SUPPLEMENTAL FIGURES

Figure S1

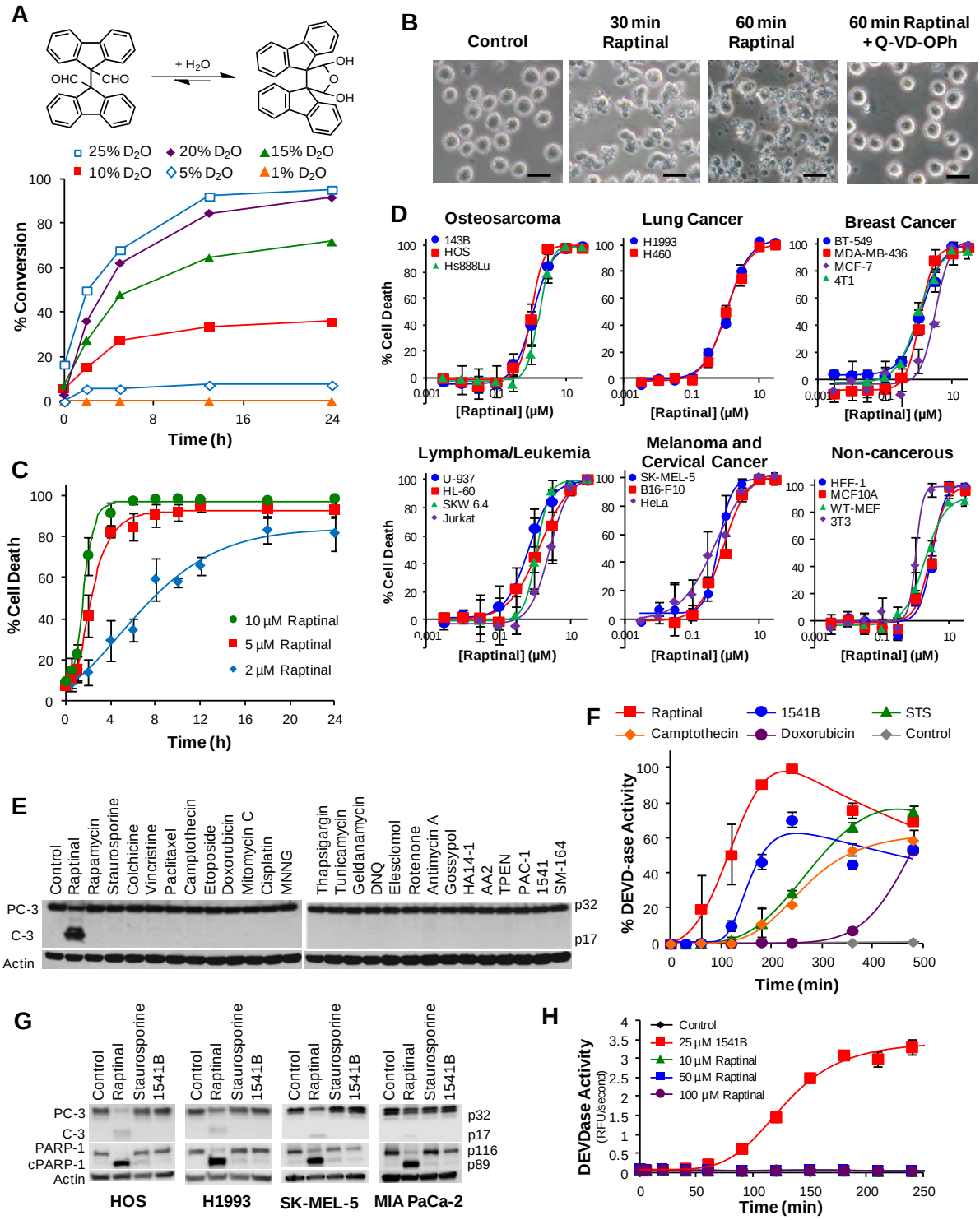


Figure S1. Raptinal Induces Rapid Apoptosis in Multiple Cell Lines, Related to **Figure 1.**

A) Raptinal exists primarily as a hydrate in solution with % conversion of **Raptinal** dialdehyde to hydrate shown as a function of time (in DMSO/D_2O mixture) by ^1H NMR spectroscopy. **B)** Light microscopy images of U-937 cells treated with 10 μM **Raptinal** for 30 and 60 min show cellular blebbing that is inhibited by the pan-caspase inhibitor Q-VD-OPh. Scale bar indicates 20 μm. **C)** Time course analysis of U-937 cells reveals that **Raptinal** induces cell death in a time- and concentration-dependent manner. Cell viability was assessed by AV/PI. Data represent the mean ± SEM from 3 independent experiments. **D)** Raptinal induces dose-dependent cell death in a variety of cancerous and non-cancerous cell lines at 24 h. **E)** Immunoblots of SKW 6.4 cells treated with various toxins for 1 hour show prominent activation of caspase-3 by 10 μM **Raptinal** versus other toxins (all tested at 10 μM). **F)** Fluorescent caspase-3/-7 activity assay in cell lysate comparing **Raptinal** to other fast-acting cytotoxins (all tested at 10 μM). **G)** Immunoblots of adherent HOS, H1993, SK-MEL-5 and MIA PaCa-2 cells show cleavage of PARP-1 and activation of procaspase-3 by 10 μM **Raptinal** but not other cytotoxins (all tested at 10 μM) after 2 hours. **H) Raptinal,** unlike 1541B (the procaspase-3 activator), does not directly activate recombinant procaspase-3 *in vitro*.

Figure S2

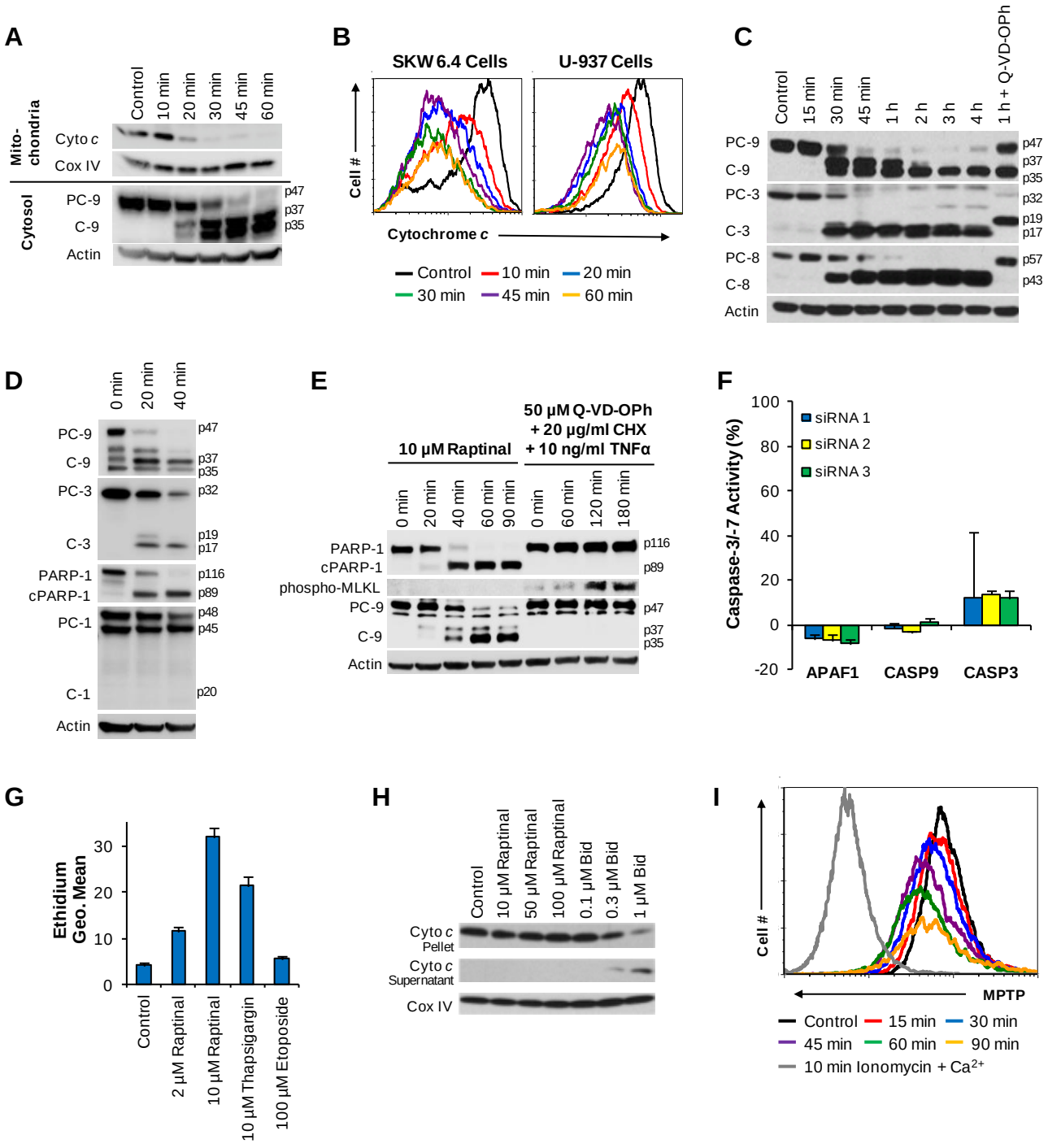


Figure S2. Raptinal Induces Apoptosis Through the Intrinsic Pathway, Related to **Figure 2.**

