Supporting information

Inhibition of the Ras/Raf Interaction and Repression of Renal Cancer Xenografts *In Vivo* by an Enantiomeric Iridium(III) Metal-Based Compound

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General Materials and Methods. Reagents, unless specified, were purchased from Sigma Aldrich (St. Louis, MO) and used as received. Iridium chloride hydrate ($IrCl_3 \cdot xH_2O$) and rhodium chloride hydrate were purchased from Precious Metals Online (Australia). The synthesis and stability experiments of compounds were described in *SI Materials and Methods*. TurboFect Transfection Reagent was purchased from Thermo Scientific (Hudson, NH, USA). Dual-luciferase reporter assay system and pRL-TK vector were obtained from Promega Corporation (Promega, Madison, WI, USA). pLuc-AP1 was purchased from SABiosciences (SABiosciences TM , MD, USA). Anti-c-myc antibody was purchased from Beyotime (Beyotime, Shanghai, China). ANTI-FLAG® M2 Magnetic Beads, Monoclonal ANTI-FLAG® M2 antibody and MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture. The human renal cell carcinoma cell line A498 was purchased from Jennio Biotech Co. Ltd (GuangZhou, China). The human embryonic kidney cell line HEK293T was acquired Prof. Simon Lee (Institute of Chinese Medical Sciences, University of Macau). A498 and HEK293T cells were cultured and maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin-glutamine. A498 and HEK293T cells were seeded in 6-well plates at a density of 1×10^6 mL^{-1} in complete medium for the further experiments.

Plasmid construction. *E. coli* strains Top10 were used for cloning expression. To construct plasmids encoding flag-fusion H-Ras-Flag gene into HaloTag®Fusion Vector (Promega), c-Myc-Ras G12V from pcDNA-c-Myc-Ras G12V was used as a PCR template for amplification. To clone Raf-1-RBD-his and Raf-1-CRD-HA in NanoLuc®-MDM2 Fusion Vector, Flag-Raf-1 from pcDNA-Flag-Raf-1 was used as a PCR template. NdeI and XhoI were used as restriction enzymes used for cloning. The forward and reverse primer set for encoding with H-Ras-Flag protein are 5′-TAGGGCTAGCGCCACCATGACGGAATATAAGCTGGTGGTGGTGGGGCGCCGGCGGTGTGG-3′ and 5′-TTGGCTCGAGCTTATCGTCGTCATCCTTGTAATCGGAGAGCACACACTTGCAGC
TCATG -3′. The forward and reverse primer set for encoding with Raf-1-RBD-his protein are 5′-TAGGGCTAGCGCCACCATGAGCAACACTACTCGTGTTTTCTTGCCGAACAAGCAA AG-3′
and 5′-GCCGCTCGAGCCGTGGTGATGGTGATGATGCAGGAAATCTACTTGAAGTTCTTCTC
C-3′. The forward and reverse primer set for encoding with Raf-1-CRD-HA protein are 5′-TAGGGCTAGCGCCACCATGCACAACTTTGCTCGGAAGACGTTCCTGAAGCTTGCC-3′ and 5′-GCCGCTCGAGCCAGCGTAATCTGGAACATCGTATGGGTAACACATAGTAGGTACTTTG
G-3′.

Pull-down assay. A498 cells co-transfected with pcDNA-Flag-Raf-1 (Sino Biological Inc.) and pcDNA-Myc-Ras G12V (Sino Biological Inc.) were incubated with 5 μM of compounds and lysed after 6 h. For evaluation of constructed domain interaction, cells were co-transfected with H-Ras-Flag-HaloTag®Fusion plasmid and NanoLuc®-Raf-1-RBD-his Fusion plasmid, or H-Ras-Flag-HaloTag®Fusion plasmid and NanoLuc®-Raf-1-CRD-HA Fusion plasmid over 48 h. For study the interactions of H-Ras-RalGDS and H-Ras-PI3K, the cells were transfected with pcDNA-Myc-Ras G12V and pEGFP-RalGDS-Flag (GeneCopoeia, Inc.) or pcDNA-Myc-Ras G12V and pEGFP-PI3K-Flag (GeneCopoeia, Inc.) over 48 h. The transfected cells were exposed to compounds (5 μM) for further 6 h and cell lysates were collected. 120 μg of each protein sample were pulled down with anti-Flag magnetic beads based on the instructions provided by manufacturer. After twice equilibriums of the beads with TBS buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) and incubation with protein samples for 1 h at room temperature, the beads were collected and the supernatant was removed. Non-specific binding proteins were wasted through twice washing with TBS buffer. The Flag-tagged proteins then were eluted with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-

PAGE) sample buffer and separated by SDS-PAGE. The amount of protein was analyzed by probing with anti-FLAG, anti-c-Myc, anti-HA and anti-His antibodies and visualized by Image Lab.

Signaling pathway detection. A498 cells were treated with 5 μM of compounds in low FBS medium for 6 h and lysed. The cell lysates were collected and 30 μg of each protein sample were subjected to SDS-PAGE and electro-transferred to anImmun-Blot® PVDF membrane (Bio-Rad, CA, USA). The membrane was blotted with 5 % non-fat milk in TBST (TBS and 0.1% Tween-20) for 1 h at room temperature. An overnight incubation of corresponding primary antibodies (anti-phospho-MEK, anti-MEK, anti-GADPH, anti-phospho-ERK and anti-ERK from Cell Signal Technology, 1:1,000 dilution) followed. The membrane was then washed with TBST buffer for 30 min and incubated with secondary antibodies for 1 h. After 30 min washing, the result was observed by Image Lab.

AP-1 luciferase reporter assay. A498 cells were co-transfected with pLuc-AP1 and pRL-TK (a transfection efficiency control) in a 10:1 ratio using TurboFect Transfection Reagent in serum-free DMEM medium. The transfected cells were then seeded in a 24-well plate and incubated with different concentrations of compounds in low FBS medium for 6 h. Cell lysates were collected by 100 μL Passive Lysis Buffer (PLB). 50 μL of each cell lysate was added and mixed with 50 μL of luciferase reporter reagent (LAR) in a 96-well plate. The activities of firefly luciferase and *Renilla* luciferase were measured subsequently after the addition of 50 μL of Stop Glo® Reagent between two 10-second measurements. The results were observed with the Molecular Device SpectraMax® M5 Microplate Reader.

MTT assay. The inhibition of cell proliferation caused by compounds was detected by MTT assay. A density of 4 X 10^3 cells was seeded in each well. The cells were then incubated with different concentrations of compounds to a total volume of 100 μL in low FBS medium for 72 h. 100 μL of 0.5 mg/mL MTT in the low FBS medium was added to each well and cultured for 4 h at 37 °C. 100 μL of DMSO was then added to dissolve the purple formazan crystals. The absorbance values were measured at 572 nm in a Molecular Device SpectraMax®M5 Microplate Reader.

Mitochondria isolation. The isolation of mitochondria was followed by the manufacturer instruction of Mitochondria Isolation Kit for Cultured Cells (Thermo Scientific). Briefly, 2 X 10^7 A498 cells were collected after treatment with each of compounds (5 μM) for 6 h. The cell samples were incubated with Mitochondria Isolation Reagent A (800 μL) on ice for 2 min after a 5-sec vortex at medium speed. 10 μL of Mitochondria Isolation Reagent B was added to each sample, following with 5-sec vortex at maximum speed. Then, Mitochondria Isolation Reagent C (800 μL) was added to and mixes with the samples. After centrifugation for 10 min at 4 °C, the cytosol fraction in supernatant were collected and Mitochondria Isolation Reagent C (500 μL) was added to the cell pellet. The mitochondrial pellet of each samples was harvested after 5-min centrifugation at 12,000 X g.

Nuclear extraction. The nuclear extraction was followed by the previous reported[1].

Inductively coupled plasma mass spectrometry (ICP-MS) study. 500 μL of the cell samples from mitochondria isolation, nuclear extraction or whole cell lysate was then combined with 1,500 μL of a 68% HNO_3: H_2O_2 (v/v=4:1) solution, while the remainder of the lysate was quantified for protein by a bicinchoninic assay (BCA). The 2% HNO3 solution was analyzed for iridium uptake levels on a Thermo iCAP Qs ICP-MS. Iridium uptake levels in each sample were normalized to the concentration of corresponding protein and calculated the each value of ng [Iridium]/mg [protein].

Thermal shift assay. The thermal shift assay was performed according to the protocol of Applied Biosystems® Protein Thermal Shift™ kit (Thermo Scientific). 12.5 μL of 0.5 μg human recombinant H-Ras protein (Sino Biological Inc.) or human recombinant Raf-1-RBD (EMD Millipore) in ddH_2O with 1-10 μM of compounds was treated with 5 μL reaction buffer and 2.5 μL diluted Protein Thermal Shift™ Dye (8x). The

reaction of each sample in triplicate was run in the Applied Biosystems™ ViiA™ 7 system. The data were exported to Excel and fluorescence signal of each samples was plotted and a significant increase in slope corresponds to the melting temperature of the protein.

UV/Vis absorbance spectroscopy. 5 μM Δ-**1** was incubated with different concentrations of BSA in phosphate-buffered saline (PBS) buffer (4.3 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, 1.4 mM KH_2PO_4, pH 7.4) for 6 h at 25 °C. The UV/Vis absorption spectra of Δ-**1** was then recorded on a DR 6000™ UV VIS Spectrophotometer.

Animal materials. In this study, the use of animals complied with the Guiding Principles in the Care and Use of Animals of the American Physiology Society and was approved by the Animal Care and Use Committee at the National Kaohsiung Medical University. Female BALB/cAnN.Cg-*Foxn1*^{nu}/CrlNarl (4–5 weeks) were purchased from BioLASCO Experimental Animal Center (Taiwan Co., Ltd). The mice were housed in Plexiglas cages in a temperature-controlled room (22 ± 1°C), on a 12 h/12 h light/dark schedule, and with free access to food and water. After one week, the mice were randomly divided into control and treatment groups.

Western blotting. We homogenized the tumor tissues from control and treatment mice on ice for 10 s using a Polytron tissue homogenizer, and then lysed these tissues by PRO-PREP™ Protein extraction solution (iNtRON BIOTECHNOLOGY, USA). We determined the protein concentration in the supernatant by the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). We separated equal amounts of proteins with SDS-PAGE, and then electro-transferred these proteins into a nitrocellulose membrane (PALL Life Science, Ann Arbor, MI, USA). We blocked the membrane for 1 h using 5% non-fat milk in TBST buffer. We incubated the membranes with corresponding primary antibodies. After washing twice with TBST buffer, we incubated the membranes with secondary antibodies against the corresponding primary antibodies, respectively. We visualized the signals with the chemiluminescence detection kit (Amersham, Piscataway, NJ, USA). The primary antibodies we used were: MEK, phosphorylation of MEK, ERK, phosphorylation of ERK, caspase 3, caspase 6, caspase 9, β-actin (Novus Biologicals, Littleton, CO, USA).

Toxicity study *in vivo*. BALB/c mice were housed and administered a intraperitoneal (i.p.) injection of different concentrations of compounds (14-280 mg/kg) or vehicle (13 % DMSO) in 0.05 mL PBS until sacrifice at 21-day. The body weight and organ weight of each group were measured. The organ index was calculated by the endpoint of organ-to-body weight ratio.

Statistical analysis. All data were presented as the mean ± standard error. For statistical analysis, all data were analyzed with one-way analysis of variance (ANOVA) followed by the Duncan's method for multiple comparisons. A significant difference was defined as $p < 0.05$.

General experiment. Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (acetone-d_6: ^{1}H, δ2.05, ^{13}C, δ29.8; dimethyl sulfoxide (DMSO)-d_6: ^{1}H, δ2.50, ^{13}C, δ39.52; chloroform ($CDCl_3$)-*d*: ^{1}H, δ7.26, ^{13}C, δ77.16). Chemical shifts (δ are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^{1}H and ±0.05 for ^{13}C. Coupling constants are typically ±0.1 Hz for ^{1}H-^{1}H and ±0.5 Hz for ^{1}H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data were acquired and processed using standard Bruker software (Topspin).

Synthesis of $[M_2(C^N)_4Cl_2]$ dimer. Cyclometalated dichloro-bridged dimers of the general formula $[M_2(C^N)_4Cl_2]$ were synthesized according to a literature method[2]. In brief, $MCl_3{\cdot}xH_2O$ was heated to 130 °C with 2.1 equivalents of C^N ligand in 3:1 methoxymethanol and deionized water under a nitrogen atmosphere overnight. The reaction was cooled to room temperature, and the product was filtered and washed with three portions of deionized water and then three portions of ether to yield the corresponding dimer.

General synthesis of $[M(C^N)_2(N^N)]PF_6$ compounds. These compounds were synthesized using a modified literature method[2]. Briefly, a suspension of $[M_2(C^N)_4Cl_2]$ (0.1 mmol) and corresponding N^N (0.21 mmol) ligands in a mixture of dichloromethane:methanol (1:1, 6 mL) was refluxed overnight under a nitrogen atmosphere. The resulting solution was allowed to cool to room temperature, and was filtered to remove unreacted cyclometallated dimer. To the filtrate, an aqueous solution of ammonium hexafluorophosphate (excess) was added and the filtrate was reduced in volume by rotary evaporation until precipitation of the crude product occurred. The precipitate was then filtered and washed with several portions of water (2 × 30 mL) followed by diethyl ether (2 × 30 mL). The solid was dissolved into acetone, and then the product was precipitated by adding diethyl ether, and filtered to yield.

