Supporting Information

Dithiocarbamation of spiro-aziridine oxindoles: a facile access to C3-functionalised 3-thiooxindoles as apoptosis inducing agents

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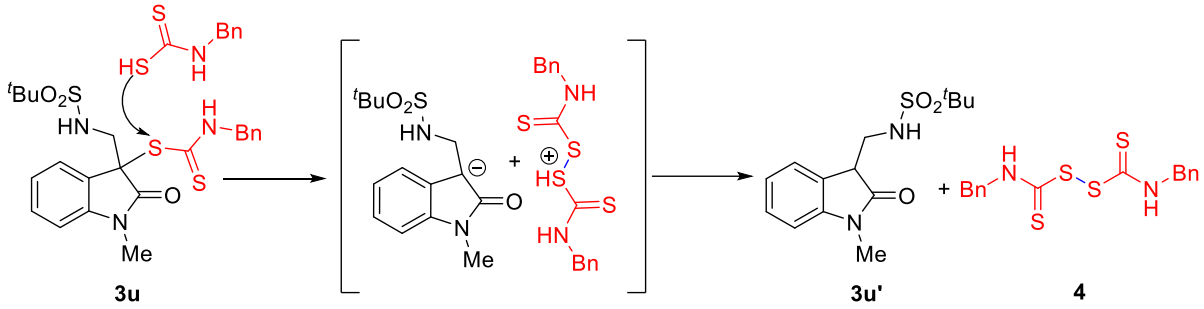
1. Mechanism for the formation of 3u'



Scheme 1: Plausible mechanism.

2. Biological Evaluation

3.1 Cell Culture: Human lung adeno cancer cell line (A549), Human colorectal cancer cell line (HCT-116), Human breast cancer cell line (MCF-7) cells were procured from National Centre for Cell Sciences (NCCS, Pune). The cells were cultured and maintained in respective DMEM (Dulbecco's Modified Eagle Medium, Hyclone) or RPMI (Roswell Park Memorial Institute, Hyclone) medium, supplemented with 10% FBS (fetal bovine serum, Hyclone), 1% pencillin-streptomycin antibiotic mixture (Hyclone). The cell lines were maintained in a CO_2 incubator with 5% CO_2, 37 °C and 90% relative humidity.

3.2 Cytotoxicity Assay: The cytotoxicity of these synthesised compounds was evaluated by MTT [(3-(4, 5- dimethylythiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. Briefly, 1 x 10^4 number of cells were plated in a flat bottom, sterile 96-well plate and incubated for 24 h at 37 °C in a CO_2 incubator. After 24 h, the cells were exposed to various concentrations of the 3-substituted-3-thiooxindoles dissolved in DMSO and incubated further for 48 h. Following the treatment, the medium containing the compounds was discarded and 100 µL of fresh media containing MTT (0.5 mg/ml) was added and incubated further for 4 h at 37 °C. Following that, the MTT media was discarded and the formazan crystals formed were dissolved in 100 µL of DMSO, kept for 20 min and absorbance was recorded at 570 nm (PerkinElmer Envision multimode plate reader). The compounds exhibiting >50% inhibition of cell viability at 20 µM in primary screening were further selected for evaluation of dose response curve (DRC) analysis. IC_{50} values were determined from the DRC analysis by linear regression method. All the values were expressed as mean ± SEM of three independent experiments.

3.3 Morphological observations in phase contrast microscopy: A549 cells were plated and treated with 2, 4 and 8 µM of compound **3u**, incubated at 37 °C in a CO_2 incubator for 48

h. The cells were washed in PBS and they were observed under phase contrast microscope (Carl Zeiss) at 10X for morphological changes and the images were captured.

3.4 Acridine Orange/Ethidium Bromide staining: A549 cells were treated with 2, 4 and 8 µM of compound **3u**, incubated at 37 °C in a CO_2 incubator for 48 h. It was followed by washing of the cells in PBS and addition of the fluorescent dyes at a concentration of 10 µg/ml into each well and visualising under the fluorescent microscope (Nikon, Inc. Japan) with excitation at 488 nm and emission at 550 nm at 200X magnification.

3.5 DAPI staining: A549 cells were treated with 2, 4 and 8 µM of compound **3u** and incubated at 37 °C in a CO_2 incubator for 48 h. Following the treatment duration, the cells were washed with PBS and fixed with 4% paraformaldehyde and 0.1% triton-X 100 for 5 min each, stained with 10 µg/ml of DAPI for each well and the images were taken in a fluorescent microscope (Nikon, Inc. Japan) with excitation at 359 nm and emission at 461 nm using DAPI filter at 200X magnification.

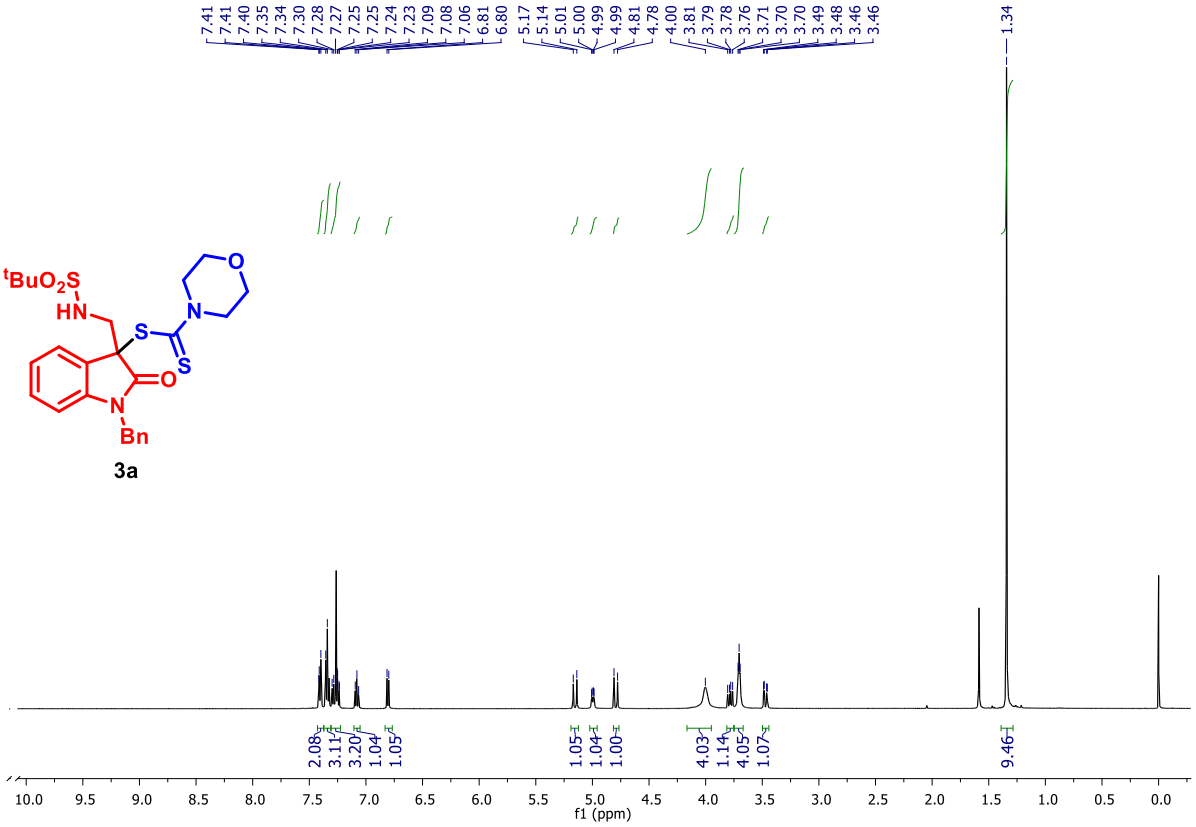
3.6 DCFDA staining: A549 cells were plated at a concentration of 1 x 10^6 cells/well in a 12 well plate and treated with 2, 4 and 8 µM of compound **3u** and incubated at 37 °C in a CO_2 incubator for 48 h. This was followed by addition of DCFDA (10 µM) for 30 min. The cells were washed with PBS for excess dye and the images were taken in a fluorescent microscope (Nikon, Inc. Japan) at 200X magnification.

3.7 Cell migration assay: A549 cells in a concentration of 5 x 10^5 cells/well were seeded in a six well plate and the cells were incubated at 37 °C in a CO_2 incubator for 24 h and allowed to form a monolayer. A small micro-pipette tip was taken to create a wound over the monolayer and the cells were washed with PBS to remove any non-adherent cell. The cells were then treated with 2, 4 and 8 µM of compound **3u** for 48 h and incubated at 37 °C in a CO_2 incubator. Images of the wounds were taken at 0^{th} h and at 48^{th} h post wound induction. The migrated cells were observed and compared with that of the control cells at both time points. The cells were photographed under Phase contrast Microscope (Carl Zeiss) at 10X objective lens and width was measured using the Zenn software and the distance/width was plotted against the concentration of the drug.

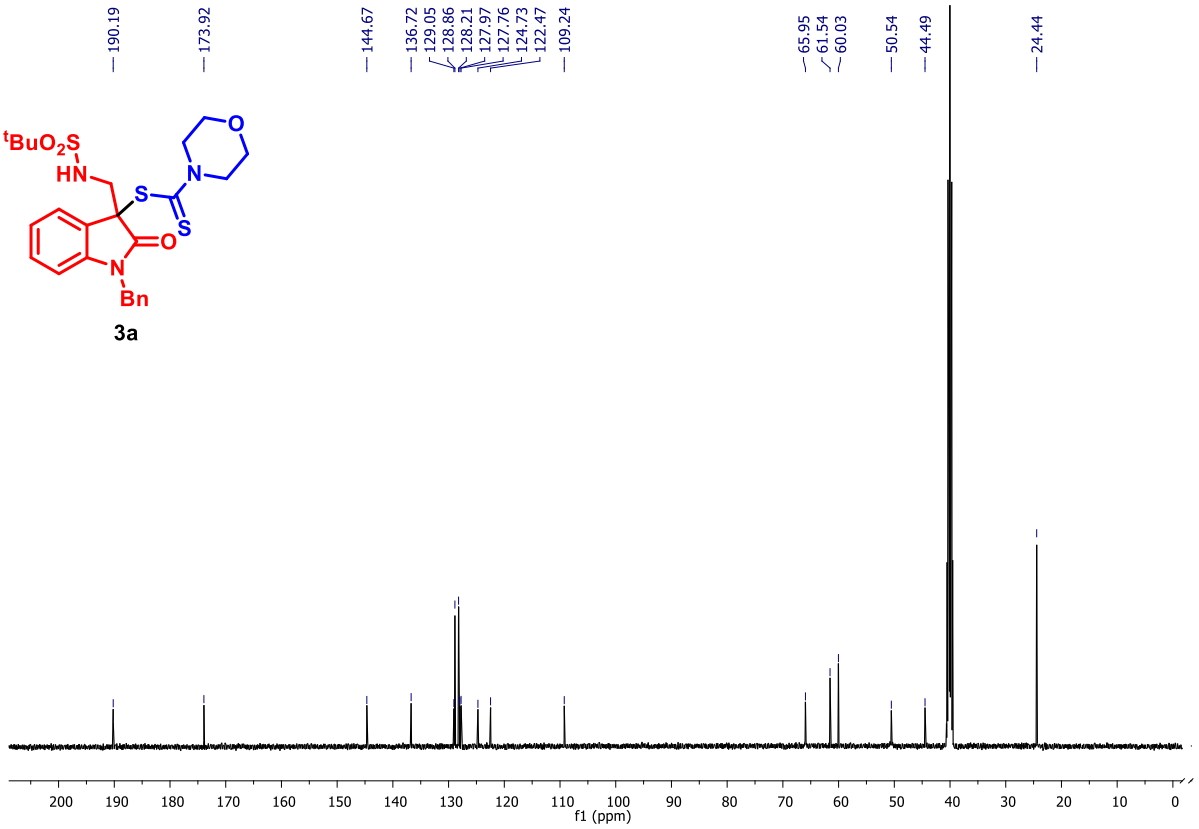
3.8 Colony formation assay: A549 cells were seeded in a 6 well plate at a density of 75-200 cells/well and incubated for 24 h at 37 °C in a CO_2 incubator. Cells were treated with 2, 4 and 8 µM of compound **3u** for 48 h. Following incubation, the media containing the treatment drug was discarded and cells were maintained in fresh complete medium for 14 days at 37 °C in a CO_2 incubator. The colonies formed after 14 days were stained with 1%

crystal violet for 3 h and washed with PBS. The wells were photographed and colonies were counted using Vilber Fusion Fx software (Vilber Lourmat, France) and the results were represented as total colony number *vs*. concentration.

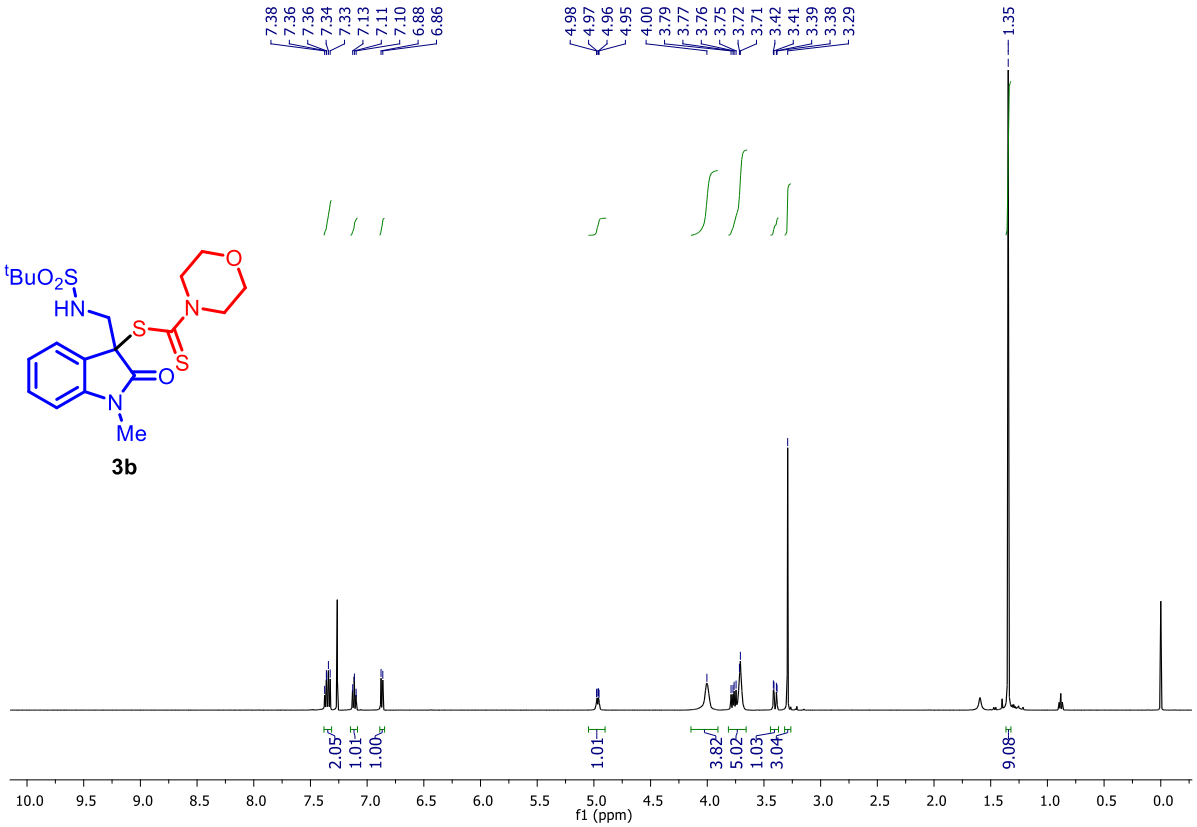
3. Copies of ^{1}H and ^{13}C NMR spectra



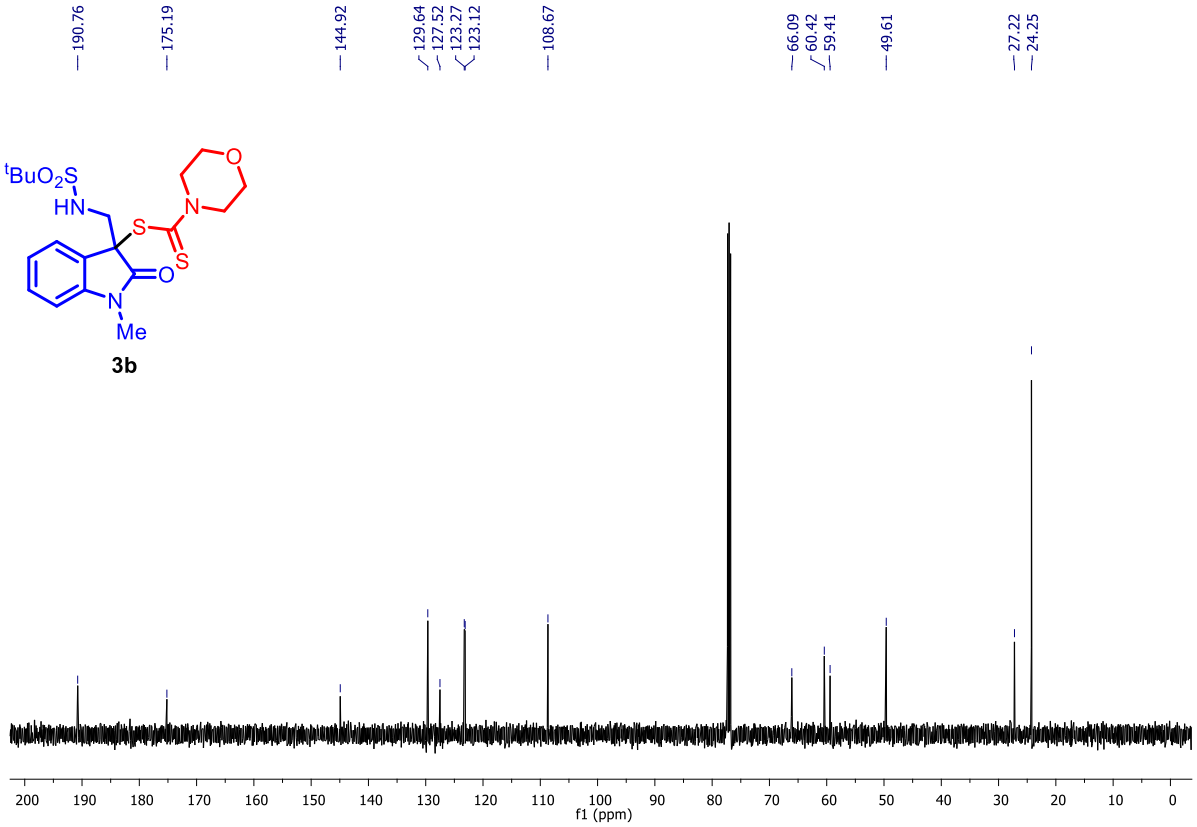
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3a**



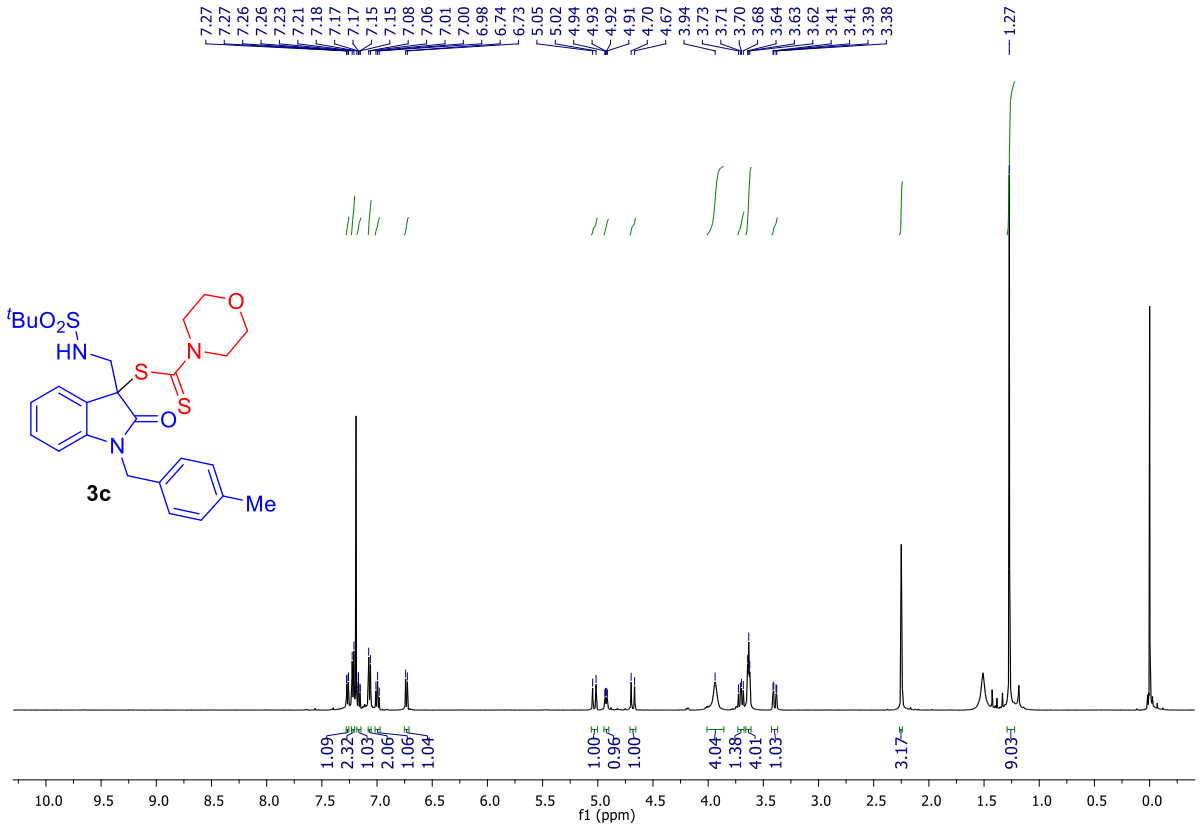
^{1}H NMR (125 MHz, DMSO-d_6) of compound **3a**



