Efficient synthesis of eudistomin U and evaluation of its cytotoxicity

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Bioorganic and Medicinal Chemistry Letters

Supporting Information

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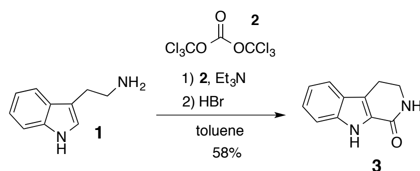
General Experimental Procedures. All reactions were performed in single-neck, oven-dried, round-bottomed flask unless otherwise noted. Each reaction flask was fitted with a rubber septum under a positive pressure of nitrogen and charged with a magnetic stir bar. Air- and moisture-sensitive liquids were transferred via syringe. Organic solutions were concentrated by rotary evaporation at 30–33 °C. Flash-column chromatography was performed as described by Still et al.,[1] employing silica gel (60 Å, 40–63 µm particle size) purchased from BDH. Analytical thin-layered chromatography (TLC) was performed using plastic plates pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV).

Materials. Chromatography solvents were used as received from Pharmco-AAPER. All other commercial reagents, including anhydrous dioxane, pyridine, toluene, and triethylamine, were used as received from Sigma-Aldrich, Strem Chemicals, or Acros Organics. Dry tetrahydrofuran was obtained using the method of Pangborn et al.[2]

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker Avance DRX 400 MHz spectrometer at 24 °C. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent (d_6-DMSO, δ 2.51; $CDCl_3$, δ 7.26). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad), integration, and coupling constant in Hertz. Proton-decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 100 MHz at 24 °C. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent (d_6-DMSO, δ 39.52; $CDCl_3$, δ 77.16). All IR spectra were measured with a PerkinElmer Spectrum One FTIR spectrometer using a thin film on NaCl plates. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). High-resolution mass spectrometry (HRMS) data were acquired on an Agilent 6530 Q-TOF mass spectrometer in positive ESI and processed with Agilent MassHunter software at Brown University.

Synthetic Procedures:

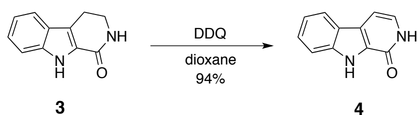
*Bischler-Napieralski cyclization of tryptamine **1***



Tryptamine **1** (3.0 g, 18.7 mmol, 1.0 equiv.) was dissolved in toluene (267 mL) and warmed to increase solubility. Once completely dissolved, the solution was cooled to room temperature and sparged with a stream of nitrogen for 5 minutes. Triethylamine (6.3 mL, 45.2 mmol, 2.4 equiv.) was then added to the reaction mixture. In an addition funnel, triphosphogene **2** (2.2 g, 7.50 mmol, 0.40 equiv.) was dissolved in toluene (11.8 mL) and added dropwise to the reaction mixture over 5 minutes. The yellowish brown solution was left to stir at room temperature for 1 hour. A solution of 48% aqueous HBr (4.3 mL) was added to the reaction mixture in a steady stream over 30 seconds. The resulting greenish yellow solution was heated at reflux for 1 hour. The solution was then cooled to roughly 4 °C and water (150 mL) was added to the product mixture dropwise. The diluted mixture was then poured into a separatory funnel and extracted with ethyl acetate (4 x 60 mL). The organic layer was then dried over magnesium sulfate, filtered, and concentrated. The residue obtained was recrystallized using ethyl acetate (15 mL) to yield the solid, pale-yellow cyclized product. The filtrate was concentrated and two additional crops of purified product were collected via recrystallization with ethyl acetate to obtain **3** (1.99 g, 58%) as a yellow solid whose spectra were consistent with a literature procedure[3]:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.60 (d, J = 8.4 Hz, 1 H), 7.45 (d, J = 8.4 Hz, 1 H), 7.32 (dd, J = 7.6 Hz, 1 H), 7.16 (dd, J = 7.6 Hz, 1 H), 3.73 (t, J = 6.8 Hz, 2 H), 3.07 (t, J = 7.2 Hz, 2 H).

*Oxidation of lactam **3***

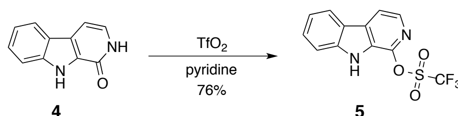


Lactam **3** (750 mg, 4.0 mmol, 1.0 equiv.) was dissolved in anhydrous dioxane (57.3 mL), cooled to 15 °C, and sparged with a stream of nitrogen for 5 minutes. In a separate flask, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.098 g, 4.839 mmol, 1.200 equiv.) was dissolved in anhydrous dioxane, sparged with a stream of nitrogen for 5 minutes, and then added to the reaction flask containing **2**. The reddish brown solution was warmed to room temperature and stirred for 25 minutes. The product mixture was then poured into a

separatory funnel containing 120 mL water. The diluted mixture was extracted with ethyl acetate (3 x 40 mL). The organic layer obtained was washed with 0.1 M sodium hydroxide (4 x 40 mL). The combined organic layers obtained were then dried over magnesium sulfate, filtered, and concentrated to provide an orange-brown solid **4** (707 mg, 94%), whose spectra were consistent with a literature procedure[4]:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 11.92 (br s, 1 H), 11.38 (br s, 1 H), 8.01 (d, J = 7.6 Hz, 1 H), 7.50 (d, J = 8.0 Hz, 1 H), 7.39 (dd, J = 6.8 Hz, 1 H), 7.16 (dd, J = 7.6 Hz, 1 H), 7.07 (dd, J = 6.8 Hz, 1 H), 6.97 (d, J = 6.8 Hz, 1 H).

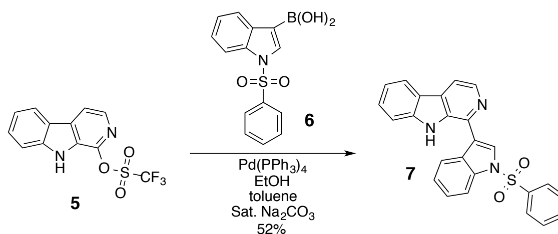
*Triflation of pyridone **4***



Pyridone **4** (300 mg, 1.6 mmol, 1.0 equiv.) was dissolved in pyridine (16.3 mL), cooled to 4° C, and sparged with a stream of nitrogen for 5 minutes. Trifluoromethanesulfonic anhydride (0.55 mL, 3.3 mmol, 2.0 equiv.) was then added to the solution dropwise over 30 minutes. The resulting orange solution was warmed to room temperature and stirred for 30 minutes. The product mixture was then poured into a separatory funnel containing 250 mL water. The diluted mixture was then extracted with ethyl acetate (3 x 125 mL). The organic layer obtained was washed with 1.0 M hydrochloric acid (30 x 40 mL). The combined organic layers were then dried over magnesium sulfate, filtered, and concentrated to provide triflate **5** as a brown film (396 mg, 76%) whose spectra were consistent with a literature procedure.[5] Triflate **5** was taken onto the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.48 (br s, 1 H), 8.16 (d, J = 5.1 Hz, 1 H), 8.14 (d, J = 7.9 Hz, 1 H), 8.01 (d, J = 5.1 Hz, 1 H), 7.67-7.58 (m, 2 H), 7.37 (dd, J = 7.4 Hz, 1 H).