Compound **1**. Yield: 50%. ^{1}H NMR (400 MHz, acetonitrile-d_3) δ 8.39 (s, 2H), 8.03-8.01 (d, *J* = 8.0 Hz, 2H), 7.85-7.81 (m, 4H), 7.72-7.70 (d, *J* = 8.0 Hz, 2H), 7.56-7.55 (d, *J* = 4.0 Hz, 2H), 7.36-7.34 (d, *J* = 8.0 Hz, 2H), 7.02-6.99 (t, *J* = 8.0 Hz, 2H), 6.91-6.89 (d, *J* = 8.0 Hz, 2H), 6.12 (s, 2H), 2.84-2.80 (t, *J* = 8.0 Hz, 2H), 2.13 (s, 6H), 1.76-1.69 (m, 4H), 1.36-1.30 (m, 26H), 0.92-0.88 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, acetonitrile-d_3) δ 167.3, 155.9, 155.3, 150.7, 149.5, 148.5, 141.1, 140.1, 137.9, 127.8, 124.4, 124.2, 34.6, 31.3, 29.5, 28.8, 28.7, 28.6, 28.5, 22.1, 20.4, 13.1; MALDI-TOF-HRMS: Calcd. for $C_{52}H_{64}IrN_4[M–PF_6]^+$: 937.4758 Found: 937.4738. Anal.: ($C_{52}H_{64}IrN_4PF_6$) C, H, N: calcd. 57.71, 5.96, 5.18; found 57.85, 6.05, 5.30.

Compound **2**. Reported[3, 4].

Compound **3**. Reported[5, 6].

Compound **4**. Reported[7].

Compound **5**. Reported[8].

Compound **6**. Yield: 42%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.23 (d, *J* = 1.6 Hz, 2H), 8.11-8.07 (m, 6H), 8.00 (d, *J* = 6.0 Hz, 2H), 7.68-7.59 (m, 8H), 7.41 (d, *J* = 7.6 Hz, 2H) , 7.22-7.16 (m, 4H), 7.08-7.04 (m, 2H), 5.06-4.99 (m, 2H), 4.79-4.72 (m, 2H), 3.69-3.62 (m, 2H), 3.22-3.14 (m, 2H); ^{13}C NMR (100 MHz, acetone-d_6) 179.4, 156.9, 151.1, 150.9, 149.6, 135.4, 132.5, 132.4, 130.5, 130.2, 129.3, 127.7, 126.5, 125,2, 121.7, 121.6, 72.4, 52.1; MALDI-TOF-HRMS: Calcd. for $C_{40}H_{32}IrN_4O_2[M–PF_6]^+$: 793.2155. Found: 793.0709; Anal.: ($C_{40}H_{32}IrN_4O_2PF_6$+$2H_2O$) C, H, N: calcd. 45.48, 3.3, 5.51; found. 45.39, 3.54, 5.74.

Compound **7**. Reported[9].

Compound **8**. Reported[10].

Compound **9**. Yield: 42%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.42 (d, *J* = 8.8 Hz, 2H), 8.75 (d, *J* = 3,2 Hz, 2H), 8.71 (d, *J* = 8.4 Hz, 2H), 8.26 (d, *J* = 8.8 Hz, 2H), 7.81-7.77 (m, 2H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.45-7.41 (m, 2H), 7.04-7.00 (m, 2H), 6.84-6.80 (m, 2H), 6.71 (t, *J* = 2.4 Hz, 2H), 6.14 (d, *J* = 7.8 Hz, 2H), 4.2 (s, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ 161.3, 157.9, 151.0, 149.2, 146.0, 142.3, 140.8, 138.4, 135.4, 132.0, 130.7, 130.6, 130.3, 129.9, 129.6, 129.4, 128.9, 128.2, 124.9, 123.3, 123.0, 121.4; MALDI-TOF-HRMS: Calcd. for $C_{40}H_{30}IrN_6O_4[M–PF_6]^+$: 851.1958 Found: 851.1940. Anal.: ($C_{40}H_{30}IrN_6O_4\,PF_6$) C, H, N: calcd. 48.24, 3.04, 8.44; found 47.96, 2.96, 8.15.

Compound **10**. Yield: 48%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.69 (dd, J_1 = 1.6 Hz, J_2 = 8.4 Hz, 2H), 8.50 (dd, J_1 = 1.2 Hz J_2 = 5.2 Hz, 2H), 8.21-8.14 (m, 4H), 8.12 (s, 2H), 7.91-7.85 (m, 6H), 7.00-6.95 (m, 4H), 6.32 (d, *J* = 1.2 Hz, 2H), 2.65 (s, 6H), 2.43 (q, *J* = 7.6 Hz, 4H), 1.04 (t, *J* = 7.6 Hz, 6H); ^{13}C NMR (100 MHz, Acetone-d_6)

168.9, 153.0, 151.0, 150.5, 147.5, 145.1, 142.8, 139.9, 139.4, 135.9, 132.2, 131.9, 129.1, 129.0, 125.9, 123.8, 123.4, 120.4, 29.9, 21.6, 17.9; MALDI-TOF-HRMS: Calcd. for $C_{46}H_{38}IrN_6$ $[M–PF_6]^+$: 867.2787 Found: 867.6509; Anal.: ($C_{44}H_{48}IrN_4PF_6$+2H_2O) C, H, N: calcd.52.72, 4.04, 8.02; found. 52.75, 4.03, 8.03.

Compound **11**. Yield: 50%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.95 (d, *J* = 8.4 Hz, 2H), 8.87 (d, *J* = 8.0 Hz, 2H), 8.58(d, *J* = 8.0 Hz, 2H), 8.51(d, *J* = 4.4 Hz, 2H), 8.10 (d, *J* = 8.4 Hz, 2H), 8.01 (d, *J* = 8.0 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 2H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.56 (m , 4H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.15 (t, *J* = 7.6 Hz, 2H), 7.01 (t, *J* = 8.4 Hz, 2H), 6.26 (d, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, acetone-d_6) 164.3, 163.9, 159.4, 155.1, 150.8, 149.0, 142.1, 140.3, 138.3, 135.2, 131.6, 131.0, 130.4, 130.2, 129.6, 129.5, 129.0, 128.4, 124.9, 123.5, 122.8, 122.4; MALDI-TOF-HRMS: Calcd. for $C_{44}H_{28}N_4Rh[M–PF_6]^+$: 715.1369 Found: 715.1312. Anal.: ($C_{44}H_{28}F_6N_4PRh$) C, H, N: calcd. 61.41, 3.28, 6.51; found 61.13, 3.44, 6.54.

Compound **12**. Yield: 53%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.14 (d, *J* = 8.0 Hz, 1H), 8.93 (d, *J* = 8.0 Hz, 1H), 8.72 (d, *J* = 8.0 Hz, 1H), 8.65-8.64 (m, 2H), 8.37 (d, *J* = 8.0 Hz, 2H), 8.22 (q, *J* = 4.0 Hz, 1H), 8.13-8.05 (m, 3H), 7.75-7.73 (m, 2H), 7.13-7.08 (m, 2H), 6.90-6.84 (m, 2H), 5.92 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (400 MHz, CD_3CN) δ 172.0, 171.8, 171.7, 171.6, 171.5, 164.8, 164.3, 162.8, 162.7, 162.2, 160.3, 160.1, 152.9, 152.5, 150.8, 147.0, 145.5, 140.8, 139.7, 137.2, 132.2, 131.7, 130.3, 128.5, 128.4, 125.1, 124.9, 124.7, 115.9, 115.8, 115.7, 101.3, 101.0, 100.7; HRMS: Calcd. for $C_{34}H_{19}ClF_4RhN_4$ $[M–PF_6]^+$: 697.0283 Found: 697.0295

Compound **13**. Reported[1].

Compound **14**. Yield: 56%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.25-8.23 (d, *J* = 8.0 Hz, 2H), 8.09 (s, 2H), 8.06-8.02 (t, *J* = 8.0 Hz, 2H), 7.92-7.90 (d, *J* = 8.0 Hz, 2H), 7.84-7.80 (t, *J* = 8.0 Hz, 4H), 7.64 (s, 10H), 7.13-7.09 (t, *J* = 8.0 Hz, 2H), 6.92-6.90 (d, *J* = 8.0 Hz, 2H), 6.19 (s, 2H), 2.84 (s, 6H), 2.35(s, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ 167.7, 167.3, 165.3, 165.2, 163.8, 150.8, 150.0, 147.1, 141.2, 139.5, 138.6, 136.3, 133.6, 129.7, 129.4, 129.0, 127.9, 127.1, 124.6, 124.3, 124.2, 122.7, 119.8, 26.2, 21.0; MALDI-TOF-HRMS: Calcd. for $C_{50}H_{40}RhN_4$ $[M–PF_6]^+$: 799.7849 Found: 799.2293; Anal. ($C_{50}H_{40}RhN_4PF_6$) C, H, N: calcd 63.57, 4.27, 5.93; found 63.32, 4.32, 5.86.

Compound **15**. Yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 2H), 8.41 (d, *J* = 4.0 Hz, 2H), 8.23-8.12 (m, 6H), 8.10 (t, *J* = 4.0 Hz, 2H),7.60-7.57 (m, 1H), 7.30-7.25 (m, 4H), 7.19 (t, *J* = 4.0 Hz, 2H), 7.10 (t, *J* = 4.0 Hz, 2H), 6.96 (t, *J* = 4.0 Hz, 2H), 6.44 (t, *J* = 4.0 Hz, 2H), 5.95-5.87 (m, 2H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.1, 177.0, 166.5, 161.0, 152.9, 148.4, 147.9, 139.7, 136.3, 134.4, 133.2, 133.0, 132.8, 131.6, 131.4, 129.4, 128.2, 127.8, 127.2, 127.0, 126.6, 125.9, 125.5, 124.4, 124.3, 122.8, 122.3, 121.7, 121.1, 116.9, 114.6, 55.4; HRMS: Calcd. for $C_{46}H_{30}RhN_6OS_2$ $[M–PF_6]^+$: 849.0978 Found: 849.0920 Anal. ($C_{46}H_{30}RhN_6OS_2PF_6$+0.5$H_2O$) C, H, N: calcd 54.82, 3.5, 8.34; found 54.69, 3.31, 8.31.

Compound **16**. Yield: 68%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.75 (s, 2H), 8.07 (d, *J* = 7.6 Hz, 2H), 7.80-7.77 (m, 6H), 7.52 (dd, *J* = 6.4, 1.6 Hz, 2H), 7.05-7.04 (m, 4H), 6.88-6.84 (m, 2H), 6.29 (dd, *J* = 7.6, 1.2 Hz, 2H), 1.72-1.66 (m, 4H), 1.37-1.25 (m, 32H), 0.87-0.83 (m, 8H); ^{13}C NMR (100 MHz, acetone-d_6) δ 166.6, 157.1, 156.6, 153.4, 150.6, 148.3, 146.6, 143.2, 133.8, 132.4, 130.2, 129.7, 129.1, 125.7, 123.6, 122.8, 36.0, 32.6, 31.0, 30.6, 23.3, 14.3; MALDI-TOF-HRMS: Calcd. for $C_{52}H_{64}IrN_4$ [M–PF6]$^+$: 937.4760; Found: 937.4763. Anal.: ($C_{52}H_{64}IrN_4PF_6$) C, H, N: calcd. 57.23, 6.00, 5.13; found 57.25, 6.14, 4.97.

Compound **17**. Reported[11-13].

Compound **18**. Reported[14, 15].

Compound **19**. Reported[7, 16].

Compound **20**. Yield: 57%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.36 (s, 2H), 8.19-8.17 (d, *J* = 8.0 Hz, 2H), 7.96-7.92 (t, *J* = 8.0 Hz, 2H), 7.87-7.84 (m, 4H), 7.80-7.78 (d, *J* = 8.0 Hz, 2H), 7.27-7.25 (d, *J* = 8.0 Hz, 2H), 7.16-7.13 (t, *J* = 8.0 Hz, 2H), 6.88-6.86 (d, *J* = 8.0 Hz, 2H), 6.20 (s, 2H), 4.10 (s, 6H), 2.10 (s, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ 168.8, 158.3, 152.3, 152.2, 147.8, 142.4, 140.9, 139.1, 133.3, 125.7, 124.1, 123.7,

120.2, 114.9, 112.2, 57.2, 21.8; MALDI-TOF-HRMS: Calcd. for $C_{36}H_{32}IrN_4O_2[M–PF_6]^+$: 745.2151 Found: 745.2129. Anal.: ($C_{36}H_{32}IrN_4O_2PF_6$) C, H, N: calcd. 48.59, 3.62, 6.30; found 48.47, 3.79, 6.19.

Compound **21**. Reported[17].

Compound **22**. Yield: 71%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.25 (s, 2H), 8.15 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.84 (d, *J* = 0.6 Hz, 2H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.73 (d, *J* = 0.8 Hz, 2H), 7.71 (d, *J* = 7.6 Hz, 2H), 6.92-6.88 (m, 4H), 6.24 (s, 2H), 2.11 (s, 6H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 168.5, 152.8, 150.5, 150.4, 148.8, 145.7, 142.5, 141.1, 139.4, 133.3, 131.0, 128.7, 126.3, 125.8, 124.7, 123.8, 120.4, 21.9; MALDI-TOF-HRMS: Calcd. for $C_{34}H_{22}Cl_2IrN_4$ $[M–PF_6]^+$: 777.1164 Found: 777.1167. Anal.: ($C_{34}H_{22}Cl_2IrN_4N_4PF_6+H_2O$) C, H, N: calcd. 45.92, 3.0, 5.96; found. 46.2, 3.09, 5.95.

Compound **23**. Yield: 46%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.96 (t, *J* = 8.0 Hz, 4H), 8.16-8.07 (m, 8H), 7.90 (t, *J* = 7.8 Hz, 2H), 7.72-7.64 (m, 4H), 7.30 (t, *J* = 8.0 Hz, 2H), 7.04 (t, *J* = 8.0 Hz, 2H), 6.87 (d, *J* = 4.0 Hz, 2H), 6.18 (s, 2H), 2.10 (s, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ 168.4, 161.0, 151.4, 149.7, 149.0, 142.1, 141.7, 141.0, 139.3, 132.7, 132.0, 130.8, 129.9, 129.6, 129.1, 125.8, 124.4, 123.6, 122.9, 120.3, 21.9. MALDI-TOF-HRMS: Calcd. for $C_{42}H_{32}IrN_4[M–PF_6]^+$: 785.2256 Found: 785.5072. Anal. ($C_{42}H_{32}IrN_4PF_6$) C, H, N: calcd 54.25, 3.47, 6.02; found 53.95, 3.4, 6.02.