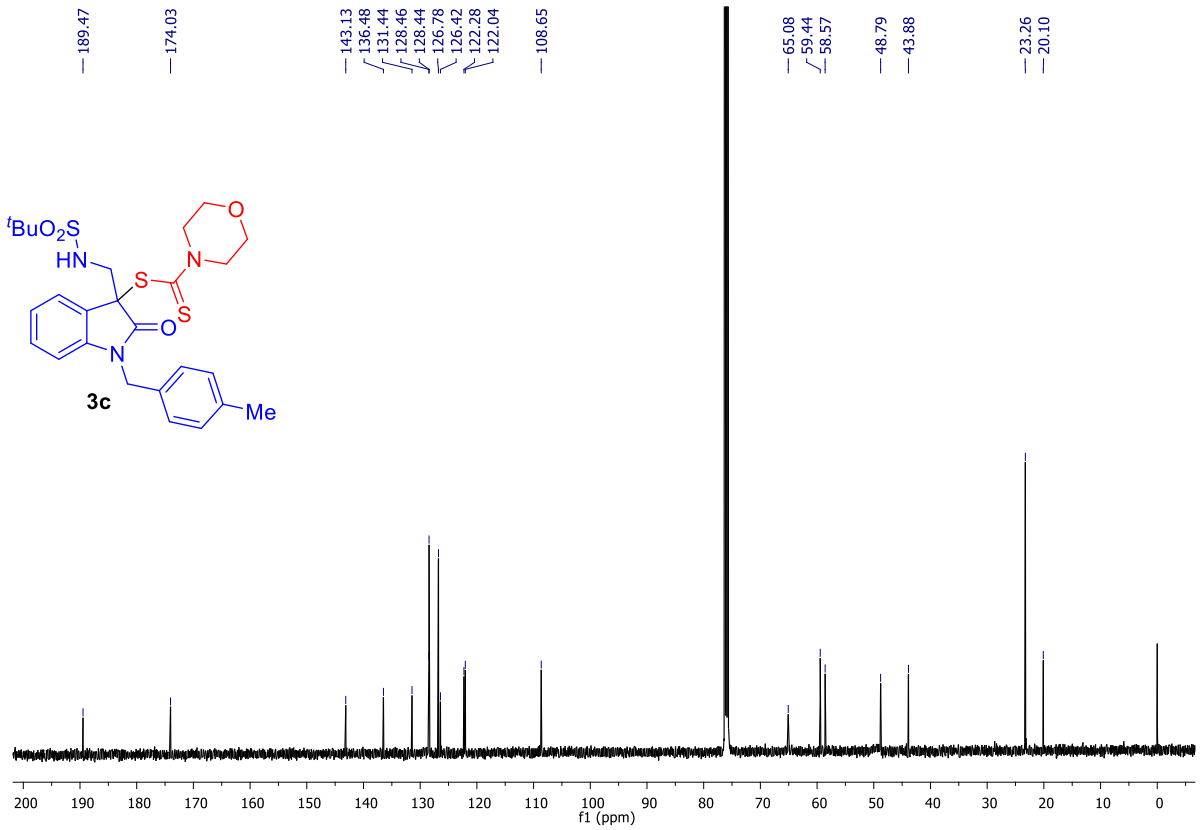
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3b**



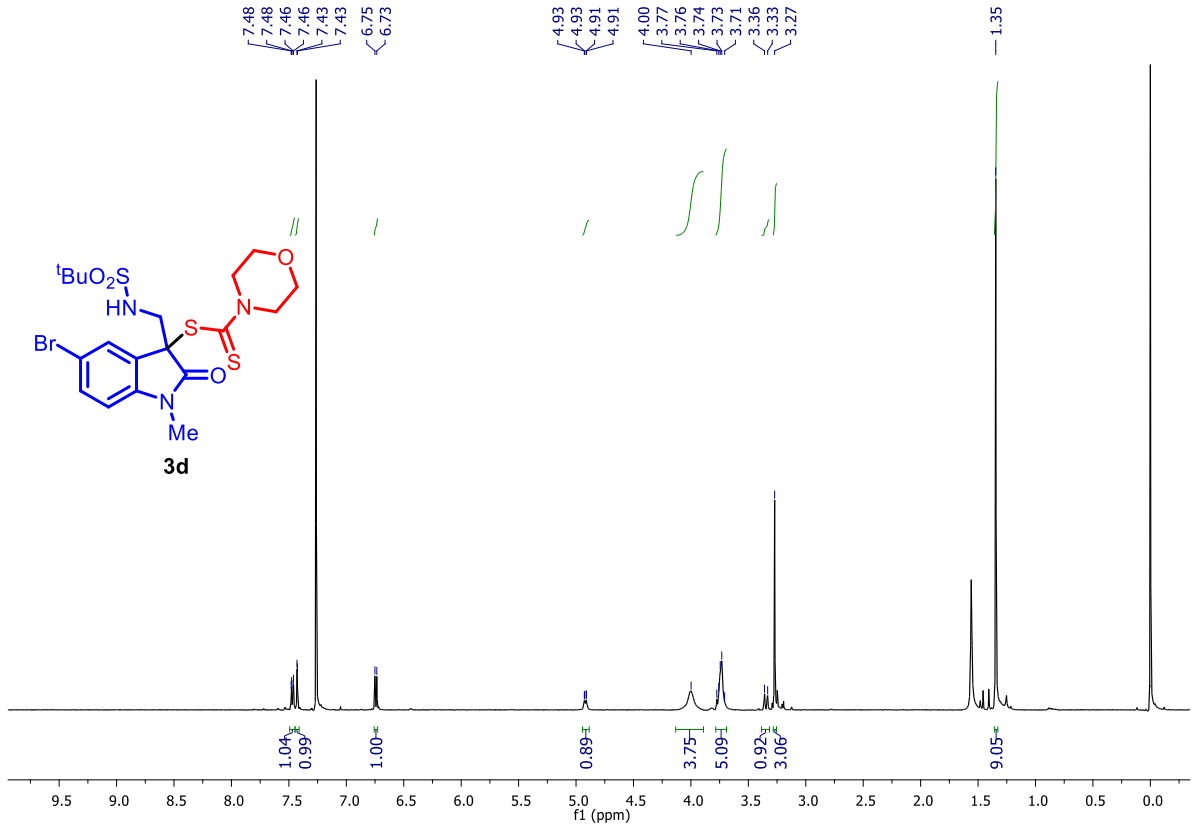
^{13}C NMR (125 MHz, $CDCl_3$) of compound **3b**



^{1}H NMR (500 MHz, $CDCl_3$) of compound **3c**



^{13}C NMR (125 MHz, $CDCl_3$) of compound **3c**



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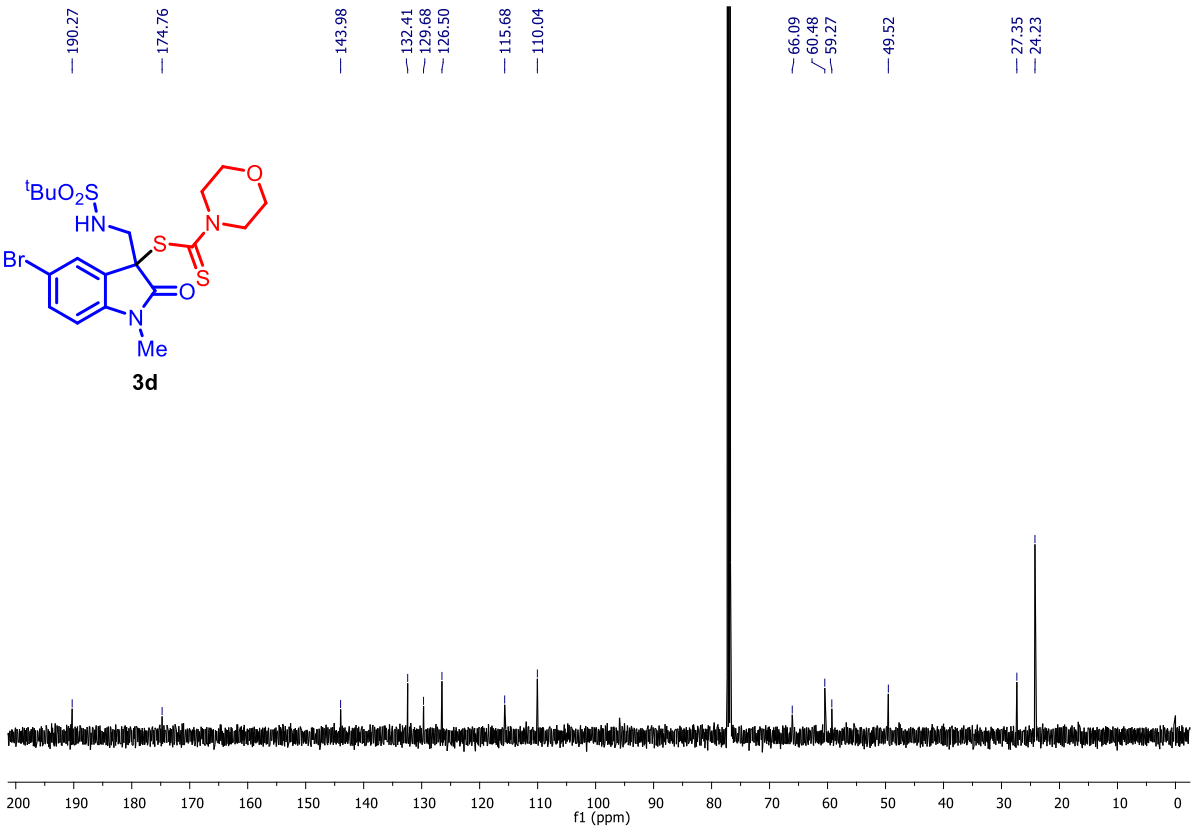

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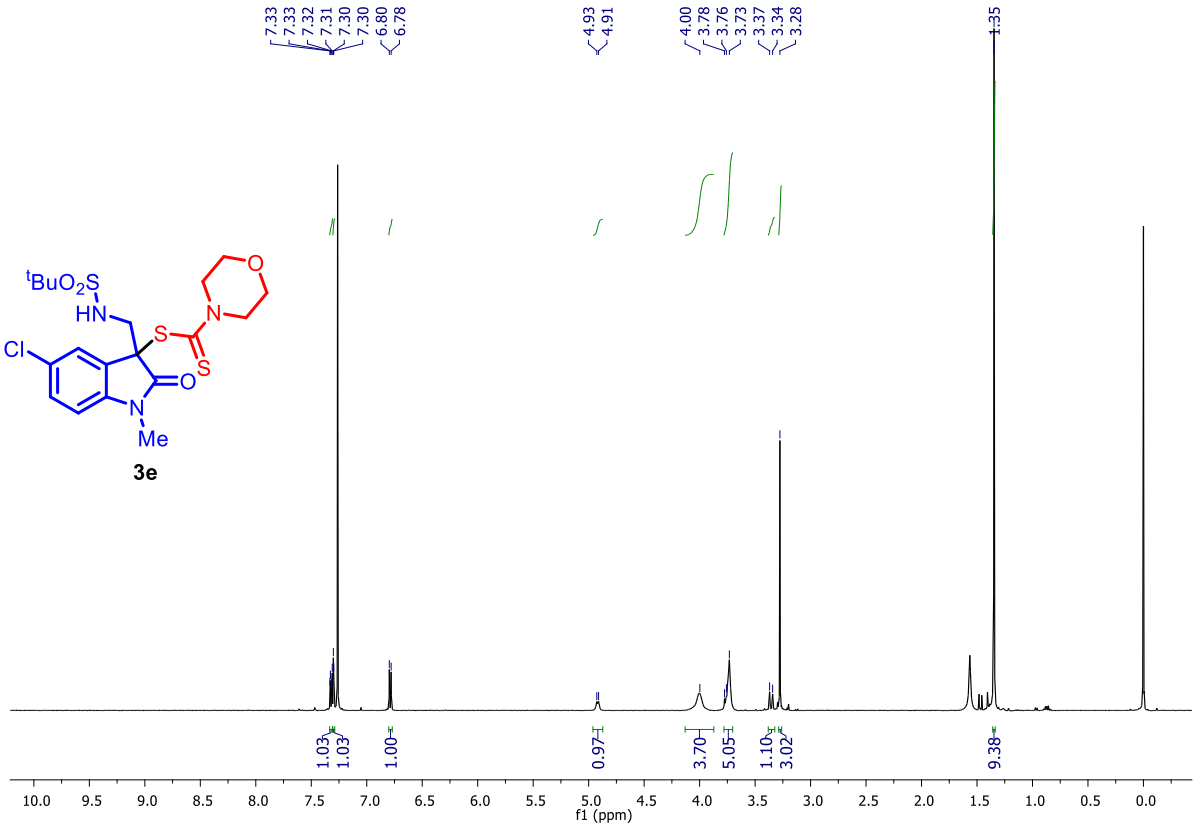

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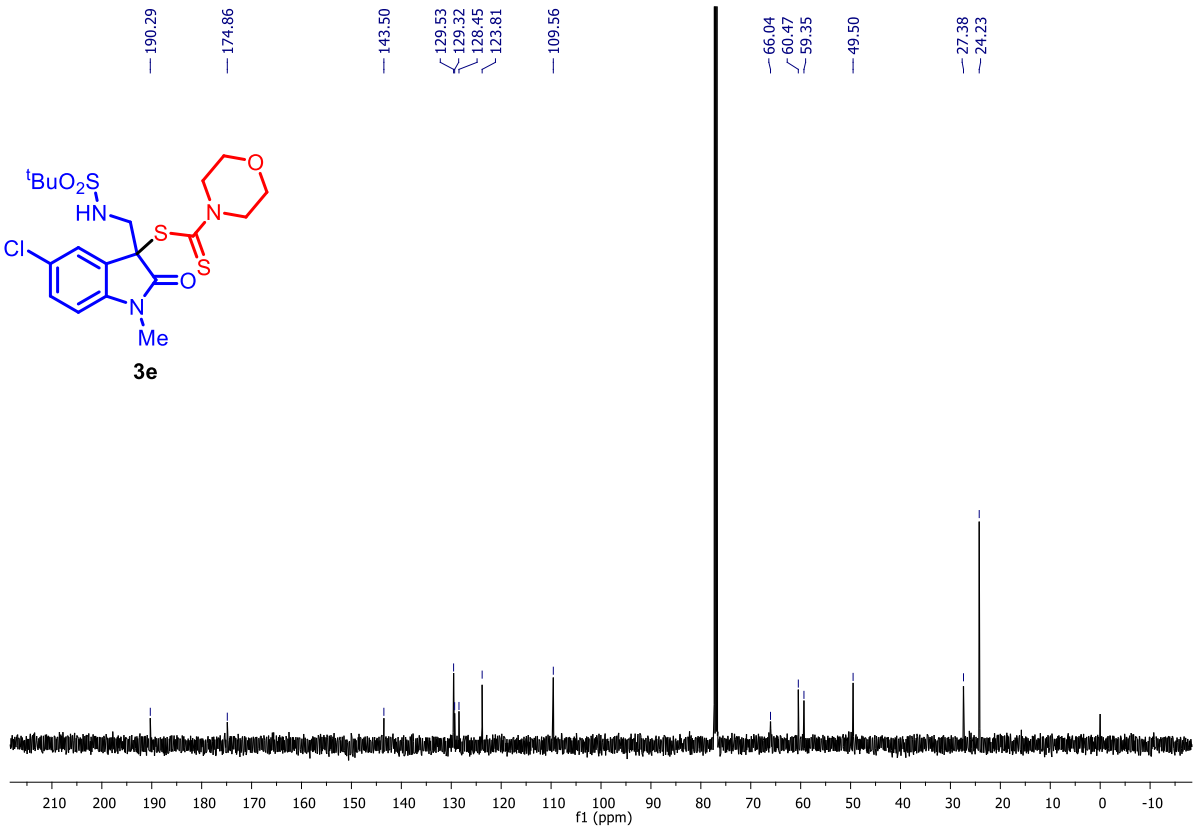
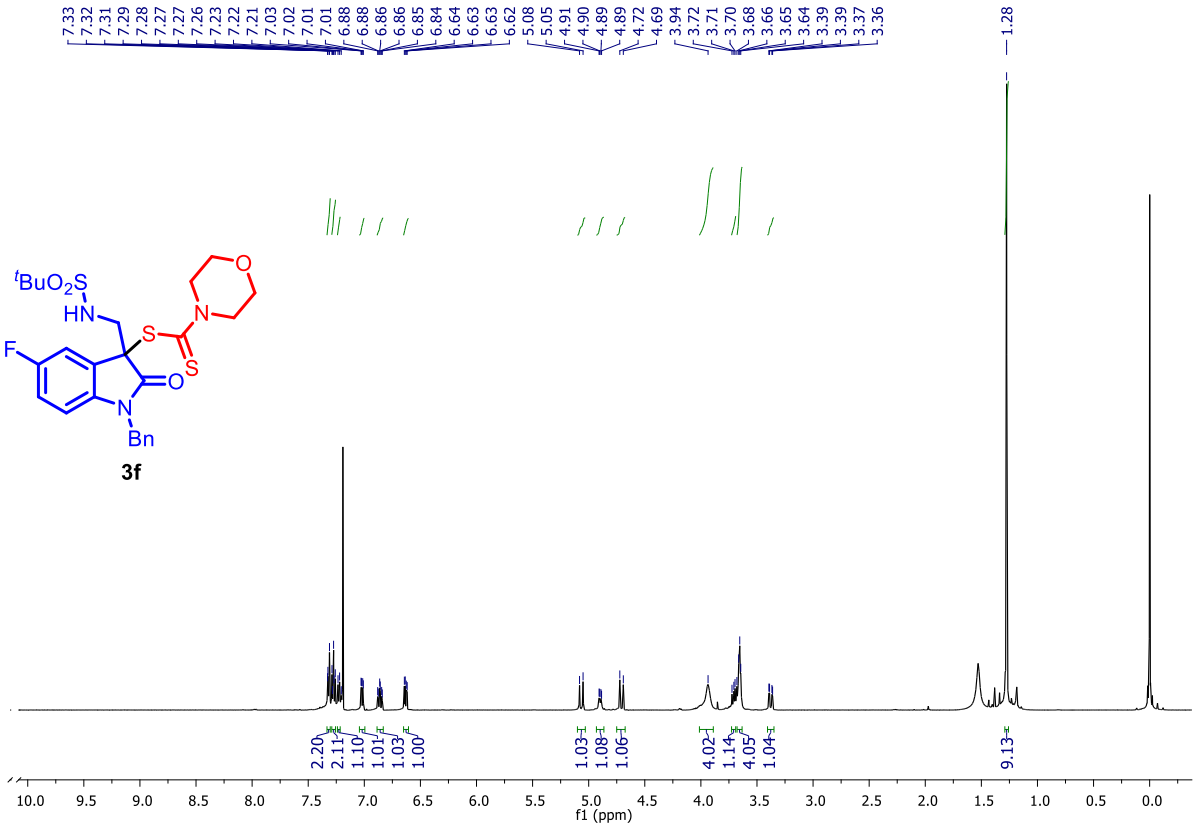
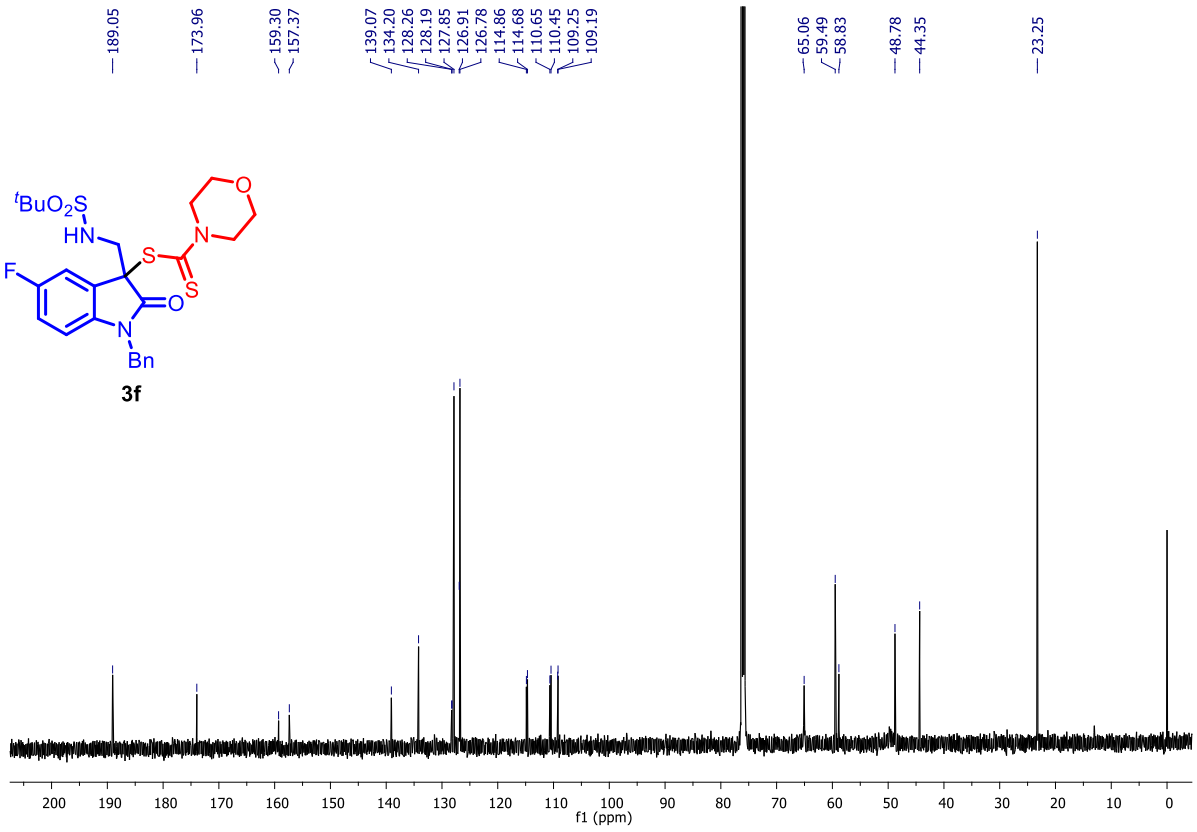


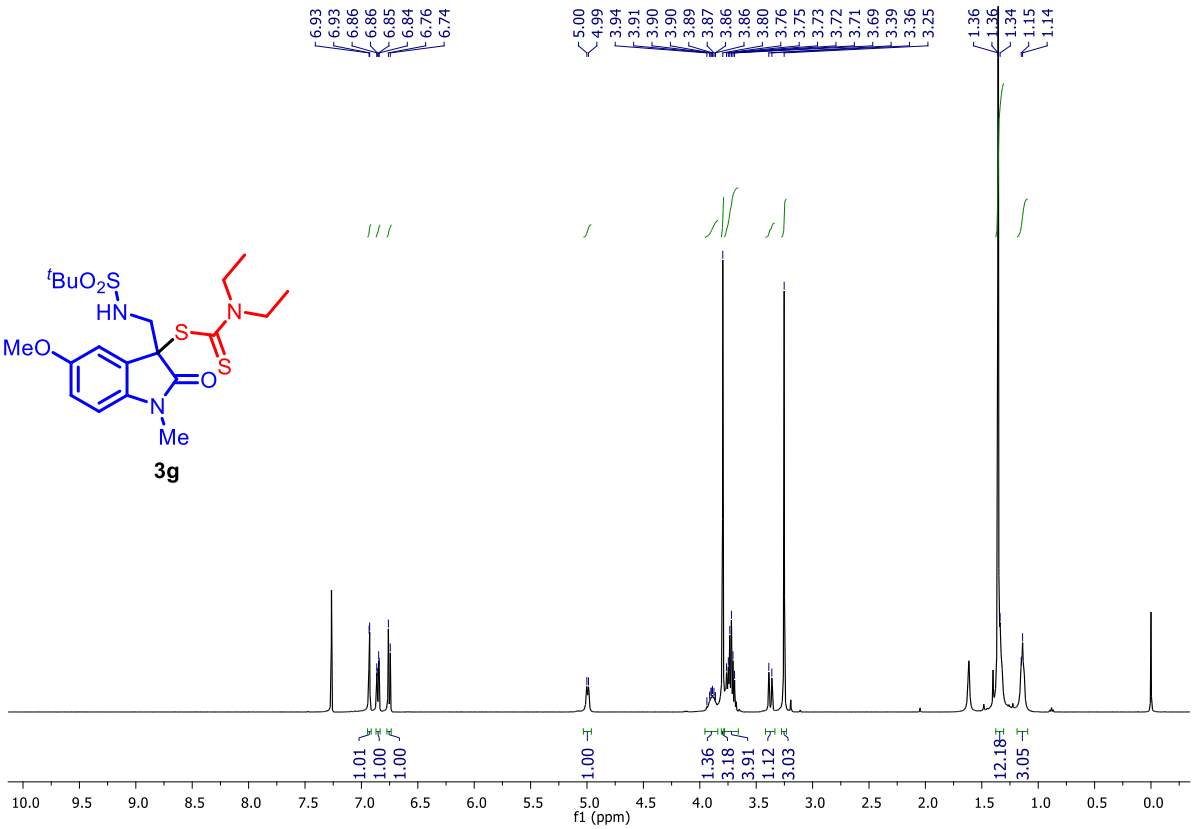
^{13}C NMR (125 MHz, $CDCl_3$) of compound **3e**



