$: 1440.61; found: 1440.40.
[Note: The presence of rotamers results in excessive splitting of the ^{13}C signals.]



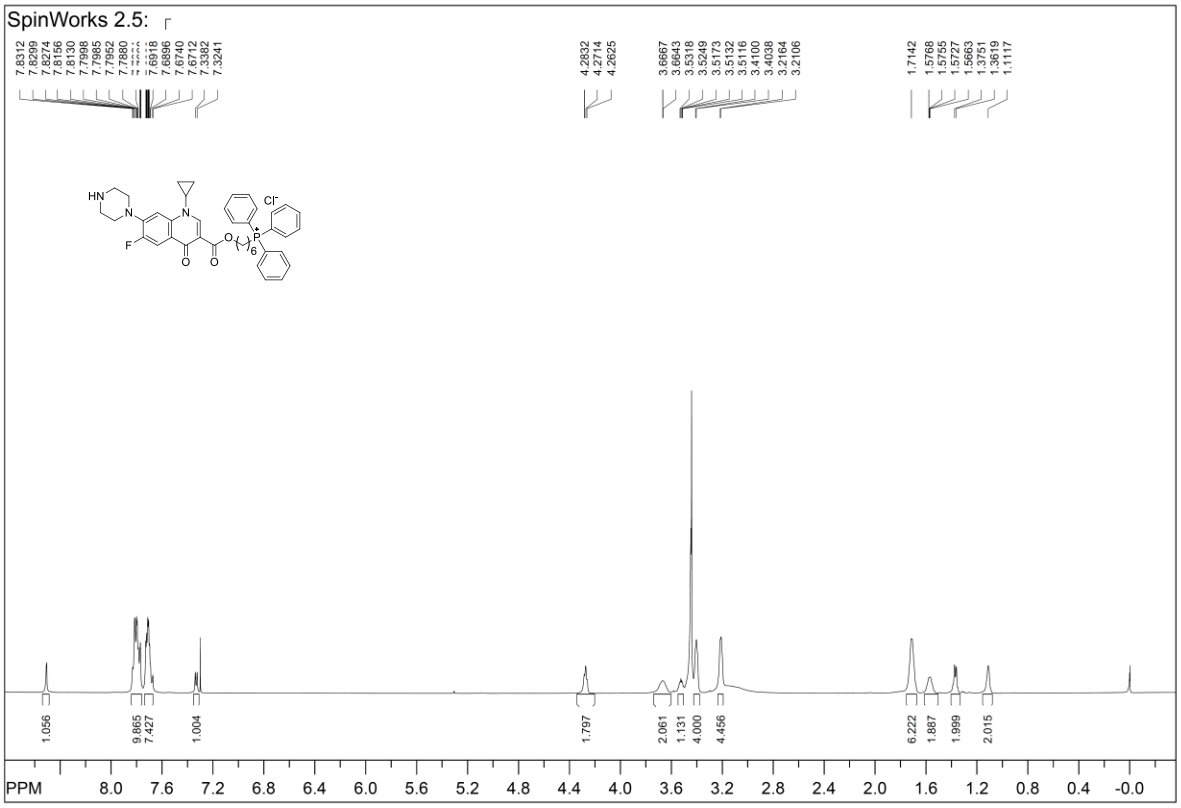