Compound **24**. Reported[18].

Compound **25**. Reported[19].

Compound **26**. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.73 (s, 2H), 8.11 (d, *J* = 8.0 Hz, 2H), 7.88 (d, *J* = 6.8 Hz, 2H), 7.84 (d, *J* = 7.6 Hz, 2H), 7.76 (d, *J* = 5.6 Hz, 2H), 7.51 (d, *J* = 6.4 Hz, 2H), 7.14-7.08 (m, 4H), 6.97-6.93 (m, 2H), 6.29 (d, *J* = 7.6 Hz, 2H), 2.84 (s, 6H), 2.79 (t, *J* = 7.6 Hz, 4H), 1.75-1.67 (m, 4H), 1.38-1.26 (m, 24H), 0.90 (t, *J* = 7.6 Hz, 6H), ^{13}C NMR (100 MHz, acetonitrile-d_3) δ 169.2, 168.9, 162.4, 156.1, 154.0, 148.8, 146.8, 145.5, 141.9, 133.0, 132.0, 128.6, 128.3, 127.2, 123.6, 122.5, 122.1, 34.6, 31.3, 29.5, 28.8, 28.7, 28.6, 28.4, 22.1, 22.0, 13.1; MALDI-TOF-HRMS: Calcd. for $C_{52}H_{64}N_4Rh$ $[M–PF_6]^+$: 847.4186; Found: 847.4473; Anal.: ($C_{52}H_{64}N_4RhPF_6+H_2O$) C, H, N: calcd. 61.78, 6.58, 5.54; found 61.79, 6.19, 5.43.

Compound **27**. Yield: 69%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.74 (s, 2H), 8.25 (d, *J* = 8.2 Hz, 2H), 8.03 (t, *J* = 8.2 Hz, 2H), 8.00 – 7.90 (m, 4H), 7.77 (d, *J* = 5.8 Hz, 2H), 7.54 (d, *J* = 5.5 Hz, 2H), 7.20 (t, *J* = 7.3 Hz, 2H), 7.10 (t, *J* = 7.5 Hz, 2H), 6.98 (td, *J* = 7.4, 1.4 Hz, 2H), 6.36 (t, *J* = 7.5 Hz, 2H), 2.88 – 2.80 (m, 4H), 1.78 – 1.65 (m, 4H), 1.38 – 1.25 (d, *J* = 5.5 Hz, 24H), 0.91 – 0.82 (m, 6H). ^{13}C NMR (101 MHz, acetone) *δ* 168.84, 168.52, 165.90, 165.88, 157.36, 155.48, 150.40, 149.93, 144.80, 139.53, 133.46, 130.87, 130.85, 128.60, 125.50, 124.99, 124.38, 124.14, 120.90, 120.88, 36.01, 32.56, 30.98, 30.16, 29.99, 29.97, 23.29, 14.35. MALDI-TOF-HRMS: Calcd. for $C_{50}H_{60}N_4Rh$ $[M–PF_6]^+$: 819.3668; found: 819.3801. Anal.: ($C_{50}H_{60}N_4RhPF_6$) C, H, N: calcd. 62.24, 6.27, 5.87; found 62.50, 6.47, 5.76.

Compound **28**. Yield: 68%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.77 (dd, *J* = 11.6, 8.8 Hz, 2H), 8.24 (d, *J* = 6.4 Hz, 2H), 7.65 (d, *J* = 7.6, 2H), 7.59 (d, *J* = 6.4 Hz, 2H), 7.20 (d, *J* = 2.0 Hz, 2H), 7.13 (t, *J* = 8.0 Hz, 2H), 6.94 (t, *J* = 7.6 Hz, 2H), 6.67 (t, *J* = 2.4 Hz, 2H), 6.32 (d, *J* = 7.6 Hz, 2H), 1.79-1.71 (m, 4H), 1.42-1.22 (m, 28H), 0.88-0.85 (m, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ 157.5, 156.0, 151.0, 149.7, 149.4, 143.5, 139.8, 134.7, 128.6, 128.3, 127.4, 124.9, 113.3, 109.7, 36.1, 23.3, 14.4; MALDI-TOF-HRMS: Calcd. for $C_{46}H_{58}N_6Rh[M–PF_6]^+$: 797.3778; Found: 797.3820. Anal.: ($C_{46}H_{58}N_6RhPF_6$) C, H, N: calcd. 58.60, 6.20, 8.91; found 58.31, 6.08, 8.86.

Compound **29**. Yield: 43%. ^{1}H NMR (400 MHz, acetone- d_6) δ 9.11 (d, *J* = 8.0 Hz, 2H), 8.79 (s, 2H), 8.48 (d, *J* = 8.0 Hz, 2H), 8.12-8.09 (m, 2H), 7.97-7.94 (m, 4H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.70-7.65 (m, 4H), 7.52 (t, *J* = 4.0 Hz, 2H), 7.23 (t, *J* = 4.0 Hz, 2H), 7.00 (t, *J* = 4.0 Hz, 2H), 6.35 (d, *J* = 4.0 Hz, 2H), 2.85-2.83 (m, 8H), 1.36-1.27 (m, 24H), 0.87 (t, *J* = 4.0 Hz, 6H); ^{13}C NMR (100 MHz, acetone- d_6) δ 171.3, 171.0, 165.7, 165.6, 156.5, 154.6, 149.5, 145.6, 140.4, 137.2, 132.8, 131.9, 130.3, 130.0, 129.3, 127.9, 127.7, 126.5, 126.3, 126.2,

124.2, 123.2, 121.7, 35.1, 31.7, 30.1, 22.4, 13.5; HRMS: Calcd. for $C_{58}H_{64}RhN_4[M–PF_6]^+$: 919.4186 Found: 919.4112 Anal. ($C_{58}H_{64}RhN_4PF_6$+0.5H_2O) C, H, N: calcd 64.86, 6.10, 5.22; found 64.59, 6.17, 5.24.

Compound **30**. Reported[19].

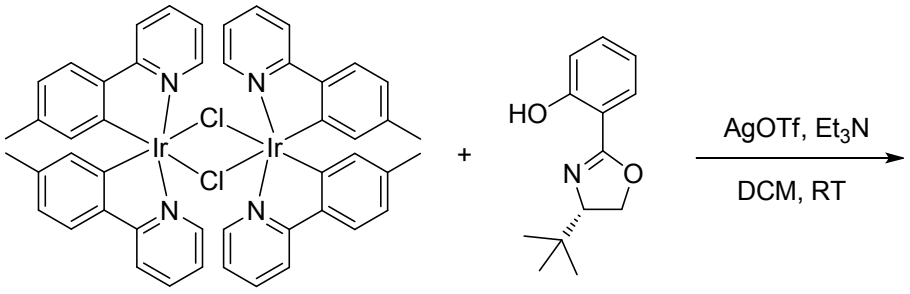
Compound **31**. Reported[19].

Compound **32**. Yield: 68%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.70 (s, 2H), 8.46 (dd, *J* = 7.6, 0.4 Hz, 2H), 8.24 (d, *J* = 7.6 Hz, 2H), 8.18 (d, *J* = 8.0 Hz, 2H), 7.97-7.92 (m, 2H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.72-7.70 (m, 2H), 6.99-6.95 (m, 4H), 6.27 (s, 2H), 2.11 (s, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ 166.9, 166.6, 164.9, 151.3, 149.5, 146.3, 145.1, 141.5, 140.0, 138.6, 133.4, 129.4, 127.4, 125.0, 124.6, 123.0, 119.7, 21.1; MALDI-TOF-HRMS: Calcd. for $C_{36}H_{26}Cl_2N_4Rh$ $[M–PF_6]^+$: 687.0590; Found: 687.0561. Anal.: ($C_{36}H_{26}Cl_2N_4RhPF_6$+$H_2O$) C, H, N: calcd. 50.78, 3.31, 6.58; found 50.59, 3.24, 6.60.

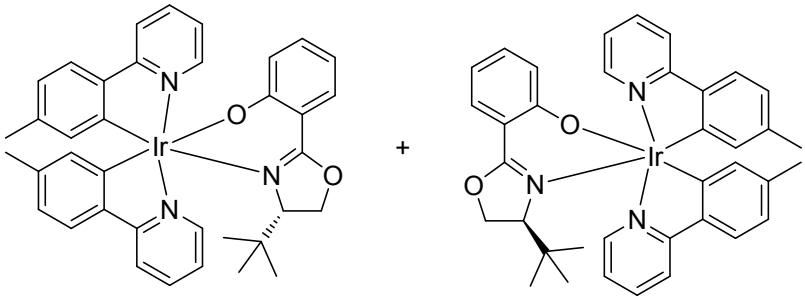
Compound **33**. Yield: 68%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.55 (dd, *J* = 14.0, 8.8 Hz, 4H), 8.16-8.04 (m, 8H), 7.94-7.89 (m, 2H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.63-7.59 (m, 2H), 7.30-7.26 (m, 2H), 7.08-7.04 (m, 2H), 6.91 (dd, *J* = 8.0, 1.2 Hz, 2H), 6.24 (s, 2H), 2.085 (s, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ 166.6, 166.3, 164.6, 158.4, 150.2, 147.8, 141.1, 140.3, 138.6, 133.2, 130.8, 129.6, 128.7, 128.5, 128.3, 124.8, 124.5, 122.8, 121.9, 119.6, 21.1; MALDI-TOF-HRMS: Calcd. for $C_{42}H_{32}N_4Rh[M–PF_6]^+$: 659.1682; Found: 659.1636. Anal.: ($C_{42}H_{32}N_4RhPF_6$) C, H, N: calcd. 60.01, 3.84, 6.67; found 59.93, 4.00, 6.56.

Compound **34**. Yield: 72%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, *J* = 5.2 Hz, 2H), 8.13 (s, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.82-7.78 (m, 2H), 7.69-7.67 (m, 4H), 7.59-7.53 (m, 10H), 7.48 (d, *J* = 5.6 Hz, 2H), 7.05-7.01 (m, 2H), 6.95 (d, *J* = 7.6 Hz, 2H), 6.23 (s, 2H), 2.17 (s, 6H); ^{13} C NMR (100 MHz, $CDCl_3$) δ 167.6, 167.2, 164.9, 151.2, 149.7, 149.0, 146.0, 141.0, 140.7, 138.1, 135.8, 133.7, 129.7, 129.6, 129.1, 129.0, 126.4, 126.0, 124.7, 124.3, 123.1, 119.3, 22.1; HRMS: calcd. for $C_{48}H_{36}RhN_4[M–PF_6]^+$: 771.1995 found: 771.2038. Anal.: ($C_{48}H_{36}RhN_4PF_6$+1.5H_2O) C, H, N: calcd. 61.09, 4.17, 5.94; found 61.24, 4.04, 6.01.

Synthesis of compound 1 isomers.



S1 **S2**

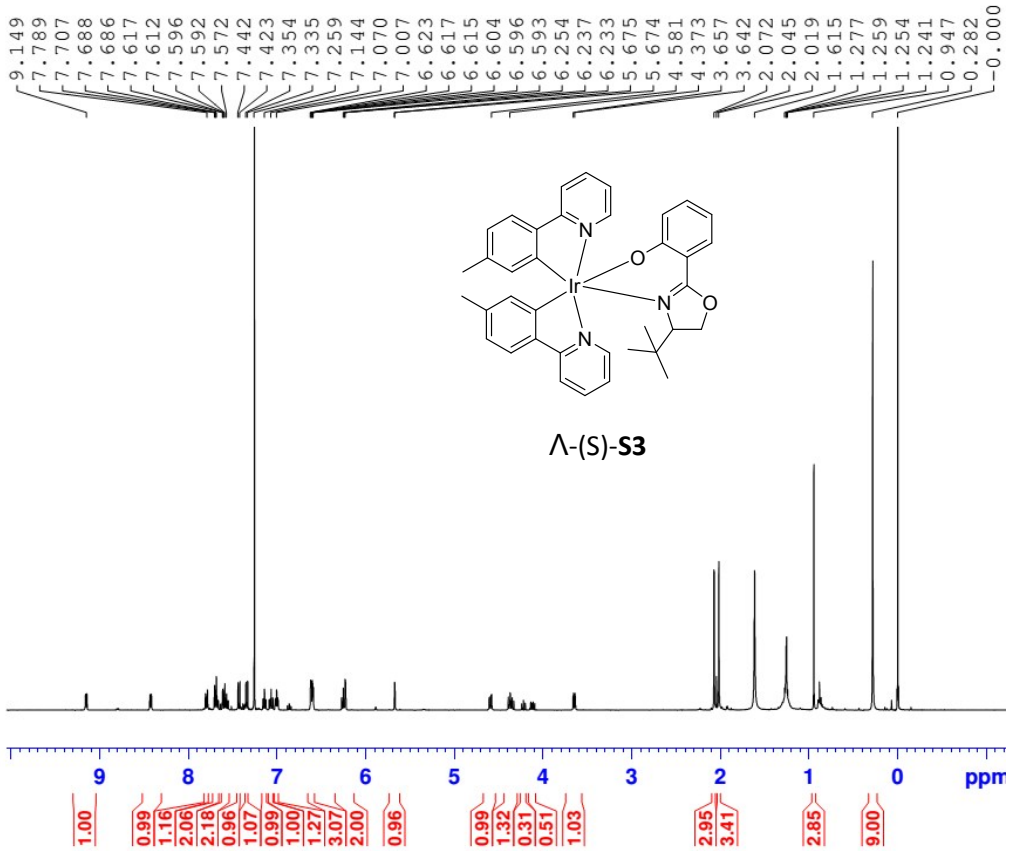


Λ-(S)-**S3** Δ-(S)-**S3**

Compounds Λ-(S)-**S3** and Δ-(S)-**S3** were synthesized using a modified literature method.[20] A suspension of dimer **S1** (116 mg, 103 µmol), (*S*)-4-*tert*-butyl-2-(2-hydroxyphenyl)-2-oxazoline **S2** (30.0 mg, 137 µmol), silver trifluoromethanesulfonate (81 mg, 315 µmol), and triethylamine (57 µL, 412 µmol) in dichloromethane (15 mL) was stirred at room temperature for 3 h. The suspension was filtered through a short plug of silica with hexane/ethyl acetate (1:2). The crude product was collected, concentrated to dryness; the residue was purified by silica gel column chromatography. The diastereomers could be well separated using hexane/ethyl acetate (3:1 to 2:1) as eluent. The resulting materials were dissolved in acetonitrile, washed five times with hexane, and concentrated to dryness to afford Λ-(*S*)-**S3** as yellow solid (30 mg, 30%) and Δ-(*S*)-**S3** as yellow solid (35.4 mg, 35%) with a *dr* of of at least 100:1 for both diastereomers. Because Λ-(S)-**S3** and Δ-(S)-**S3** were somewhat unstable during silica gel column chromatography, their ^{1}H NMR and ^{13}C NMR spectra could not be recorded. However, HRMS analysis indicated that the desired products had formed with sufficietn purity to be used in the next step without further purification.