A) Time course analysis of cytochrome *c* in SKW 6.4 cells by Western Blot shows partial cytochrome *c* release at 10 min and complete release by 20 min of treatment with 10 μM **Raptinal**. **B)** Time course analysis of cytochrome *c* in SKW6.4 (left) and U-937 (right) cells by flow cytometry shows cytochrome *c* release is partial at 10 minutes and complete release by 20 minutes of treatment with 10 μM of **Raptinal**. **C)** Time course immunoblot analysis of caspase-9, -3, and -8 activation in SKW 6.4 cells treated with 10 μM **Raptinal. D)** Time course immunoblot analysis of caspase-9, -3, and -1 activation and PARP-1 cleavage in U-937 cells treated with 10 μM **Raptinal. E)** Time course immunoblot analysis of PARP-1 cleavage, caspase-9 activation and MLKL phosphorylation in **Raptinal** or Q-VD-OPh/CHX/TNFα treated U-937 cells. **F)** Relative percent caspase-3/-7 activity of MIA PaCa-2 cells treated with 10 μM **Raptinal** for 1 hour upon siRNA knockdown of APAF1, CASP9 and CASP3 with 3 distinct siRNA constructs. **G)** U-937 cells exhibit a rapid accumulation of superoxide anion radicals as assessed by dihydroethidium staining and flow cytometry upon 20 minute treatment with **Raptinal** or thapsigargin but not with etoposide, a topoisomerase II inhibitor. Data represent the mean ± SD from 3 independent experiments. **H) Raptinal** does not induce cytochrome *c* release from the mitochondrial pellet into the supernatant of isolated mitochondria treated with **Raptinal** *in vitro* under respiring conditions. The positive control, pro-apoptotic recombinant Bid protein is able to induce cytochrome *c* release. **I) Raptinal** does not induce mitochondrial permeability transition pore (MPTP) in U-937 cells as assessed by a calcein-cobalt quenching assay. Ionomycin in the presence of calcium, a positive control, is able to induce MPTP.

Figure S3

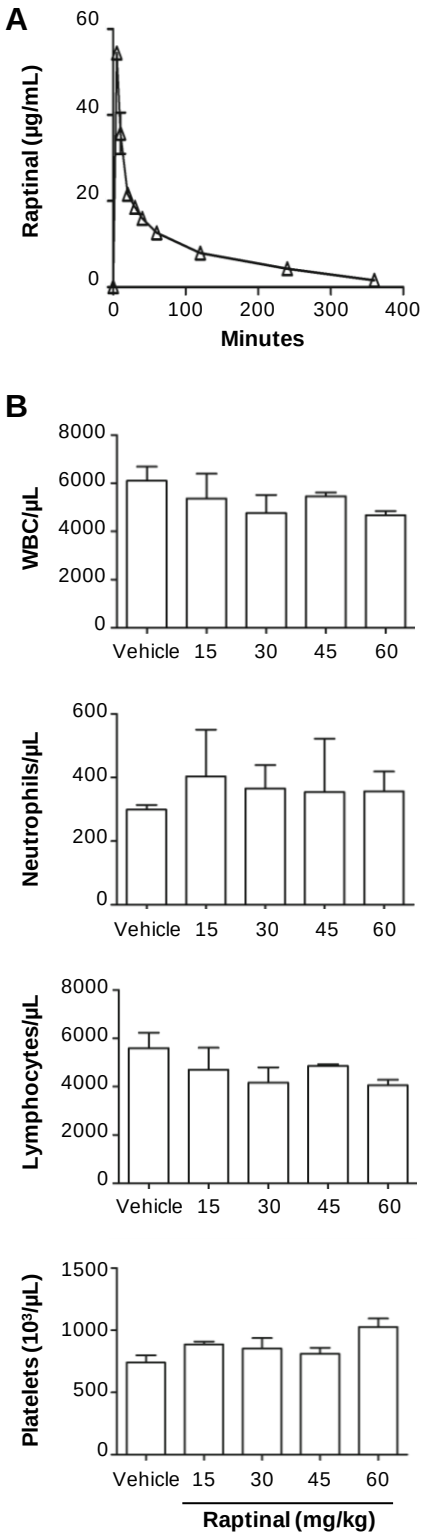


Figure S3. Bioavailability and Biological Activity of Raptinal *in vivo*, Related to **Figure 3.**

A) Pharmacokinetic analysis of **Raptinal** administered to mice reveals a peak plasma concentration and elimination half-life of 54.4 ± 0.9 µg/ml and 92.1 ± 5.8 minutes, respectively. Data represent the mean ± SD from n=3 mice/group. **B)** Single-dose, intravenous administration of **Raptinal** across a 4-fold dose range (15-60 mg/kg) does not induce any hematologic toxicity (evaluated 7 days following treatment with **Raptinal**). Data represent the mean ± SD from n=3 mice/group.

SUPPLEMENTAL TABLES

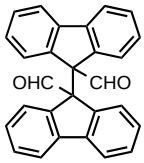
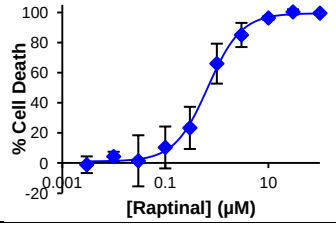
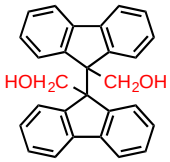
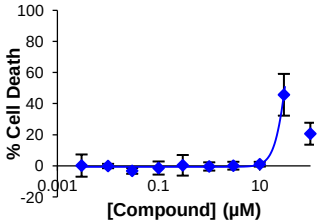
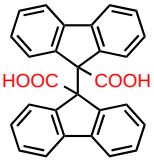
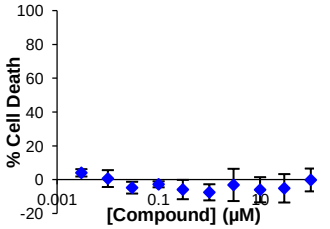
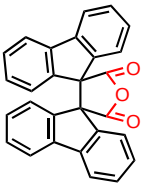
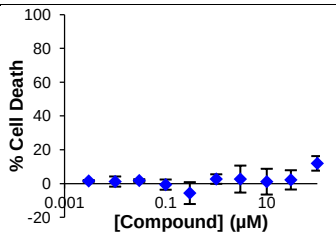
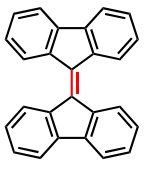
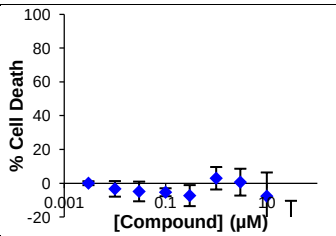
Table S1. Time to 50% Cell Death for Various Cytotoxins, Related to **Figure 1.**

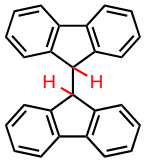
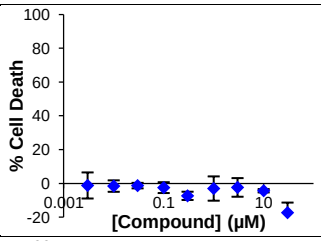
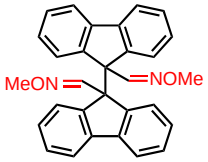
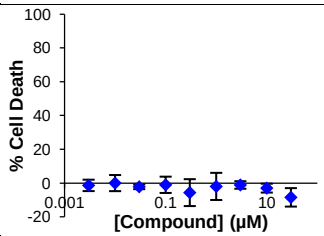
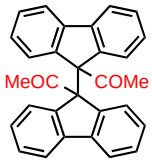
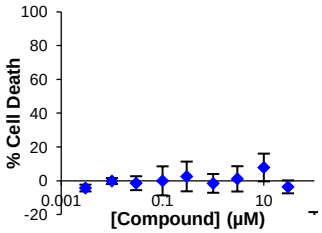
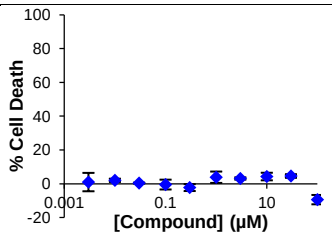
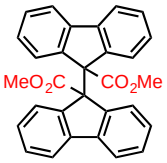
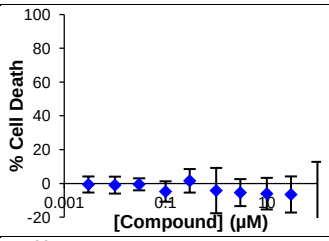
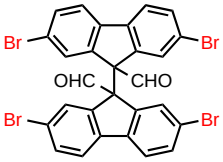
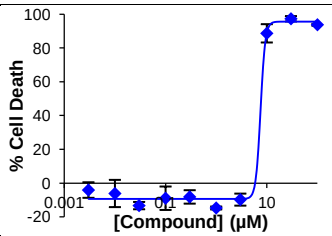
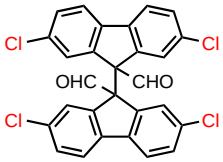
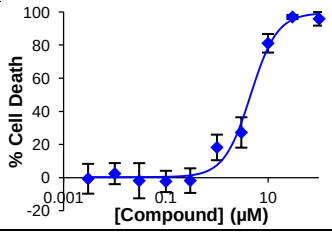
The time to 50% cell death in U-937 cells and mechanism of action for the agents tested based on the data in **Figure 1F**.