^{1}H NMR spectrum of compound **3**



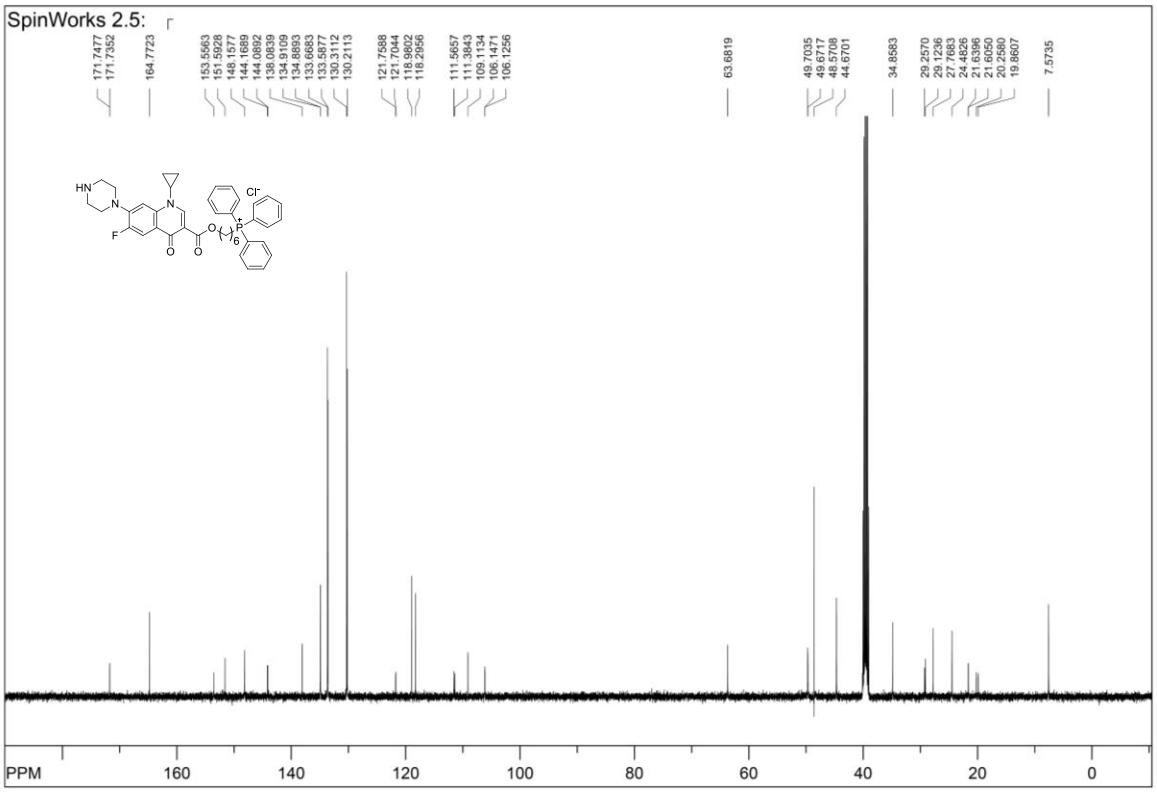
^{13}C NMR spectrum of compound **3**



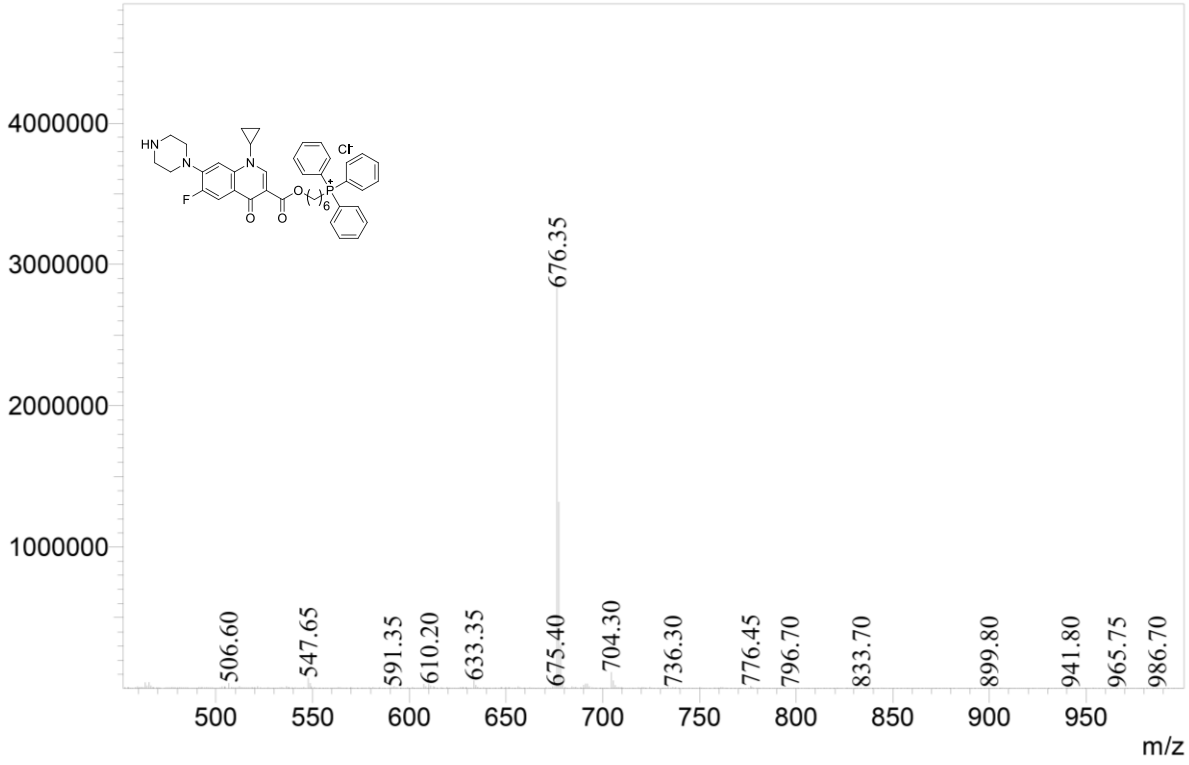