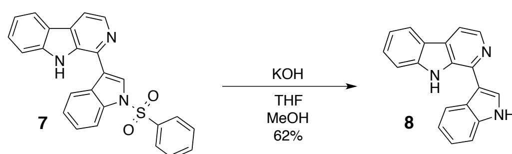
*Suzuki Cross Coupling of triflate substituted β-Carboline **5** with 1-(Phenylsulfonyl)-3-indolylboronic acid **6***



Triflate-substituted β-carboline **5** (20.0 mg, 0.06 mmol, 1.00 equiv.) was dissolved in toluene (0.63 mL) and sparged with a stream of nitrogen for 5 minutes. In a separate flask, 1-(phenylsulfonyl)-3-indolylboronic acid **6** (26.7 mg, 0.09 mmol, 1.4 equiv.) was dissolved in ethanol (0.42 mL) and sparged with nitrogen for 5 minutes. The resulting solution was added to the reaction mixture containing **5**. Saturated sodium carbonate (0.21 mL) was added to the reaction mixture, followed by addition of tris(dibenzylideneacetone)dipalladium(0) (2.89 mg, 0.003 mmol, 0.050 equiv.) and triphenylphosphine (1.65 mg, 0.006 mmol, 0.100 equiv.). The yellowish brown reaction mixture was stirred at room temperature and sparged with a stream of nitrogen for 5 min. The solution was then heated to 80 °C for 1 h. The product-mixture was then poured into a separatory funnel containing 20 mL water. The diluted mixture was then extracted with ethyl acetate (4 x 25 mL). The organic layer was then dried over magnesium sulfate, filtered, and concentrated. The residue obtained was purified by flash-column chromatography (eluting with 100 mL 5% ethyl acetate–petroleum ether initially, grading to 25% ethyl acetate–petroleum ether, six steps) to provide the benzenesulfonyl-protected eudistomin U **7**, as a bluish-green foam (19.5 mg, 73%).

Characterization of **7**:
R_f = 0.63 (50% ethyl acetate–petroleum ether; UV). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.96 (s, 1 H), 8.55 (d, J = 5.2 Hz, 1 H), 8.21 (s, 1 H), 8.15 (d, J = 7.6 Hz, 1 H), 8.11 (t, J = 12.8 Hz, 2 H), 7.93 (m, 3 H), 7.55 (d, J = 4.0 Hz, 2 H), 7.51 (t, J = 7.6 Hz, 1 H), 7.40 (t, J = 7.6 Hz, 3 H), 7.32 (m, 2 H). ^{13}C NMR (100 MHz, d_6-DMSO): δ (ppm) 141.0, 138.1, 136.8, 136.7, 134.8, 134.4, 133.0, 129.9, 129.4, 129.1, 128.4, 127.1, 125.9, 125.5, 124.0, 123.4, 121.7, 121.0, 119.9, 119.8, 113.8, 113.1, 112.5. IR (FT-IR) cm^{-1}: 3442 (s), 3055 (s), 2885 (s), 1732 (s), 1626 (s), 1570 (s), 1447 (s), 1373 (s), 1264 (s), 1174 (s), 744 (s). HR-MS (*m/z*): $[M + H]^+$ calcd for $C_{25}H_{18}N_3O_2S$, 424.1049; found, 424.1118.

Deprotection of benzenesulfonylated eudistomin U 7



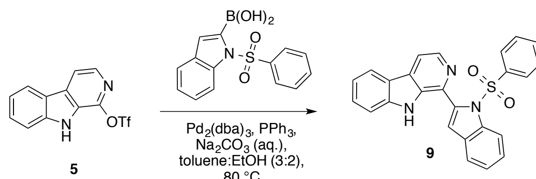
Benzenesulfonyl-protected eudistomin U **7** (12.2 mg, 0.03 mmols, 1.00 equiv) was dissolved in a 1:1 mixture of MeOH (0.24 mL) and dry THF (0.24 mL) and sparged with stream of nitrogen for 5 minutes. Solid KOH (22.7 mg, 0.40 mmol, 14.00 equiv.) was then added to the solution. The yellow reaction mixture was sparged with a stream of nitrogen for 5 minutes, heated to 80 °C, and stirred for 40 minutes under inert atmosphere. The product mixture was then poured into a separatory funnel containing 15 mL water. The diluted mixture was then extracted with ethyl acetate (3 x 20 mL). The combined organic layers were then dried over magnesium sulfate, filtered, and

concentrated. The residue obtained was purified by flash-column chromatography (eluting with 100 mL 10% ethyl acetate–hexanes initially, grading to 25% ethyl acetate–hexanes, 4 steps) to provide the naturally occurring product eudistomin U **8** as a yellow granular solid (5.10 mg, 62%) whose spectra were consistent with the literature[6]:

Comparison of synthetic eudistomin U to previously synthesized material

<table>
<tr><th>Our synthetic material</th><th>Reference material[7]</th></tr>
<tr><td>11.70 (s, 1 H)</td><td>11.69 (s, 1 H)</td></tr>
<tr><td>11.29 (s, 1 H)</td><td>11.27 (s, 1 H)</td></tr>
<tr><td>8.56 (d, J = 7.6 Hz, 1 H)</td><td>8.56 (d, J = 7.9 Hz, 1 H)</td></tr>
<tr><td>8.45 (d, J = 5.2 Hz, 1 H)</td><td>8.45 (d, J = 5.1 Hz, 1 H)</td></tr>
<tr><td>8.29 (d, J = 2.8 Hz, 1 H)</td><td>8.29 (d, J = 2.7 Hz, 1 H)</td></tr>
<tr><td>8.24 (d, J = 8.0 Hz, 1 H)</td><td>8.23 (d, J = 7.8 Hz, 1 H)</td></tr>
<tr><td>7.96 (d, J =4.8 Hz, 1 H)</td><td>7.95 (d, J = 5.2 Hz, 1 H)</td></tr>
<tr><td>7.69 (d, J = 8.4 Hz, 1 H)</td><td>7.69 (d, J = 8.3 Hz, 1 H)</td></tr>
<tr><td rowspan="2">7.56-7.51 (m, 2 H)</td><td>7.55 (d, J = 7.1 Hz, 1 H)</td></tr>
<tr><td>7.52 (t, J = 7.9 Hz, 1 H)</td></tr>
<tr><td rowspan="2">7.27-7.20 (m, 2 H)</td><td>7.25 (td, J = 0.9, 7.3 Hz, 1 H)</td></tr>
<tr><td>7.22 (td, J = 1.2, 7.0 Hz, 1 H)</td></tr>
<tr><td>7.14 (dd, J = 7.2 Hz, 1 H)</td><td>7.14 (td, J = 1.0, 7.6 Hz 1 H)</td></tr>
</table>