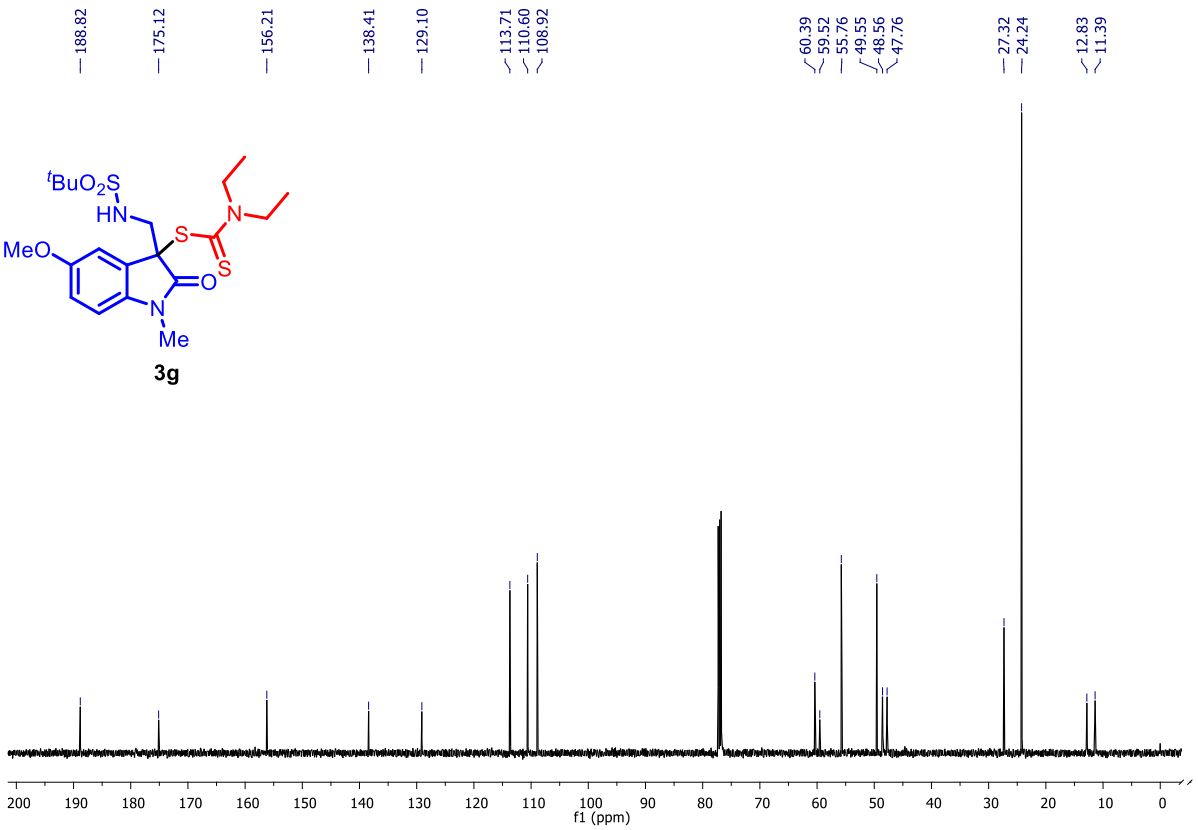
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3f**



^{13}C NMR (125 MHz, $CDCl_3$) of compound **3f**



^{1}H NMR (500 MHz, $CDCl_3$) of compound **3g**



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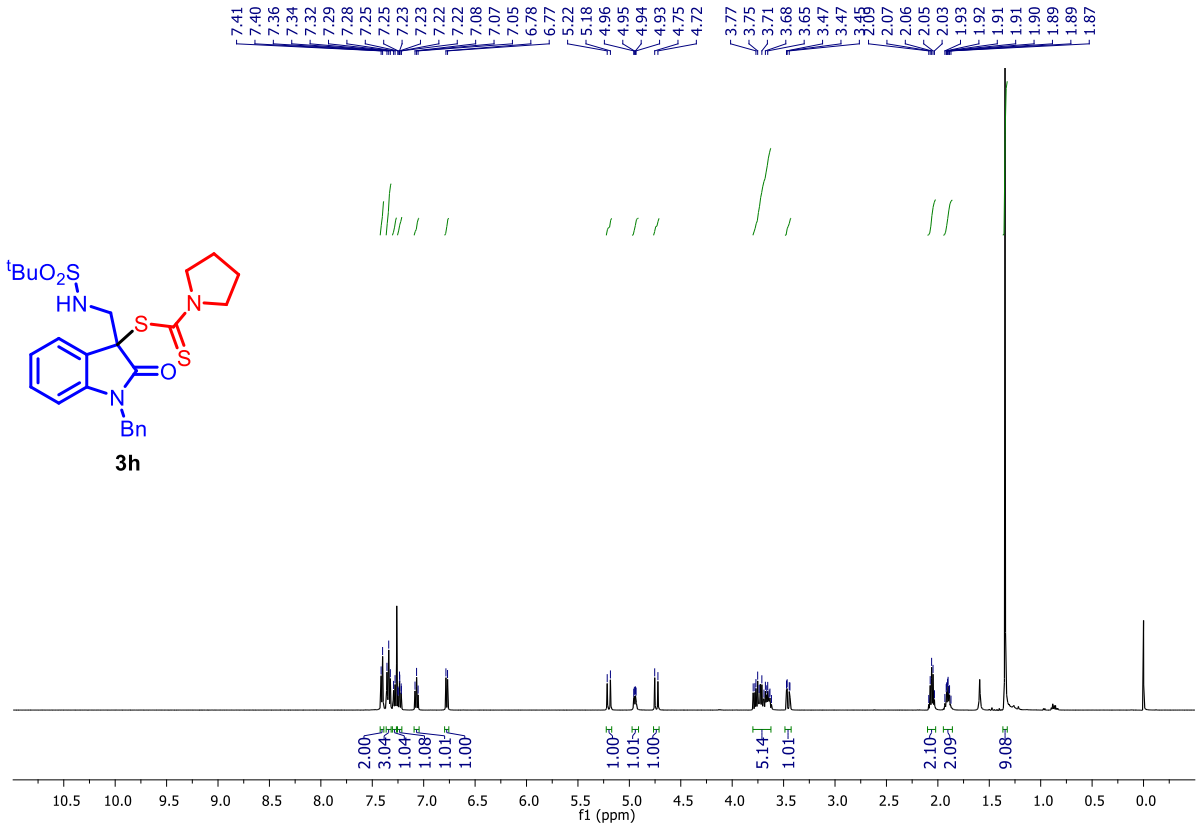

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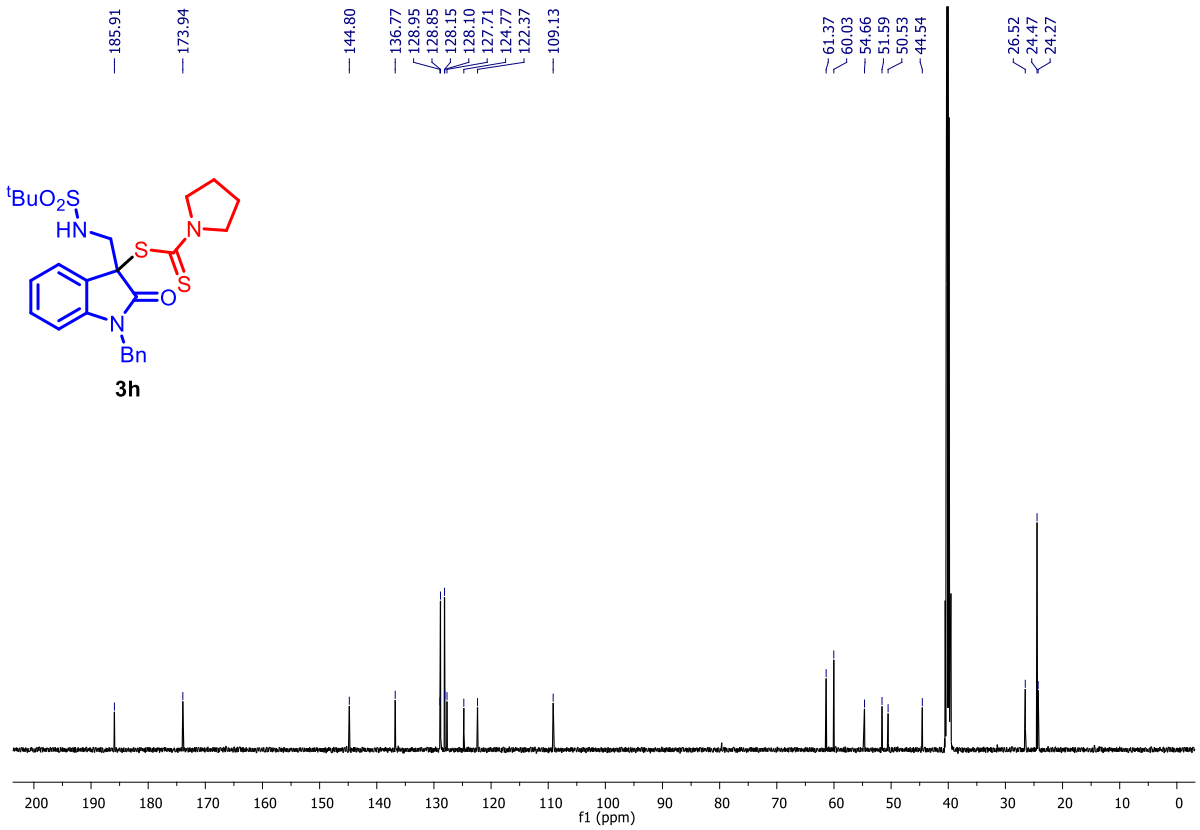
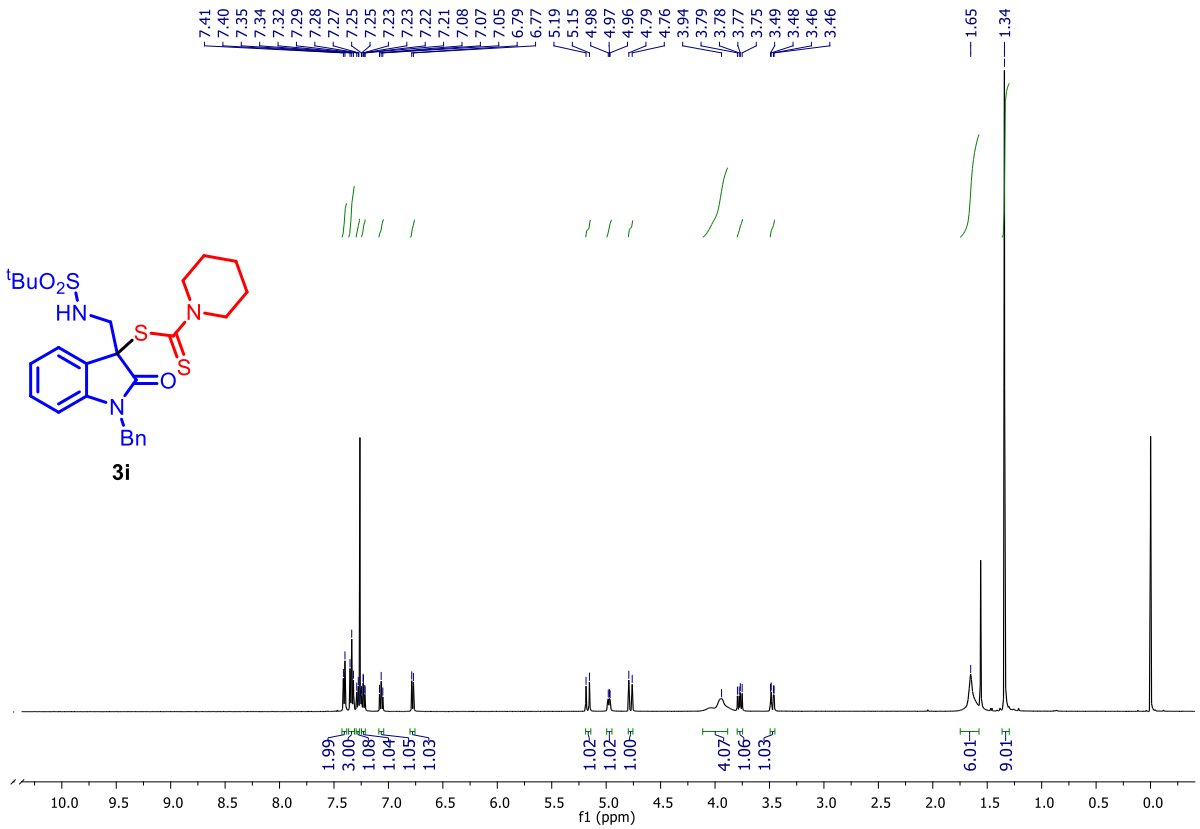


^{13}C NMR (125 MHz, DMSO-d_6) of compound **3h**



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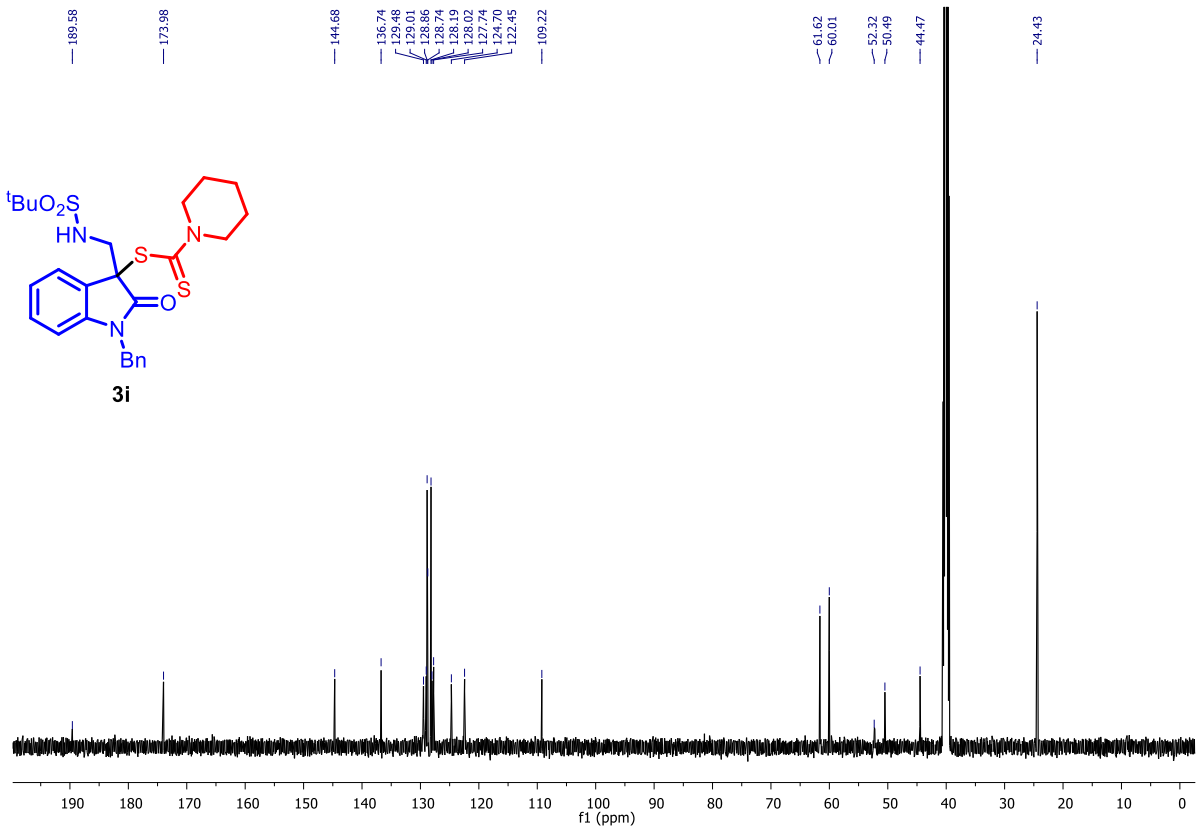
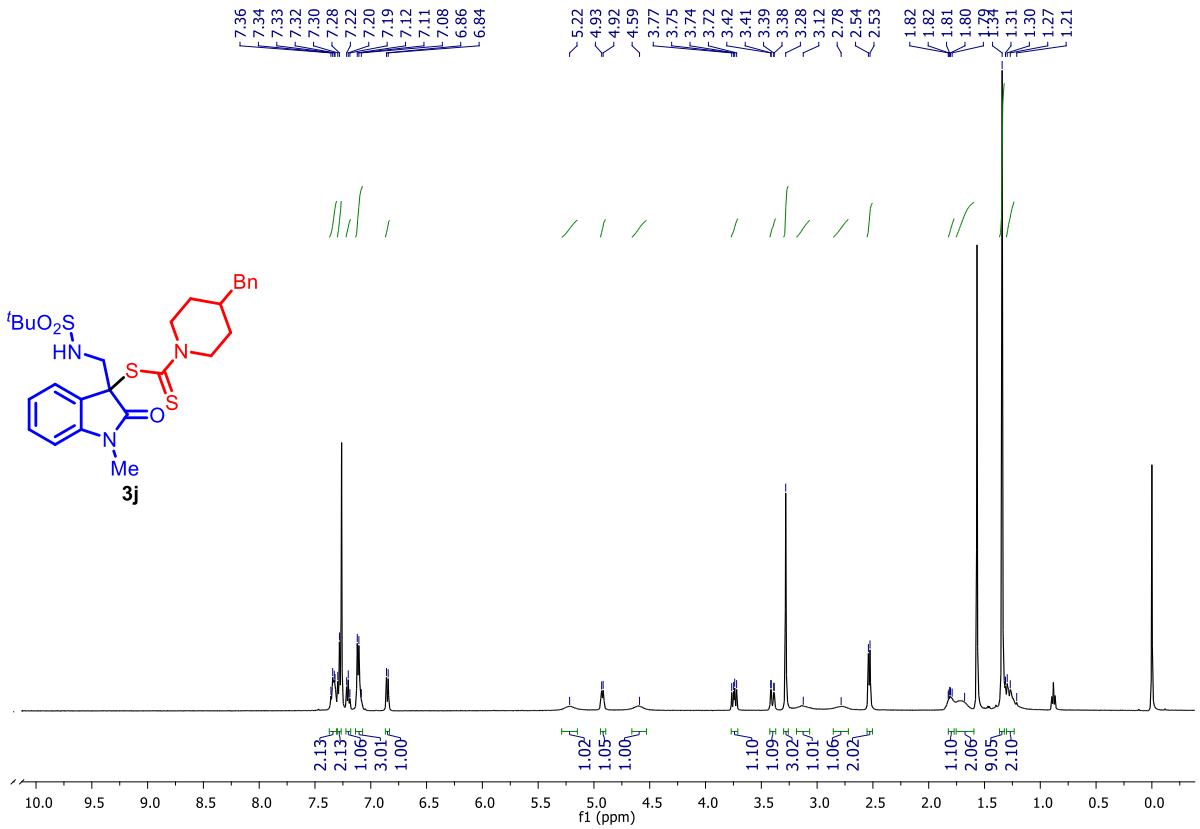