ESI-MS spectrum of compound **3**



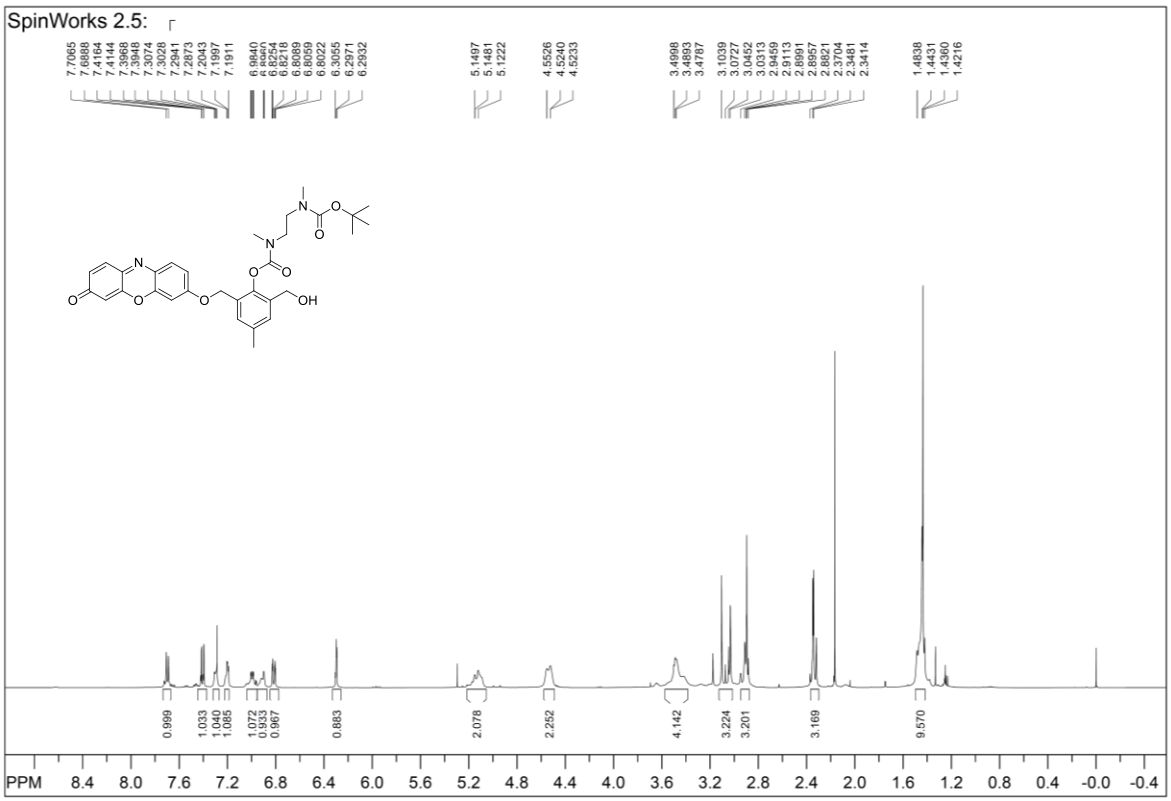
^{1}H NMR spectrum of **Mt-CFX**



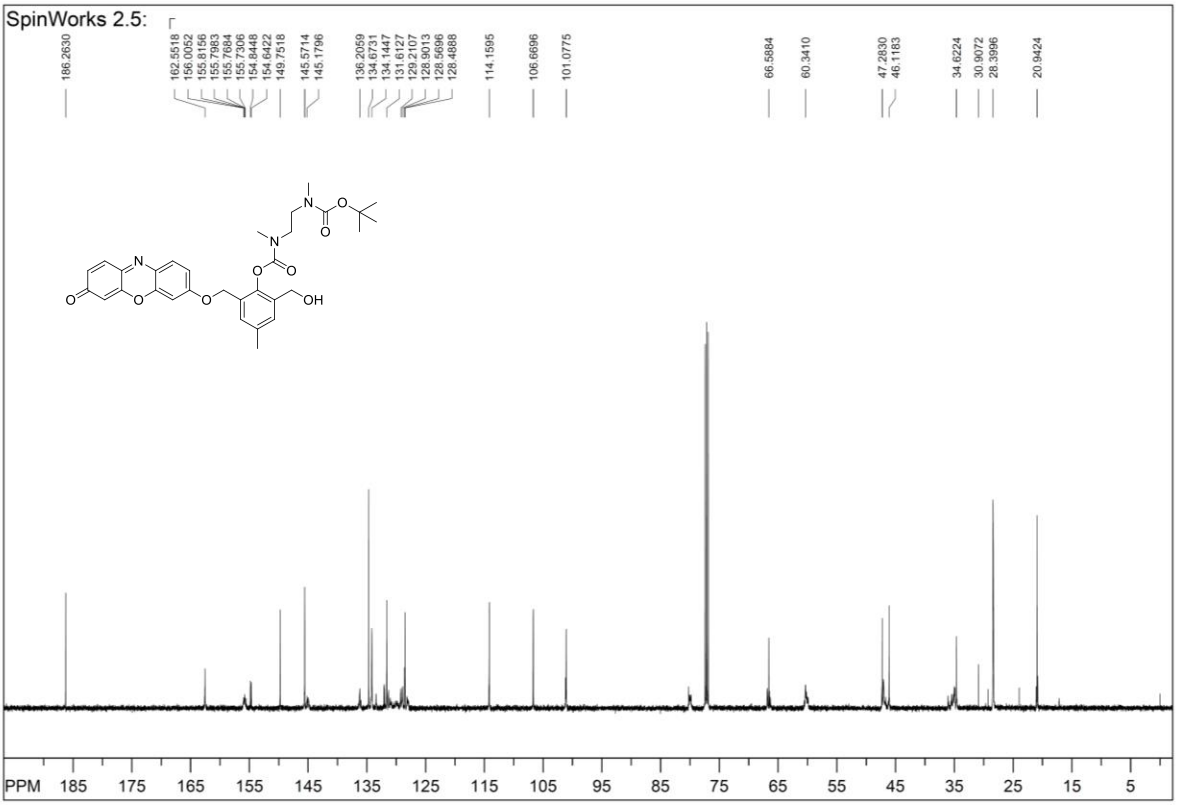