Molecule	Mechanism	Time to 50% death (hours)
Raptinal	Intrinsic pathway inducer	1.5
Staurosporine	Kinase inhibitor	5
Rapamycin	Kinase inhibitor	> 36
Colchicine	Tubulin inhibitor	15
Vincristine	Tubulin inhibitor	14
Paclitaxel	Tubulin stabilizer	18
Camptothecin	Topoisomerase inhibitor	5
Etoposide	Topoisomerase inhibitor	5
Doxorubicin	Topoisomerase inhibitor	6
Mitomycin C	DNA alkylator	20
Cisplatin	DNA cross-linker	> 36
MNNG	DNA alkylator	> 36
Thapsigargin	ER/proteasome inhibitor	14
Tunicamycin	ER/proteasome inhibitor	24
Geldanamycin	ER/proteasome inhibitor	> 36
DNQ	ROS inducer	6
Elesclomol	ROS inducer	> 36
Rotenone	Mitochondrial Complex I inhibitor	20
Antimycin A	Mitochondrial Complex III inhibitor	> 36
Gossypol	Bcl-2 inhibitor	36
HA-141	Bcl-2 inhibitor	> 36
AA2	Apoptosome promoter	> 36
TPEN	Zinc chelator	12
PAC-1	Procaspase-3 activator/ Zinc chelator	> 36
1541	Procaspase-3 activator	2
SM-164	XIAP inhibitor	> 36

Table S2. Activity of Raptinal and derivatives Related to **Figure 1.**

The IC_{50} values for **Raptinal** and synthesized derivatives of **Raptinal** after 24 hour treatment of U-937 cells. Structures are shown under the Chemistry section of the Detailed Experimental Procedures. Data represent the mean ± SD from 3 independent experiments. N.D. indicates not determined.

Compound	24 h IC_{50} (μM)	24 h IC_{50} Curve	% Cell Death at 2 h (10 μM treatment)
 **Raptinal**	**0.7 ± 0.3**		**84.9 ± 17.7**
 S1	33 ± 7		N.D.
 S2	> 100		N.D.
 S3	> 100		N.D.
 S4	> 100		N.D.

 S5	> 100		N.D.
 S6	> 100		N.D.
 S7	> 100		N.D.
 S8	> 100		N.D.
 S9	> 100		N.D.
 S10	6.5 ± 1.6		6.3 ± 0.8
 S11	4.6 ± 0.9		7.6 ± 1.9

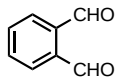
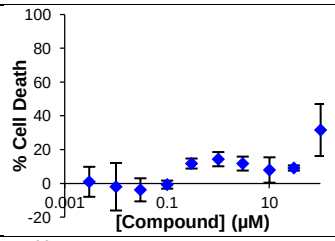
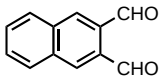
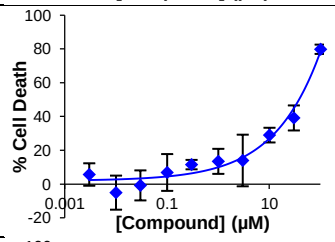
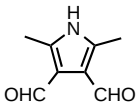
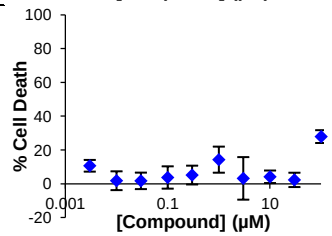
 S12	> 100		N.D.
 S13	38 ± 12		N.D.
 S14	> 100		N.D.

Table S3. Sequences of siRNAs used in these studies. Related to **Figure 2.**

Table S4. Concentrations of cytoprotective agents and methods for determining working concentrations in cytoprotective assays. Related to **Figure 2.**

Inhibitor	Concentration	Method
Q-VD-OPh	50 μM	Optimized (Liu and Cohen, 2014)
DIDS	2 mM	Optimized (Himi et al., 2002)
Oligomycin A	10 μM	Optimized (Cai et al., 2012; Goldstein et al., 2000)
FCCP	10 μM	Based on literature (Kim et al., 2009)
Rotenone	100 μM	Optimized
TTFA	1 mM	Based on literature (Lemarie et al., 2011; Suzuki et al., 1999)
Atpenin A5	1 μM	Based on literature (Quinlan et al., 2012)
Antimycin A	100 μM	Optimized
Sodium Azide	1 mM	Optimized (Duewelhenke et al., 2007; Goldstein et al., 2000)
Potassium Cyanide	1 mM	Optimized (Ricci et al., 2003)
Cyclosporine A	10 μM	Based on literature (Eskes et al., 1998)
Sodium Fluoride	1 mM	Based on literature (Nishikimi et al., 2000)
Sodium Pyruvate	5 mM	Based on literature (Yoo et al., 2004)
Tempol	10 mM	Based on literature (Dohare et al., 2014)
MnTBAP	20 μM	Based on literature (Madesh et al., 2009)
Dicoumerol	50 μM	Based on literature (Blanco et al., 2010)
Tiron	10 mM	Based on literature (White et al., 2005)
DPI	10 μM	Based on literature (Manosalva et al., 2015)
Allopurinol	1 mM	Based on literature (Kacimi et al., 2011)
N-Acetyl-L-Cysteine	10 mM	Based on literature (Spagnuolo et al., 2006)
Ru360	10 μM	Based on literature (Perveen et al., 2014)
Ferrostatin-1	2 μM	Based on literature (Dixon et al., 2012)
Calpain/Cathepsin Inhibitor I	50 μM	Used at high conc. (K_i-value 0.12-0.23 μM)
Granzyme B Inhibitor I	50 μM	Used at high conc. (K_i-value 300 nM)
Necrostatin-1	30 μM	Based on literature (Degterev et al., 2005)
Actinomycin D	2 μM	Based on literature (Jao and Salic, 2008)
Cycloheximide	10 μM	Based on literature (Selvarajah et al., 2013)

DETAILED EXPERIMENTAL PROCEDURES

Biology

Reagents for Biological Experiments

All immunoblotting antibodies (for human PARP-1, caspase-3, caspase-8, caspase-9, caspase-1, cytochrome *c*, phosphor-MLKL, actin and cox IV) were purchased from Cell Signaling Technologies. Full-length recombinant Bid was purchased from R&D Systems. Human TNFα was purchased from PeproTech. Annexin V-FITC conjugate was obtained from Southern Biotech. Propidium iodide, sulforhodamine B, staurosporine, rapamycin, colchicine, vincristine, paclitaxel, camptothecin, etoposide, doxorubicin, mitomycin C, cisplatin, MNNG, thapsigargin, tunicamycin, rotenone, antimycin A, gossypol, oligomycin A, potassium cyanide, sodium azide, TTFA, HA14-1, TPEN, FCCP, CCCP, DIDS, cyclosporine A, sodium fluoride, tempol, dicoumarol, tiron, diphenyleneiodonium chloride (DPI), allopurinol, N-acetyl-L-cysteine (NAC), calpain inhibitor I, actinomycin D, cycloheximide, dihydroethidium, acridine orange, rhodamine 123 and cobalt (II) chloride were obtained from Sigma Aldrich. Ru360, Q-VD-OPh and granzyme B inhibitor I were obtained from Calbiochem. MnTBAP hydrochloride were purchased from Santa Cruz Biotechnology. Apoptosis activator 2 (AA2) was purchased from Tocris Biosciences. Atpenin A5 was purchased from Cayman Chemical. Calcein-AM was purchased from Invitrogen. MTS was obtained from Promega Inc. BALB/c and C57Bl/6 female mice (6-8 weeks old) were purchased from Charles River.

Cell culture conditions

All cells were grown in RPMI 1640, DMEM or EMEM media supplemented with 10% FBS, 1% penicillin-streptomycin and incubated at 37 ºC in 5% CO_2, 95% humidity incubator.

High-throughput cell death screen

HL-60 cells were plated into 384 well plates (50 μL of 2 x 10^6 cells/mL per well) in RPMI cell culture media. Library compounds were transferred using a 384-pin transfer apparatus (V & P Scientific, San Diego) which transferred 0.1 μL from 10 mM DMSO stock solutions to achieve a final concentration of 20 μM. Controls containing DMSO only and 10 μM etoposide were used to serve as live and dead controls respectively. The cells were incubated for 24 hours. A solution containing the soluble tetrazolium salt ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and the electron coupling reagent, phenazine methosulfate (PMS) was prepared according to the manufacturer's instructions (Promega, Madison WI) and 10 μL added to each well. The plates were incubated at 37 ºC for approximately an hour after which the formation of the reduced formazan product by viable cells was assessed at 490 nm using a SpectraMax Plus 384 well plate reader (Molecular Devices, Sunnyvale CA). The top 220 toxic compounds were selected and re-tested at 1 μM. **Raptinal** induced quantitative cell death under these conditions.