^{13}C NMR (125 MHz, DMSO-d_6) of compound **3i**



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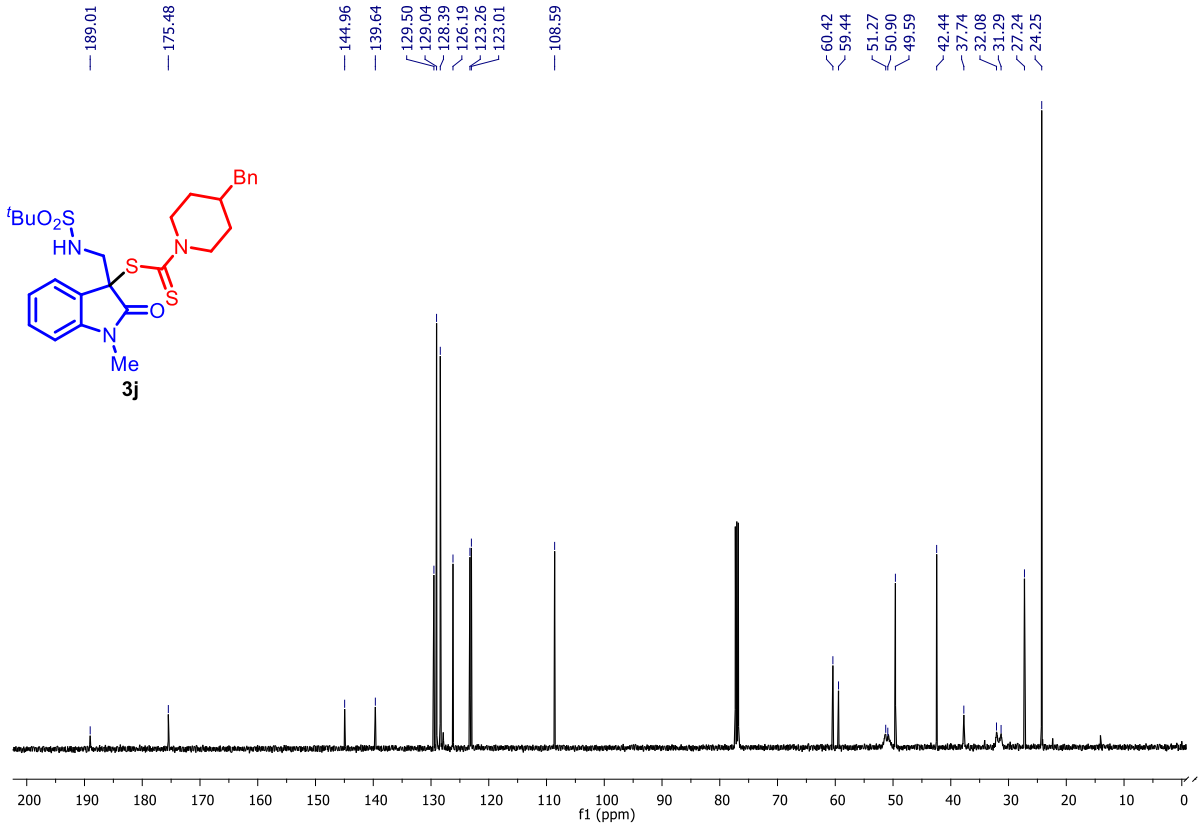
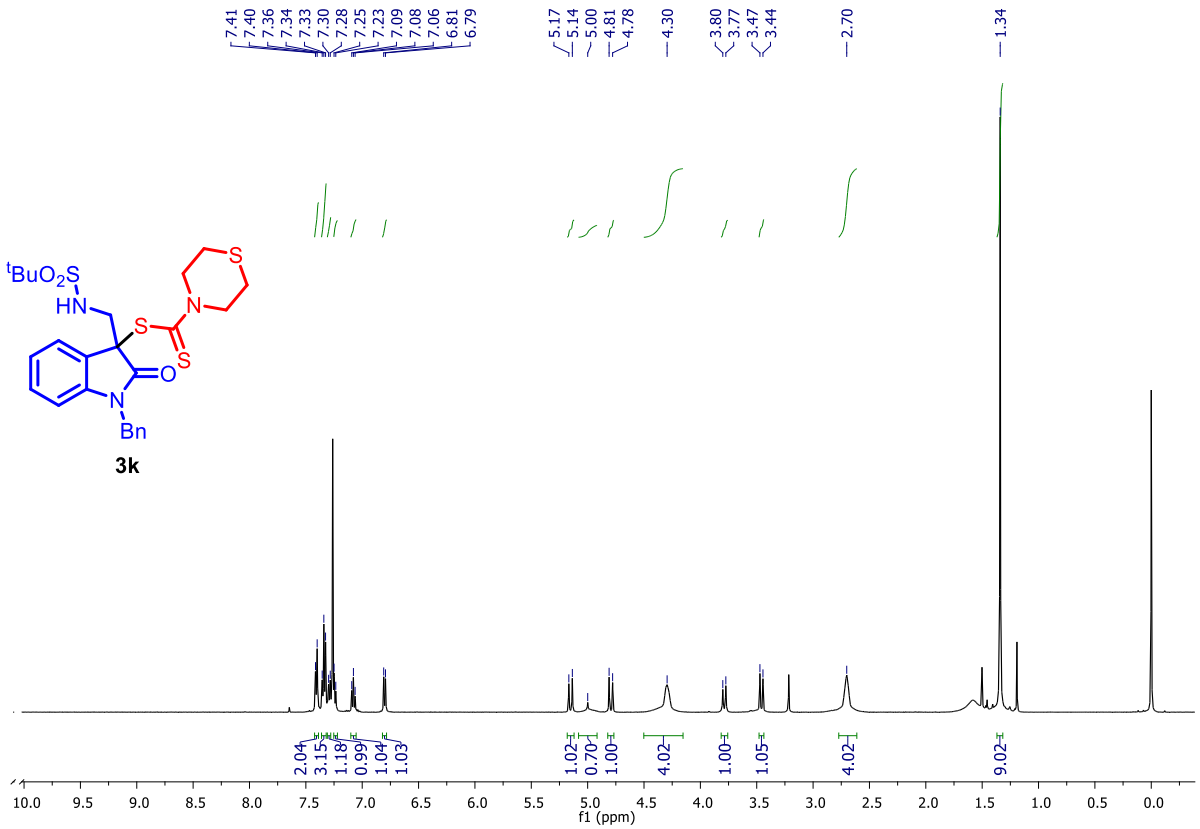


^{13}C NMR (125 MHz, $CDCl_3$) of compound **3j**



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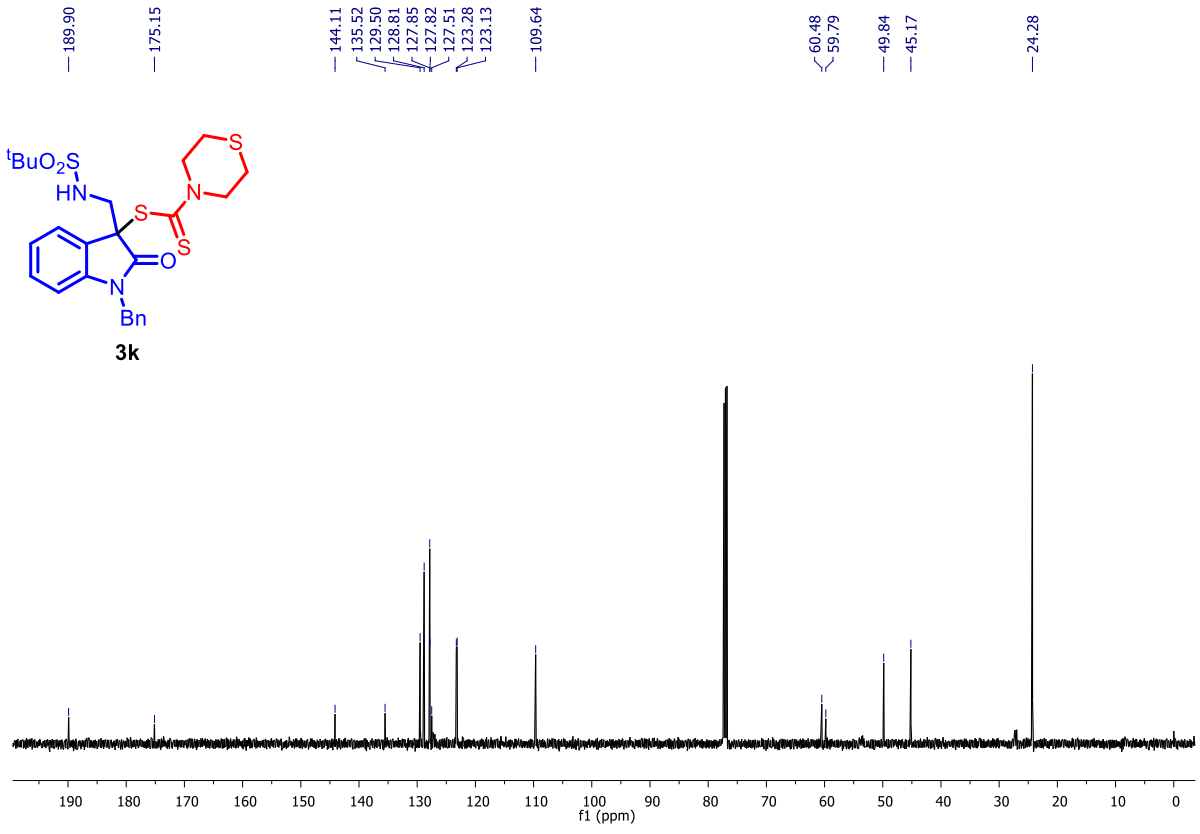

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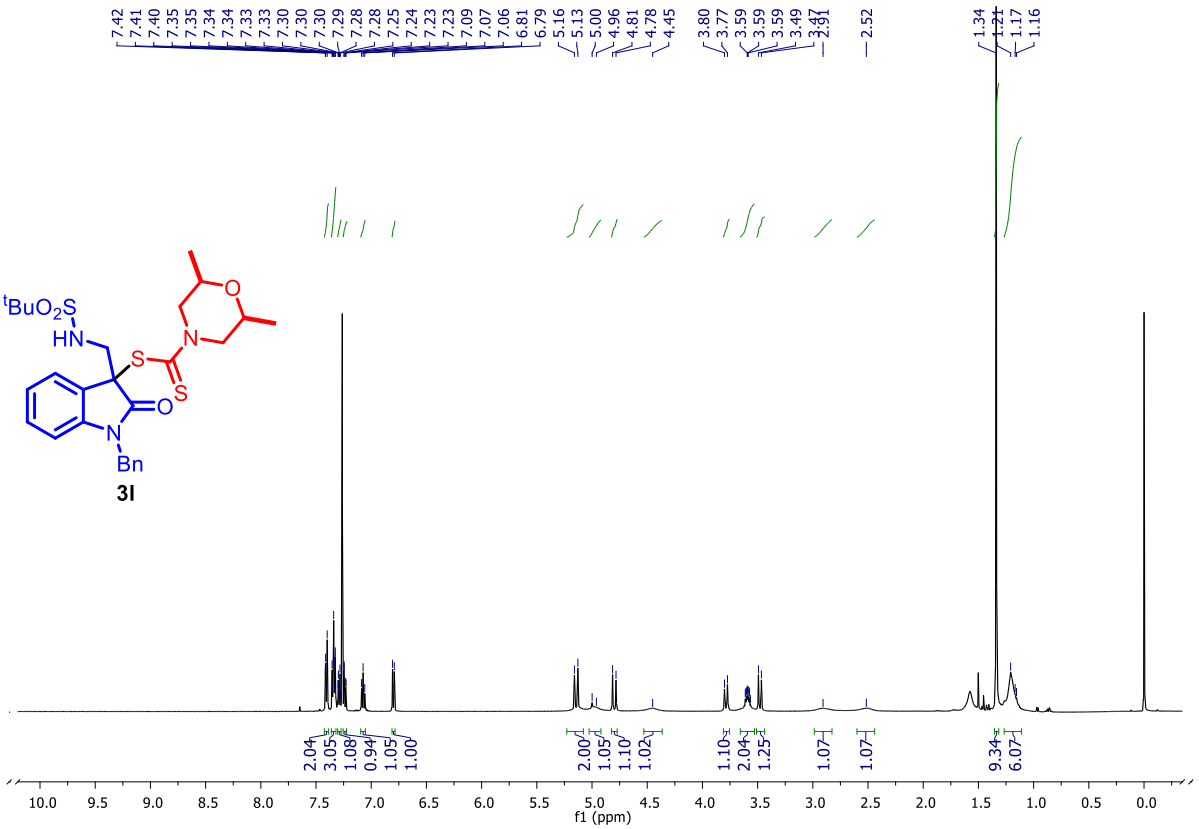

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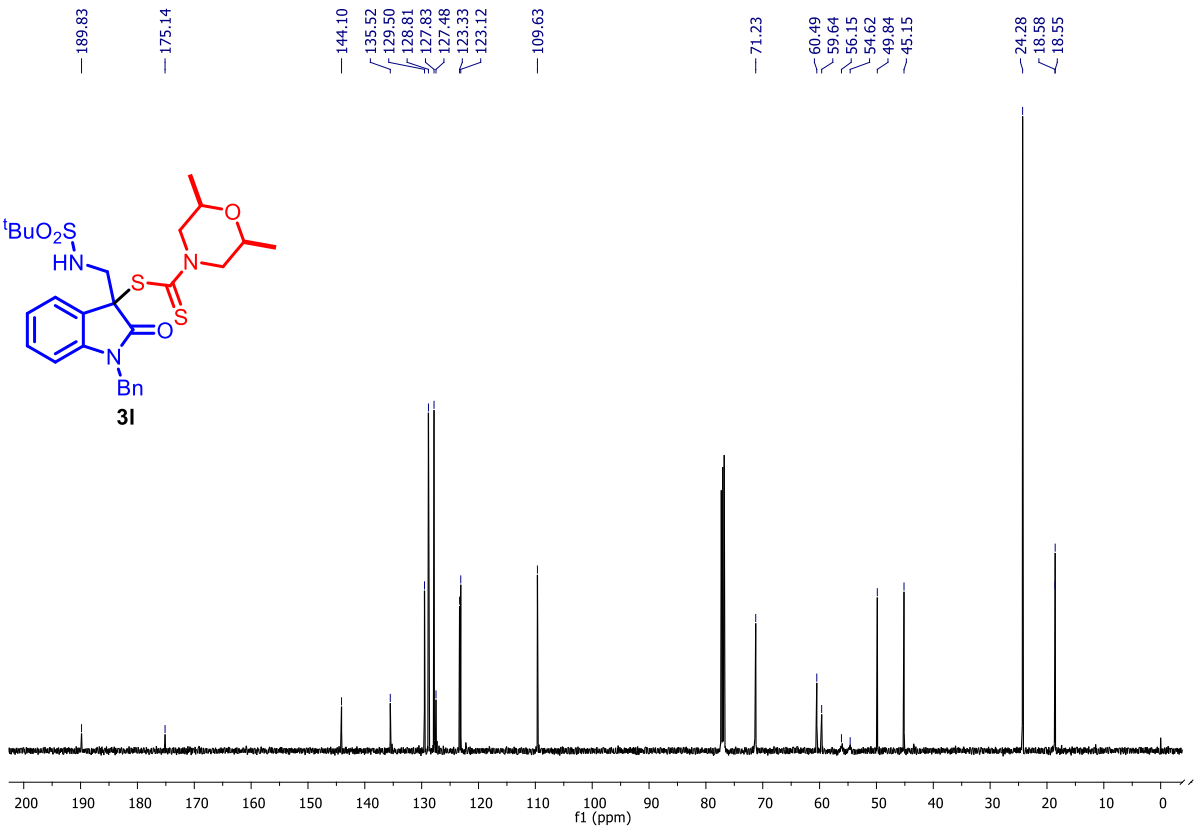

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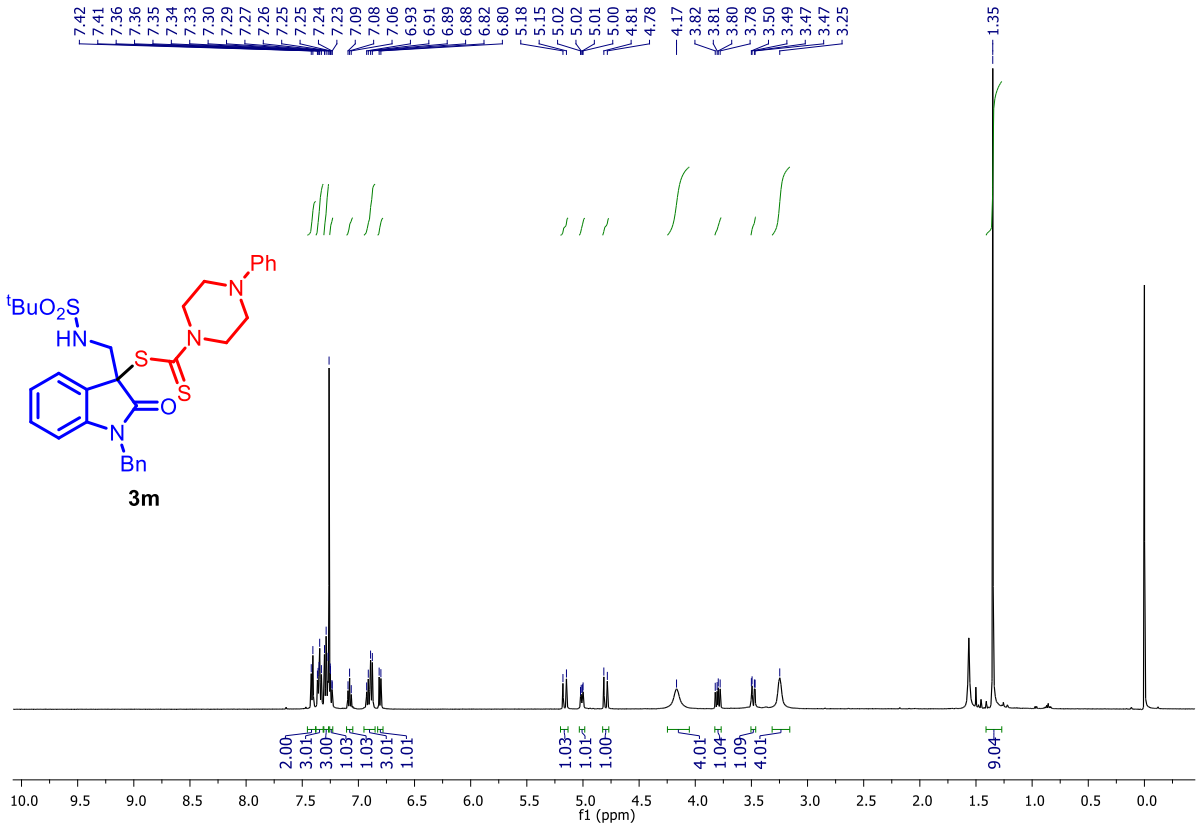

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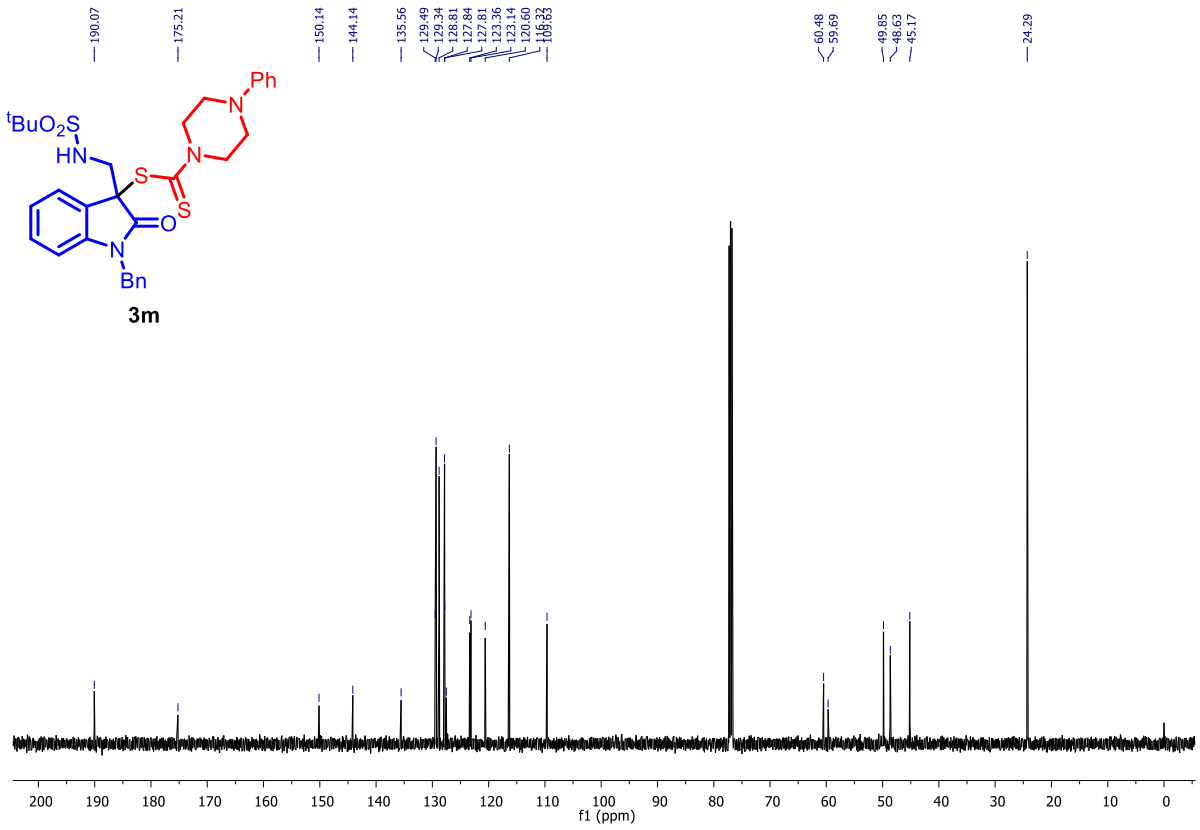

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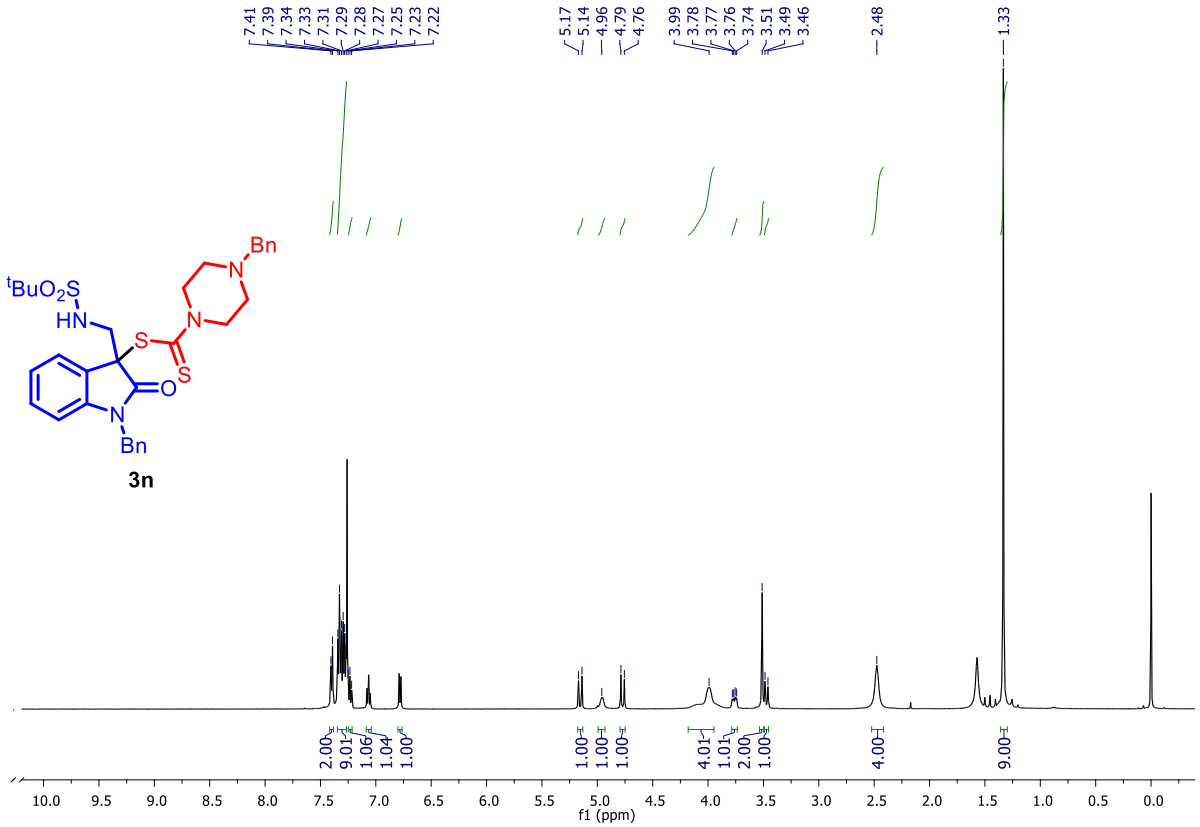