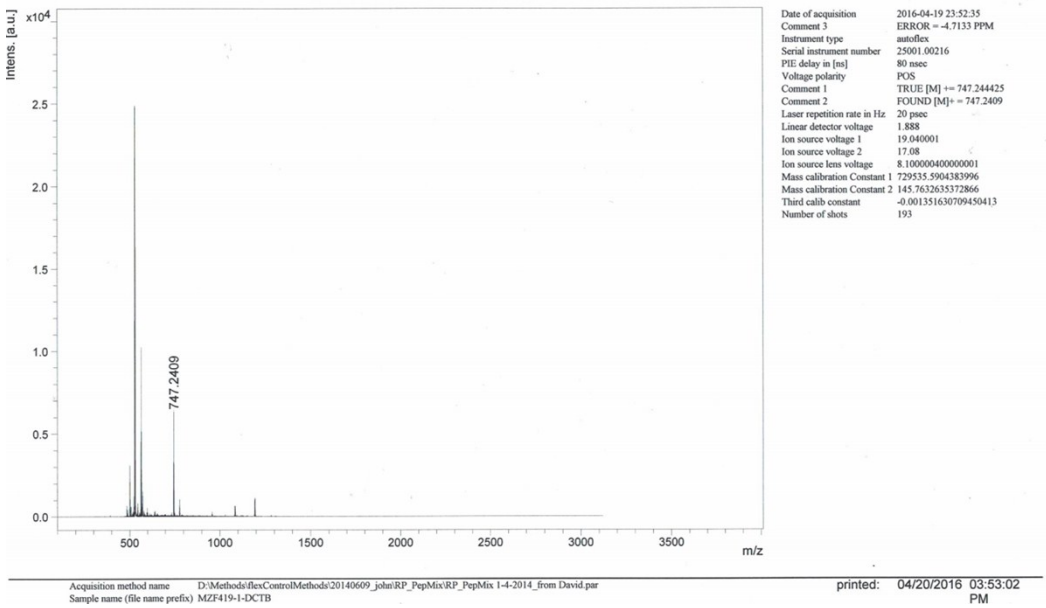
Λ-(*S*)-**3** (first eluted diastereomer): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.16 (dd, *J* = 6.0, 1.2 Hz, 1H), 8.43 (dd, *J* = 6.0, 0.8 Hz, 1H), 7.80 (d, *J* = 7.6, 1H), 7.70 (dd, *J* = 7.6, 1.6 Hz, 2H), 7.67-7.55 (m, 2H), 7.43 (d, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.26-7.13 (m, 1H), 7.09-7.04 (m, 1H), 7.03-6.84 (m, 1 H), 6.62-6.59 (m, 3H), 6.27-6.23 (m, 2H), 5.68 (s, 1H). 4.60 (dd, *J* = 9.2, 2.0 Hz, 1H), 4.39-4.33 (m, 1H), 3.65 (dd, *J* = 8.0, 2.0 Hz, 1H), 2.07 (s, 3H), 2.02 (s, 3H), 0.28(s, 9H) ppm. HRMS: calcd. for $C_{37}H_{36}N_3O_2Ir$ M^+ 747.2437; found 747.2409.

HKBU_PROTON



Λ-(S)-**S3**

HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)



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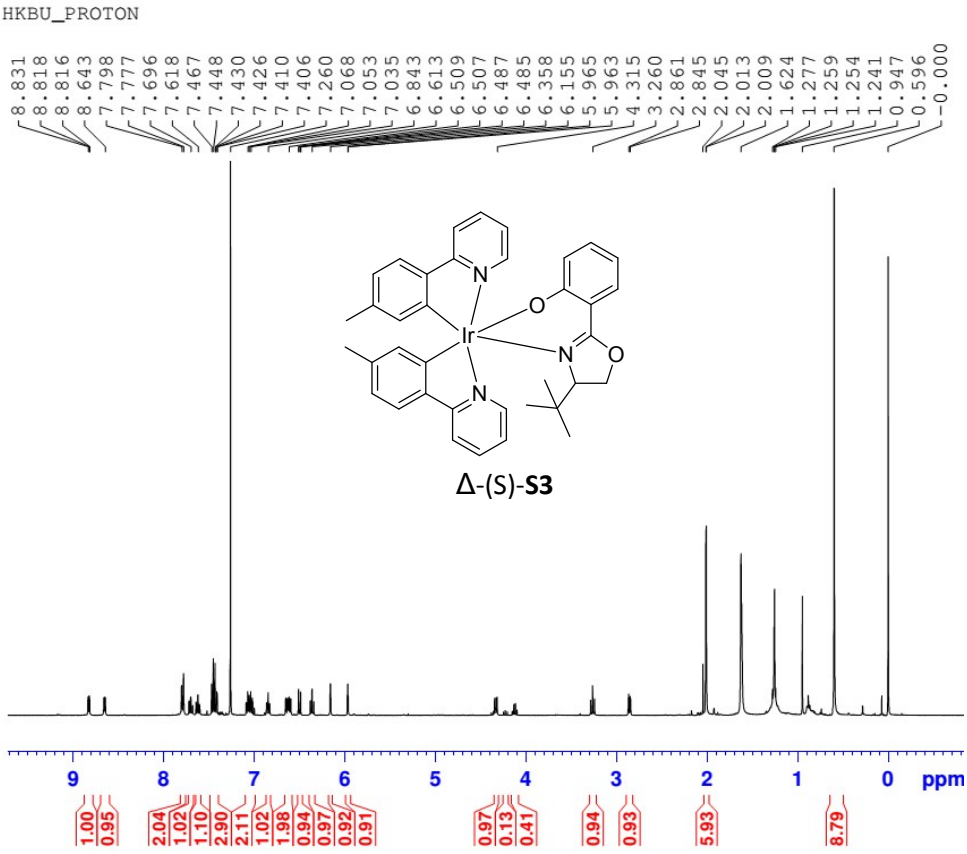
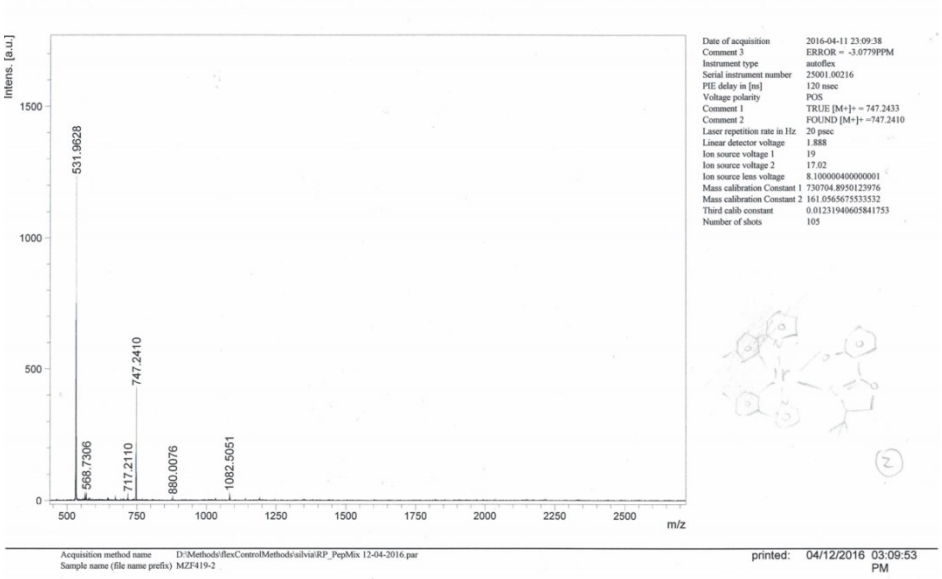
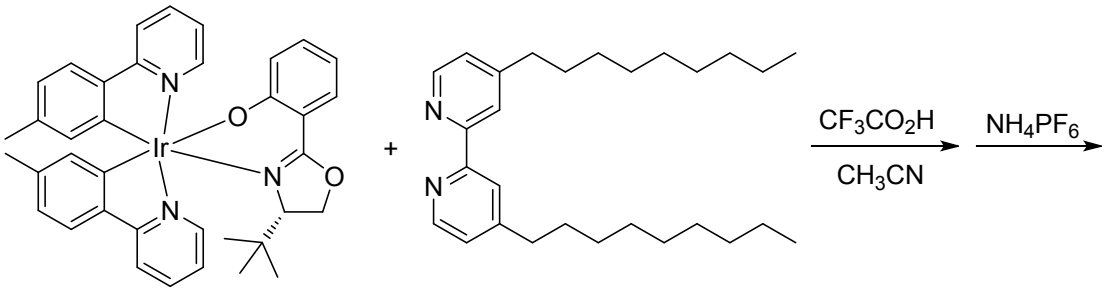
Δ-(*S*)-**3** (second eluted diastereomer): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.82 (dd, *J* = 5.2, 0.8 Hz, 1H), 8.65 (dd, *J* = 5.6, 0.8 Hz, 2H), 7.79 (d, *J* = 5.6, 2H), 7.72-7.68 (m, 1H), 7.64-7.60 (m, 1H), 7.47-7.41 (m, 3H), 7.09-7.00 (m, 2H), 6.86-6.83 (m, 2H), 6.65-6.59 (m, 2H), 6.50 (dd, *J* = 8.8, 1.2 Hz, 1H), 6.38-6.34 (m, 1H), 6.16 (s, 1H), 5.96 (s, 1H), 4.33 (dd, *J* = 6.8, 2.0 Hz, 1H), 3.26 (t, *J* = 8.8, 1H), 2.85 (dd, *J* = 8.4, 2.0 Hz, 1H), 2.01 (s, 6H), 0.60 (s, 9 H) ppm. HRMS: calcd. for $C_{37}H_{36}N_3O_2Ir$ M^+ 747.2437; found 747.2410.