MTS assay for suspension cells

Serial dilutions of compound in 100% DMSO were added in triplicate (2 μL to each well) to empty wells of a 96-well plate. Suspension cells (HL-60, U-937, SKW 6.4, Jurkat WT, Jurkat CASP8$^{-/-}$, Jurkat FADD$^{-/-}$, Jurkat Bcl-2) cells in RPMI 1640 media were added to 96-well plates (198 μL containing 4 x 10^4 cells) and the cells incubated for 24 hours. A solution containing the soluble tetrazolium salt ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and the electron coupling reagent, phenazine methosulfate (PMS) was prepared according to the manufacturer's instructions (Promega) and 20 μL added to each well. The plates were incubated at 37 ºC for 15-45 min and the absorbance at 490 nm was measured using a

SpectraMax Plus 384 well plate reader (Molecular Devices, Sunnyvale CA). The mean IC_{50} values and standard deviations were determined from three independent experiments.

Sulforhodamine B assay for adherent cells

Serial dilutions of compound in 100% DMSO were added in triplicate (2 μL to each well) to empty wells of a 96-well plate. Adherent cells (3T3, Hs888Lu, MCF-7, HeLa, WT-MEF, 4T1, B16-F10, SK-MEL-5, MIA PaCa2, BT-549, MDA-MB-436, 143B, HOS, H460, H1993) cells were added to each well (5 x 10^3 cells in 198 μL of RPMI 1640, DMEM or EMEM media). The plates were incubated for 24 hours. The media was removed from the plate and ice-cold trichloroacetic acid (100 μL of 10% w/v trichloroacetic acid) was added to the plates which were then incubated at 4 ºC overnight to fix the cells. The trichloroacetic acid was removed and the wells washed with 200 μL of de-ionized water 5 times. Sulforhodamine B (200 μL of 0.04 % sodium salt dissolved in 1% acetic acid) was added to each well and the plates incubated at room temperature for 30 min. Excess sulforhodamine B was removed by washing the plates 5 times with 1% acetic acid. The bound dye was released by the addition of unbuffered Tris-base (200 μL of 10 mM solution) and after a 30 min incubation at room temperature the absorbance at 510 nm was measured (Molecular Devices SpectraMax Plus 384 plate reader). The mean IC_{50} values and standard deviations were determined from three independent experiments.

Immunoblotting

U-937 cells (3 x 10^6 cells in 2 mL RPMI) or SKW 6.4 cells (5 x 10^6 cells in 2 mL RPMI) were treated with compound (1% final DMSO v/v) for the appropriate period of time. After centrifugation and washing with PBS, cells were lysed with RIPA lysis buffer containing protease cocktail inhibitor III (Calbiochem) and cell debris removed by centrifugation (16000x*g* 5 min). The lysate concentrations

were normalized after determination of protein concentration by the Bradford assay and whole cell lysate (40-60 μg) was resolved by 4-20% gradient SDS-PAGE gel electrophoresis at 120 V for 70 min after which proteins were transferred onto PVDF membranes (60 V for 2 hours) and blocked in 5% BSA or fat-free milk in TBST (as per primary antibody manufacturer's instructions) overnight at 4 ºC. The membranes were blotted for molecules of interest with primary antibody (1:1000 in 5% BSA or milk in TBST) overnight at 4 ºC. The bound primary antibodies were detected using appropriate secondary HRP conjugated antibodies (1:5000 in TBST) for 1 hour at room temperature and visualized by ECL autoradiography or with an Image Quant LAS 4010. The membranes were stripped in acidic methanol, blocked and re-probed as necessary.

Induction of Necroptosis and Observation of Phospho-MLKL by Immunoblot

U-937 cells (1 x 10^6 cells in 1 mL RPMI) were pre-treated with Q-VD-OPh (50 μM) for 30 minutes. Cells were then treated with TNFα (10 ng/ml) in the presence of cycloheximide (20 μg/ml) for (1% final DMSO v/v) for the appropriate period of time. After centrifugation and washing with PBS, cells were lysed with RIPA lysis buffer containing protease cocktail inhibitor III (Calbiochem) and Phosphatase Inhibitor IV (BioVision). Samples were further processed for immunblot analysis as described above.

Cell Morphology by light microscopy

Phase contrast images of U-937 cells treated with 10 μM **Raptinal** were taken with an Olympus DP-21 microscope digital camera. The cells were kept warm between images by returning the plate to the 37°C incubator.

Scanning electron microscopy

U-937 cells (4 x 10^6 cells in 8 mL 0.45 micron-filtered cell culture media) were treated with 1% DMSO) or 10 μM **Raptinal** in 10 cm culture dishes for 60 minutes . The cells were centrifuged (250x*g*, 5 min) and the media aspirated. Ice-cold Karnovsky's fixative (1 mL of 0.45 micron filtered) was added to the cell pellet which was resuspended by gently agitation. The cells were fixed overnight at 4 ºC and submitted for scanning electron microscopy (SEM) sample processing. The cells were stained with OsO_4, placed on filter paper and dehydrated. After mounting, the cells were coated with Pd/Au and scanning electron microscopy was performed.

Transmission electron microscopy

U-937 cells (4 mL of 5 x 10^5 cells/ml) were treated with DMSO or 10 μM of **Raptinal** for 5, 30 and 60 minutes. The cells were centrifuged 400xg for 5 min and the media aspirated. The cells were fixed by the addition of Karnovsky's fixing solution (0.5 mL) and placed in a 4 ºC fridge overnight. The next day, the cells were microwave fixed, stained with 2% OsO_4, washed with 3% KCN and enbloc stained with uranyl acetate. The samples were dehydrated with acetonitrile and ethanol, suspended in pure epoxy, and embedded overnight at 85 ºC. The embedded samples were cut into thick and then thin sections and these were mounted and viewed.

***In Vitro* Activation of Procaspase-3**

Procaspase-3 was expressed as described previously and purified with Qiagen nickel-NTA resin (Hsu et al., 2012). Increasing concentrations of **Raptinal** (0, 10, 25, 50 and 100 μM) and 25 μM 1541B were assessed for their capacity to enhance the activity of procaspase-3 over time. 250 nM procaspase-3 was treated with compounds in caspase activity buffer (50 mM HEPES, 50 mM KCl, 0.1 mM EDTA, 10

mM DTT, 0.01% Triton X-100, pH 7.4) and activity was assessed by cleavage of Ac-DEVD-AFC (50 μM) at designated time points.

Protection assays using small molecule inhibitors

For protection assays, U-937 cells (0.5 x 10^6 cells/mL) were pretreated with the prospective protective agents for 2 hours at the following concentrations: Sodium fluoride (1 mM), cyclosporine A (10 μM), oligomycin A (10 μM), FCCP (10 μM), cycloheximide (10 μM), TTFA (1 mM), atpenin A5 (1 μM), actinomycin D (2 μM), rotenone (200 μM), potassium cyanide (1 mM), sodium azide (1 mM) antimycin A (100 μM), sodium pyruvate (5 mM), tempol (10 mM), granzyme B inhibitor I (50 μM), calpain inhibitor I (50 μM), MnTBAP (20 μM), DPI (10 μM), allopurinol (1 mM), dicoumarol (50 μM), tiron (10 mM), Ru360 (10 μM), ferrostatin-1 (2 μM), necrostatin-1 (30 μM), NAC (10 mM), DIDS (2 mM), and Q-VD-OPh (50 μM). The cells were then co-treated with **Raptinal** at 10 μM for 2 hours prior to analysis by propidium iodide/FITC-annexin V staining and flow cytometry.

Cytochrome *c* release by immunoblot

U-937 cells (3 x 10^6 cells in 2 mL RPMI) or SKW 6.4 cells (5 x 10^6 cells in 2 mL RPMI) were treated with 10 μM **Raptinal** for 10, 20, 30, 45 or 60 minutes in a 12-well plate. Cells were centrifuged (1000x*g*. 2 min), washed with ice-cold PBS and resuspended in cold 200 μL digitonin permeabilization buffer (75 mM NaCl, 1 mM Sodium phosphate monobasic, 8 mM sodium phosphate dibasic, 250 mM sucrose, 190 μg/mL digitonin, protease cocktail inhibitor, pH 7.5) and placed on ice for 5 min. Cell permeability (> 95%) was confirmed by trypan blue. The permeabilized cells were centrifuged (14000x*g*, 5 min) and 150 μL of supernatant (cytosolic fraction) was saved. The pellet (mitochondrial fraction) was washed in 200 μL digitonin permeabilization buffer and lysed in 25 μL RIPA lysis buffer.

After normalizing samples for protein concentration, 40 µg cytosolic fraction and 50 µg mitochondrial fraction were resolved by electrophoresis on 4-20% SDS-Page gels and immunoblotted for caspase-9 and cytochrome *c* respectively. Cytosolic immunoblots were re-probed for actin while mitochondrial immunoblots were re-probed with cox IV to confirm equal loading. For protection assays against cytochrome *c* release, cells were pre-treated with protective agents for 2 hours and co-treated with 10 µM **Raptinal** for 2 hours. The protective agents were used at the same concentration as listed in the annexin V/propidium iodide assays.