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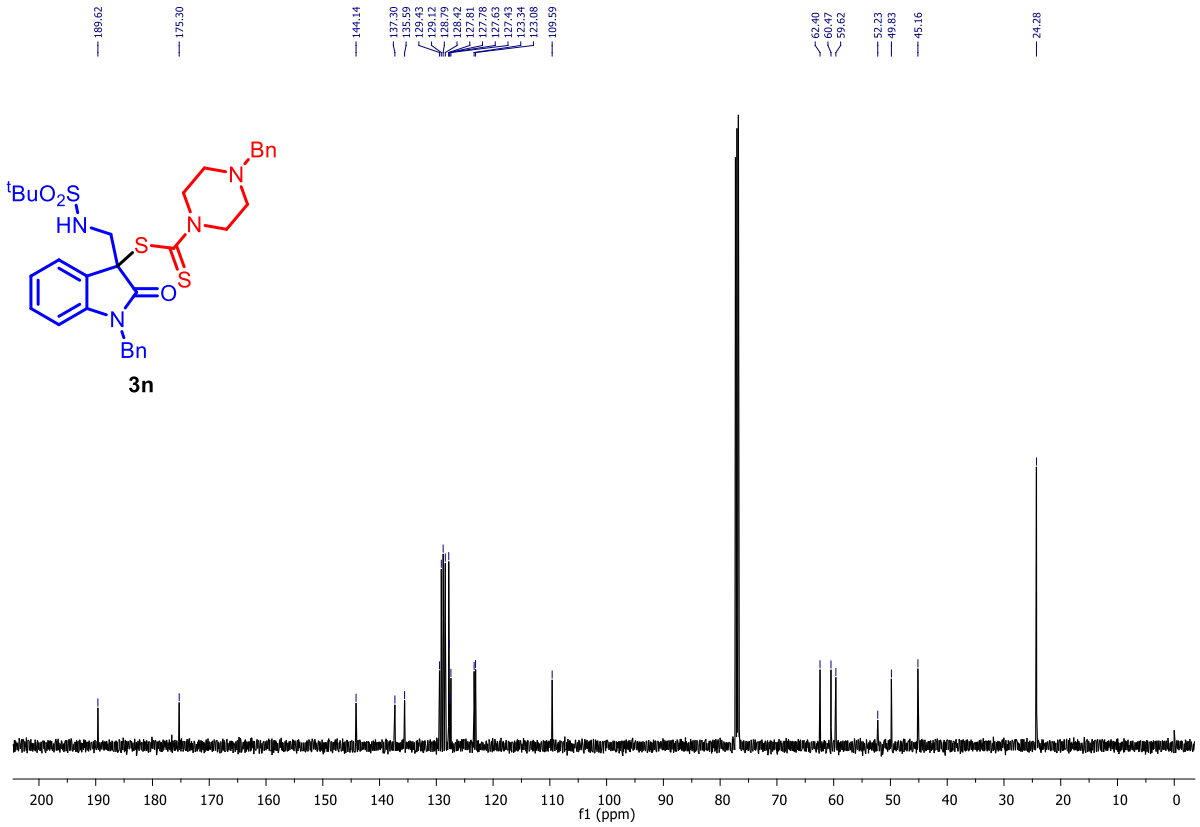

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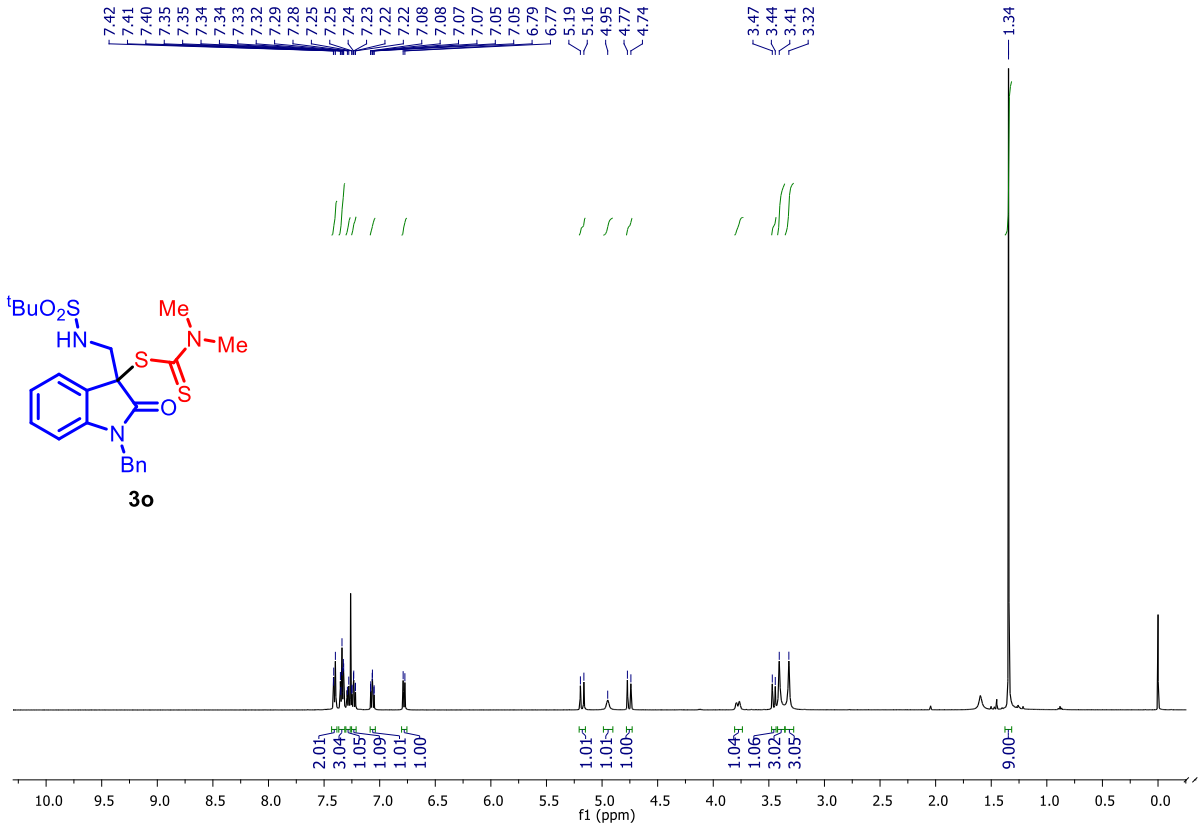

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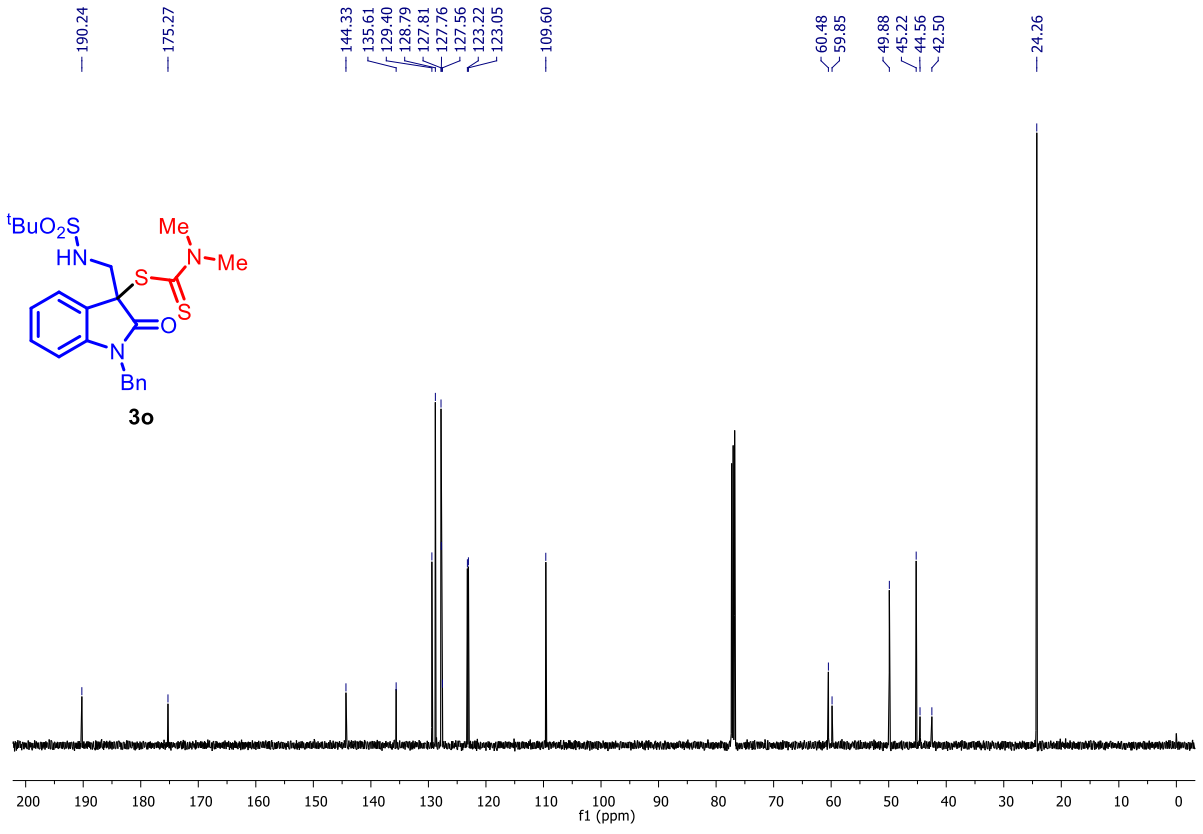

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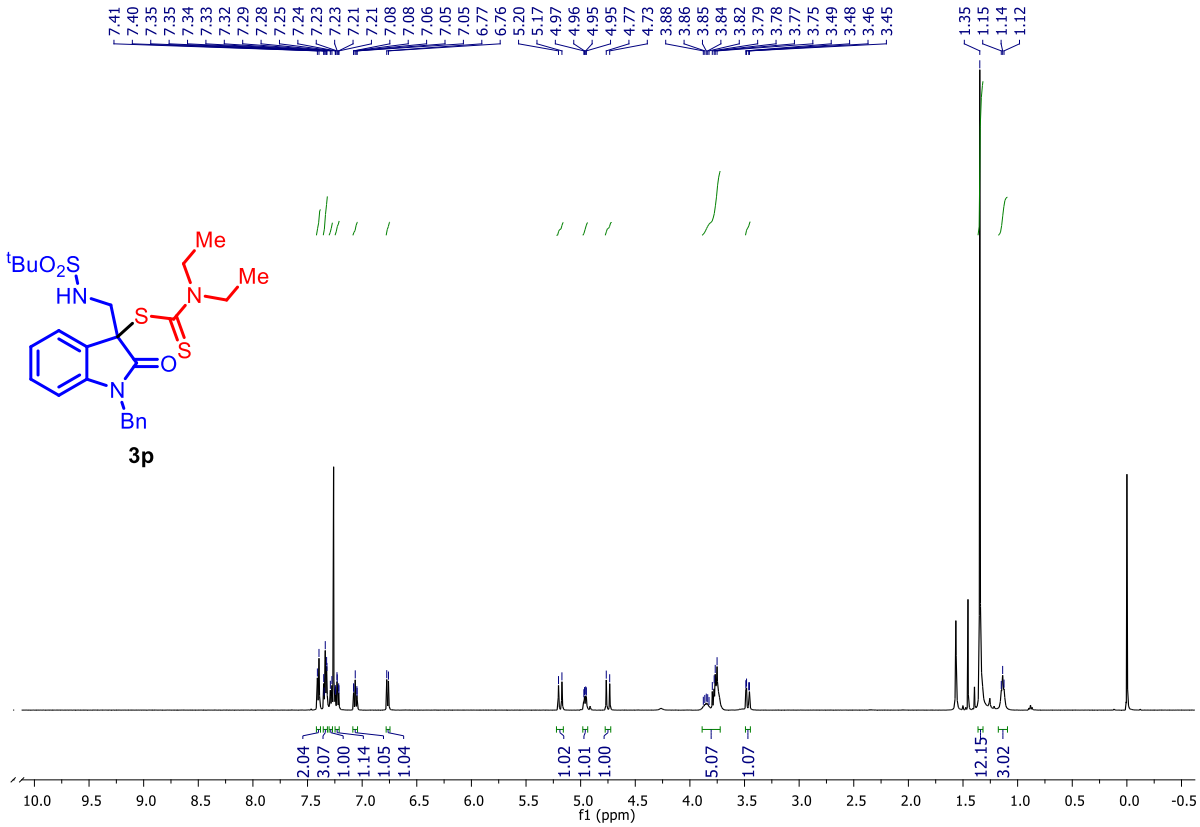

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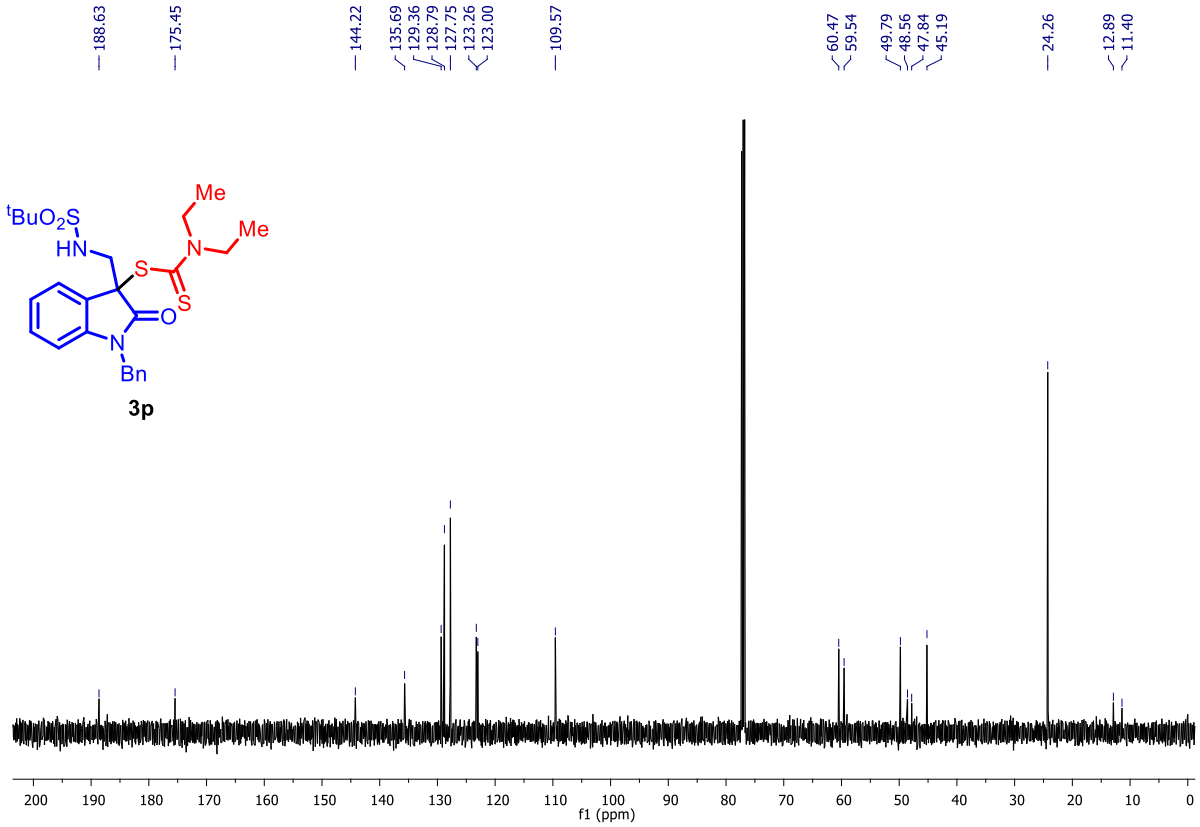

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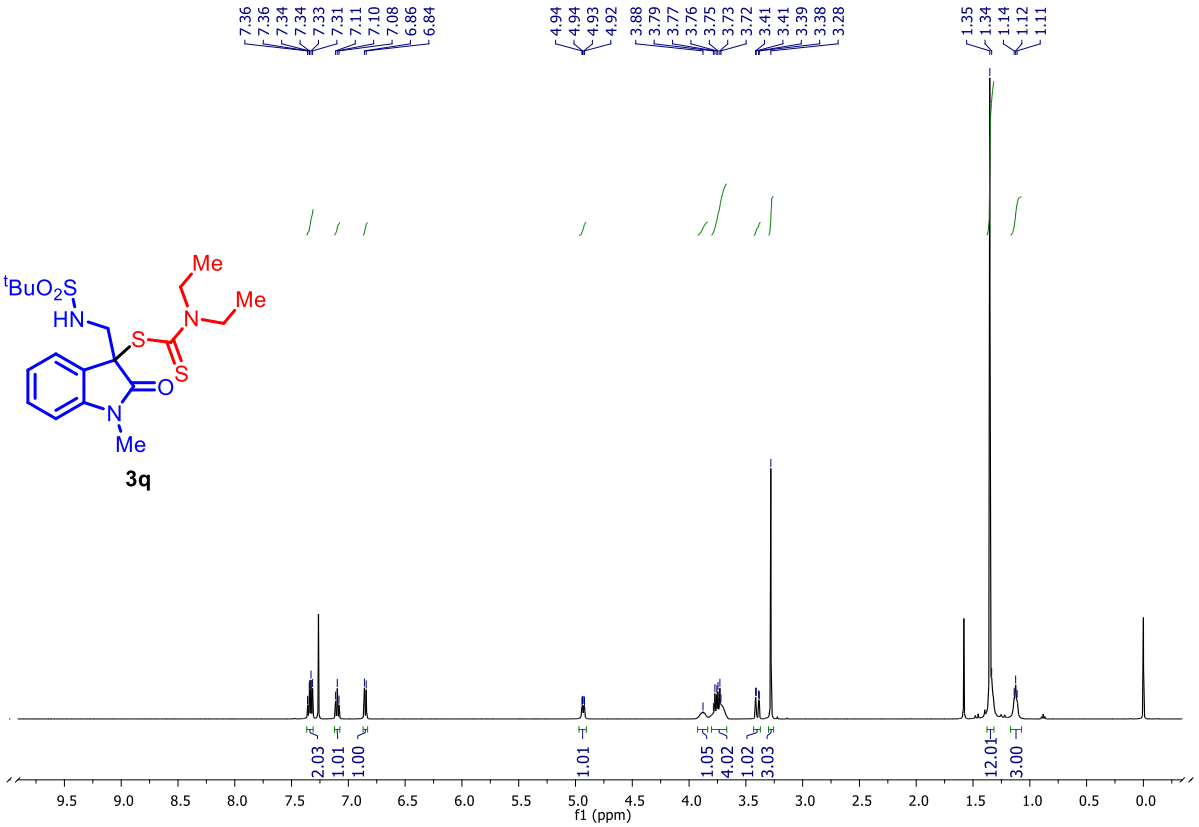

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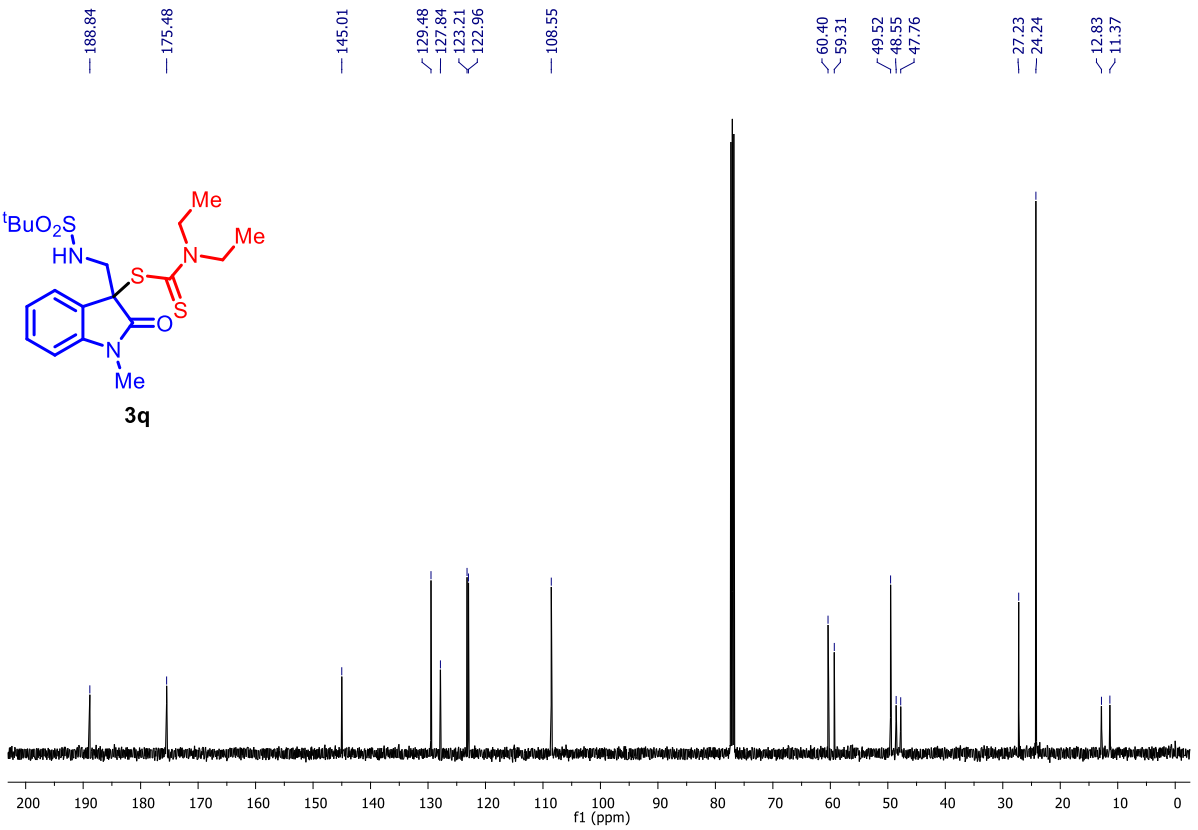