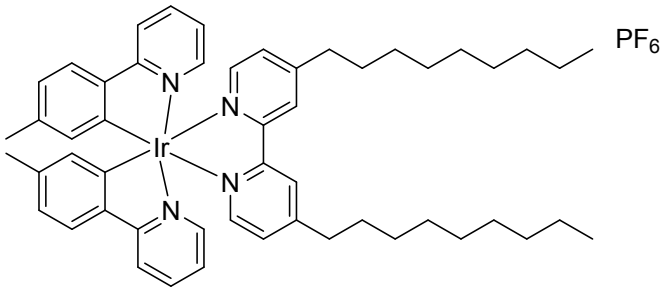


Δ-(S)-**S3**

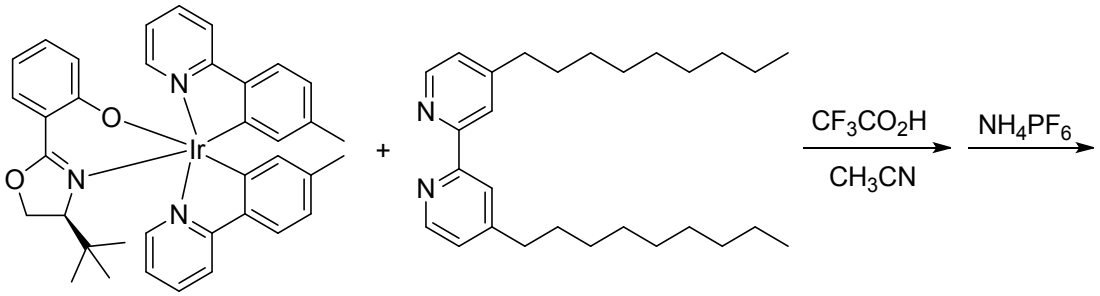




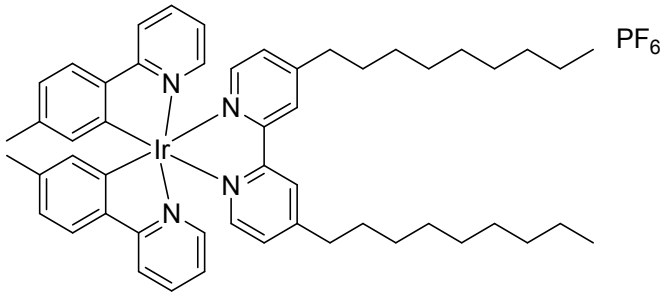
Λ-(S)-**S3**



Λ-1



Δ-(S)-**S3**



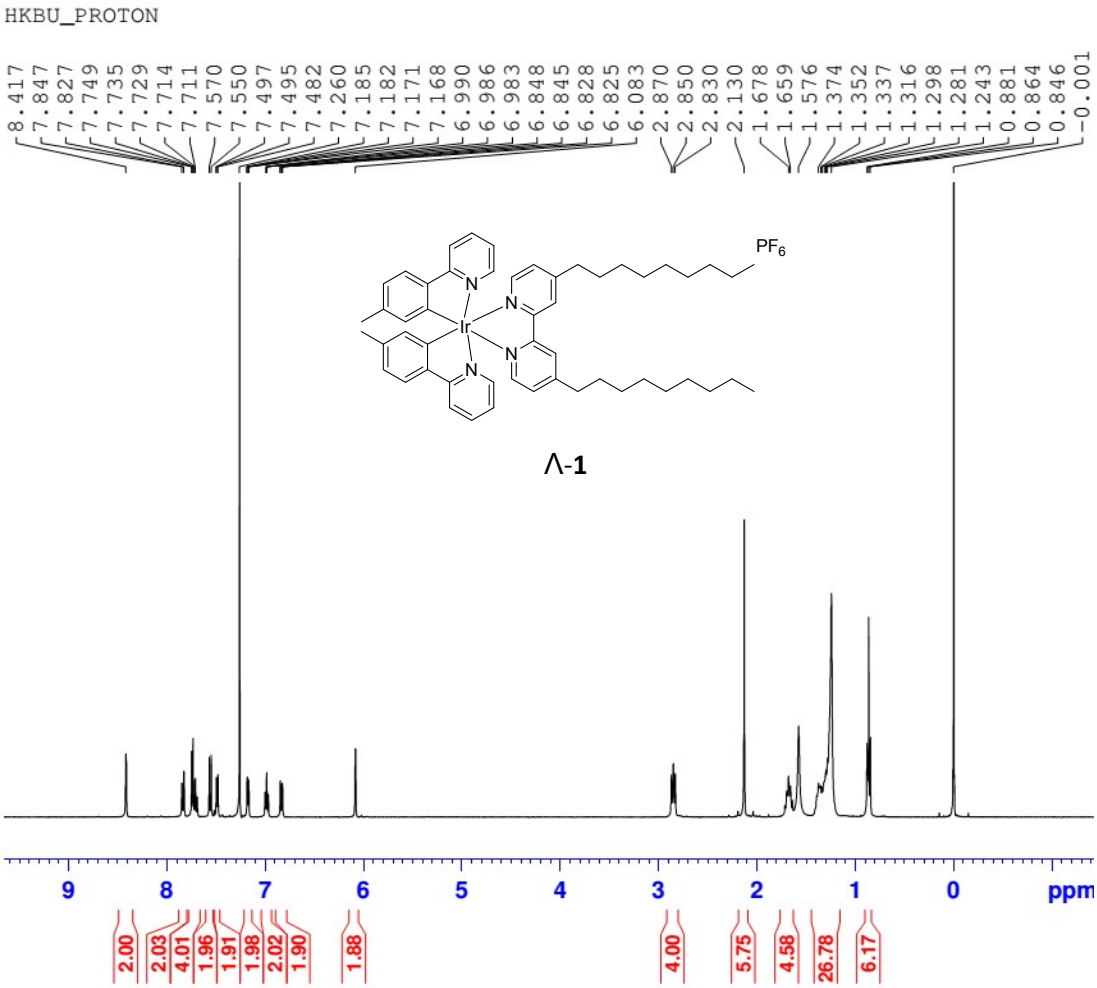
Δ-1

Compounds Λ-**1** and Δ-**1** were synthesized using a modified literature method[20].

Compound Λ-**1:** A suspension of Δ-(*S*)-**S3** (30 mg, 40.1 µmol), 4,4'-dinonyl-2,2'-bipyridine (24.6 mg, 60.2 µmol), and trifluoroacetic acid (15 µL, 200 µmol) in acetonitrile (3.8 mL) was stirred at room temperature for 30 min. The reaction mixture was concentrated to dryness and subjected to silica gel chromatography with dichloromethane/methanol (20:1). The combined product eluents were again concentrated to

dryness, and the resulting material was dissolved in a minimum amount of acetonitrile. The product was precipitated by the addition of a few drops of a saturated, aqueous solution of NH_4PF_6, and water (8 mL) was added. The yellow precipitate was collected by centrifugation, washed twice with water, and dried under high vacuum to afford Λ-**1** as its PF_6 salt (20.0 mg, 46%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (s, 2H), 7.84 (d, J = 8.0, 2H), 7.75-7.69 (m, 4H), 7.56 (d, J = 8.0, 2H), 7.49 (dd, J = 5.2, 0.8 Hz, 2H), 7.18 (dd, J = 5.6, 1.2 Hz, 2H), 7.00-6.97 (m, 2 H), 6.84 (dd, J = 6.8, 1.2 Hz, 2H), 6.08 (s, 2H), 2.85 (t, J = 8.0, 4H), 2.13 (s, 6 H), 1.72-1.64 (m, 4H), 1.39-1.24 (m, 26 H), 0.86 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.8, 156.7, 155.5, 151.1, 149.5, 148.6, 140.9, 140.8, 137.7, 132.5, 127.7, 125.2, 124.5, 123.5, 122.7, 119.0, 35.4, 31.9, 30.1, 29.5, 29.4, 29.3, 22.7, 21.9, 14.1. ppm. CD (MeCN): λ ($\Delta\varepsilon$/m–1cm–1) 414 (-4), 327 (+6), 282 (-10), 253 (+7), 225 (-9). HRMS: Calcd. for $C_{52}H_{64}IrN_4$[M–PF_6]+: 937.4760 Found: 937.4720. Anal.: ($C_{52}H_{64}IrN_4PF_6$) C, H, N: calcd. 57.71, 5.96, 5.18; found 56.86, 5.92, 5.13.



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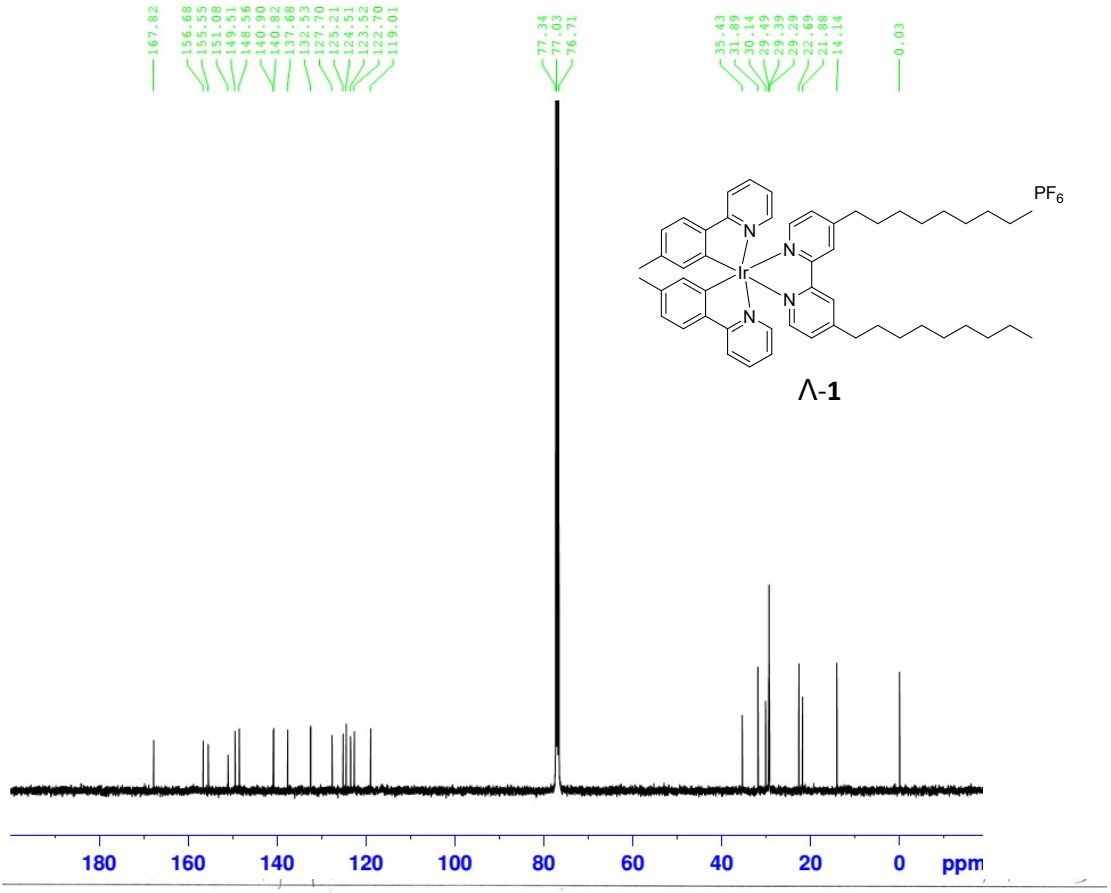
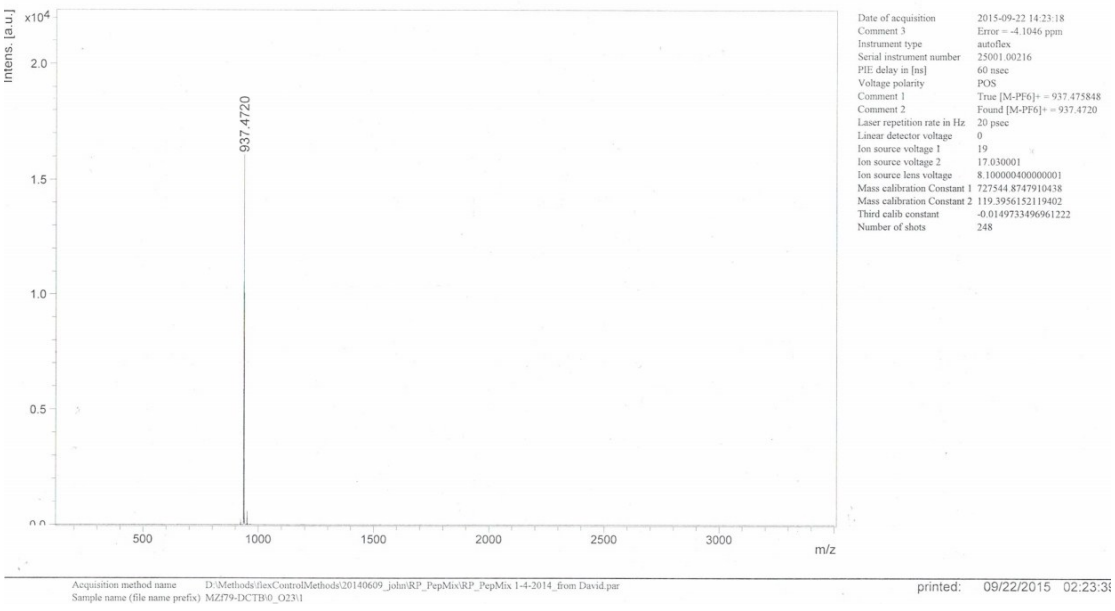


Λ-1

HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)



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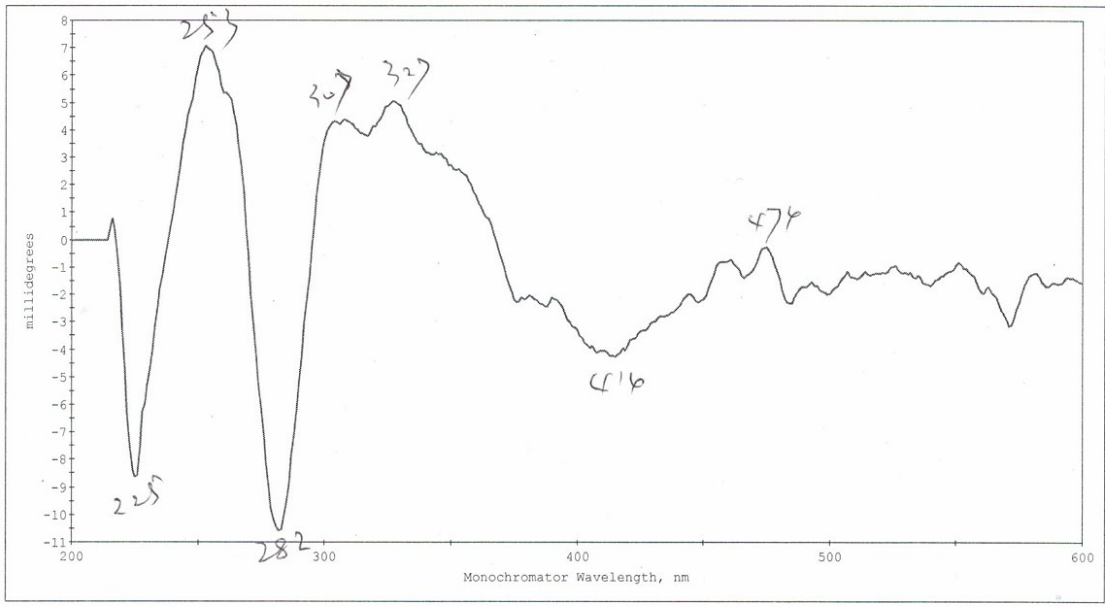