Cytochrome *c* release by flow cytometry

Suspension cells (2.5 x 10^6 SKW 6.4 cells or 1.5 x 10^6 U-937 cells in 1 mL RPMI) were treated with 10 µM **Raptinal** in a 24 well plate for 10, 20, 30, 45 and 60 min. The cells were centrifuged (1000x*g*, 2 min), washed with cold PBS and resuspended in cold permeabilization buffer (PBS containing 50 µg/mL digitonin and 100 mM KCl) and placed on ice for 5 min after which > 95% of cells were permeabilized as determined by trypan blue. The cells were fixed by addition of paraformaldehyde (0.5 mL of 8% solution in PBS) by incubating at room temperature for 20 min. The fixed cells were washed three times with 1 mL PBS and blocked in 200 µL blocking buffer (3% BSA, 0.05% saponin in PBS) for 1 hour at room temperature. Anti-cytochrome *c* monoclonal antibody (Clone 6H2.B4, BD Pharmingen, San Diego, CA) was added (200 µL of 1:100 dilution in blocking buffer) to achieve a final antibody dilution of 1:200 and the cells incubated at 4 ºC overnight. The cells were pelleted and washed with PBS (1 mL) and secondary antibody added (200 µL of 1:200 dilution of AlexaFluor 488 anti-mouse antibody in blocking buffer) and the cells incubated at room temperature in the dark. The cells were pelleted and washed with PBS (3 mL) and resuspended in PBS (1 mL) and analyzed by flow

cytometry. During acquisition whole intact cells were gated for analysis based on side scatter and forward scatter characteristics.

In vitro cytochrome *c* release from isolated mitochondria

U-937 cells (120 x 10^6) were washed twice in PBS and resuspended in 800 µL ice-cold lysis buffer (50 mM HEPES, 5 mM DTT, 5 mM PMSF, 25 mM $MgCl_2$, protease cocktail inhibitor, pH 7.5) and the cells were passed through a 26 gauge needle 26 times using a 1mL syringe until >95% cells were lysed as determined by trypan blue. Isolation buffer (0.25 M sucrose in lysis buffer pH 7.5) was added to the lysed cells (2.4 mL) and mixed by inversion. Debris was removed by centrifugation (500x*g*. 10 min at 4 ºC) and the mitochondria in the supernatant were pelleted by centrifugation (10000x*g*, 5 min at 4 ºC). The mitochondrial pellet was resuspended in washing buffer (2 mL of 50 mM HEPES, 250 mM KCl, 25 mM EDTA, pH 7.5) and incubated on ice for 2 min and centrifuged (12500x*g*, 5 min). The mitochondrial pellet was resuspended in either non-respiration (300 µL of 50 mM HEPES, 5 mM DTT, 5 mM PMSF, 25 mM $MgCl_2$, protease inhibitor, 250 mM KCl, 25 mM EDTA, pH 7.5) or respiration buffer (300 µL of 50 mM HEPES, 1250 mM sucrose, 5 mM ATP, 0.4 mM ADP, 25 mM sodium succinate, 10 mM potassium phosphate dibasic, pH 7.5). The mitochondria were aliquoted into 18 µL aliquots and **Raptinal** or recombinant Bid protein added to achieve a final volume of 20 µL. The samples were incubated in a 37 ºC water bath for 30 min and then the mitochondria were pelleted (16500x*g*, 5 min at 4 ºC). The supernatant was saved (15 µL) and the pellet washed with washing buffer (100 µL), and finally lysed in RIPA lysis buffer (20 µL) and debris removed by centrifugation. Supernatant and pellet lysates were loaded and run on SDS-PAGE gels and processed for immunoblotting of cytochrome *c* and cox IV (loading control).

Measurement of cellular ROS production

U-937 cells (1 mL of 5 x 10^5 cells/mL) in Hank's buffered salt solution (HBSS) containing 5 mM glucose were treated with DMSO, 2 or 10 μM **Raptinal**, 10 μM thapsigargin, 100 μM etoposide (1% DMSO final) and incubated in the presence of 5 μM dihydroethidium a 37 ºC, 5% CO_2 incubator for 20 minutes. Dihydroethidium is oxidized in the presence of superoxide anion radicals to ethidium, which intercalates DNA and experiences a 24 fold increase in fluorescence. The levels of ethidium were assessed by flow cytometry. Live cells were analyzed based on gating on the forward and side scatter properties and the geometric mean of ethidium staining was determined using the software FCS Express.

Mitochondrial permeability transition pore (MPTP) calcein-cobalt assay

U-937 cells (1 x 10^6 cells/mL in HBSS) were loaded with calcein-AM (20 nM final concentration) for 45 minutes at 37 ºC. The cells were washed with HBSS and resuspended in RPMI and plated into a 24-well plate (1 x 10^6 cells in 1 mL). The cells were treated with **Raptinal** (10 μM) for 15, 30, 45, 60 and 90 minutes. Cobalt(II) chloride was added (10 μL of 80 mM stock in HBSS) to the cells 10 minutes prior to the end of the time points. The cells were pelleted and resuspended in HBSS (0.5 mL) and calcein fluorescence was measured by flow cytometry. As a positive control for MPTP, ionomycin and calcium were added to calcein-labeled cells in HBSS 10 minutes prior to analysis by flow cytometry.

Transfection and screening of siRNA constructs

All RNA interference reagents were purchased from Life Technologies (Carlsbad, CA) and a complete list of siRNAs used can be found in supplemental excel file named Table S3. The optimal transfection conditions (i.e., siRNA concentration, time, cell number, etc.) were determined using the

KDalert GAPDH Assay Kit according to the manufacturer's instructions. Human Silencer Select siRNA was purchased for gene targets of interest and diluted in DEPC water. The siRNA (5 nM final conc.) was complexed in OptiMEM media with RNAiMAX lipofectamine (0.3 µL lipofectamine/pmol siRNA) for 5 min in the well prior to addition of cells. MIA PaCa-2 cells were trypsinized, washed in PBS, resuspended at 50 cells/µL in OptiMEM containing 4% FBS, added to the well (100 µL/96-well or 2.5 mL/6-well), and grown for 4 days. Silencer® Negative Control siRNA was used in control wells. After silencing, fresh OptiMEM with 4% FBS was supplied to the cells (half immediately and half at time of compound addition).

Pharmacokinetics and toxicity profiling in mice

All animal studies were performed with prior approval by the University of Illinois at Urbana-Champaign IACUC committee. Eight week old female C57BL/6 mice were used for all pharmacokinetic and toxicity experiments. For pharmacokinetic profiling, **Raptinal** was administered at 37.5 mg/kg intravenously and mice were sacrificed in cohorts of 3 at 0, 5, 10, 20, 30, 40, 60, 120, 240, and 360 minutes post-injection, and plasma concentrations of **Raptinal** were determined by LCMS (n=3 mice/group). For toxicity assessment, **Raptinal** was administered intravenously as a single-dose injection across a dose range of 15-60 mg/kg, and mice were sacrificed 7 days post-injection for evidence of hematologic toxicity (n=3 mice/group).

Chemistry

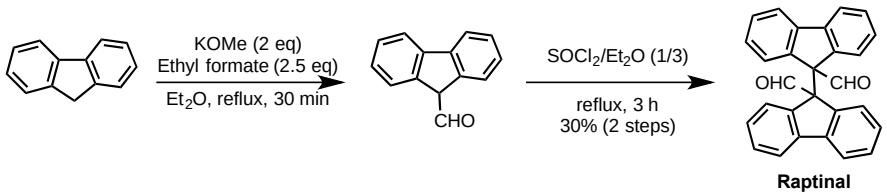
General

Compounds **S4**, **S5**, **S12**, **S13** and **S15** are commercially available, were purchased and were used as received. All reactions were run in flame or oven dried glassware under an atmosphere of dry nitrogen unless otherwise noted. Acetonitrile, tetrahydrofuran, methanol and methylene chloride used in reactions were obtained from a solvent dispensing system. Diethyl ether was distilled from sodium metal. 4 Å molecular sieves were dried at 200 °C on high vacuum overnight. Pyridine was distilled from CaH_2 and stored on 4 Å molecular sieves. All other reagents were of standard commercial purity and were used as received.

Analytical thin-layer chromatography was performed on EMD Merck silica gel plates with F254 indicator. Plates were visualized with UV light (254 nm) or staining with p-anisaldehyde. Silica gel for column chromatography was purchased from Sorbent Technologies (40-75 μm particle size).

Unless otherwise indicated, ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were recorded at 500, 125, 470 and 203 MHz, respectively. ^{1}H and ^{13}C NMR spectra were referenced to tetramethylsilane or the residual solvent peak. ^{19}F NMR spectra were referenced using C_6F_6 as an internal standard (-164.9 ppm). Chemical shifts are reported in ppm and multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), h (hextet), hep (heptet), m (multiplet), and b (broad). Mass spectrometry analysis was performed by the University of Illinois Mass Spectrometry Center.

Procedures for Chemical Synthesis

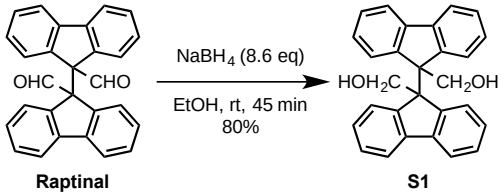


Raptinal: A modified version of the procedure of Curtin and co-workers was followed (Curtin et al., 1965). To a solution of fluorene (4.4 g, 26.4 mmol, 1 eq) and potassium methoxide (3.7 g, 52.8 mmol, 2 eq) in diethyl ether (50 mL) was added ethyl formate (5.3 mL, 66.0 mmol, 2.5 eq) and the reaction was heated at reflux for 30 min. The reaction was poured into 1M KOH, and the organic layer removed. The aqueous layer was acidified with concentrated HCl until the solution tested acidic to pH paper (at which time a white precipitate was formed). The solution was extracted with diethyl ether, dried through Na_2SO_4, and concentrated. The crude material was dissolved in diethyl ether (25 mL), thionyl chloride (8 mL) was added and the solution was heated to reflux for 3 h. The reaction was quenched by careful addition to ice water. The solution was extracted three times with methylene chloride, washed with water and saturated aqueous NaCl, dried through Na_2SO_4, and concentrated. The crude material was recrystallized from acetic acid, the crystals were collected and washed with water until the washing solution tested neutral to pH paper, affording **Raptinal** as white crystals (1.52 g, 30%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.89 (s, 2H), 7.53 (dt, *J* = 7.6, 1.0 Hz, 4H), 7.33 (td, *J* = 7.5, 1.1 Hz, 4H), 7.16 – 7.06 (m, 4H), 6.97 (bd, *J* = 7.8 Hz, 4H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 197.6, 142.5, 139.6, 129.2, 127.2, 126.8, 119.9, 71.2.