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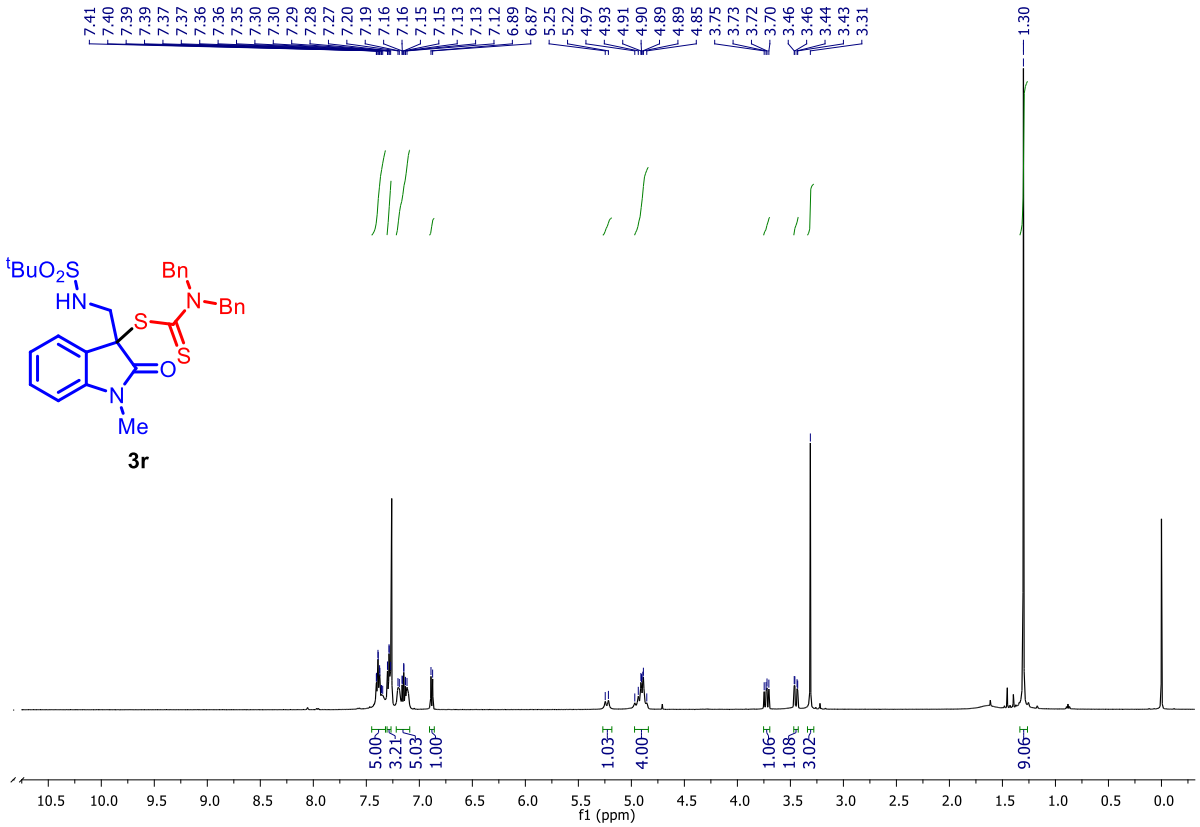

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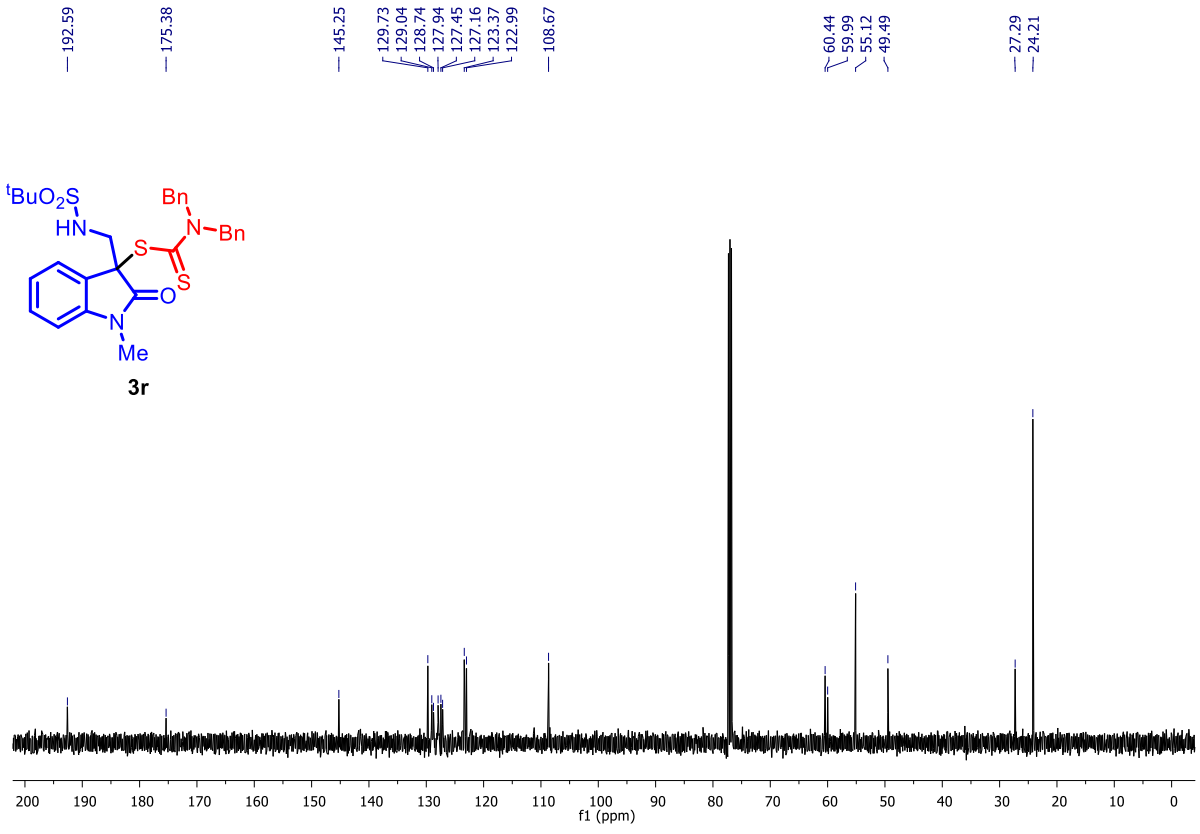

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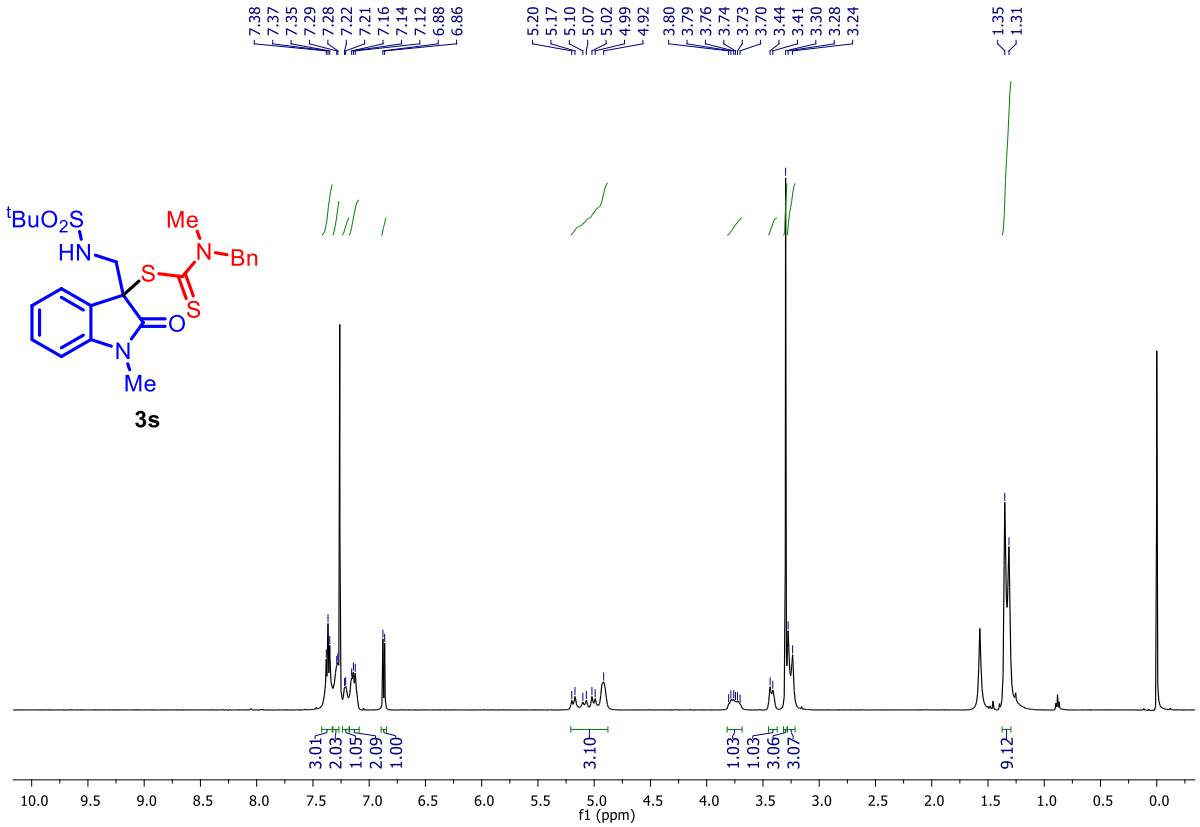

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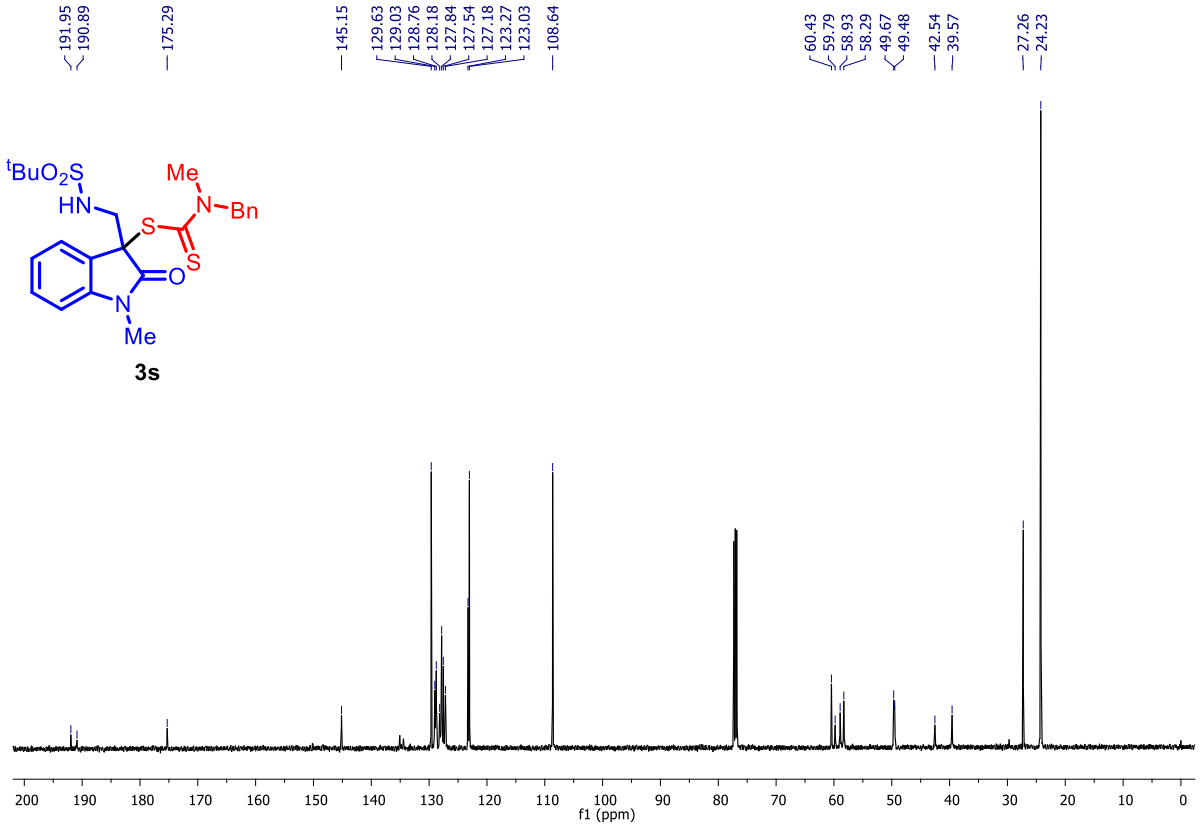

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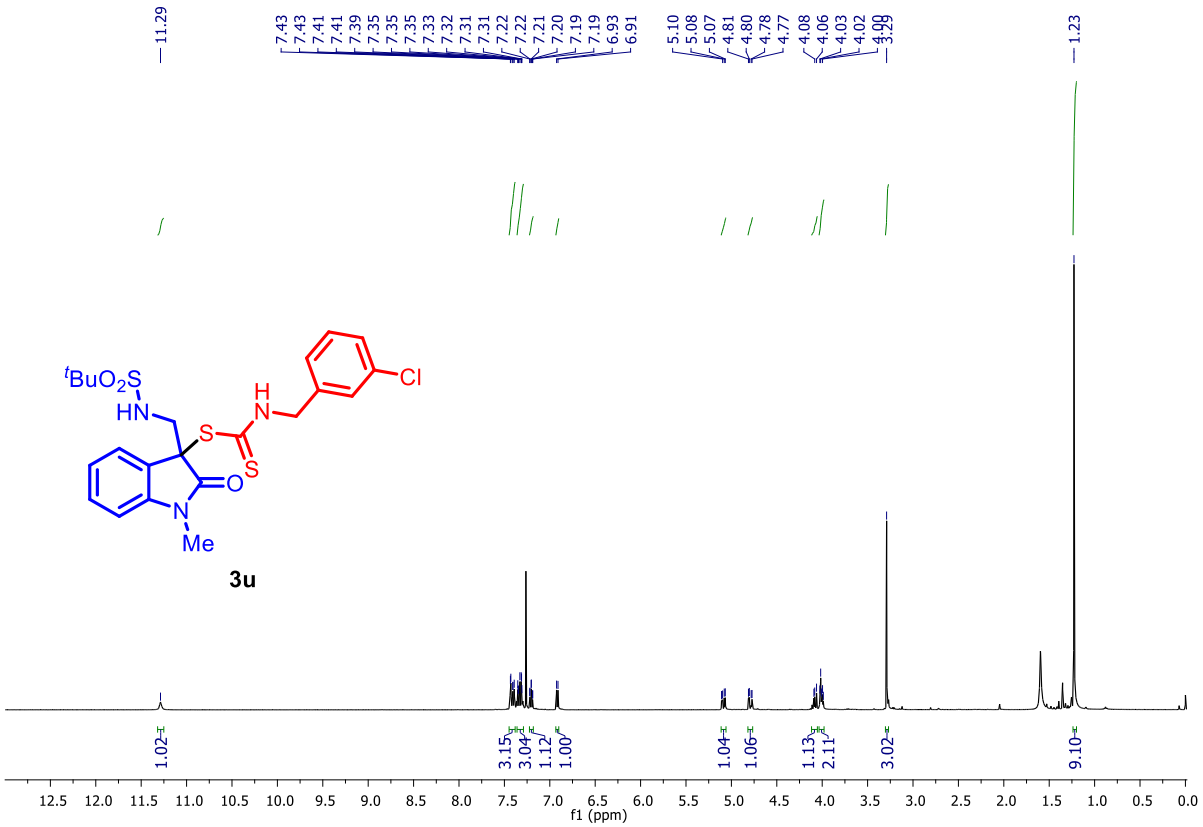

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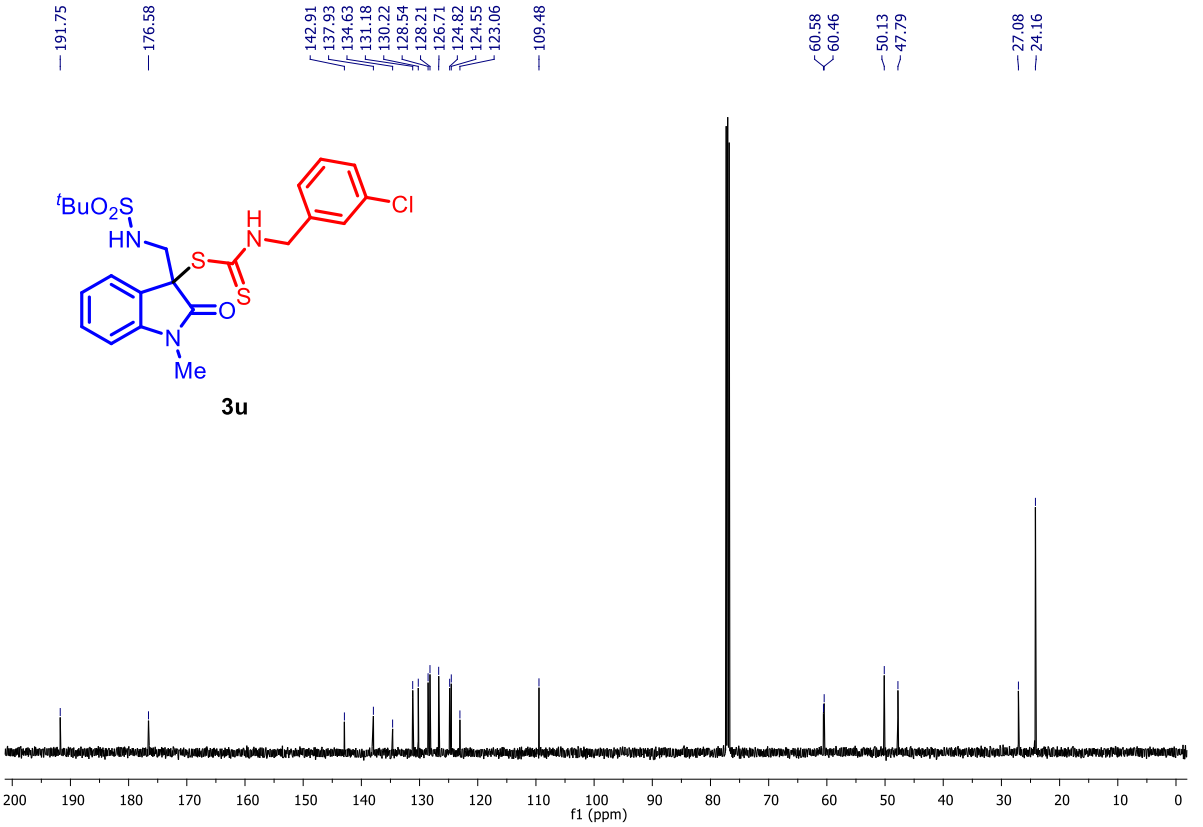

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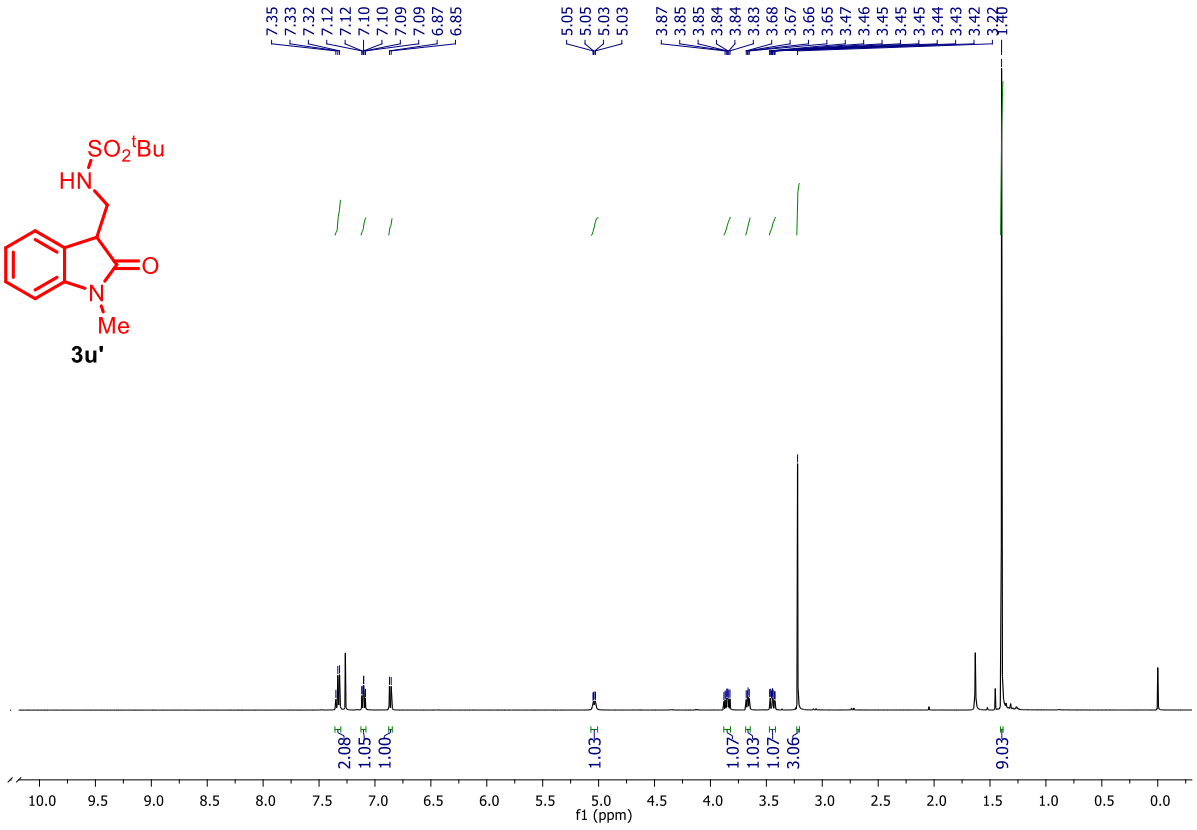