Suzuki Cross Coupling of triflate substituted β-Carboline ***5*** *with 1-(Phenylsulfonyl)-2-indolylboronic acid*

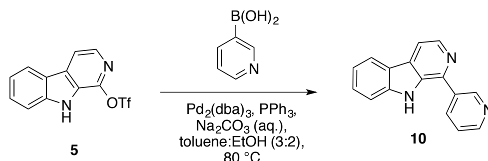


Triflate-substituted β-carboline **5** (50.0 mg, 0.158 mmol, 1.00 equiv.) was dissolved in toluene (1.58 mL) and sparged with a stream of nitrogen for 5 minutes. In a separate flask, 1-(phenylsulfonyl)-2-indolylboronic acid (66.6 mg, 0.222 mmol, 1.4 equiv.) was dissolved in ethanol (1.05 mL) and sparged with nitrogen for 5 minutes. The resulting solution was added to the reaction mixture containing **5**. Tris(dibenzylideneacetone)dipalladium(0) (7.2 mg, 0.008 mmol, 0.050 equiv.) was added to the reaction mixture, followed by addition of triphenylphosphine (4.14 mg, 0.016 mmol, 0.100 equiv.) and saturated sodium carbonate (0.5 mL). The yellowish brown reaction mixture was stirred at room temperature and sparged with a stream of nitrogen for 5 min. The solution was then heated to 80 °C for 2.5 h. The product-mixture was then poured into a separatory funnel containing 20 mL ethyl acetate. The diluted mixture was then washed with water (2 x 20 mL). The organic layer was then dried over sodium sulfate, filtered, and concentrated. The residue obtained was purified by flash-column

chromatography (eluting with 100 mL 5% ethyl acetate–petroleum ether initially, grading to 25% ethyl acetate–petroleum ether, five steps) to provide the benzenesulfonyl-protected analog **9** as a yellow film (36 mg, 54%).

Characterization of **9**:
R_f = 0.18 (20% ethyl acetate–petroleum ether; UV). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.68 (br s, 1 H), 5.58 (d, J = 5.2 Hz, 1 H), 8.27 (d, J = 8.0 Hz, 1 H), 8.18, (d, J = 8.0 Hz, 1 H), 8.07 (d, J = 5.2 Hz, 1 H), 7.71 (d, J = 8.0 Hz, 2 H), 7.58-7.51 (m, 3 H), 7.47-7.40 (m, 2 H), 7.35-7.28 (m, 4 H), 7.03 (br s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 140.6, 138.7, 138.2, 136.5, 136.0, 135.6, 134.0, 132.3, 131.0, 129.5, 128.9, 128.8, 127.4, 125.8, 124.9, 122.0, 121.8, 121.7, 120.5, 116.5, 116.2, 115.4, 112.0. IR (FT-IR) cm^{-1}: 3446 (s), 3064 (s), 1628 (s), 1448 (s),, 1425 (s), 1373 (s), 1320 (s), 1265 (s), 1176 (s), 729 (s). HR-MS (*m/z*): $[M + H]^+$ calcd for $C_{25}H_{18}N_3O_2S$, 424.1049; found, 424.1111.

Suzuki Cross Coupling of triflate substituted β-Carboline ***5*** *with 3-pyridyl boronic acid*



Triflate-substituted β-carboline **5** (50.0 mg, 0.158 mmol, 1.00 equiv.), 3-pyridyl boronic acid (27.2 mg, 0.221 mmol, 1.4 equiv.), tris(dibenzylideneacetone)dipalladium(0) (7.2 mg, 0.008 mmol, 0.050 equiv.), and triphenylphosphine (4.14 mg, 0.016 mmol, 0.100 equiv.) were dissolved in toluene (1.58 mL) and ethanol (1.05 mL) and sparged with nitrogen for 5 minutes. A solution of saturated sodium carbonate (0.5 mL) was then added to the reaction mixture. The yellowish brown reaction mixture was stirred at room temperature and sparged with a stream of nitrogen for 5 min. The solution was then heated to 80 °C for 1.5 h. The product-mixture was then poured into a separatory funnel containing 50 mL ethyl acetate. The diluted mixture was then washed with water (2 x 20 mL). The organic layer was then dried over sodium sulfate, filtered, and concentrated. The residue obtained was purified by flash-column chromatography (eluting with 100 mL 10% ethyl acetate–petroleum ether initially, grading to 100% ethyl acetate, four steps) to provide the analog **10** as an orange film (36 mg, 94%) whose spectra were consistent with the literature[8]:

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.96 (br s, 1 H), 9.47 (s, 1 H), 8.59 (d, J = 5.3 Hz, 2 H), 8.37 (d, J = 7.9 Hz, 1 H), 8.17 (d, J = 7.9 Hz, 1 H), 8.01 (d, J = 5.3 Hz, 1 H), 7.57-7.51 (m, 2 H), 7.49-7.39 (m, 2 H), 7.32-7.28 (m, 1 H).

Biological Assays:

General procedures: Optical density measurements were taken on a Genesys 10 UV-Vis spectrophotometer. Absorbance measurements in 96-well plates were taken on an OpsysMR plate reader by Dynex Techologies. Caspofungin was obtained from Professor Nicanor Austriaco in the Department of Biology at Providence College. For stock solution preparation, an 8 mg sample of pure eudistomin U was dissolved in dimethyl sulfoxide (DMSO) to obtain a final concentration of 0.3 M. The sample was carefully mixed using a pipette and sonicated to achieve homogeneity, then stored at 4 °C. An aliquot of this stock solution was used to prepare a serial dilution in which the final assay concentrations were: 300 μM, 100 μM, 30 μM, 10 μM, 3 μM, 1 μM, 0.3 μM, and 0.1 μM. The concentration of DMSO in the final assay buffer was 0.3% for prokaryotic and fungi cells, and 0.1% for human cancer cell lines.