^{13}C NMR spectrum of **Mt-CFX**



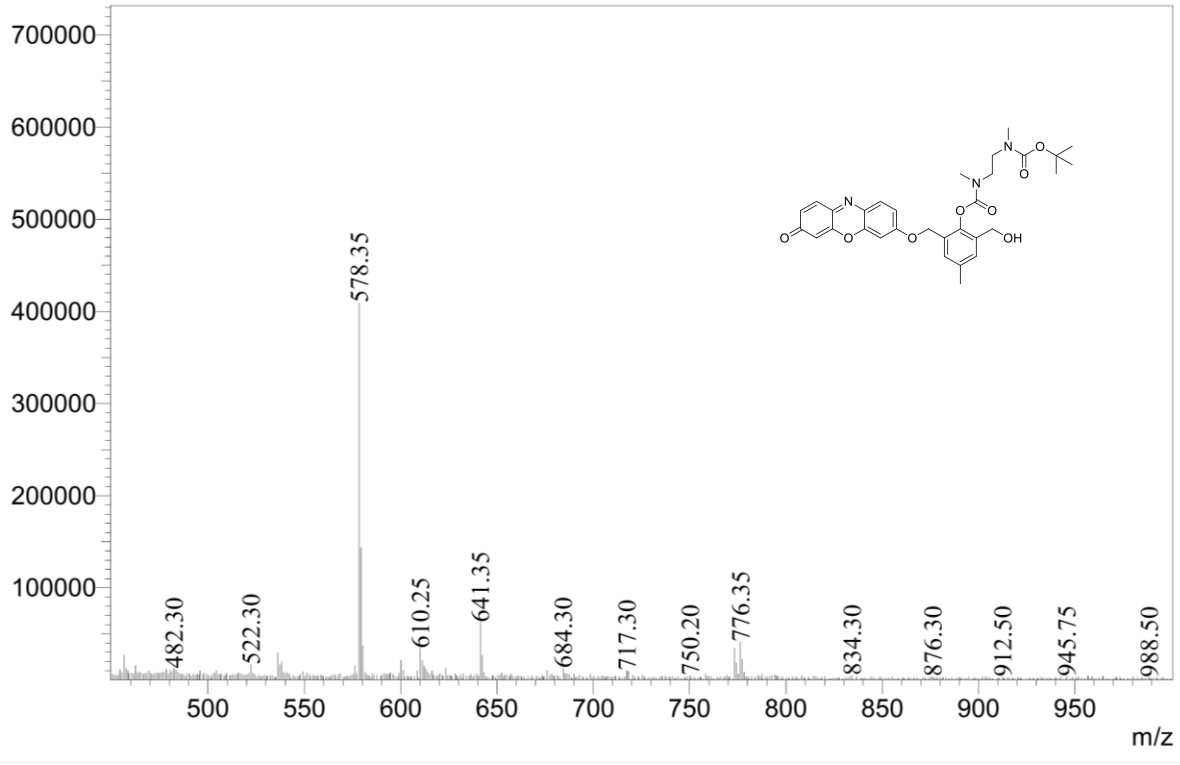
ESI-MS spectrum of **Mt-CFX**



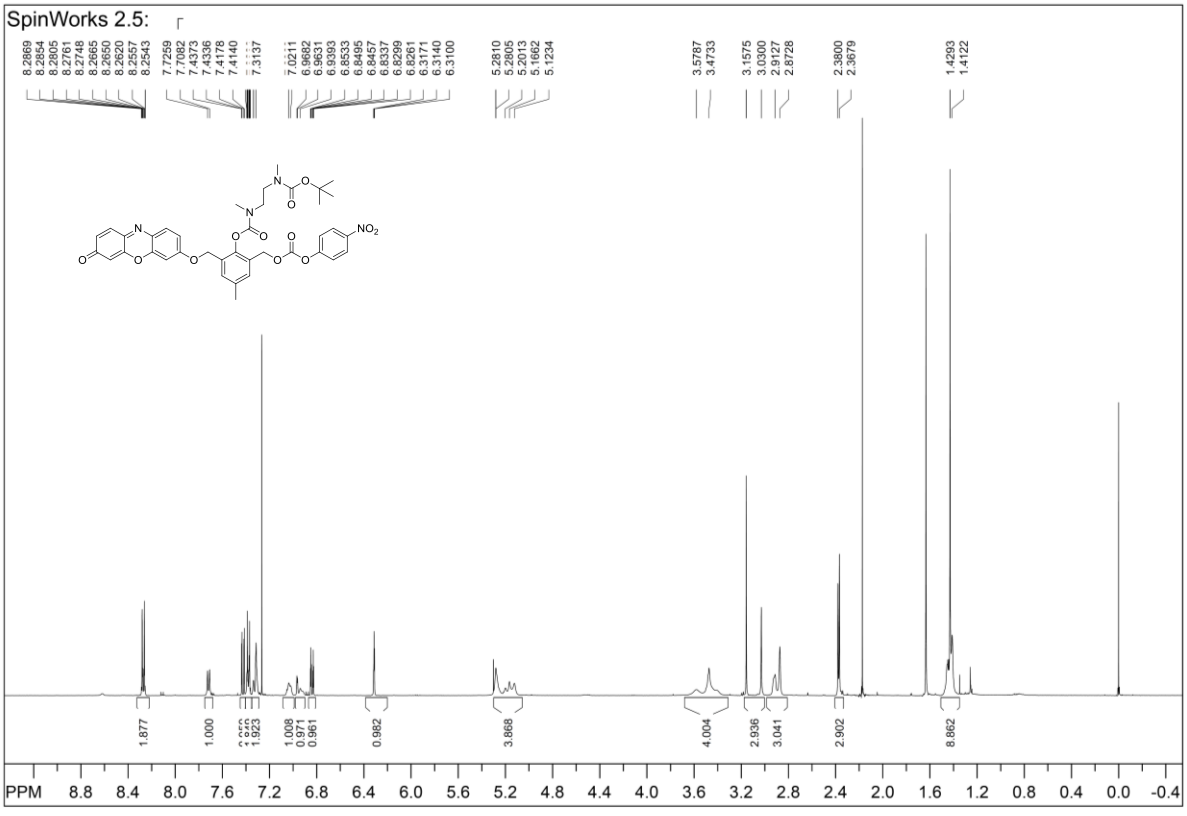
^{1}H NMR spectrum of compound **8**