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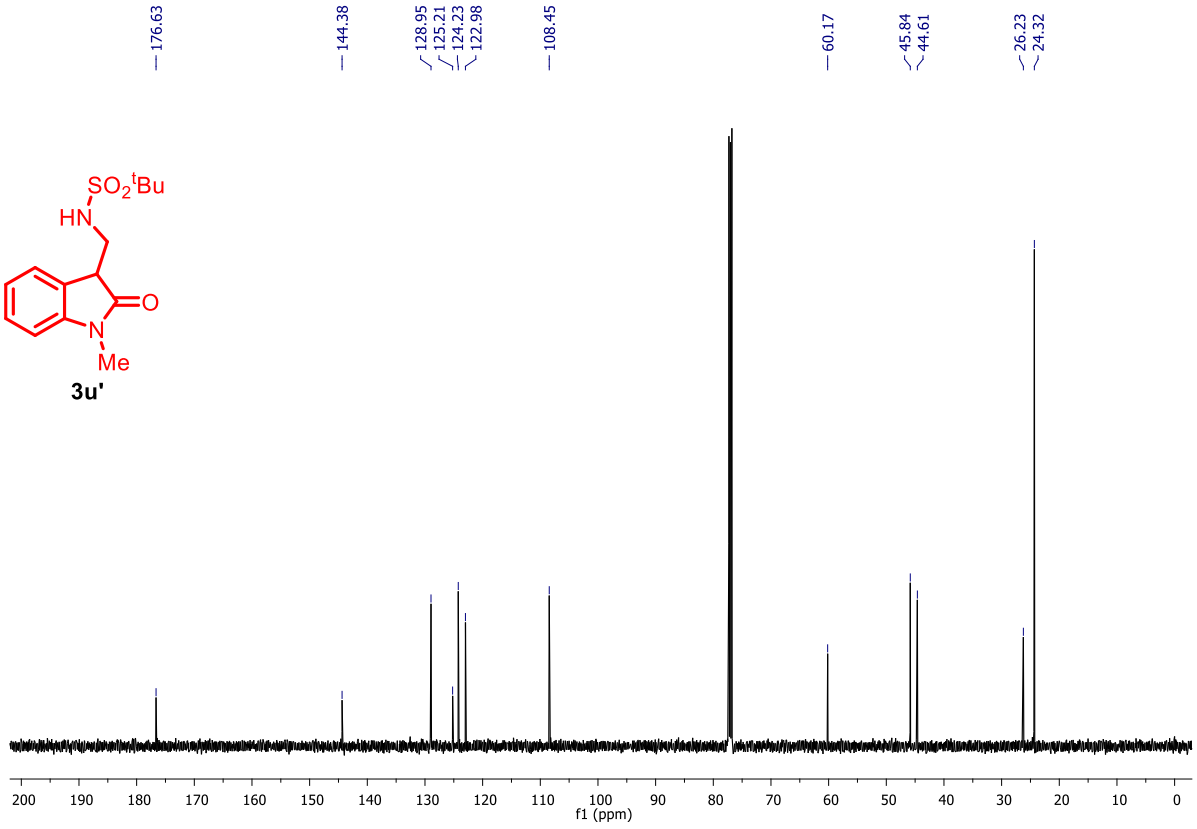
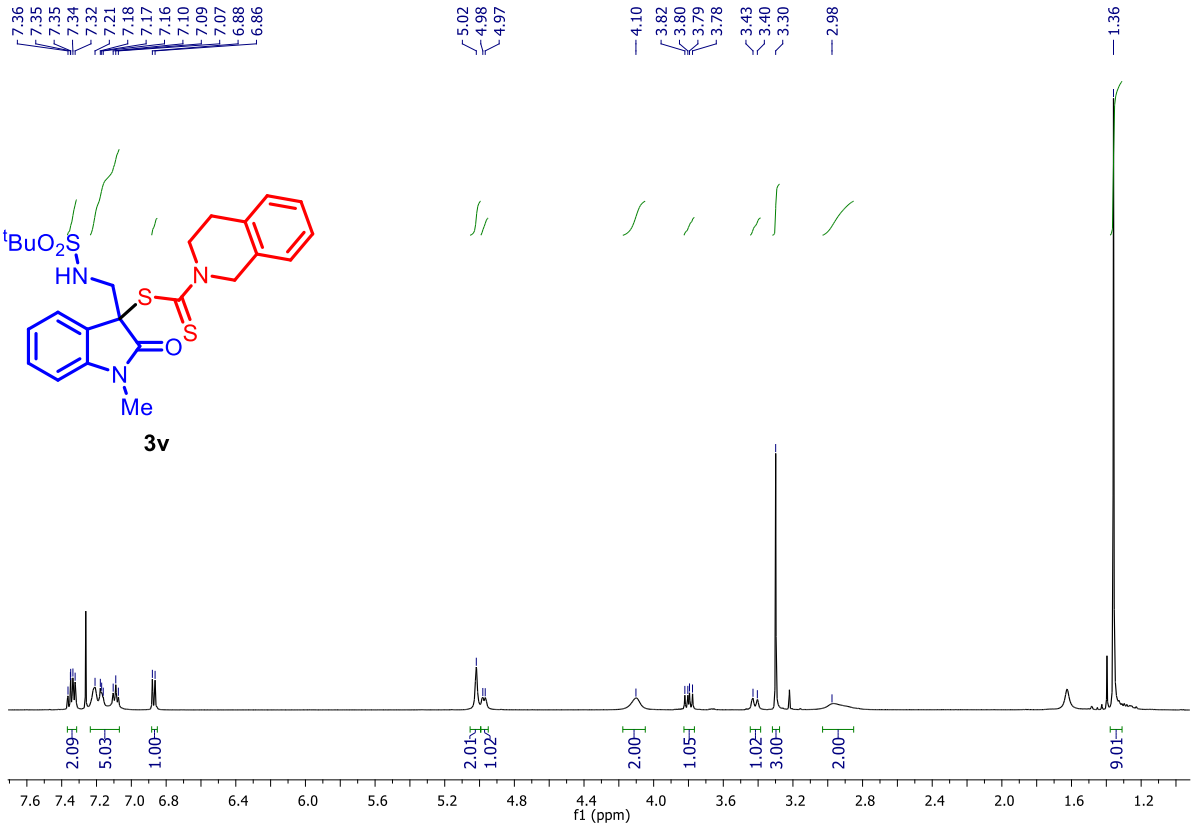
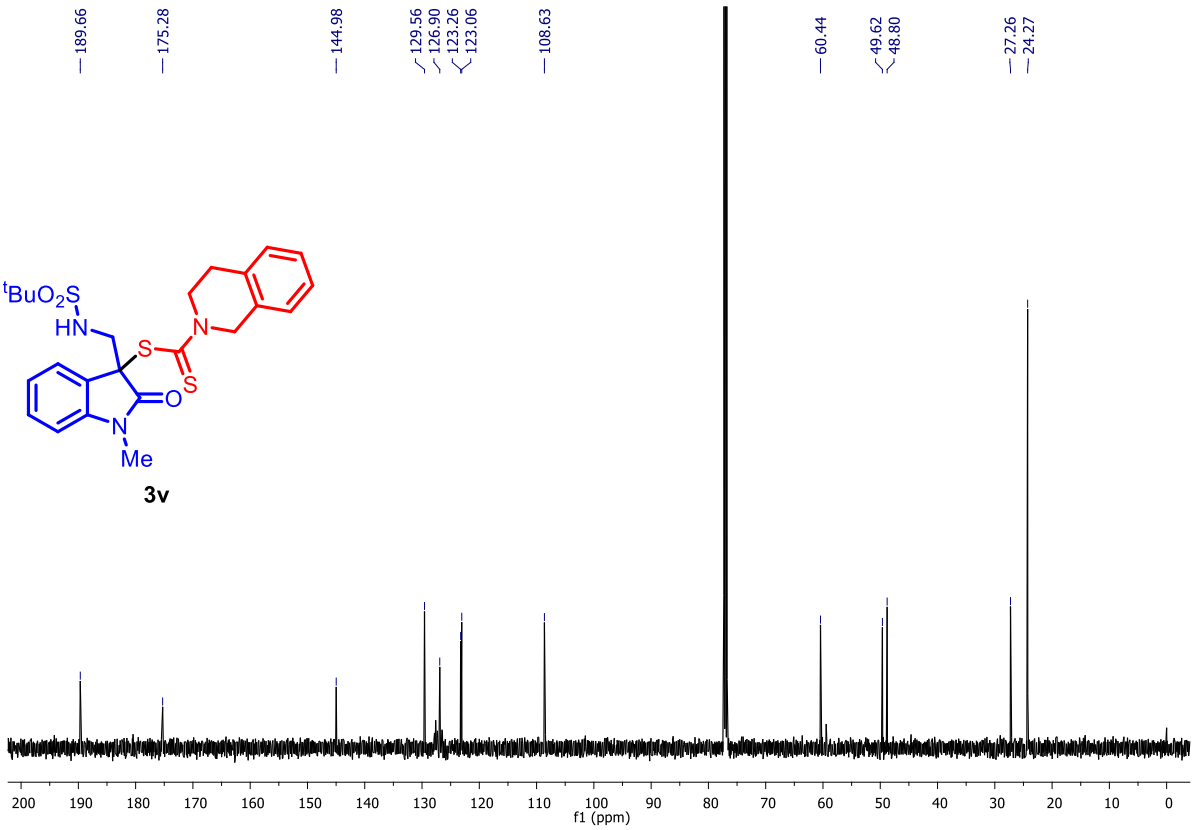


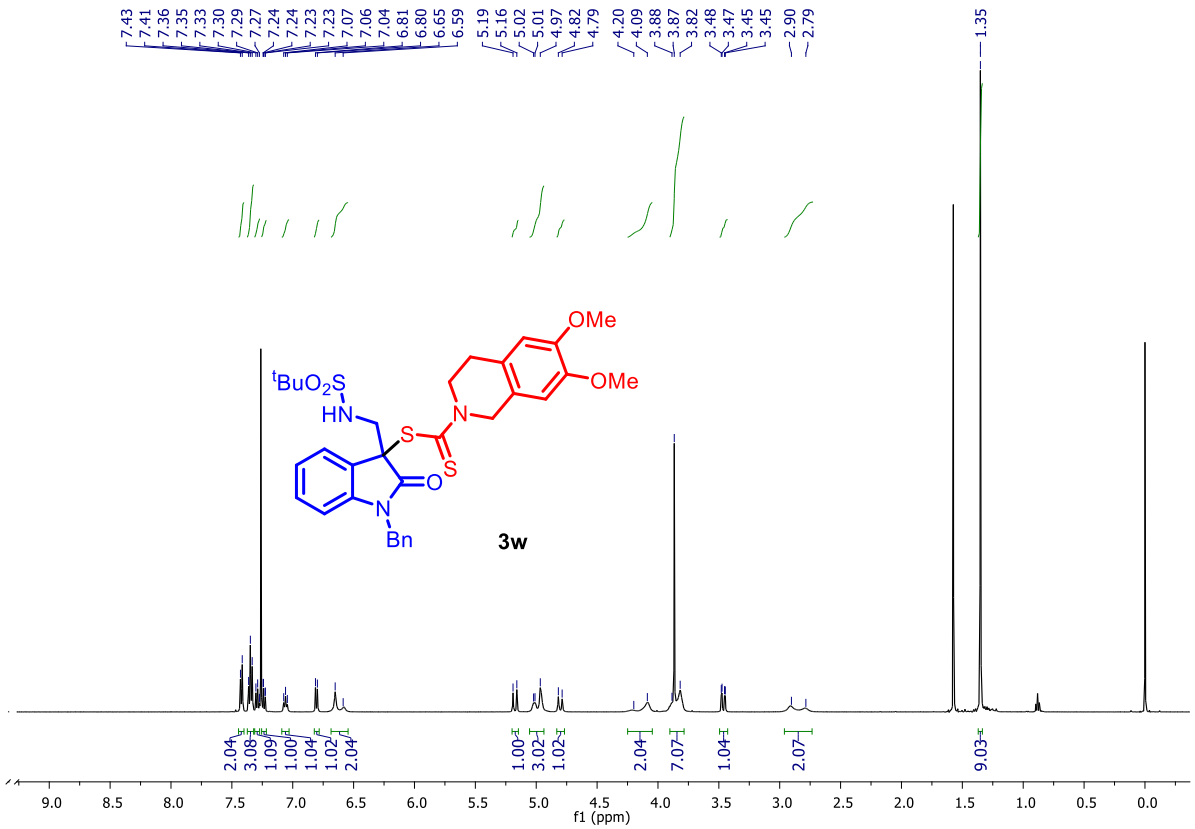
^{13}C NMR (125 MHz, $CDCl_3$) of compound **3u'**



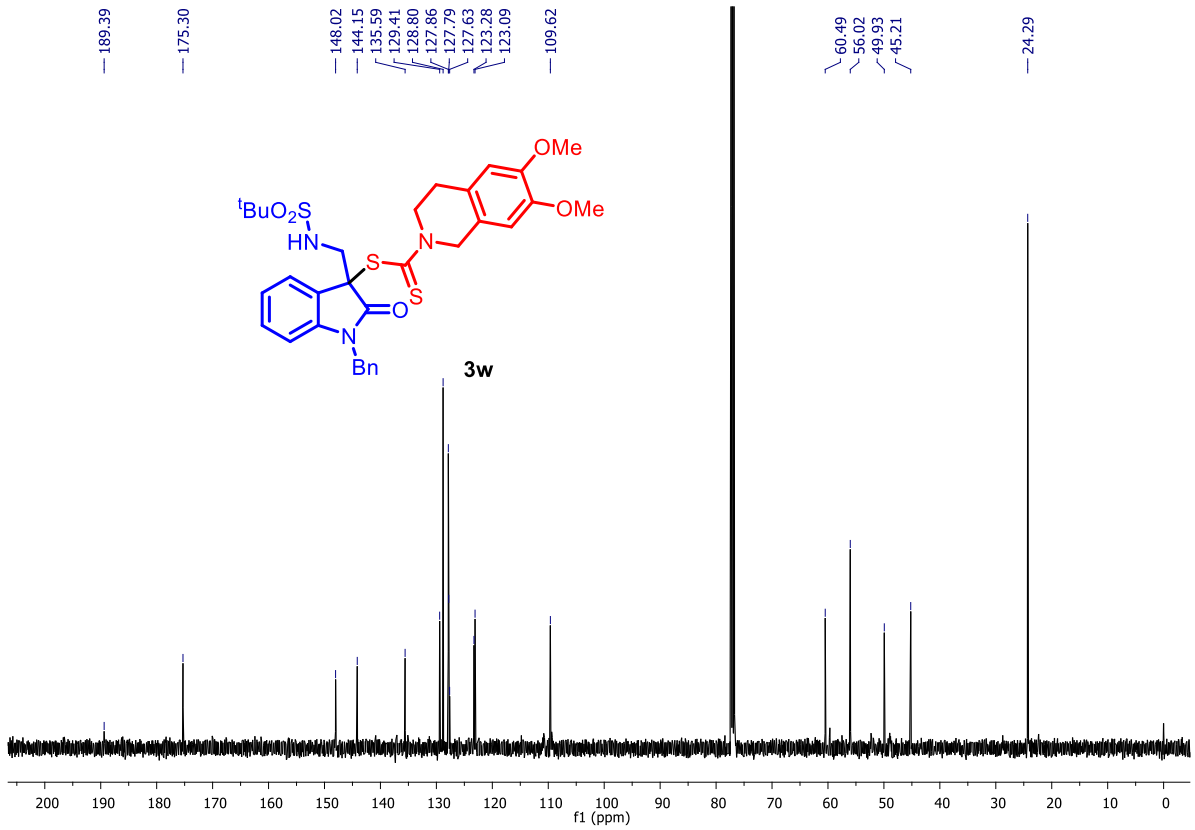
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3v**



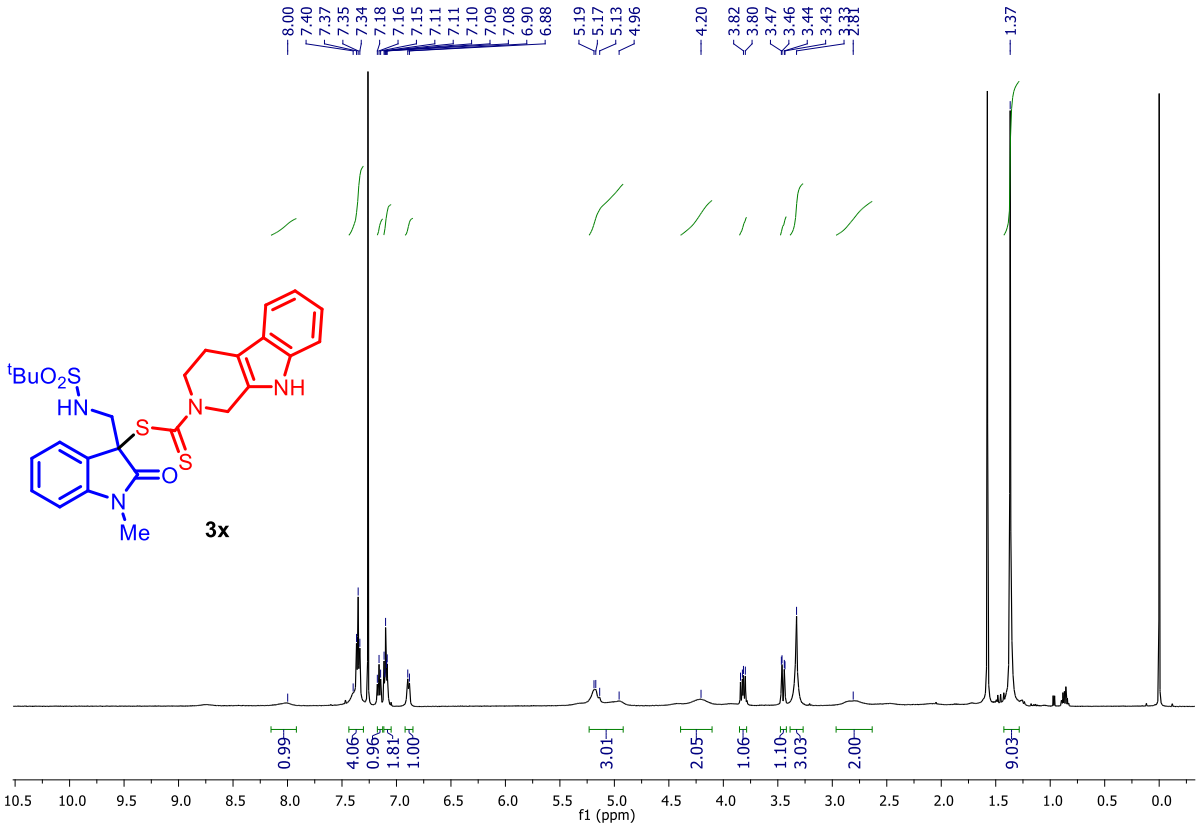
^{13}C NMR (125 MHz, $CDCl_3$) of compound **3v**



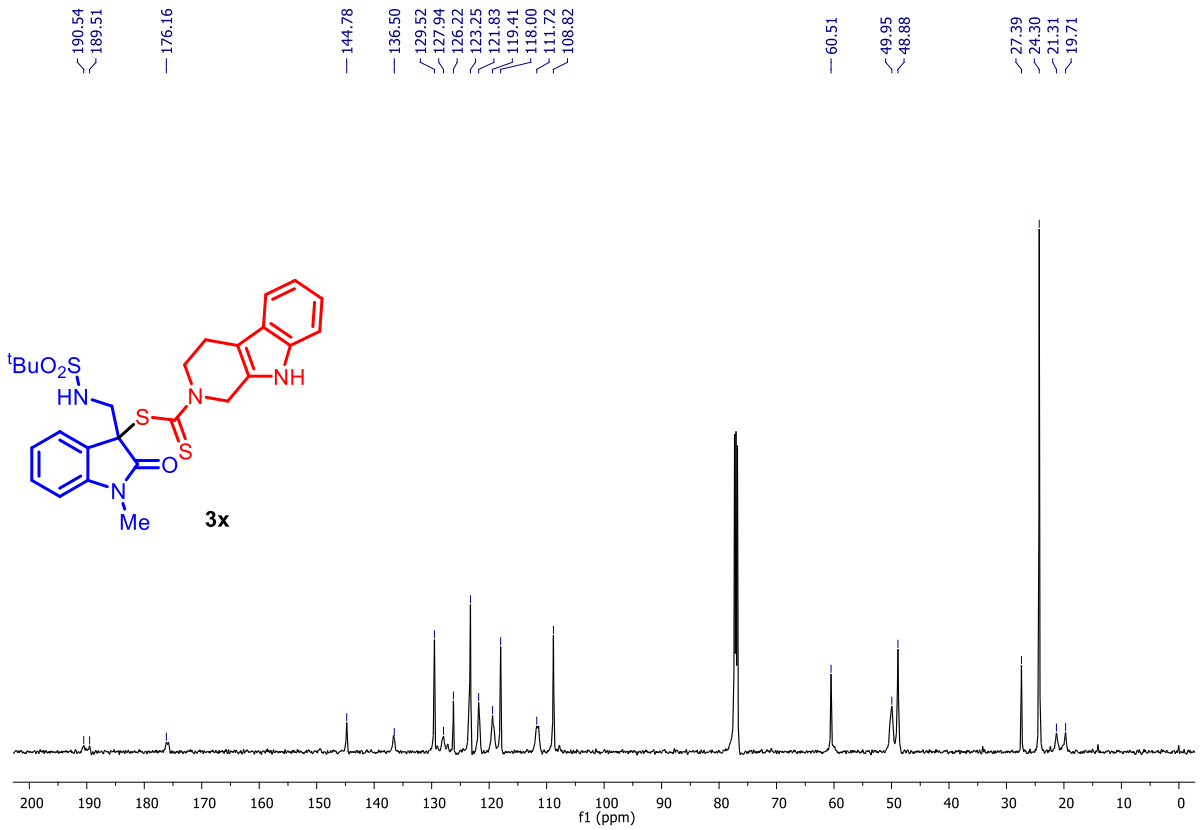
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3w**



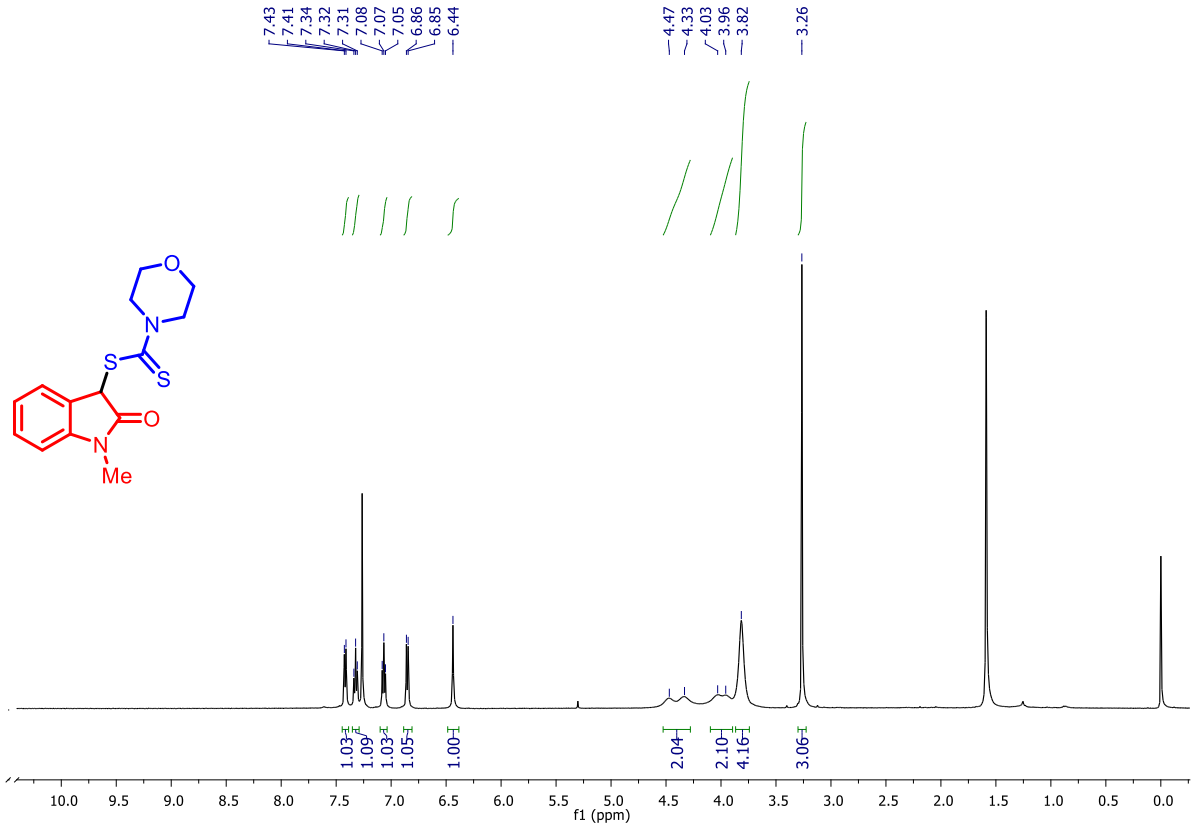
^{13}C NMR (125 MHz, $CDCl_3$) of compound **3w**