HRMS (ESI): m/z 387.1381, [calculated for $C_{28}H_{19}O_2$ $(M+H)^+$: 387.1385]



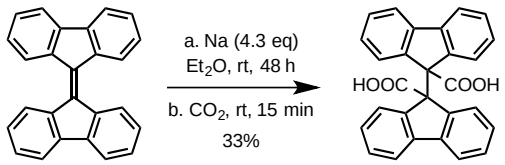
Compound S1: To a solution of **Raptinal** (32 mg, 0.0828 mmol, 1 eq) in EtOH (0.8 mL) was added $NaBH_4$ (27 mg, 0.714 mmol, 8.6 eq) and the reaction was stirred for 45 min. The reaction was quenched

by careful addition of 1M HCl. The reaction was extracted three times with $CHCl_3$, dried through Na_2SO_4, and concentrated. The compound was purified by silica column chromatography to give compound **S1** (26 mg, 80%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.5 Hz, 4H), 7.27 (t, *J* = 7.5 Hz, 4H), 7.07 (t, *J* = 7.5 Hz, 4H), 6.96 (bs, 4H), 4.10 (s, 4H), 3.45 (bs, 2H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 145.3, 141.3, 127.9, 126.6, 125.1, 119.7, 67.0, 60.4.

HRMS (ESI): m/z 413.1510, [calculated for $C_{28}H_{22}O_2Na$ $(M+Na)^+$: 413.1512]

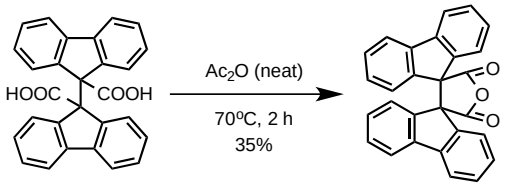


Compound S2: To a solution of compound **S4** (100 mg, 0.304 mmol, 1 eq) in diethyl ether (6 mL) was added sodium metal (30 mg, 1.30 mmol, 4.3 eq). The solution was stirred at room temperature for 48 h, at which point CO_2 (g) was bubbled through the solution. The crude reaction was poured into 2.5% NaOH, and washed once with diethyl ether. The aqueous layer was acidified until complete precipitation at which point it was extracted three times with methylene chloride and once with diethyl ether. The organic extracts were combined, dried through Na_2SO_4, concentrated and purified by silica column chromatography (with 1% formic acid) to afford compound S2 (42 mg, 33%).

^{1}H NMR (500 MHz, CD_3OD) δ 7.42 (d, *J* = 7.5 Hz, 4H), 7.23 (t, *J* = 7.5 Hz, 4H), 7.08 (bs, 4H), 7.00 (t, *J* = 7.5 Hz, 4H)

^{13}C NMR (125 MHz, CD_3OD) δ 174.7, 143.9, 143.1, 129.2, 128.6, 126.9, 119.8, 67.7.

HRMS (ESI): m/z 417.1125, [calculated for $C_{28}H_{17}O_4$ $(M-H)^-$: 417.1127]



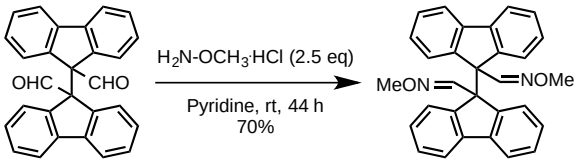
S2 **S3**

Compound S3: To a reaction vial was added compound **S2** (50 mg, 0.12 mmol, 1 eq) and acetic anhydride (2 mL). The reaction was heated to 70 °C for 2 h, after which the solvent was evaporated. The compound was purified by silica column chromatography to give 16.6 mg (35%) of compound **S3**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8 Hz, 4H), 7.39 (bt, *J* = 8 Hz, 4H), 7.20 (bs, 8H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 141.7, 138.4, 130.3, 127.8, 124.7, 120.9, 68.1

HRMS (ESI): m/z 423.1008, [calculated for $C_{28}H_{16}O_3Na$ $(M+Na)^+$: 423.0997]



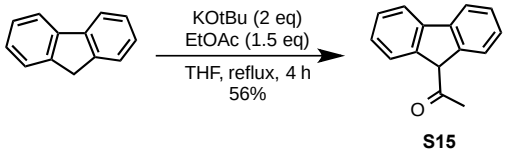
Raptinal **S6**

Compound S6: To a solution of **Raptinal** (32 mg, 0.083 mmol, 1 eq) in pyridine (0.8 mL) was added methoxyamine hydrochloride (17 mg, 0.207 mmol, 2.5 eq) and the reaction was stirred at room temperature for 44 h. The reaction was diluted with chloroform and washed twice with 1M HCl. The organic layer was dried through Na_2SO_4, concentrated, and purified by silica column chromatography to afford compound **S6** (26 mg, 70%) as a white foam.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (s, 2H), 7.45 (d, *J* = 7.6 Hz, 4H), 7.25 (t, *J* = 7.7 Hz, 4H), 7.06 (t, *J* = 7.0 Hz, 4H), 6.83 (s, 4H), 3.88 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 150.1, 143.0, 141.2, 128.2, 126.3, 126.3, 119.4, 62.1, 60.8.

HRMS (ESI): 445.1912 [calculated for $C_{30}H_{25}N_2O_2$ $(M+H)^+$ 445.1916]



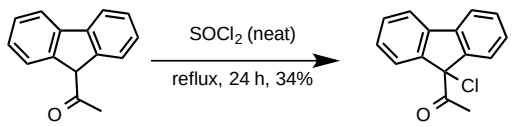
S15

Compound S15: The procedure of Borowiecki and co-workers was followed and the product matches the known compound (Borowiecki et al., 2013). To a 50 mL round-bottom flask was added fluorene (1.0 g, 6.02 mmol), and potassium t-butoxide (1.01 g, 9.03 mmol, 1.5 eq). The solids were dissolved in THF (20 mL) and ethyl acetate (0.89 mL, 9.03 mmol, 1.5 eq) was added dropwise to the solution. The reaction was heated at reflux under nitrogen for 4 h. The reaction mixture was poured into a saturated solution of NH_4Cl and which was extracted three times with diethyl ether. The combined ether extracts were washed with a saturated NaCl solution, dried over Na_2SO_4, and concentrated *in vacuo*. The compound was purified by silicia column chromatography to give compound **S15** as a yellow solid (699 mg, 56%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, J=8 Hz, 2H), 7.51 (d, J=7.5 Hz, 2H), 7.46 (t J=7.5 Hz, 2H), 7.35 (t, J=7.5 Hz, 2H), 4.80 (s, 1H), 1.62 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 207.1, 142.6, 141.6, 128.9, 128.2, 125.6, 120.9, 64.0, 25.6

HRMS (ESI): 209.0970 [calculated mass for $C_{15}H_{13}O$ $(M+H)^+$ 209.0966]



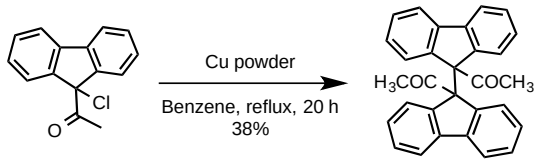
S15 **S16**

Compound S16: A modified version of the procedure of Greenhow and co-workers was followed (Greenhow et al., 1954). To a 10 mL round bottom flask containing compound **S15** (1.0 g, 4.8 mmol) was added thionyl chloride (4 mL). The reaction was heated at reflux for 24 h. Following removal of thionyl chloride, the product was purified by silica column chromatography (10:1 hexane/ethyl acetate) and recrystallized from ethanol, affording compound **S16** (395 mg, 34%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J=7.2 Hz, 2H), 7.51 (d, J=7.2 Hz, 2H), 7.49 (td, J=7.6 Hz, 0.8 Hz, 2H), 7.39 (td, J=7.6 Hz, 1.2 Hz, 2H), 1.770 (s, 3H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 198.8, 144.2, 141.0, 130.8, 129.4, 125.6, 121.3, 110.0, 24.9

HRMS (ESI): 265.0399 [calculated mass for $C_{15}H_{11}OClNa$ $(M+Na)^+$ 265.0396]



S16 **S7**

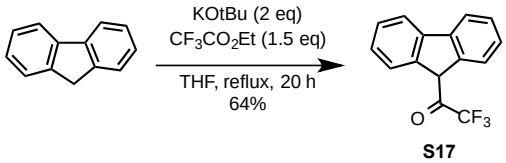
Compound S7: A modified version of the procedure of Greenhow and co-workers was followed (Greenhow et al., 1954). To a 25 mL round bottom flask containing **S16** (169 mg, 0.69 mmol, 1 eq) was added 274 mg of copper powder and 15 mL of benzene. The reaction was heated at reflux for 20 h, the copper was filtered and the solvent was evaporated. The product was purified by silicia column chromatography (10:1 hexane/ethyl acetate) to give compound **S7** (55.3 mg, 38%). Note: NMR analysis

of the product at room temperature resulted in very poor NMR signal, presumably due to limited rotation about the C9-C9' single bond. NMR spectroscopic analysis was therefore carried out at -40 °C.