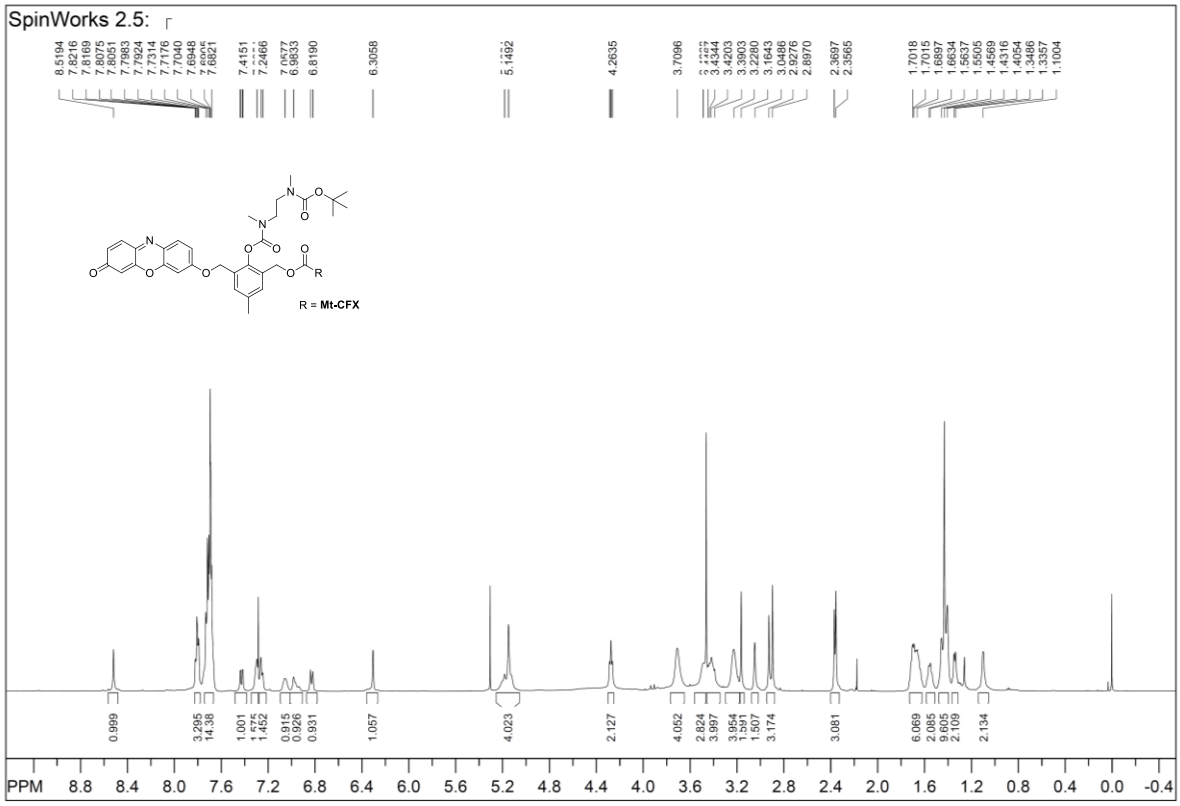
^{13}C NMR spectrum of compound **8**



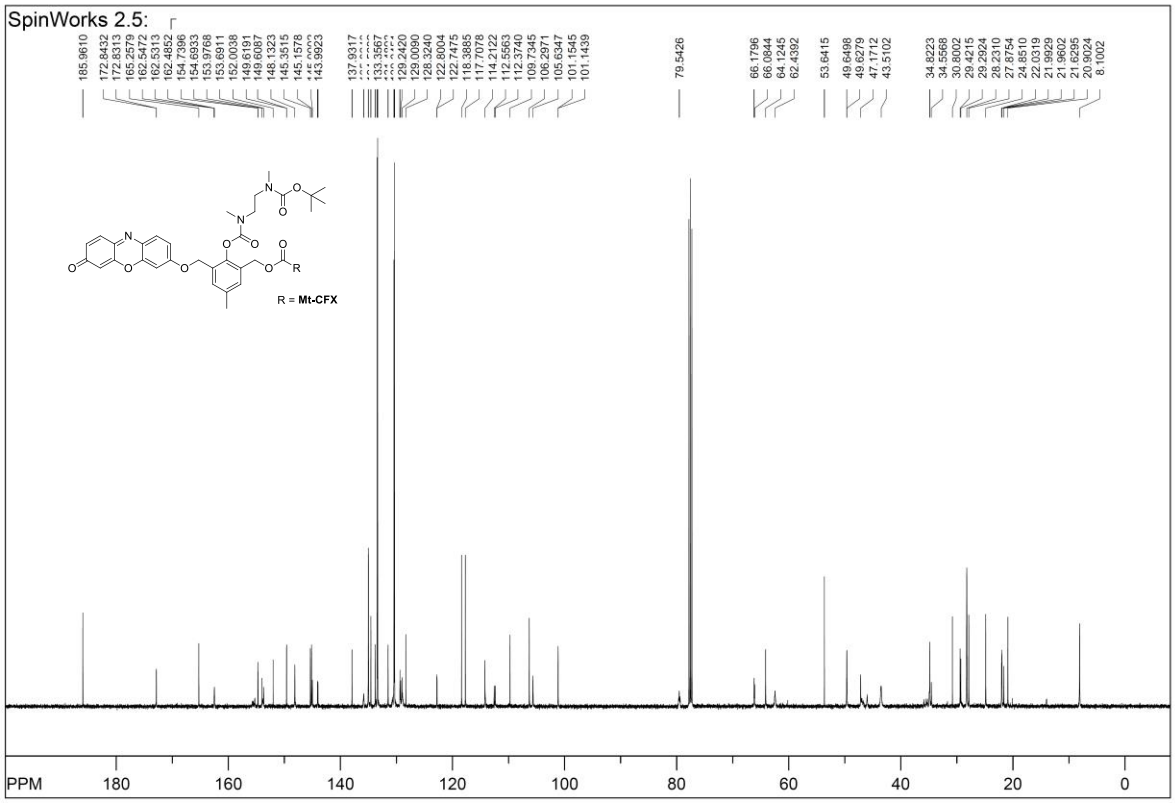
ESI-MS spectrum of compound **8**