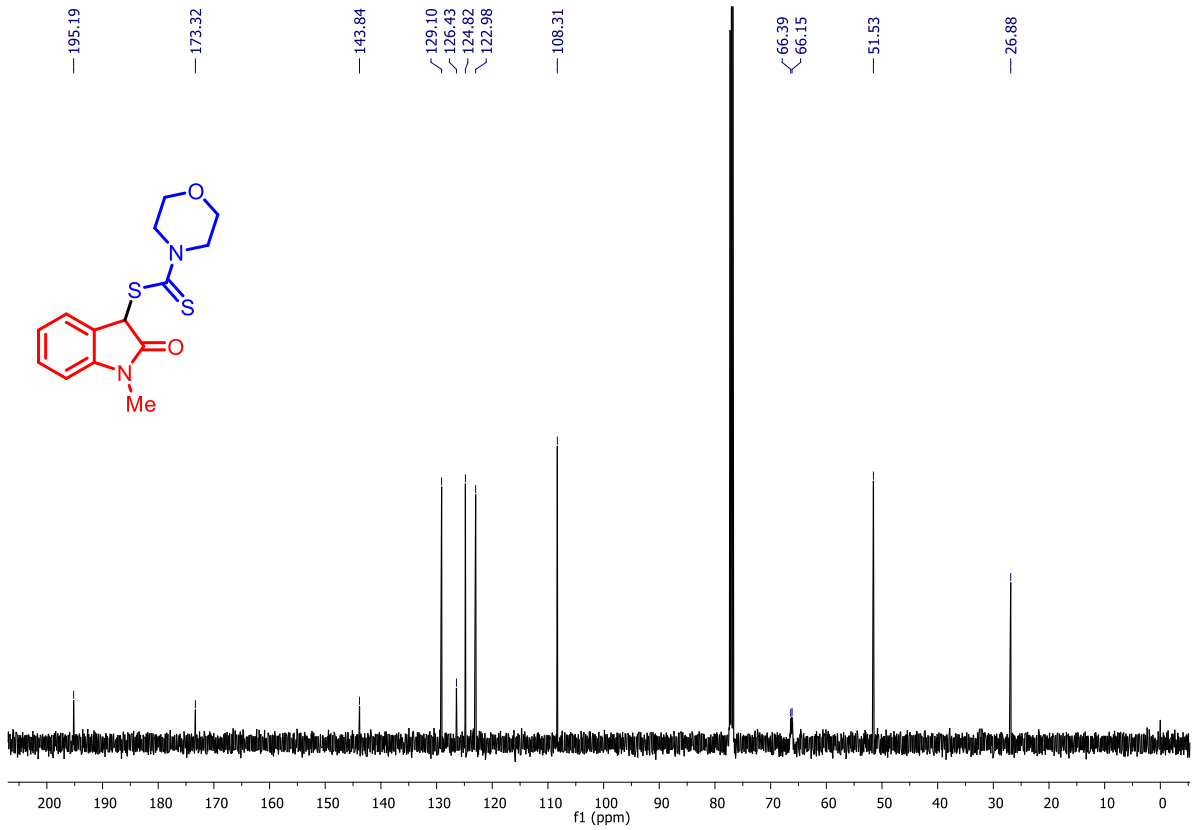
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3x**



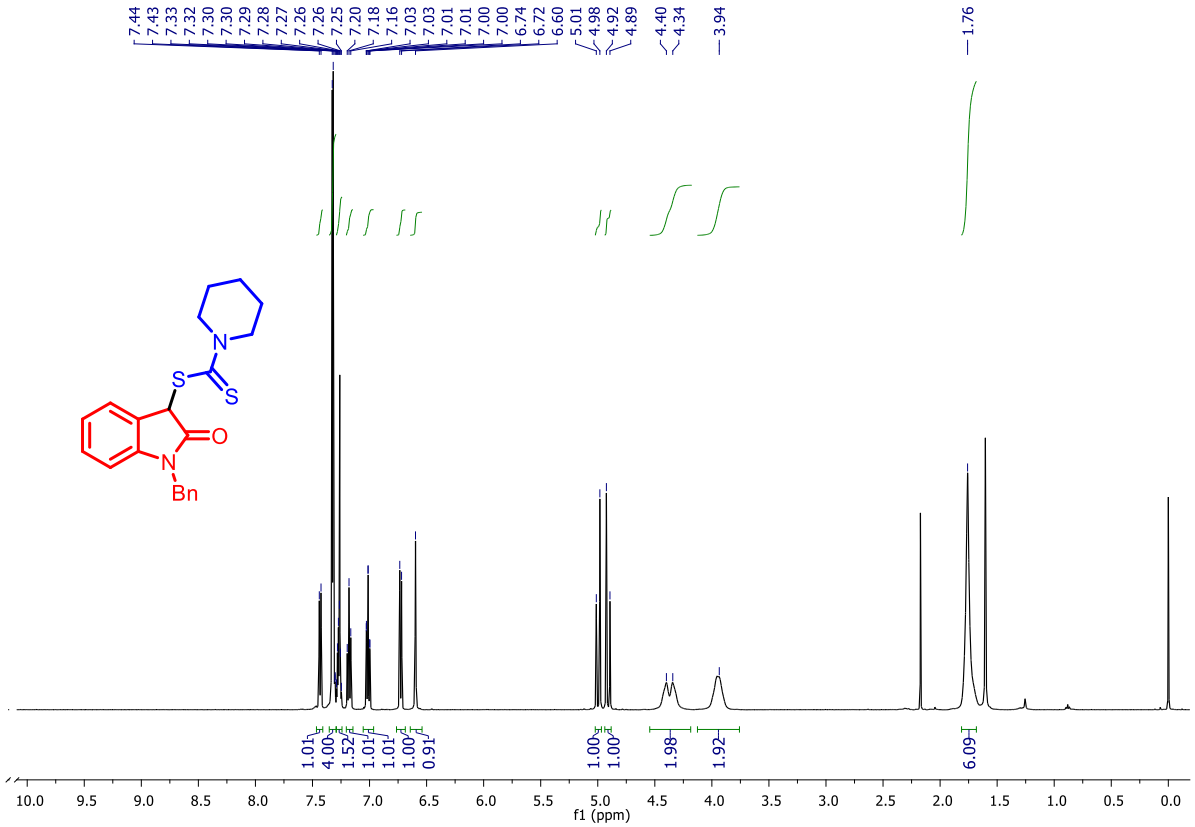
^{13}C NMR (125 MHz, $CDCl_3$) of compound **3x**



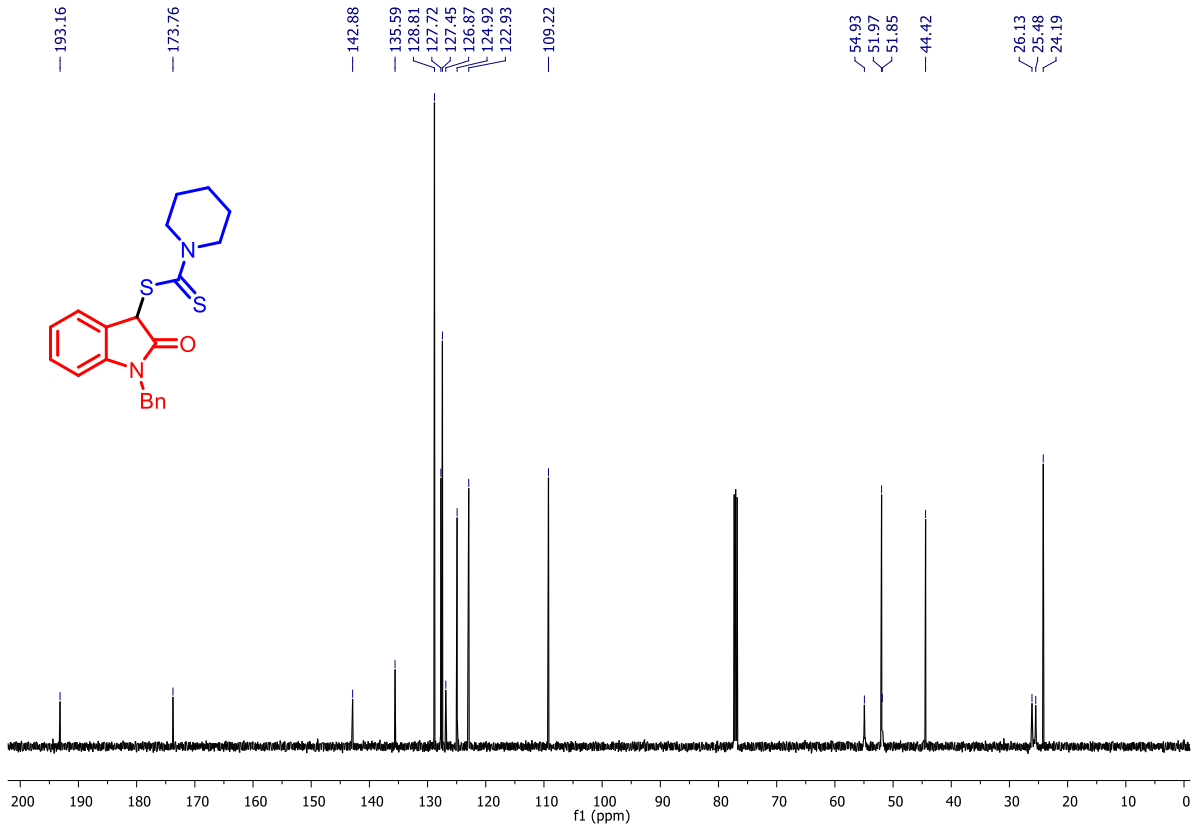
^{1}H NMR (500 MHz, $CDCl_3$) of compound **4a**



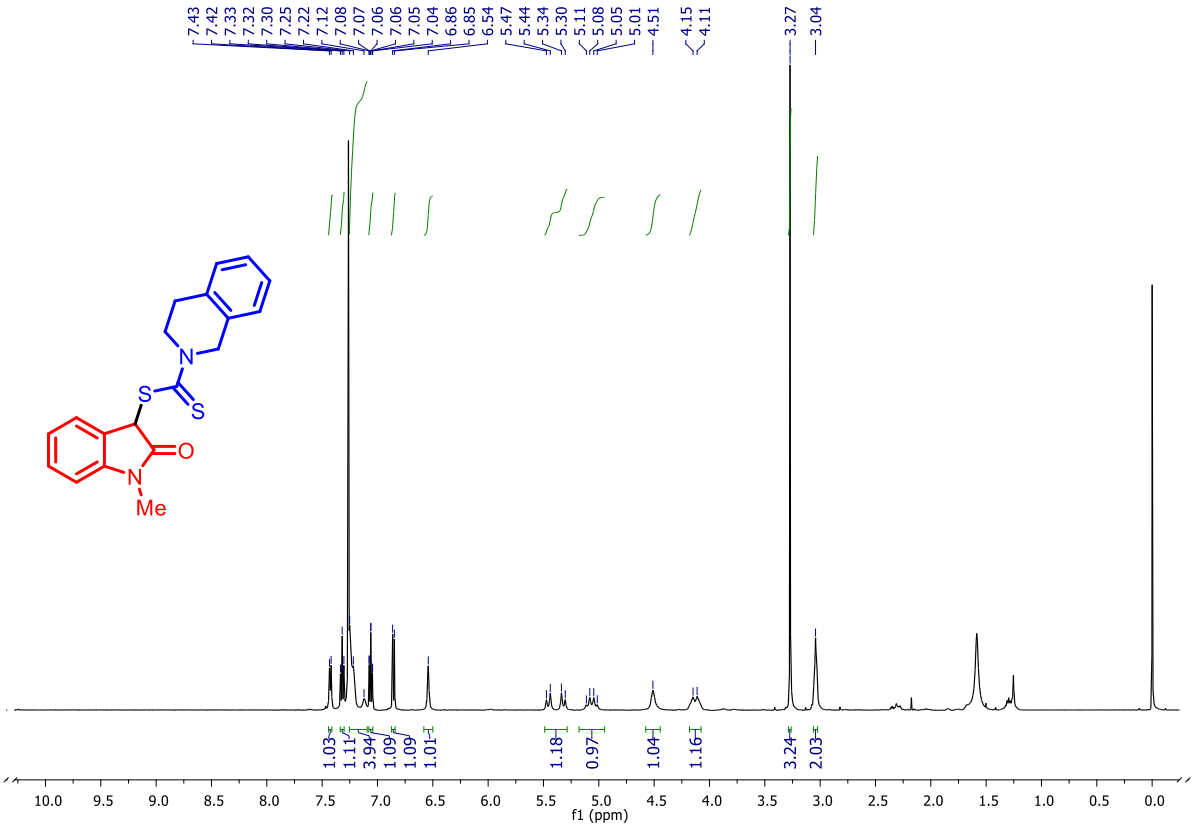
^{1}H NMR (125 MHz, $CDCl_3$) of compound **4a**



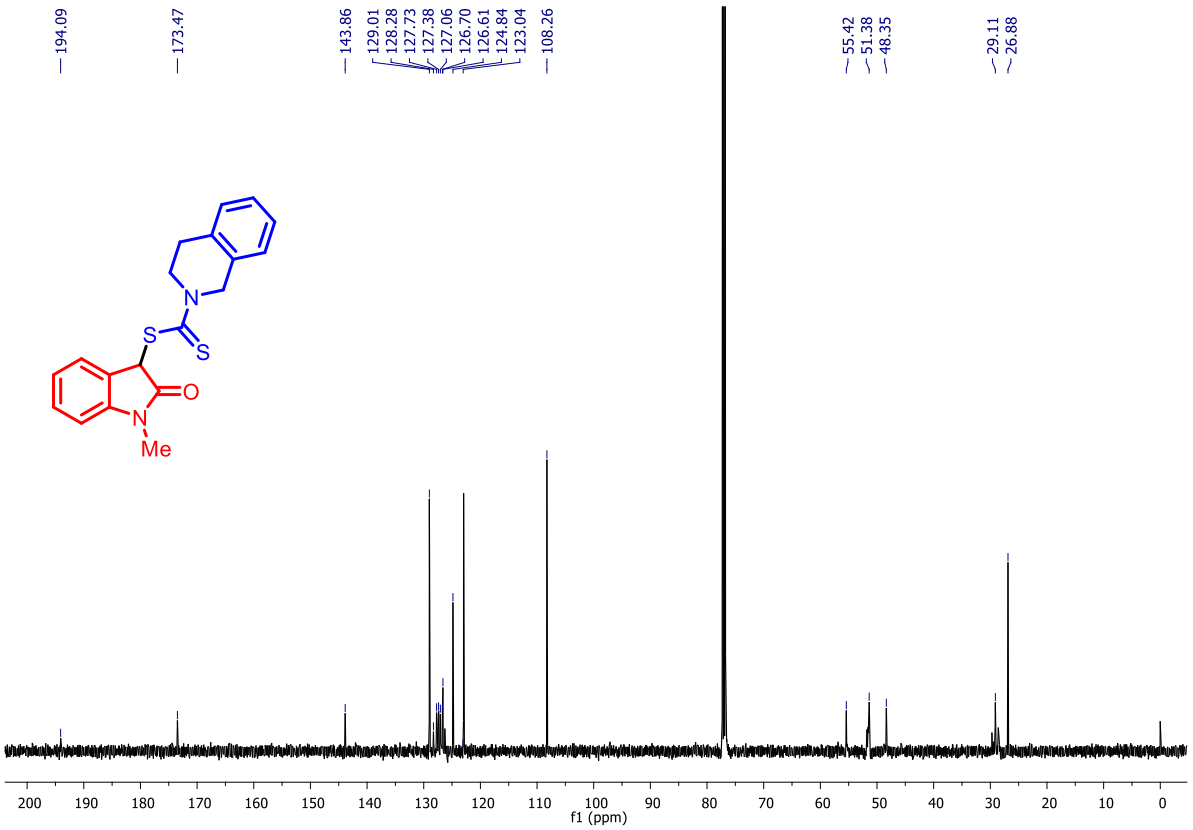
^{1}H NMR (500 MHz, $CDCl_3$) of compound **4b**



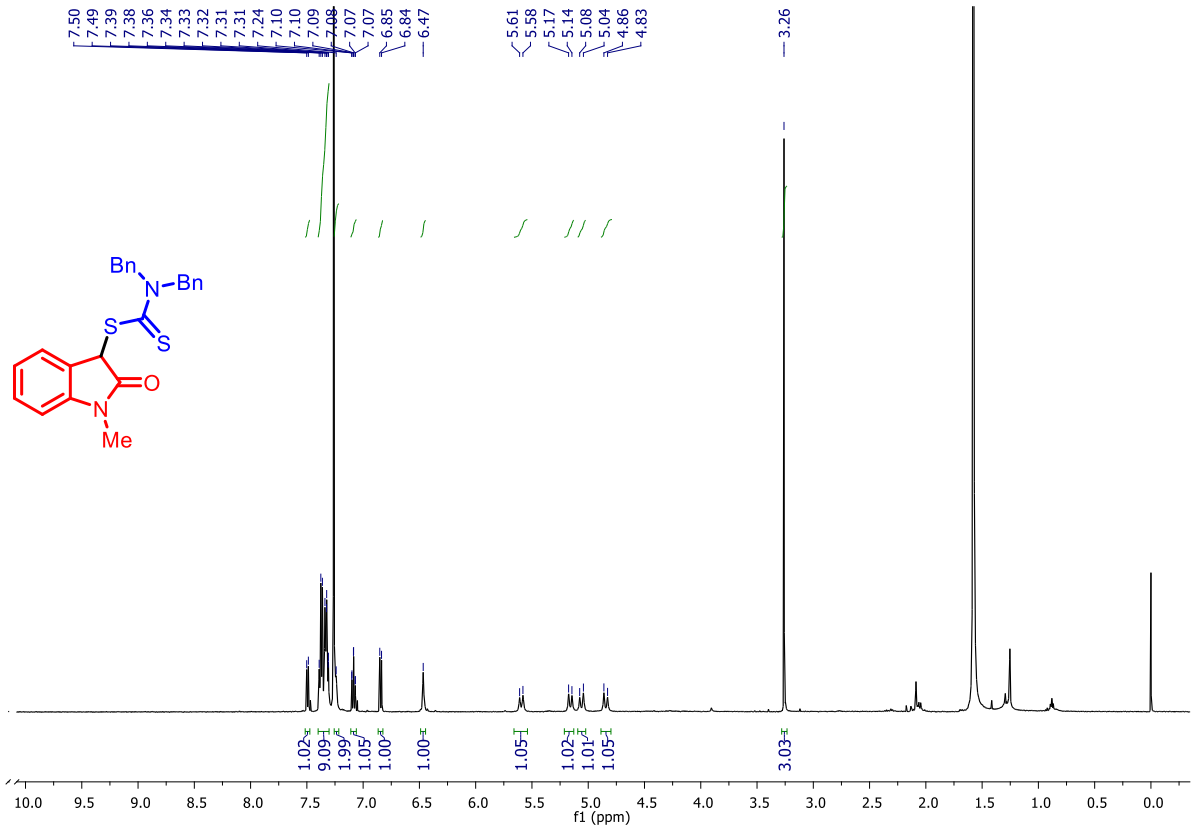
^{13}C NMR (125 MHz, $CDCl_3$) of compound **4b**



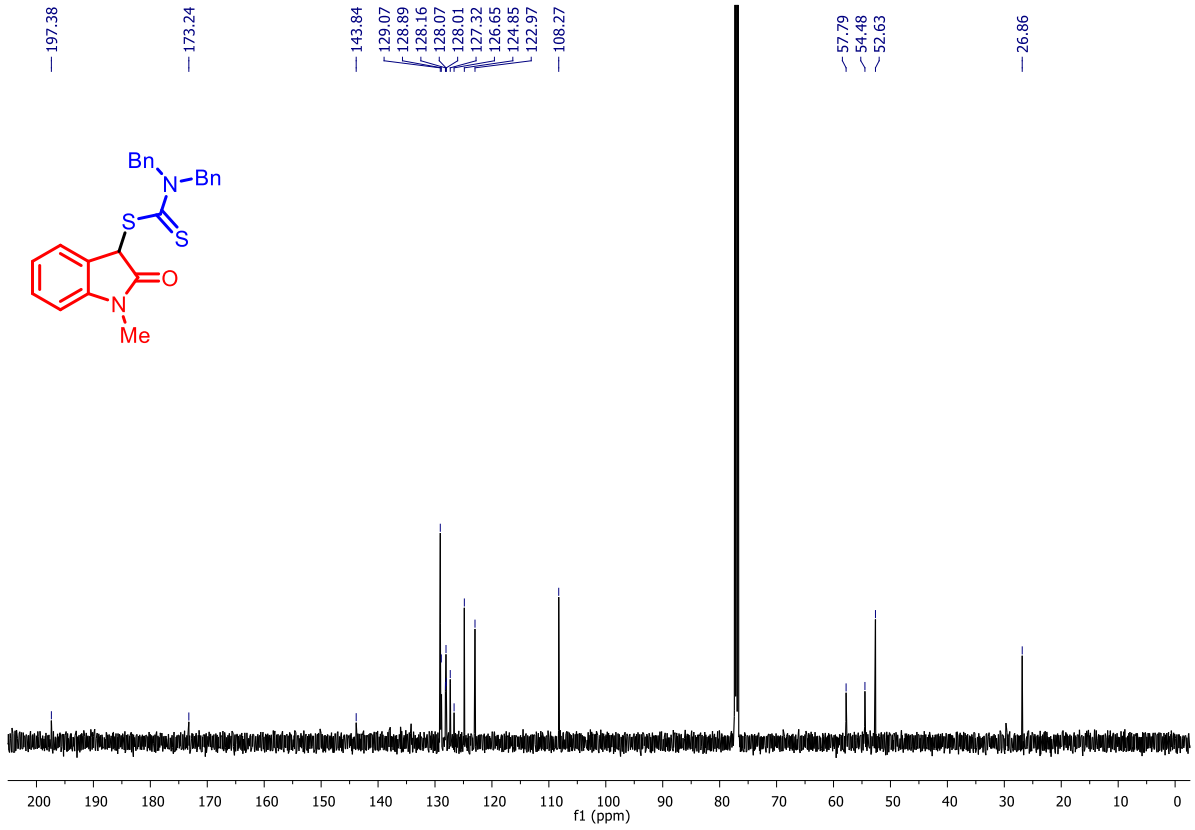
^{1}H NMR (500 MHz, $CDCl_3$) of compound **4c**



^{13}C NMR (125 MHz, $CDCl_3$) of compound **4c**



^{1}H NMR (500 MHz, $CDCl_3$) of compound **4d**



^{13}C NMR (125 MHz, $CDCl_3$) of compound **4d**