Optical Density-Based Assays

1. Antibacterial:

The following is a general procedure used to evaluate cytotoxicity against the following bacterial cell lines: *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli*. A sterile wooden stick was used to pick a frozen stock of *S.aureus* and was mixed aseptically into a glass cell culture tube containing 5 mL Luria based liquid medium. The cell culture was placed in roller drum and spun overnight at 37 °C. The optical density of the saturated culture was measured and then sub-cultured to a final volume of 5 mL and an optical density of 0.1. The diluted culture was placed in roller drum for 2 hours at 37 °C. A volume of 10 μL of cell culture was added to a set of 8 plastic cuvettes containing a different concentration of eudistomin U (prepared as previously above), plus a negative (DMSO) and positive control (streptomycin, see below for concentrations). A second set of 10 plastic cuvettes with identical labels were prepared in the same manner but excluded the cell culture. All 20 samples were placed on a shaker in a 37 °C incubator at low velocity and the optical density of each sample was recorded every 2.5 hours in duplicate. After each measurement, the samples were placed back on the shaker at low velocity at 37 °C. Once bacterial growth exited log phase approximately 7.5 hours after t=0, a percent growth graph was constructed. Inhibitory concentration at 50% was constructed using the computer program Origin. A sigmoidal curve was fitted to the data and the IC_{50} was calculated using the slope of the curve. The IC_{50} values reported are an average of three independent assay trials.

Streptomycin concentration in antibacterial assay

Organism	Concentration
S.aureus	0.0625 μg/mL
S.pyogenes	0.0625 μg/mL
P.aeruginosa	0.500 μg/mL
E.coli	0.0625 μg/mL

Mycobacterium smegmatis

A single colony was picked from a frozen plate of pure colonies of MC^2155 *Mycobacterium smegmatis* and aseptically added to a 10 mL flask containing 7H9 complete medium with tween. The flask was placed on a shaker at 37 °C for 48 hours. A 1:1000 dilution was performed in a separate 10 mL flask containing the appropriate volume of 7H9 complete medium with Tween. The flask was placed on a shaker at 37 °C incubator overnight. The optical density of the saturated culture was measured and the cells were sub-cultured for a final volume of 5 mL and final optical density of 0.1, and then was placed in roller drum for 2 hours at 37 °C. A volume of 10 µL of cell culture was added to a set of 8 plastic cuvettes containing a different concentration of eudistomin U (prepared as previously above), plus a negative (DMSO) and positive control (streptomycin, 0.250 µg/mL). A second set of 10 plastic cuvettes with identical labels were prepared in the same manner but excluded the cell culture. All 20 samples were placed on a shaker in a 37 °C incubator at low velocity and the optical density of each sample was recorded every 2.5 hours in duplicate. After each measurement, the samples were placed back on the shaker at low velocity at 37 °C. Once bacterial growth exited log phase approximately 7.5 hours after t=0, a percent growth graph was constructed. Inhibitory concentration at 50% was constructed using the computer program Origin. A sigmoidal curve was fitted to the data and the IC_{50} was calculated using the slope of the curve. The IC_{50} values reported are an average of three independent assay trials.

2. Antifungal:

Saccharomyces cerevisiae

A sterile wooden stick was used to pick a frozen stock of *S. cerevisiae* and was mixed aseptically into a glass cell culture tube containing 5 mL synthetic dextrose liquid media. The cell culture was placed in roller drum and spun overnight at 30 °C. The optical density of the saturated culture was measured and then sub-cultured to a final volume of 5 mL and an optical density of 0.1. The diluted culture was placed in roller drum for 2 hours at 30 °C. A volume of 10 µL of cell culture was added to a set of 8 plastic cuvettes containing a different concentration of eudistomin U (prepared as previously above), plus a negative (DMSO) and positive control (caspofungin, 1 µg/mL). A second set of 10 plastic cuvettes with identical labels were prepared in the same manner but excluded the cell culture. All 20 samples were placed on a shaker in a 30 °C incubator at low velocity and the optical density of each sample was recorded every 2.5 hours in duplicate. After each measurement, the samples were placed back on the shaker at low velocity at 30 °C. Once bacterial growth exited log phase approximately 7.5 hours after t=0, a percent growth graph was constructed. Inhibitory concentration at 50% was constructed using the computer program Origin. A sigmoidal curve was fitted to the data and the IC_{50} was calculated using the slope of the curve. The IC_{50} values reported are an average of three independent assay trials.

Candida albicans

A sterile wooden stick was used to pick a frozen stock of *C.albicans* wildtype diploid and was mixed aseptically into a glass cell culture tube containing 5 mL WYPD with glucose. The cell culture was placed in roller-drum and spun overnight at 30 °C. The optical density of the saturated culture was measured and then sub-cultured to a final

volume of 5 mL and an optical density of 0.1. The diluted culture was placed in roller drum for 2 hours at 30 °C. A volume of 10 µL of cell culture was added to a set of 8 plastic cuvettes containing a different concentration of eudistomin U (prepared as previously above), plus a negative (DMSO) and positive control (caspofungin, 1 µg/mL). A second set of 10 plastic cuvettes with identical labels were prepared in the same manner but excluded the cell culture. All 20 samples were placed on a shaker in a 30 °C incubator at low velocity and the optical density of each sample was recorded every 2.5 hours in duplicate. After each measurement, the samples were placed back on the shaker at low velocity at 30 °C. Once bacterial growth exited log phase approximately 7.5 hours after t=0, a percent growth graph was constructed. Inhibitory concentration at 50% was constructed using the computer program Origin. A sigmoidal curve was fitted to the data and the IC_{50} was calculated using the slope of the curve. The IC_{50} values reported are an average of three independent assay trials.

MTT-based Cytotoxicity Assays

C-19 Human Leukemia Cell Line:

A frozen stock of C-19 human leukemia cells was thawed and added to a cell culture petri dish containing 10 mL warmed IMDM medium containing penicillin, streptomycin, and *L*-glutamate. The plate was labeled and placed in a 37 °C incubator with 5% CO_2 for 48 hours, after which a hemocytometer was used to count the cells. The cells were suspended into a sterile 96-well plate at a concentration of 10,000 cells/well and a final volume of 100 µL in IMDM media. The edges of the plate were filled with 100µL media so that only the central 10 x 6 matrix was used for the cell-based assay. Once all of the cells were plated, the assay plate was labeled and placed in the 37 °C incubator with 5% CO_2 for 24 hours. A 2X dilution series of eudistomin U was prepared in a separate sterile 96-well plate (to obtain the final concentrations of eudistomin U as described previously. A volume of 100µL of each concentration was added to the central 10 x 6 matrix so that five rows (50 wells) were used for assaying eudistomin U at varying concentrations while the sixth row (10 wells) was a negative control (0.1% DMSO in IMDM media). The treated plate was placed back in the 37 °C incubator with 5% CO_2 for 24 hours. A 5 mL MTT solution was prepared in PBS buffer at a final concentration of 5 mg/mL. The solution was vortexed to ensure homogeneity. A volume of 20µL of this solution was added to each well and the plate was placed back in the incubator for 2 hours. At this point, a volume of 150 µL of media was carefully removed from each well and 155 µL of DMSO was added. The absorbance was measured at 490 nm relative to background. Treated cells were compared to the control to construct a percent survival graph. An IC_{50} plot was constructed using the computer program Origin's sigmoidal fitting applications. A sigmoidal curve was fitted to the data and the IC_{50} was calculated using the slope of the curve. The IC_{50} values reported are an average of three independent assay trials.