^{1}H NMR (500 MHz, $CDCl_3$, -40°C) δ 8.24 (d, *J* = 7.5 Hz, 2H), 7.56 (d, *J* = 7.3 Hz, 2H), 7.53 – 7.41 (m, 4H), 7.29 (d, *J* = 7.7 Hz, 2H), 7.04 (t, *J* = 7.5 Hz, 2H), 6.63 (t, *J* = 7.5 Hz, 2H), 5.86 (d, *J* = 7.8 Hz, 2H), 1.71 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$, -40°C) δ 206.4, 144.4, 143.4, 141.3, 141.1, 129.7, 128.9, 128.1, 127.1, 126.1, 125.4, 119.8, 118.9, 72.9, 29.0.

HRMS (ESI): 415.1690 [calculated mass for $C_{30}H_{23}O_2$ $(M+H)^+$ 415.1698]



S17

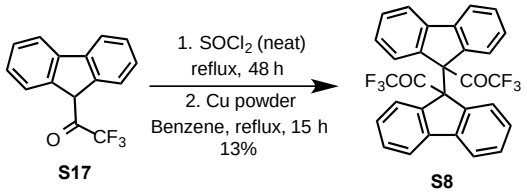
Compound S17: To an oven-dried 50 mL round-bottom flask with a stir bar was added fluorene (1.0 g, 6.02 mmol) and potassium t-butoxide (1.01 g, 9.03 mmol, 1.5 eq). The solids were dissolved in THF (25 mL) and ethyl trifluoroacetate (1.1 mL, 9.03 mmol, 1.5 eq) was added dropwise. The reaction was heated at reflux under nitrogen for 20 h. The reaction mixture was poured into a saturated NH_4Cl solution and extracted three times with diethyl ether. The combined ether extracts were washed with water, dried over Na_2SO_4, and the solvent was removed *in vacuo* to give a yellow oil which slowly solidified. The compound was purified by silicia column chromatography (6:1 hexanes/ethyl acetate) to give compound **S17** (1.002 g, 64%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, J=7.5 Hz, 2H), 7.50 (m, 4H), 7.37 (t, J=7 Hz, 2H), 5.25 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 190.4 (q, J=33.9 Hz), 142.8, 139.1, 129.6, 127.6, 125.7, 121.2, 116.2 (q, J=292.1 Hz), 57.3.

^{19}F NMR (470 MHz, $CDCl_3$, C_6F_6) δ: -78.30

HRMS (ESI): m/z 263.0691 [calculated mass for $C_{15}H_{10}OF_3$ $(M+H)^+$ 263.0684]



S17 **S8**

Compound S8: To a 50 mL round bottom flask containing compound **S17** (500 mg, 1.91 mmol) was added thionyl chloride (20 mL). The reaction was heated at reflux for 48 h. The thionyl chloride was removed. 1H NMR showed a compound consistent with the α-chloro trifluoromethyl ketone with partial conversion to the desired dimer. The crude material (426 mg) was added to a 10 mL round bottom flask containing copper powder (827 mg) and benzene (40 mL). The reaction was heated at reflux for 15 h after which the copper was filtered and the solvent evaporated. The product was purified by silica column chromatography (20:1 hexane/ethyl acetate) to give compound **S8** (63.4 mg, 13%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (bs, 2H), 7.55-7.49 (m, 8H), 7.10 (bs, 2H), 6.66 (bs, 2H), 5.76 (bs, 2H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 144.5, 142.2, 140.3, 136.6, 130.2, 129.7, 129.4, 127.7, 126.4, 125.6, 120.3, 119.4, 115.7 (q, J = 294.5 Hz), 70.0.

^{19}F NMR (470 MHz, $CDCl_3$, C_6F_6) δ -74.80

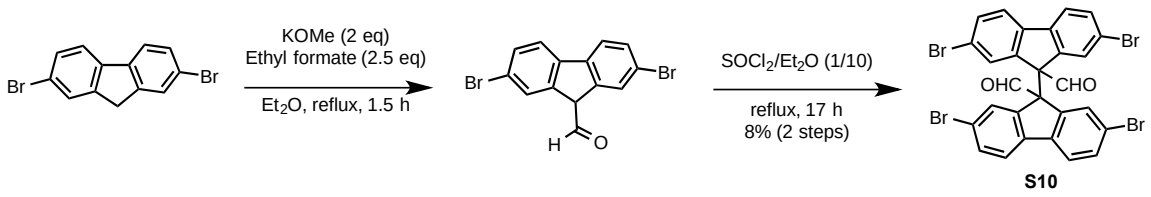
HRMS (ESI) 523.1140 [calculated for $C_{30}H_{17}F_6O_2$ $(M+H)^+$ 523.1133]

Compound S9: To a room temperature solution of compound **S2** (10 mg, 0.0239 mmol, 1 eq) in methanol (0.3 mL) was added TMS-diazomethane (0.036 mL of a 2M solution in diethyl ether, 0.717 mmol, 3 eq) at room temperature. The reaction was stirred at room temperature for 2 h. The reaction was concentrated and purified by silica column chromatography to afford compound **S9** (5.3 mg, 50%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 7.6, 1.2 Hz, 4H), 7.25 (dd, *J* = 7.8, 6.6 Hz, 4H), 7.08 – 7.01 (m, 4H), 6.97 (bd, *J* = 7.9 Hz, 4H), 3.77 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 141.7, 141.5, 128.6, 127.4, 126.7, 126.3, 119.0, 66.4, 52.9.

HRMS (ESI) 447.1603 [calculated for $C_{30}H_{23}O_4$ $(M+H)^+$ 447.1596]



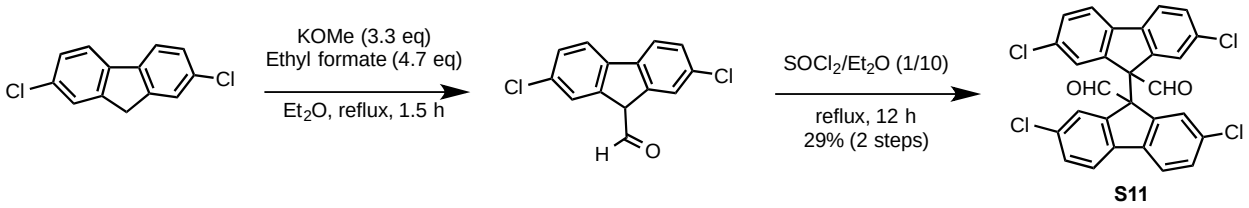
Compound S10: To a solution of 2,7-dibromofluorene (500 mg, 1.5 mmol, 1 eq) and potassium methoxide (210 mg, 3.0 mmol, 2 eq) in diethyl ether (10 mL) was added ethyl formate (0.3 mL, 3.75 mmol, 2.5 eq) and the reaction was heated to reflux for 1.5 h. The reaction was poured into water and washed with diethyl ether. The aqueous layer was acidified with concentrated HCl until precipitation was observed. The aqueous layer was extracted twice with diethyl ether, washed once with water, dried through Na_2SO_4 and concentrated. To the crude material was added diethyl ether (10 mL) and thionyl

chloride (1 mL) and the reaction was heated at reflux for 17 h. The product was precipitated and washed with diethyl ether to afford compound **S10** (42 mg, 8%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.80 (s, 2H), 7.49 (d, *J* = 6.6 Hz, 4H), 7.37 (d, *J* = 8.1 Hz, 4H), 7.03 (bs, 4H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 196.2, 141.0, 140.8, 133.4, 130.6, 121.9, 121.8, 71.5.

HRMS (ESI) 724.7595, [calculated for $C_{28}H_{14}O_2Br_4Na$ $(M+Na)^+$ 724.7581]



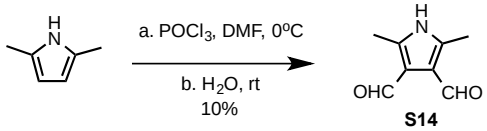
S11

Compound S11: To a solution of 2,7-dichlorofluorene (1.0 g, 4.25 mmol, 1 eq) and potassium methoxide (1.0 g, 14.2 mmol, 3.3 eq) in diethyl ether (30 mL) was added ethyl formate (1.6 mL, 19.8 mmol, 4.7 eq) and the reaction was heated to reflux for 2.5 h. The reaction was poured into water and extracted twice with hexane. The aqueous layer was acidified with concentrated HCl until precipitation was observed. The aqueous layer was extracted three times with ethyl acetate, the combined organic extracts were washed with saturated aqueous NaCl, dried through Na_2SO_4 and concentrated. The crude product was dissolved in diethyl ether (5 mL) and thionyl chloride (0.62 mL) was added. The reaction was heated at reflux for 12 h. The precipitate was collected and washed with diethyl ether to give compound **S11** (76 mg, 29%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.82 (s, 2H), 7.44 (d, *J* = 8.1 Hz, 4H), 7.36 (d, *J* = 8.2 Hz, 4H), 6.91 bs, 4H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 196.2, 140.9, 140.4, 133.9, 130.5, 127.7, 121.5, 71.3.