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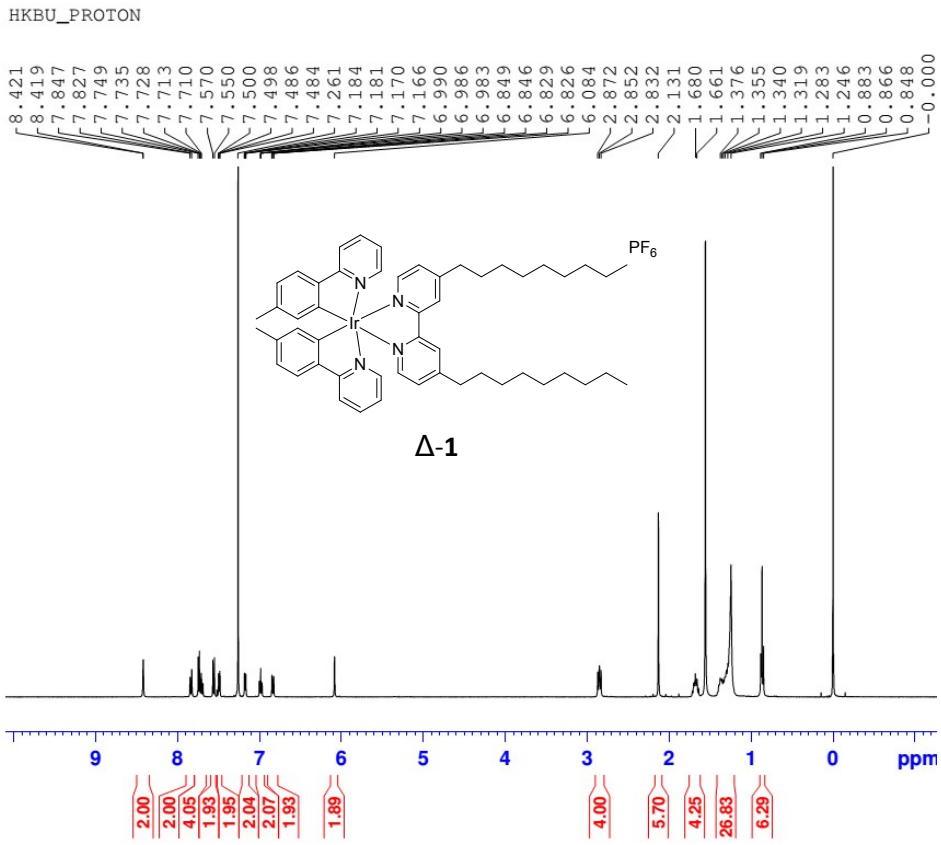
Compound Δ-**1:** Compound Δ-**1** was prepared in an analogous manner to Λ-**1** from Λ-(*S*)-**S3**. After purification and precipitation, Δ-**1** was obtained as its PF_6 salt (25 mg, 57%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (s, 2H), 7.84 (d, *J* = 8.0, 2H), 7.75-7.69 (m, 4H), 7.56 (d, *J* = 8.0, 2H), 7.49 (dd, *J* = 5.2, 0.8 Hz, 2H), 7.18 (dd, *J* = 5.6, 1.2 Hz, 2H), 7.00-6.97 (m, 2 H), 6.84 (dd, *J* = 6.8, 1.2 Hz, 2H), 6.08 (s, 2H), 2.85 (t, *J* = 8.0, 4H), 2.13 (s, 6 H), 1.72-1.64 (m, 4H), 1.39-1.25 (m, 26 H), 0.87 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.9, 156.7, 155.6, 151.1, 149.5, 148.6, 140.9, 140.8, 137.7, 132.5, 127.7, 125.2, 124.5, 123.5, 122.7, 119.0, 35.4, 31.9, 30.1, 29.5, 29.4, 29.3, 22.7, 21.9, 14.1. ppm. CD (MeCN): λ ($\Delta\varepsilon$/m–1cm–1) 414 (+1), 327 (-9), 282 (+7), 253 (-10), 225 (+6). HRMS: Calcd. for $C_{52}H_{64}IrN_4$[M–PF_6]+: 937.4760 Found: 937.4785. Anal.: ($C_{52}H_{64}IrN_4PF_6$) C, H, N: calcd. 57.71, 5.96, 5.18; found 57.67, 6.03, 5.18.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0.848
0.000
PF6
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Ir
N
N
N
Δ-1
9
8
7
6
5
4
3
2
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2.00
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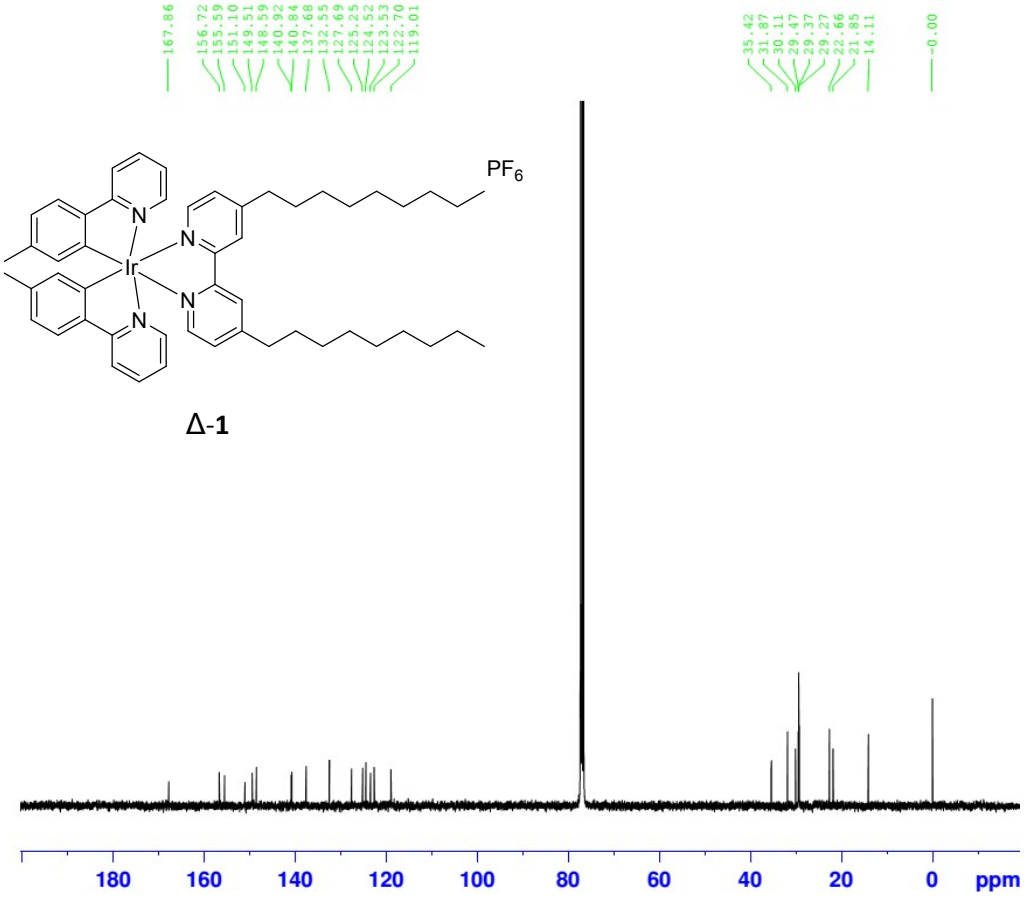
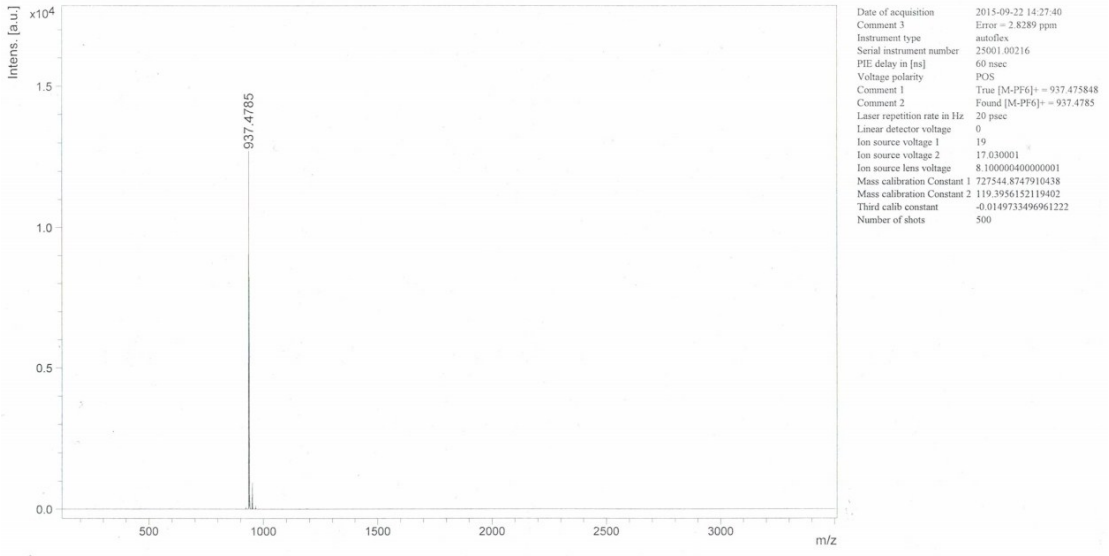
HKBU_CARBON



Δ-1

HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)



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Ion source voltage 1	19
Ion source voltage 2	17.030001
Ion source lens voltage	8.100000400000001
Mass calibration Constant 1	727544.8747910438
Mass calibration Constant 2	119.3956152119402
Third calib constant	-0.0149733496961222
Number of shots	500

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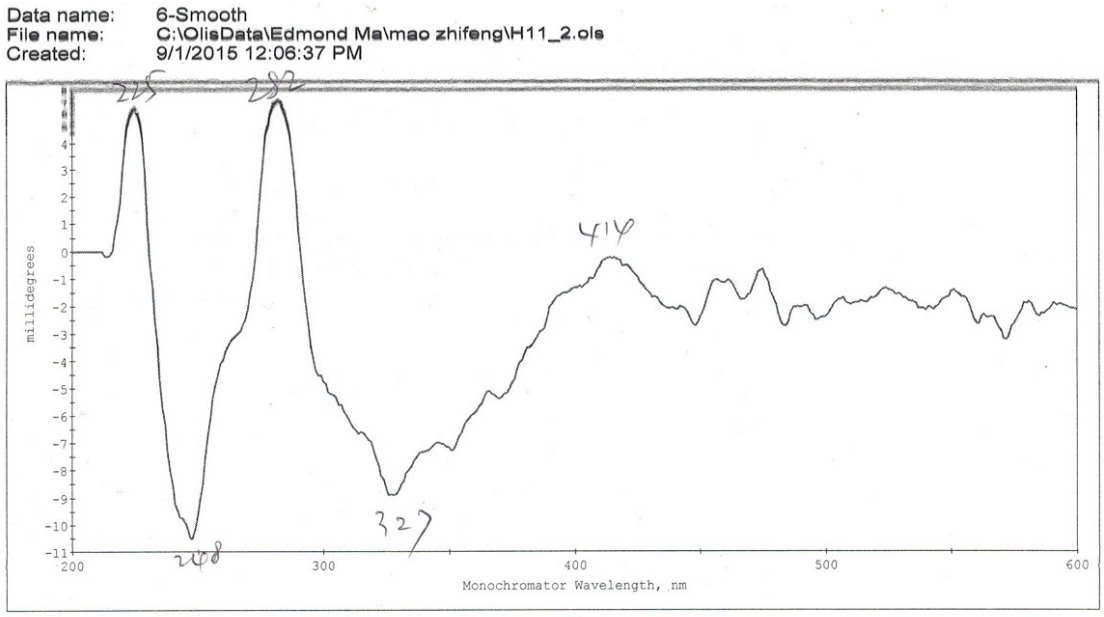

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Reduction mode: Circular Dichroism
Scan mode: Fixed bandwidth
(Bandwidth = 5 nm)
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Stability experiments. 5 mM compound **1** was stored in DMSO-d6/D2O (v/v = 9:1) at 298 K for 48 h, and ^{1}H NMR spectra were recorded. ^{1}H NMR experiments were carried out on a 400 MHz Bruker instrument. Additionally, 10 μM compound **1** was also stored in 80% acetonitrile/20% 20 mM Tris-HCl (20 mM NaCl, pH 7.4) buffer or cell culture DMEM medium for 48 h. Absorption spectra were recorded on Cary UV-100 Spectrophotometer. The absorbance of compound **1** in Tris-HCl buffer or culture medium was corrected by subtraction of the background absorbance.

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Table S1. IC_{50} Values (mean ± SD μM) of compound **1** and its enantiomers in different cancer cell lines *via* MTT assay.

Cell lines	**1**	Λ-**1**	Δ-**1**	Wild-type or mutant H-Ras/K-Ras
A498	17.5 ± 1.1	29.2 ± 1.4	9.2 ± 1.1	mutant H-Ras/K-Ras
HEK293	23.9 ± 1.1	27.7 ± 1.6	19.6 ± 1.0	wild-type Ras
MDA-MB-231	14.7 ± 1.1	58.1 ± 1.5	25.3 ± 1.2	mutant K-Ras
MCF7	20.6 ± 1.5	36.8 ± 1.3	23.3 ± 1.3	-
T47D	29.7 ± 1.2	33.8 ± 1.0	20.8 ± 1.0	-
MCF10A	25.8 ± 1.3	40.2 ± 1.3	26.5 ± 1.3	Wt and mutant K-Ras
A549	30.9 ± 1.5	>50	15.0 ± 1.2	mutant K-Ras
H1299	6.3 ± 1.1	15.3 ± 1.1	10.3 ± 1.2	Wt and mutant K-Ras
DU145	35.3 ± 2.3	>50	28.1 ± 1.6	wild-type Ras
A431	25.3 ± 1.3	30.1 ± 1.7	18.8 ± 1.2	mutant H-Ras and wild-type K-Ras
A357	27.7 ± 1.8	37.8 ± 1.1	21.9 ± 1.0	-
A2780	29.6 ± 1.1	32.1 ± 1.4	29.8 ± 1.1	mutant K-Ras
K562	38.5 ± 1.0	39.6 ± 1.1	37.1 ± 1.2	wild-type K-Ras