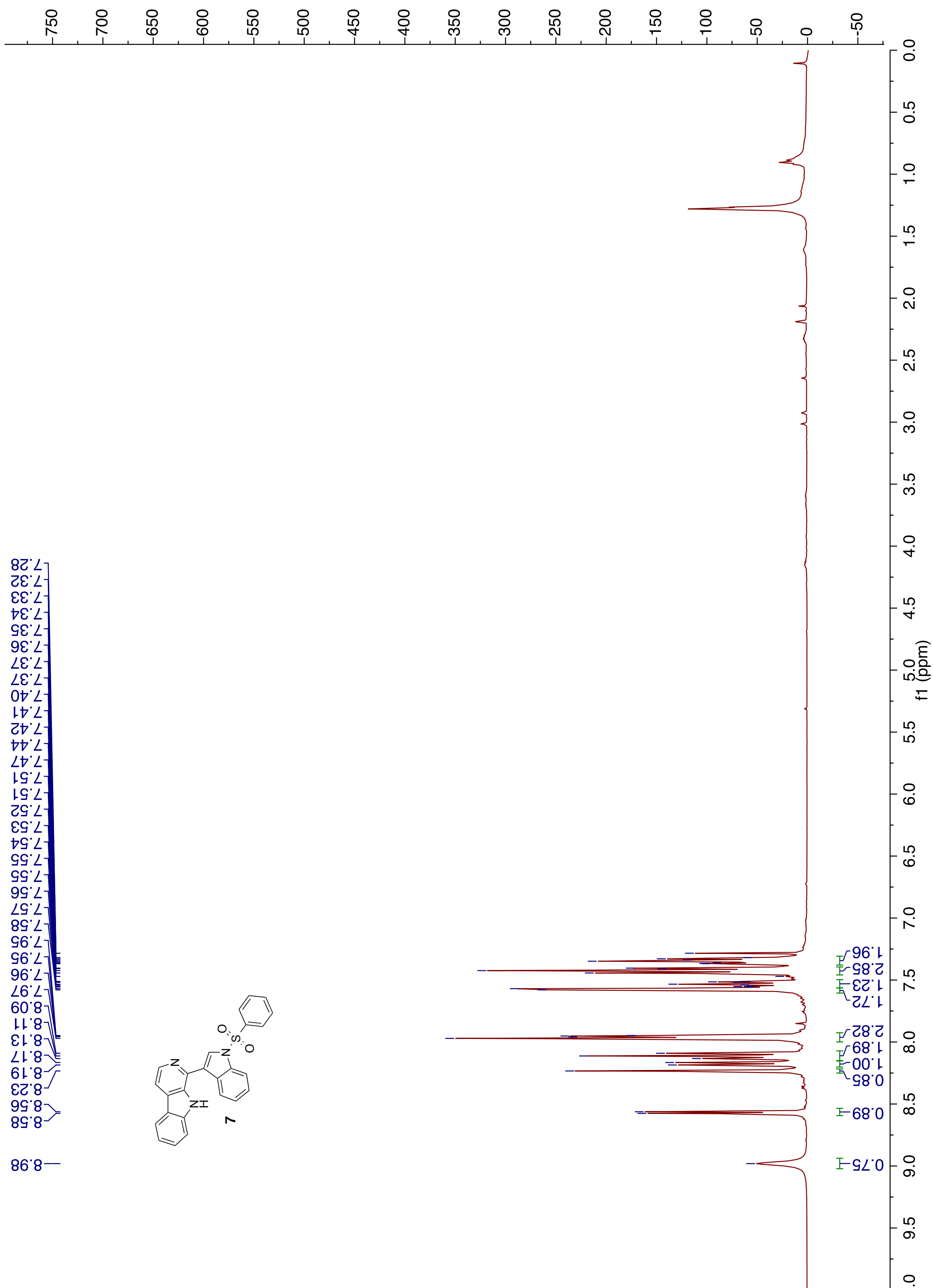
CaOV3 Human Ovarian and WM266-4 Human Melanoma Cell Lines:

The following is a general procedure used to evaluate the cytotoxicity against CaOV3 human ovarian and WM266-4 human melanoma cell lines. A frozen stock of CaOV3 cells was thawed and added to a 25 mL cell culture flask containing 10 mL