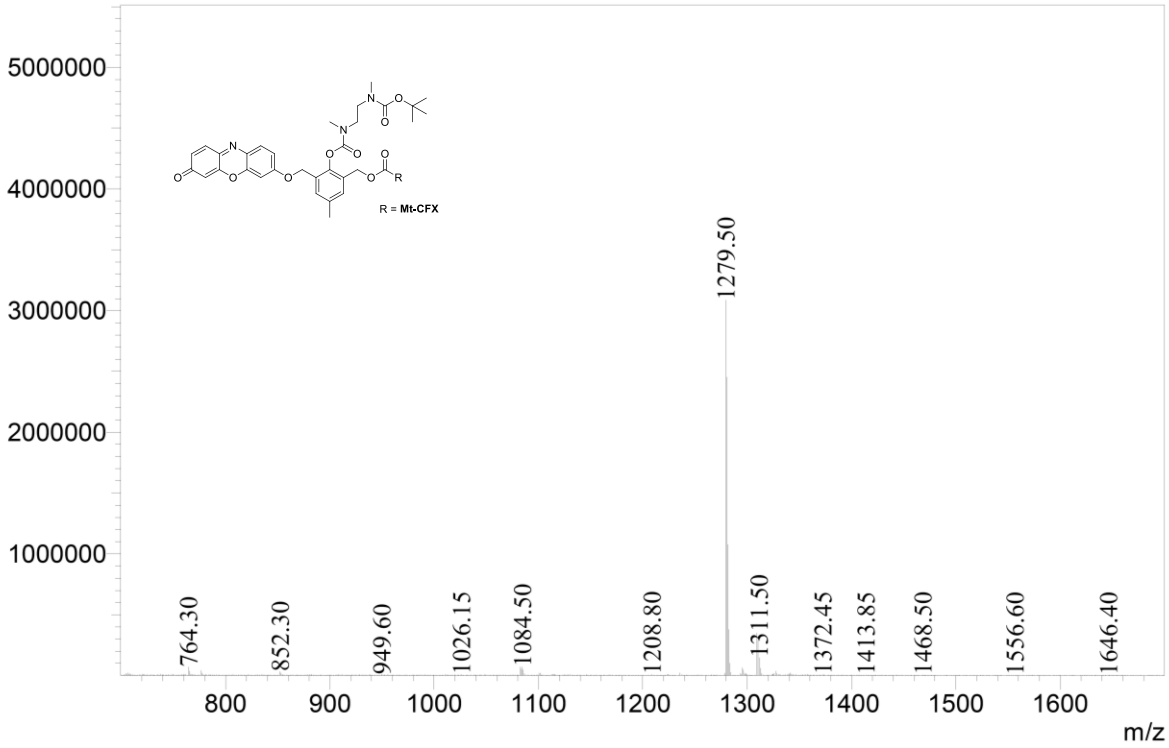
^{1}H NMR spectrum of compound **9**



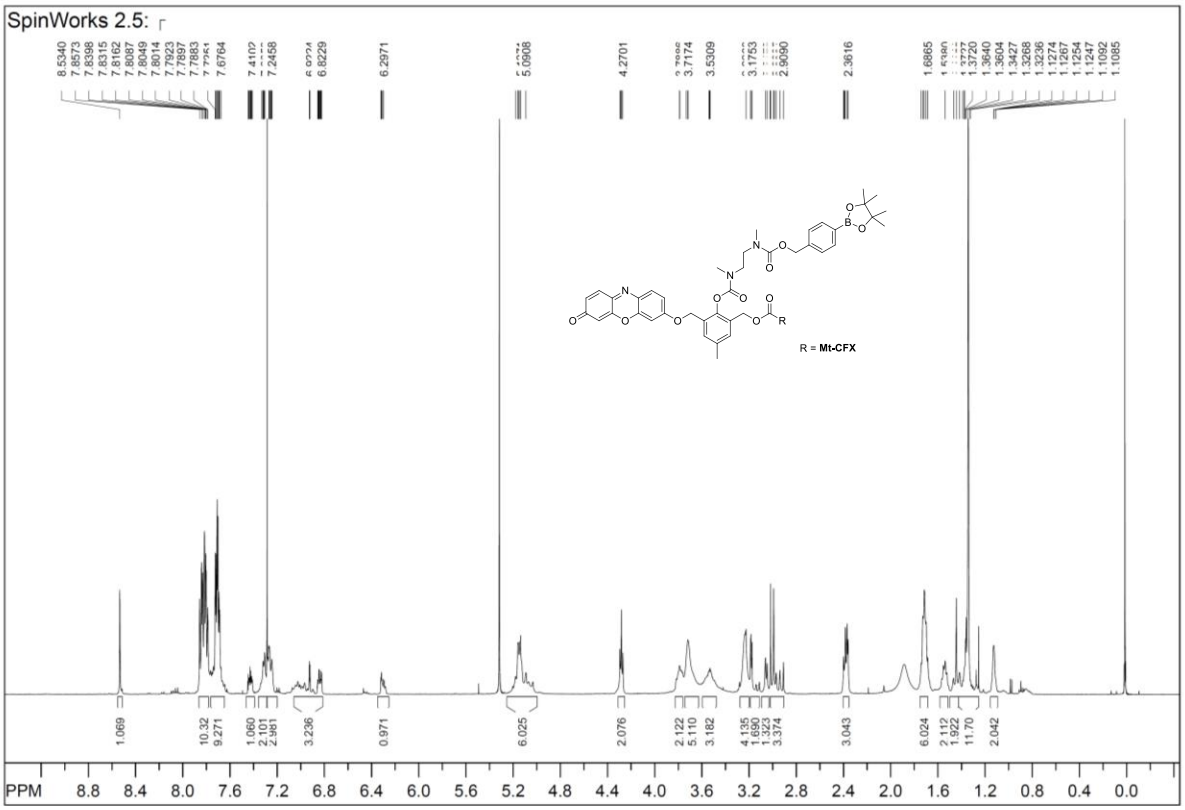
^{1}H NMR spectrum of compound **10**