HRMS (ESI) 522.9849 [calculated for $C_{28}H_{15}O_2Cl_4$ $(M+H)^+$ 522.9826]



S14

Compound S14: A modified version of the procedure of Boudif and Momenteau was followed (Boudif and Momenteau, 1996). Briefly, $POCl_3$ (0.33 mL) was added dropwise to DMF (0.5 mL) at 0°C and stirred for 1 h. Next, 2,5-dimethyl-1H-pyrrole (100 mg, 1.05 mmol, 1 eq) was added at room temperature and heated to 50°C for 2 h. The reaction was cooled to room temperature and slowly quenched by addition to H_2O. The reaction was extracted three times with EtOAc and washed with saturated aqueous NaCl. The solution was dried through Na_2SO_4, concentrated and purified by silica column chromatography to afford compound **S14** (16.4 mg, 10%) as a white solid.

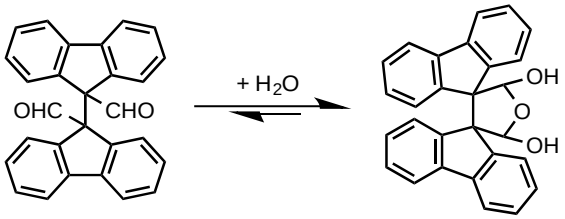
^{1}H NMR (500 MHz, d_6-Acetone) δ 10.26 (s, 2H), 2.50 (s, 6H).

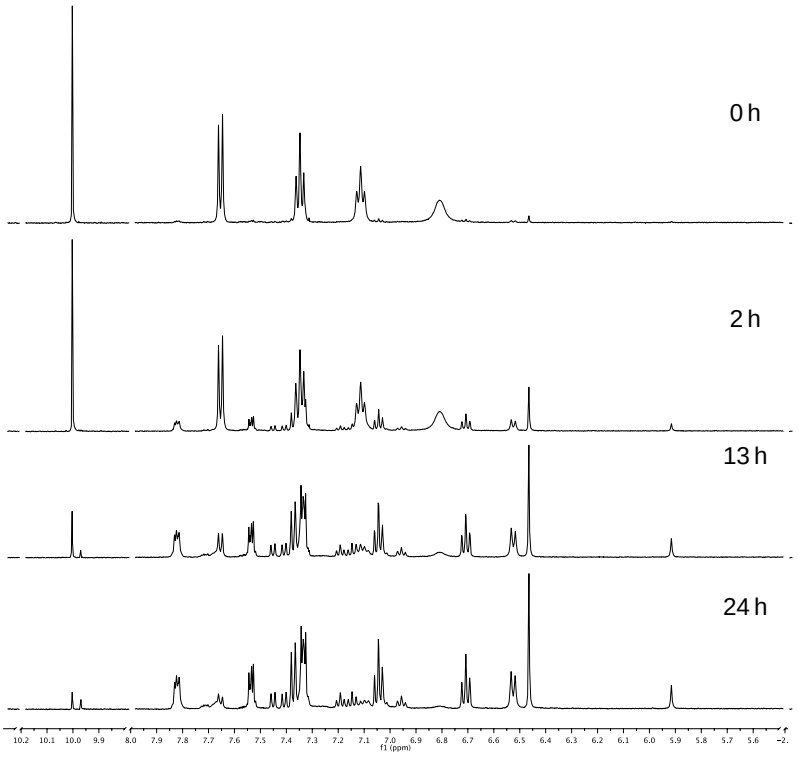
^{13}C NMR (125 MHz, d_6-Acetone) δ 186.5, 137.9, 120.4, 11.1.

HRMS (ESI) 152.0716 [calculated for $C_8H_{10}NO_2$ $(M+H)^+$ 152.0712]

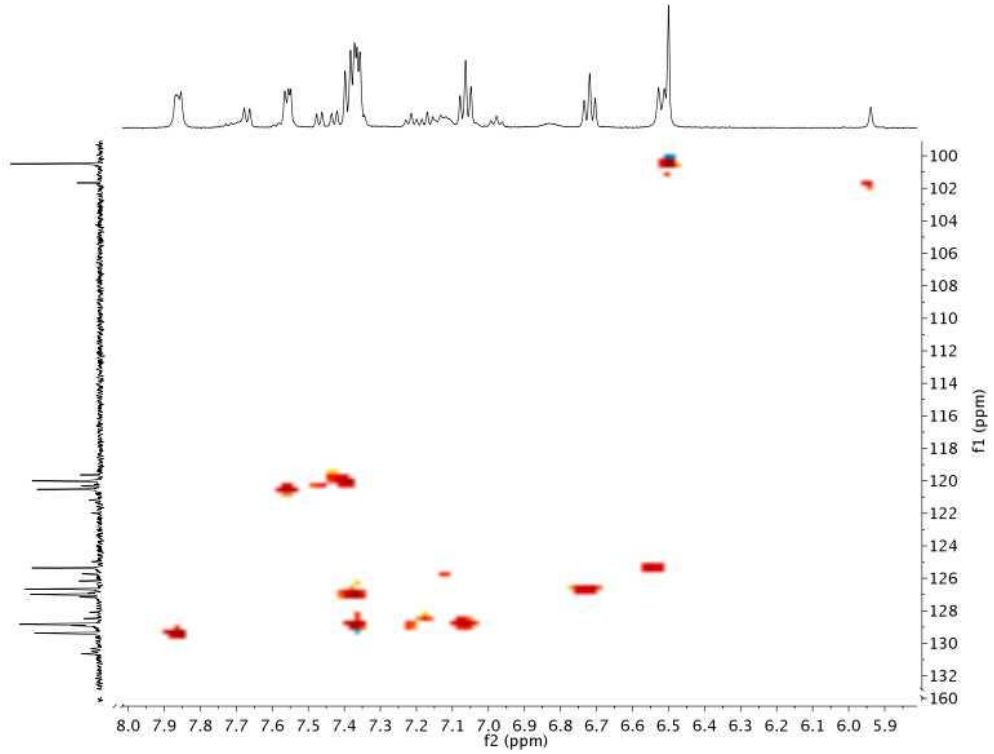
Characterization of Hydrate:

^{1}H NMR Time Course (20% D_2O in d_6-DMSO):

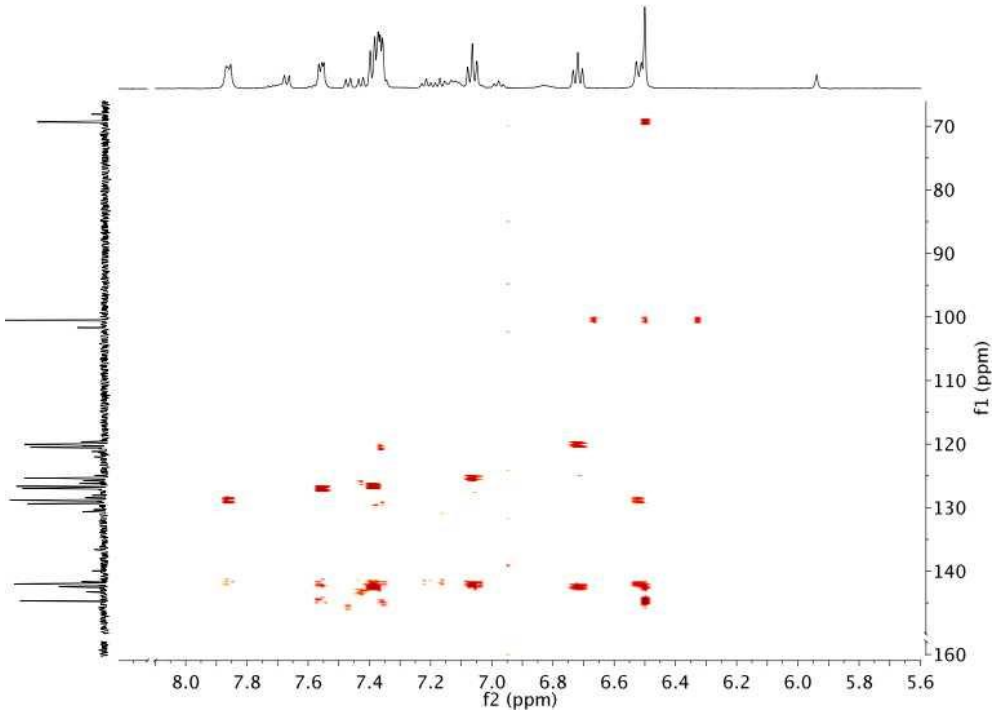




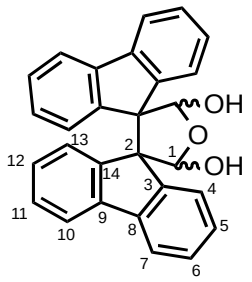
HMQC (500 MHz):



HMBC (500 MHz):



Signal	^{1}H	^{13}C
1	6.50	100.5
2	-	69.3
3	-	142.4
4	7.40	120.0
5	6.73	126.6
6	7.07	128.7
7	6.55	125.3
8	-	142.0
9	-	141.9
10	7.37	127.0
11	7.56	120.5
12	7.86	129.3
13	7.37	128.8
14	-	144.7



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