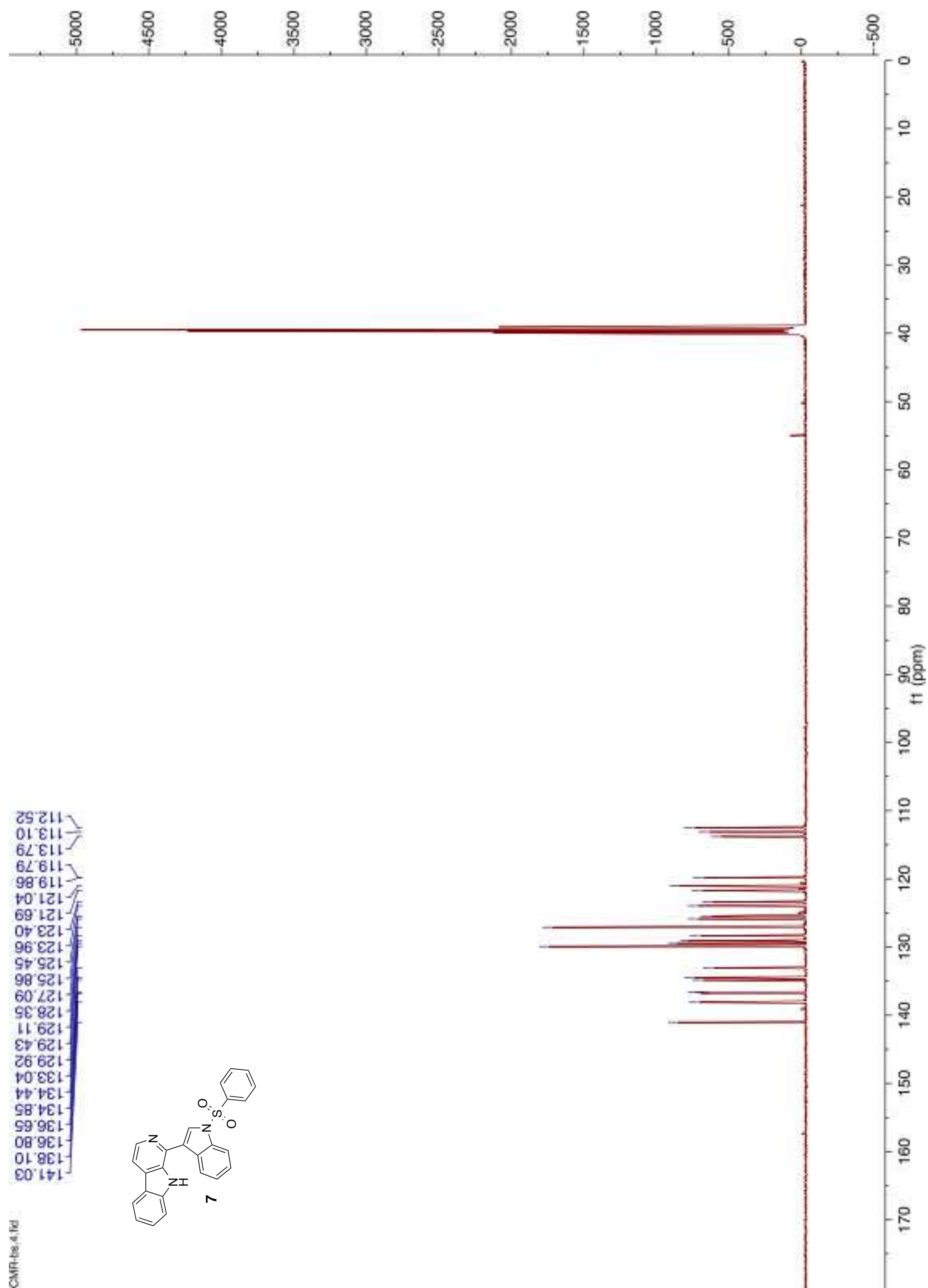
warmed DMEM/F12 medium containing penicillin, streptomycin, and *L*-glutamate. The flask was labeled and placed in a 37 °C incubator with 5% CO_2 for 72 hours. The media was aspirated out of the flask, and 5 mL PBS buffer was added to the flask. The PBS buffer was aspirated and then 2 mL trypsin was added to the flask. The flask was placed in the incubator for 1 minute. A microscope ensured the cells were in suspension. A volume of 10 mL warm DMEM/F12 media was added to the flask and mixed, then a volume of 10 mL was transferred to a 25 mL conical tube. To the original flask containing 2 mL cell culture, a volume of 8 mL DMEM/F12 media was added. The flask was placed back in the incubator and labeled appropriately. Using the cell culture in the 25 mL conical tube, a hemocytometer was used to count the cells. The cells were suspended into a sterile 96-well plate at a concentration of 10,000 cells/well and a final volume of 100 µL in IMDM media. The edges of the plate were filled with 100µL media so that only the central 10 x 6 matrix was used for the cell-based assay. Once all of the cells were plated, the assay plate was labeled and placed in the 37 °C incubator with 5% CO_2 for 24 hours. A 2X dilution series of eudistomin U was prepared in a separate sterile 96-well plate (to obtain the final concentrations of eudistomin U as described previously. A volume of 100µL of each concentration was added to the central 10 x 6 matrix so that five rows (50 wells) were used for assaying eudistomin U at varying concentrations while the sixth row (10 wells) was a negative control (0.1% DMSO in IMDM media). The treated plate was placed back in the 37 °C incubator with 5% CO_2 for 24 hours. A 5 mL MTT solution was prepared in PBS buffer at a final concentration of 5 mg/mL. The solution was vortexed to ensure homogeneity. A volume of 20µL of this solution was added to each well and the plate was placed back in the incubator for 2 hours. At this point, a volume of 150 µL of media was carefully removed from each well and 155 µL of DMSO was added. The absorbance was measured at 490 nm relative to background. Treated cells were compared to the control to construct a percent survival graph. An IC_{50} plot was constructed using the computer program Origin's sigmoidal fitting applications. A sigmoidal curve was fitted to the data and the IC_{50} was calculated using the slope of the curve. The IC_{50} values reported are an average of three independent assay trials.

Catalog of Nuclear Magnetic Resonance and Infrared Spectra:

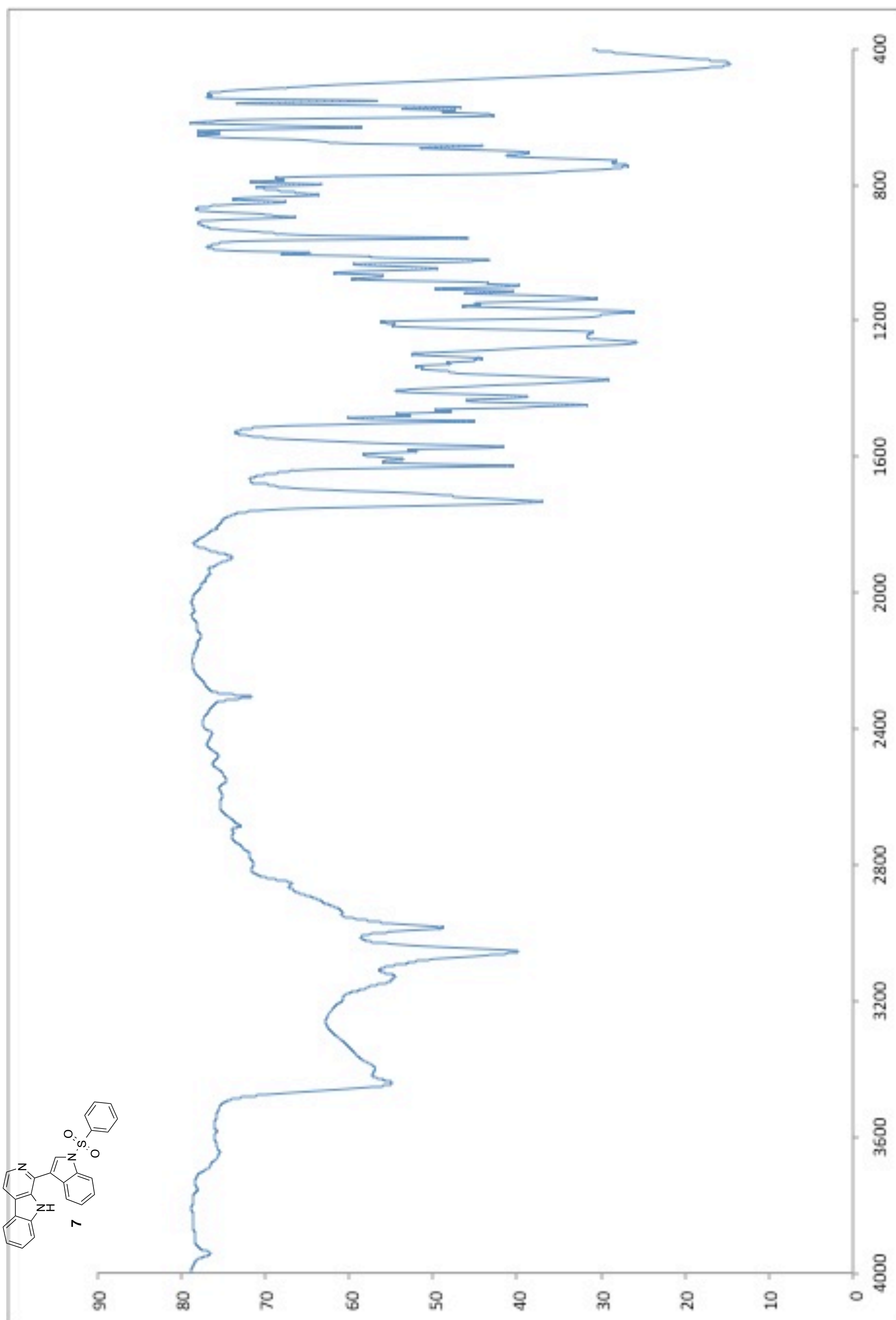
^{1}H NMR of **7**:



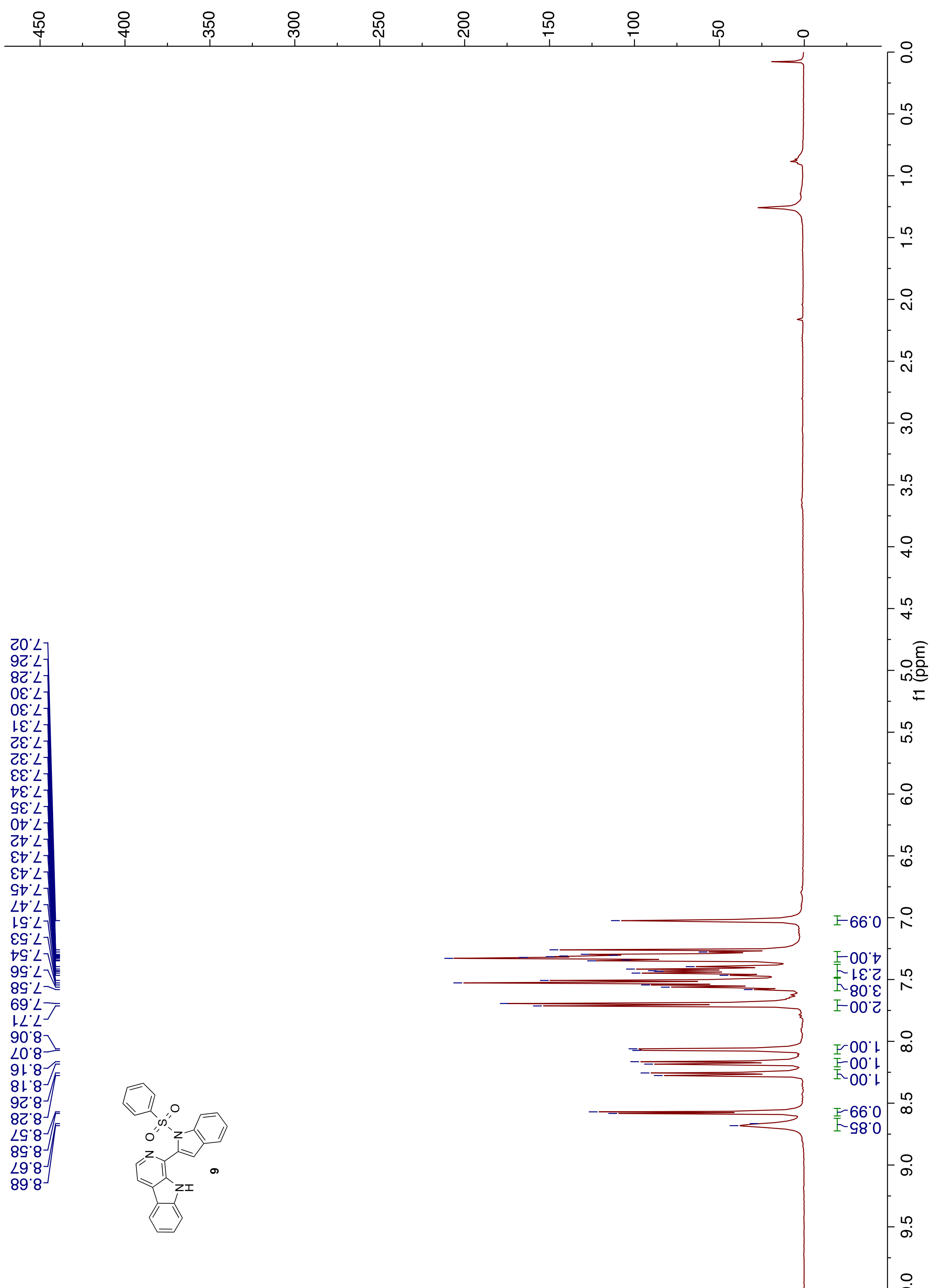
^{13}C NMR of **7**:



IR of **7**:



^{1}H NMR of **9**:



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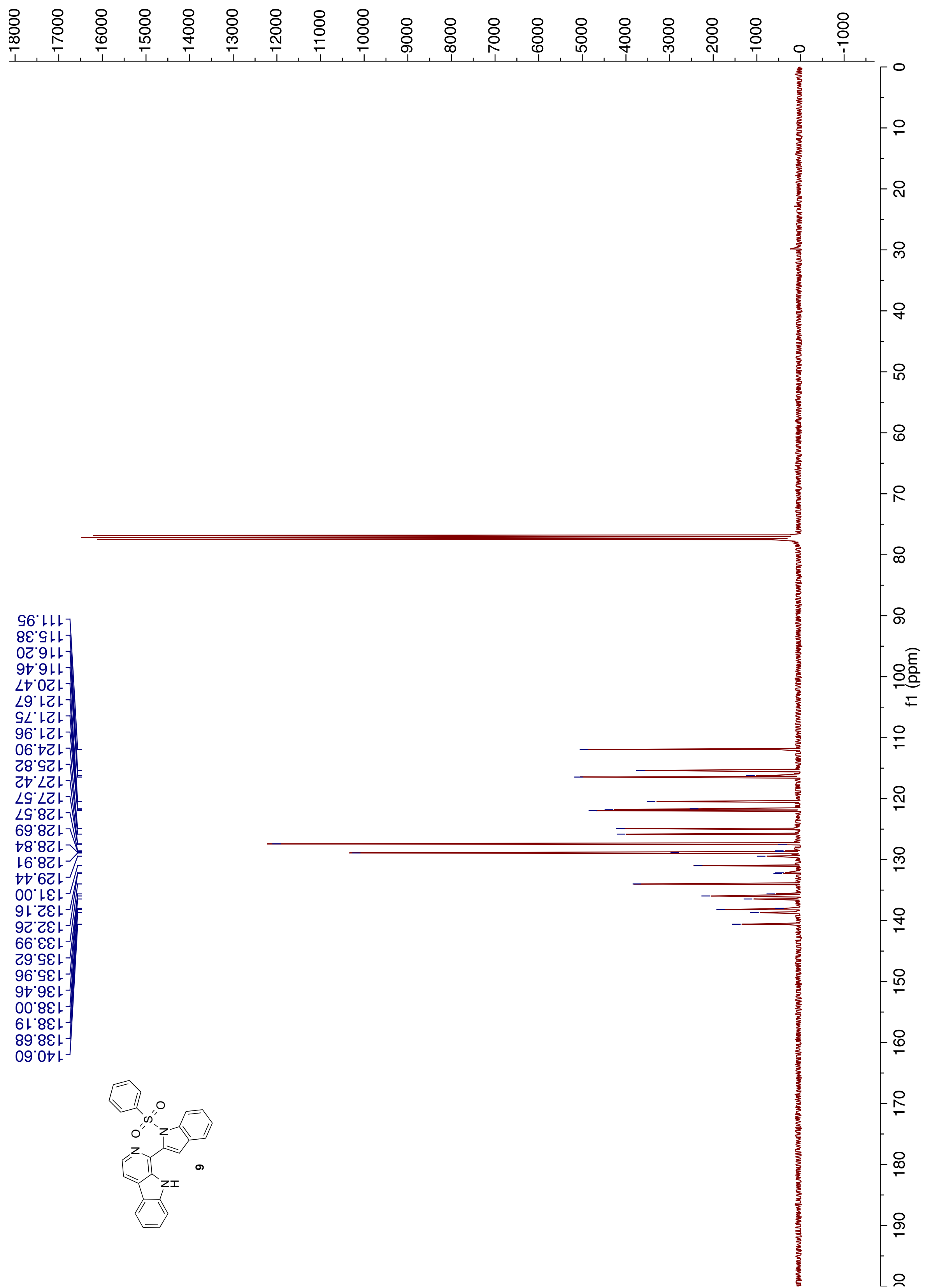

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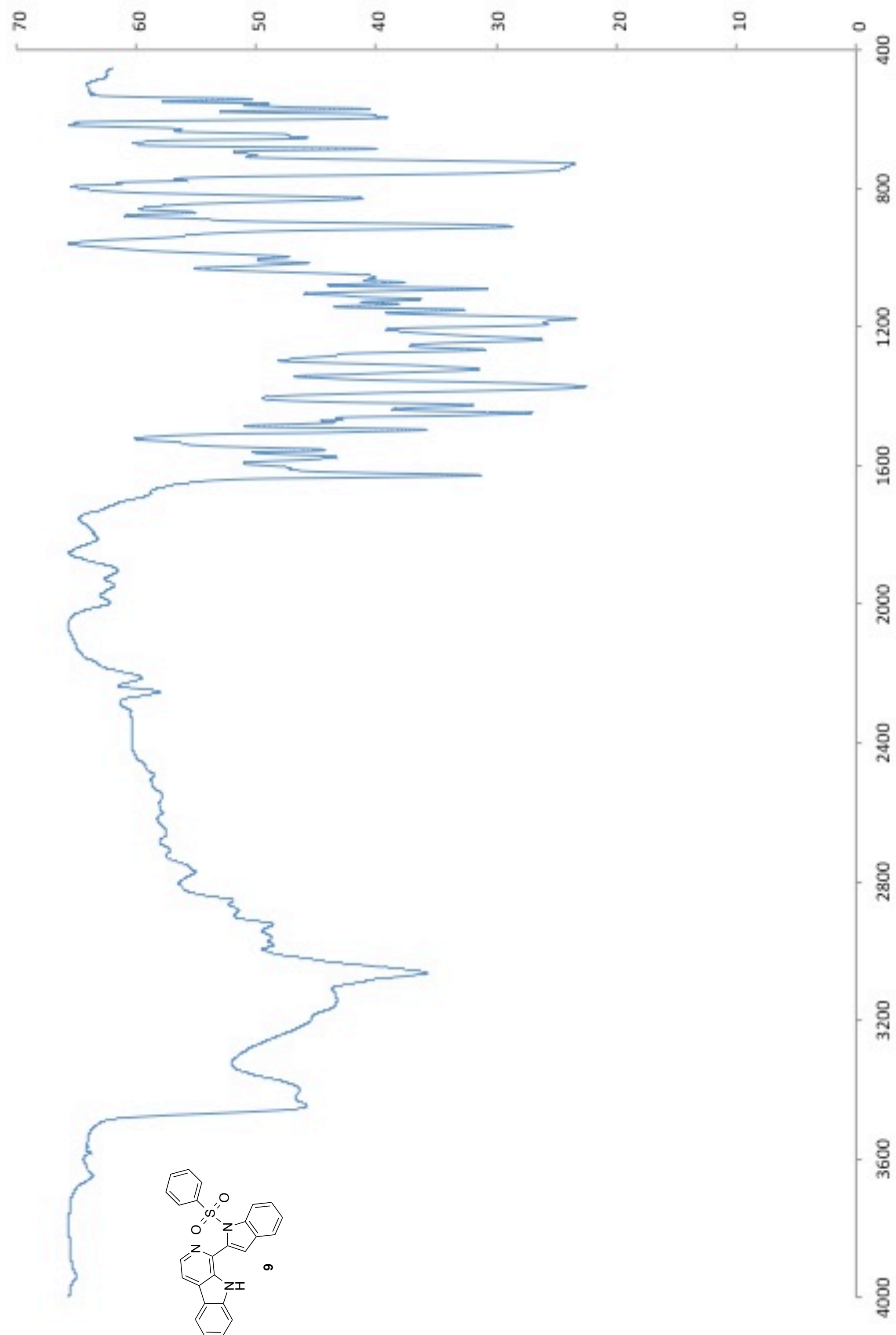

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