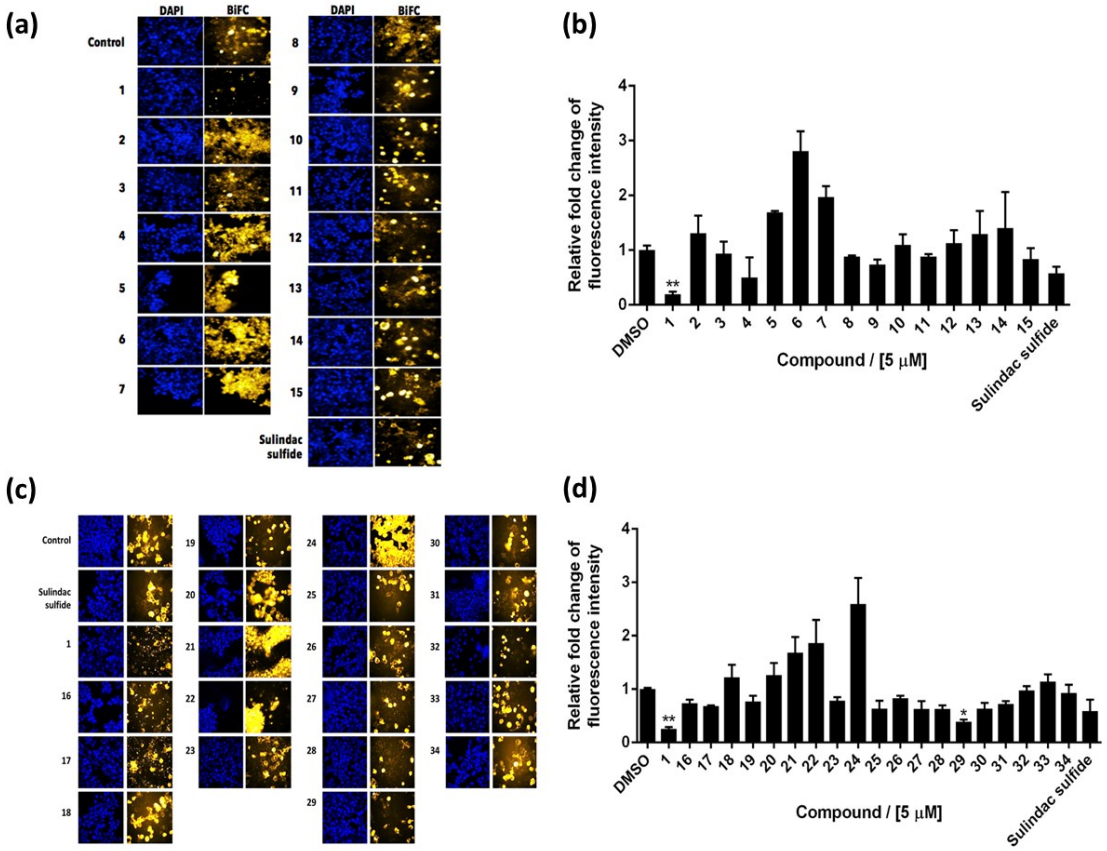

Fig. S1. Effects of 35 metal-based compounds on the H-Ras/Raf-1 interaction in HEK293T cells as determined by the BiFC assay. Metal-based compounds and sulindac sulfide (5 μM) were incubated with HEK293T cells for 6 h and the fluorescence was monitored by confocal imaging. (a) Effect of 15 metal-based compounds (**1**–**15**) and sulindac sulfide on the H-Ras/Raf-1 interaction in HEK293T cells as determined by the BiFC assay. (b) Quantification of fluorescence intensity of cell samples treated with compounds **1**–**15** and sulindac sulfide using ImageJ. Significantly different from DMSO control at $*p < 0.05$, $**p < 0.005$. (c) Effect of 20 metal-based compounds (**1**, **16**–**35**) and sulindac sulfide on the H-Ras/Raf-1 interaction in HEK293T cells as determined by BiFC assay. (d) Quantification of fluorescence intensity of cell samples treated with compounds **1**, **16**–**35** and sulindac sulfide using ImageJ. Significantly different from DMSO control at $*p < 0.05$, $**p < 0.005$.

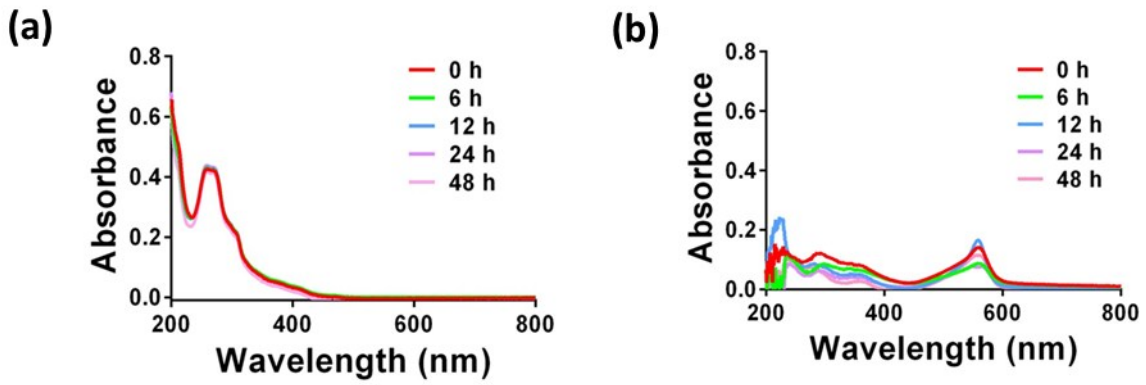

Fig. S2. UV/Vis absorption of the compound **1** at a concentration of 10 µM in: (a) 80% acetonitrile / 20% 20 mM Tris-HCl buffer (20 mM NaCl, pH 7.4); (b) cell culture DMEM medium.

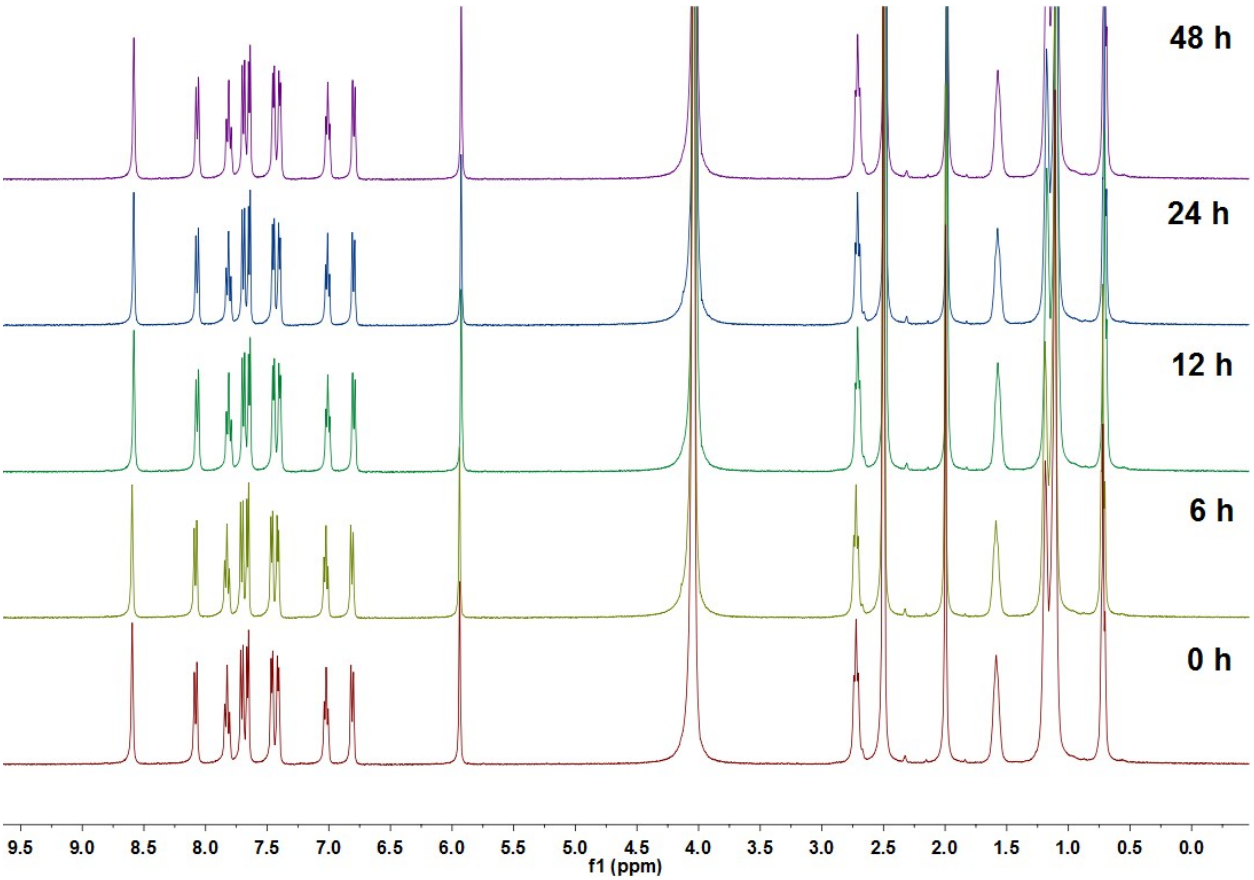

Fig. S3. The ^{1}H NMR spectra of the compound **1** at a concentration of 5 mM in 90% DMSO-d_6/10% D_2O at 298 K over 48 h.

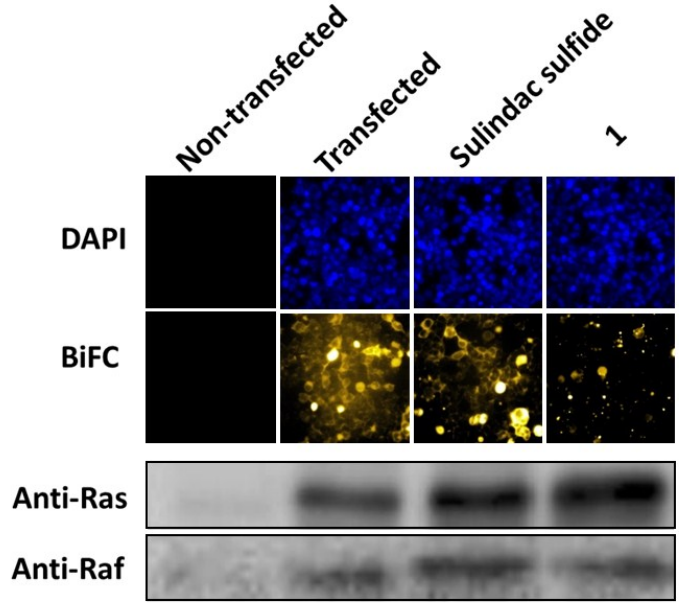

Fig. S4. Compound **1** has no significant effect on H-Ras and Raf-1 protein expression. HEK293T cells were incubated with compound for 6 h and the yellow fluorescence signal that was generated by interaction of Ras/Raf was detected by confocal imaging. The protein expression of H-Ras and Raf-1 was detected and analyzed by Western blotting.

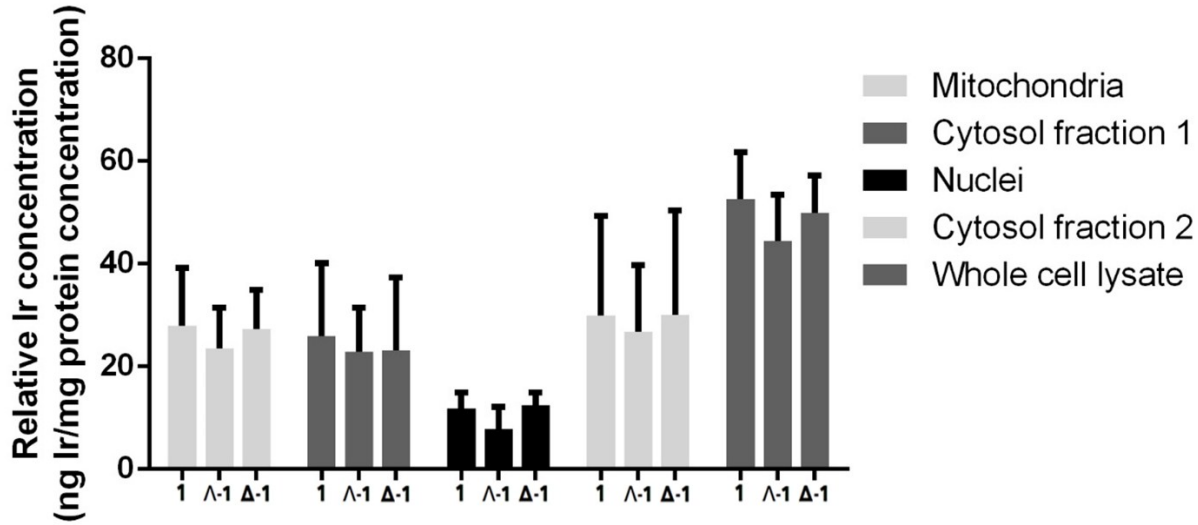

Fig. S5. ICP-MS assay for mitochondrial, nuclear and whole cell lysate iridium accumulation. A498 cells were treated with racemic **1**, Λ-**1** or Δ-**1** (5 μM) for 6 h and the cells were harvested by trypsinization and appropriate organelle isolation procedures performed. Cytosol fraction 1 was the isolate from the the separation of mitochondria. Cytosol fraction 2 was cytoplasmic extract from the nuclear protein

extraction. The iridium counts were normalized to protein concentration of indicated organelles, which was determined by a BCA assay.

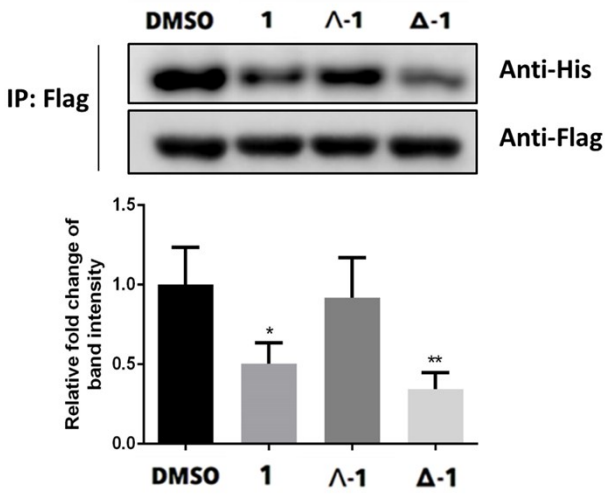

Fig. S6. Racemic **1**, Λ-**1** and Δ-**1** (5 μM) disrupt the interaction of wtRas-His and Raf-1-Flag in A498 cells as revealed by a pull-down assay. Protein complexes were immunoprecipitated by Flag antibody and analyzed with His antibody.