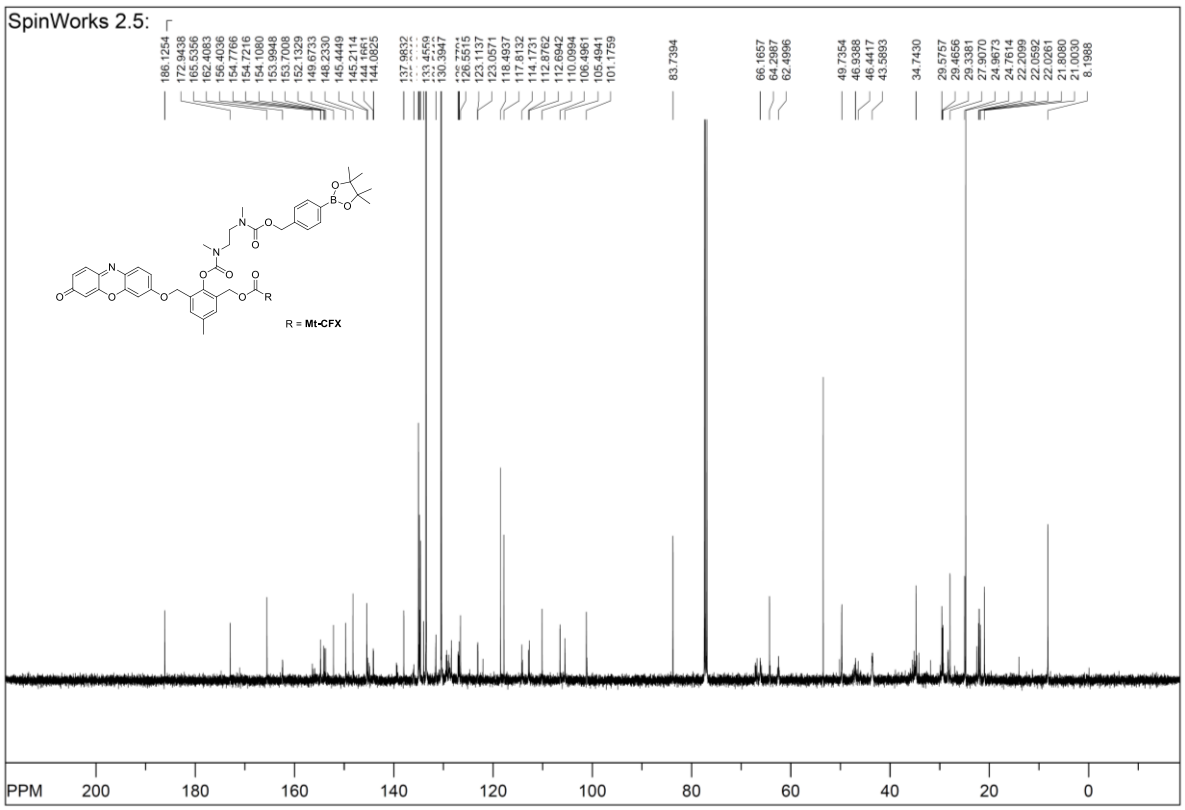
^{13}C NMR spectrum of compound **10**



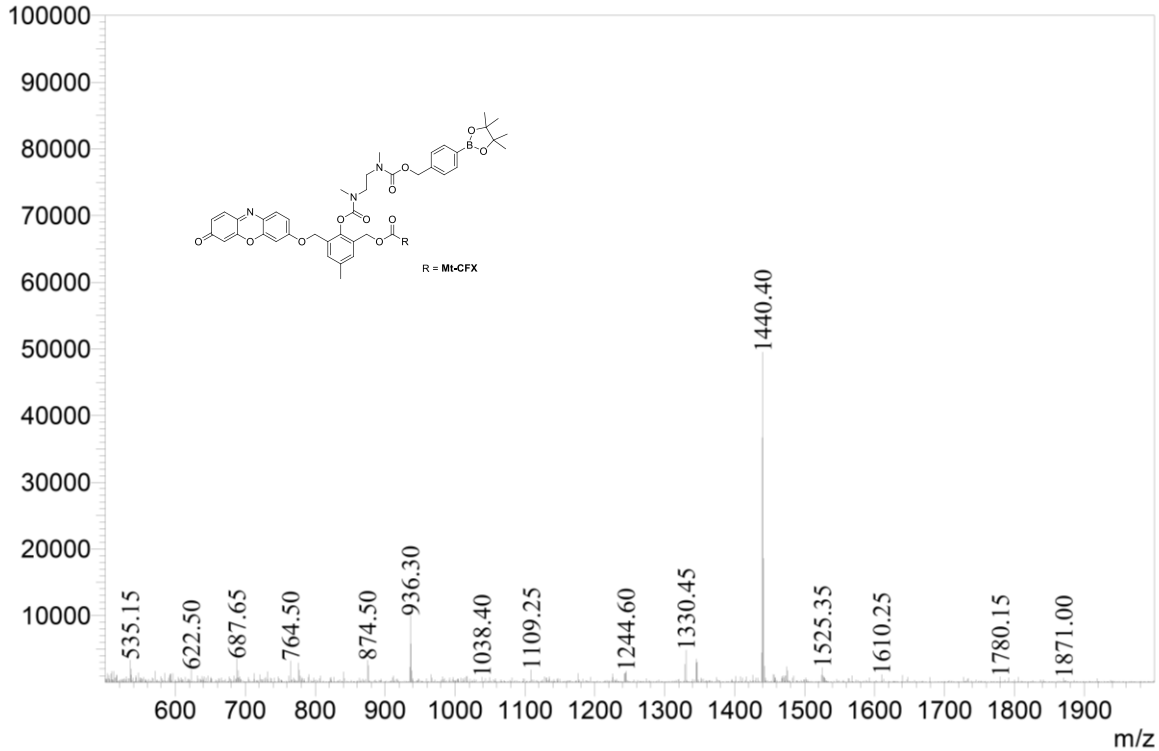
ESI-MS spectrum of compound **10**



^{1}H NMR spectrum of **Bo-Mt-CFX**



^{13}C NMR spectrum of **Bo-Mt-CFX**



ESI-MS spectrum of **Bo-Mt-CFX**

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