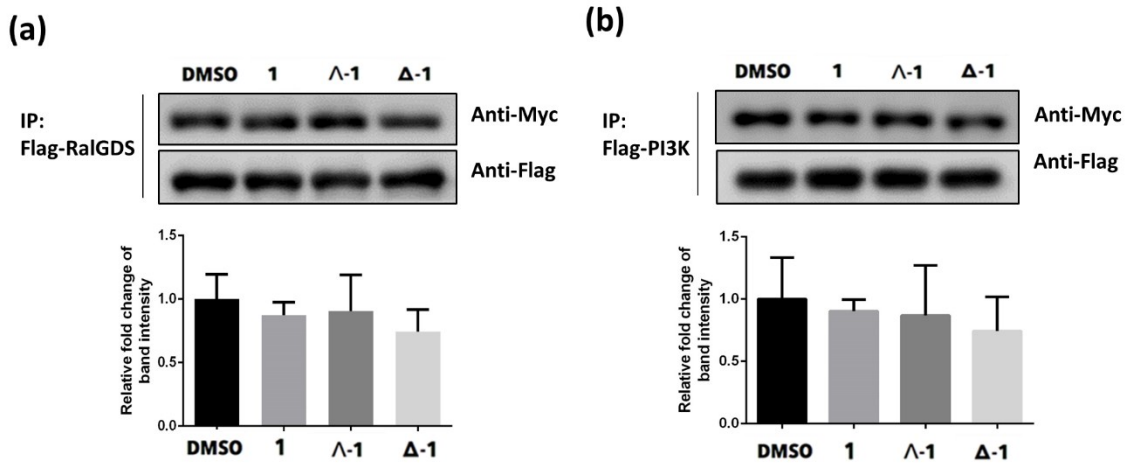

Fig. S7. Effect of racemic **1**, Λ-**1** and Δ-**1** (5 μM) on interaction of HRas-Myc-RalGDS-Flag (a) and HRas-Myc-PI3K-Flag (b) in A498 cells as revealed by a pull-down assay. Protein complexes were immunoprecipitated by Flag antibody and analyzed with Myc antibody.

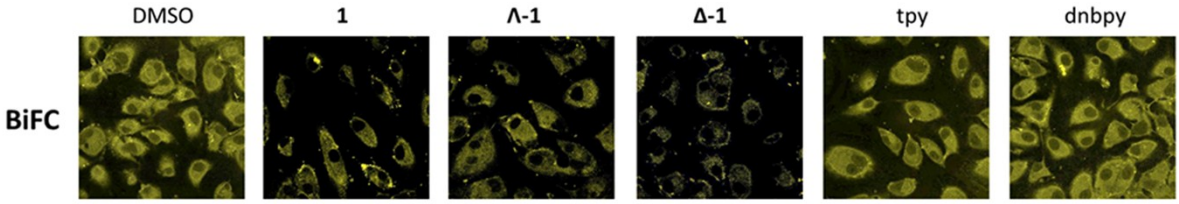

Fig. S8. Effects of racemic **1**, Λ-**1**, Δ-**1**, tpy and dnbpy (5 μM) on inhibiting the H-Ras/Raf-1 PPI as determined by the BiFC assay. A498 cells were incubated with compounds at the concentration of 5 μM for 6 h and the yellow fluorescence signal that was generated by the interaction of H-Ras/Raf-1 was detected by confocal imaging.

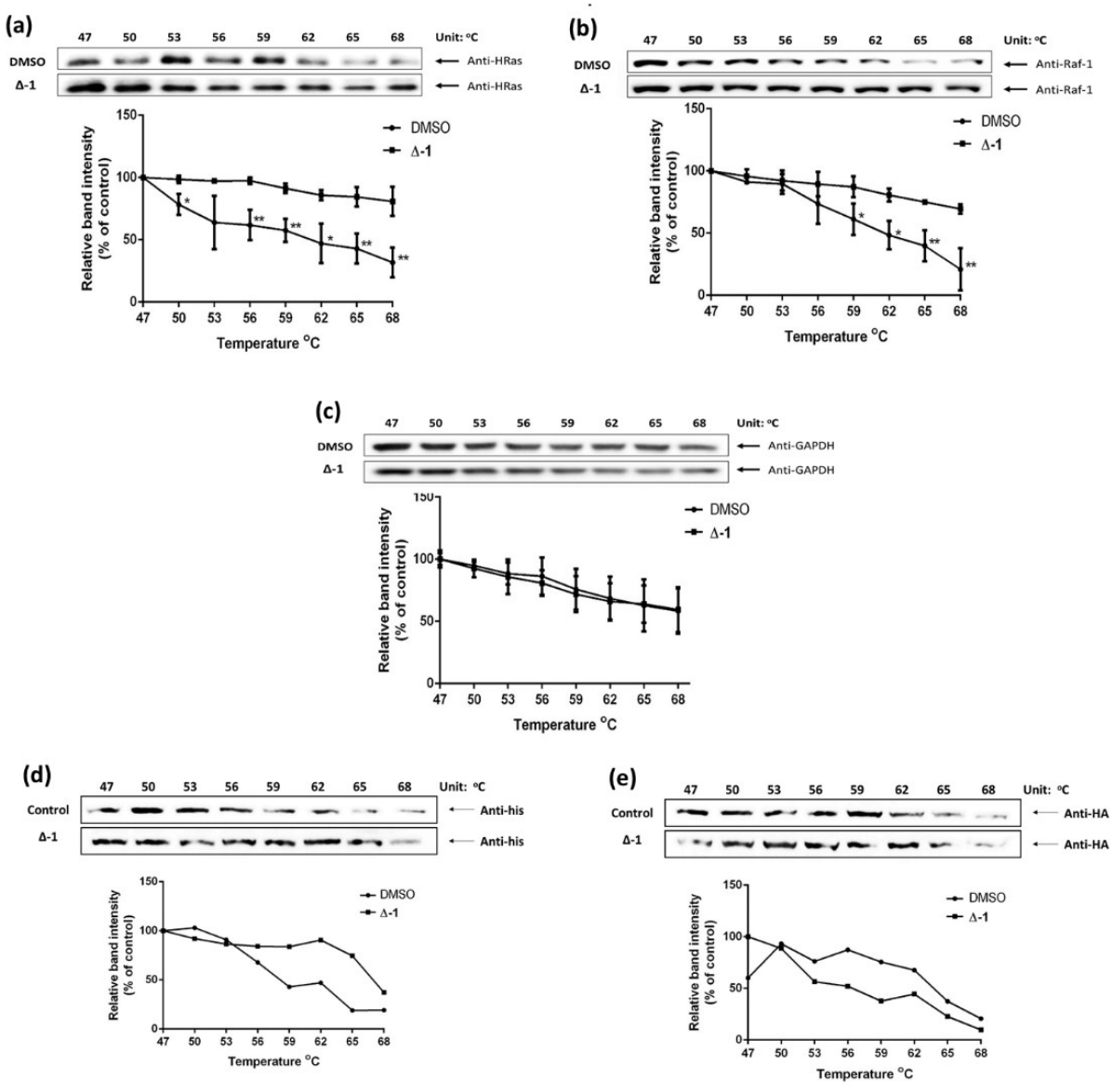

Fig. S9. Enantiomer Δ-**1** engages H-Ras and Raf-1 in cell lysates. Effect of enantiomer Δ-**1** on the thermal stability of H-Ras (a), Raf-1 (b), GAPDH (c), Raf-1-RBD-His (d) and Raf-1-CRD-HA (e) in A498 cell lysates. A498 cell lysates were treated with Δ-1 (5 μM) at room temperature for 30 min and then heated at different temperature ranging from 47 °C to 68 °C for 5 min. The protein samples were detected by Western blotting and analyzed by probing anti-Ras, anti-Raf-1 and anti-GAPDH. Significantly different from control at *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, ****$p < 0.0001$.

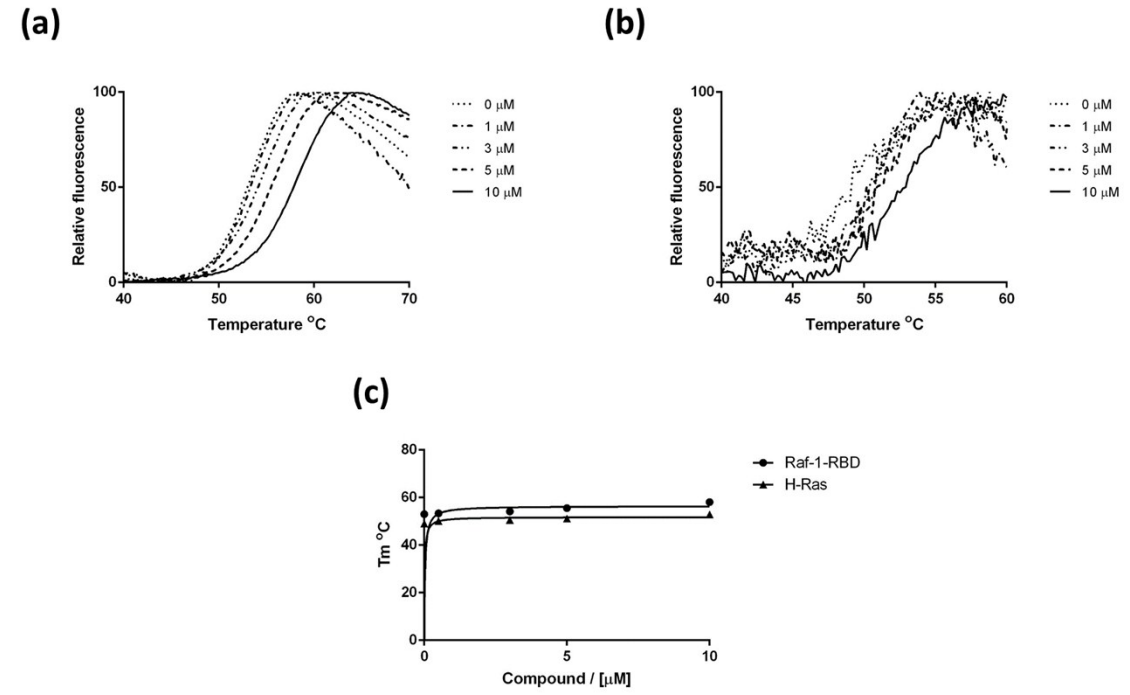

Fig. S10. Melt profile of (a) Raf-1-RBD (25 mg/L) and (b) H-Ras (25 mg/L) in the absence or presence of Δ-**1** (0-10 μM). Shift in the melt curve of Raf-1 RBD and H-Ras upon addition of 1-10 μM of Δ-**1** suggests a stabilization effect due to binding. (c) Concentration effect of Δ-**1** and proteins on T*m*. T*m* of 25 mg/L Raf-1-RBD in 0, 1, 3 5 and 10 μM of Δ-**1** was 53.0, 53.4, 54.2, 55.5, and 58.1°C, respectively. T*m* of 25 mg/L H-Ras in 0, 1, 3 5 and 10 μM of Δ-**1** was 49.0, 50.2, 50.6, 51.2, and 53.0°C, respectively. The K_d of Raf-1-RBD and H-Ras was 4.40 and 2.88 μM, respectively.

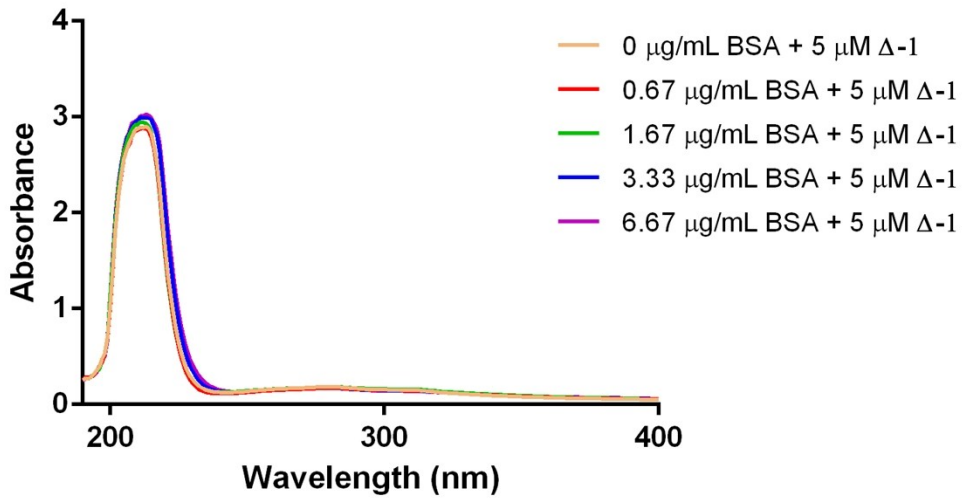

Fig. S11. UV/Vis absorption of Δ-**1** (5 μM) in the presence of the indicated concentrations of BSA from 0.67 - 6.67 μg/mL in PBS buffer.

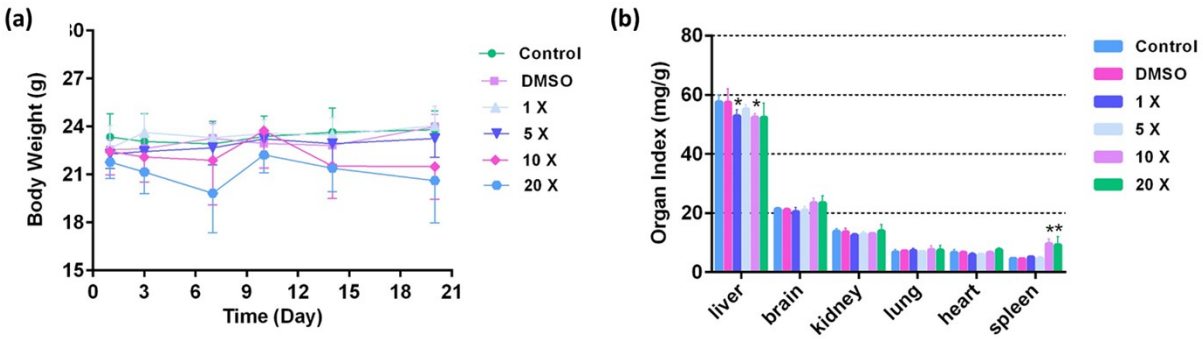

Fig. S12. Toxicity effect of racemic **1** at different concentrations (X = 14 mg/kg) on mice body weight (a) and organs (b). Significantly different from control at *p < 0.05.