Electronic Supplementary Material (ESI) for Dalton Transactions.

SUPPORTING INFORMATION

Iridium(III) Polypyridine Artificial Metalloenzymes with Tunable Photophysical Properties: a New Platform for Visible Light Photocatalysis in Aqueous Solution

Bingqing Liu, Yasmine S. Zubi, Jared C. Lewis*

*To whom correspondence should be addressed

Supplemental Figures and Tables

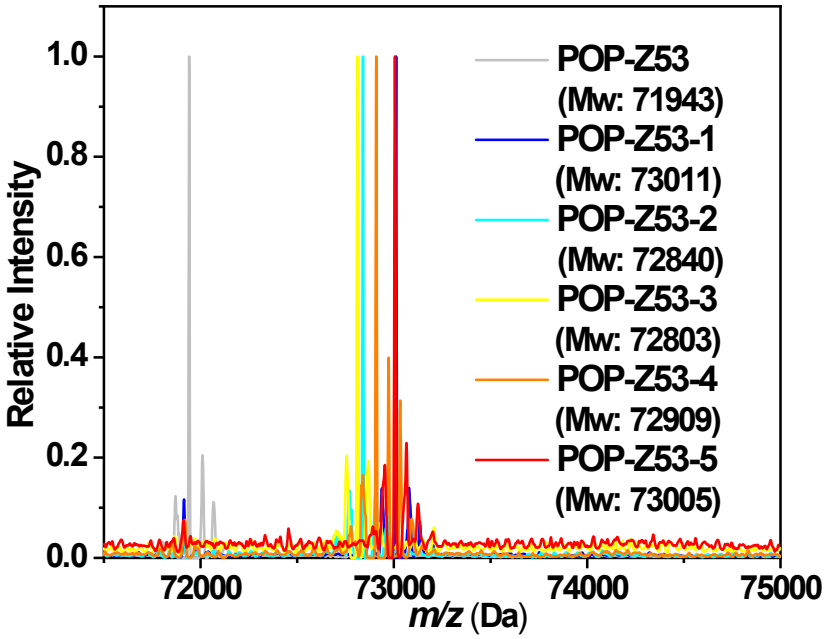


Figure S1. Intact ESI-MS characterization of apo (POP-Z53) before and after bioconjugation with the corresponding cofactor.

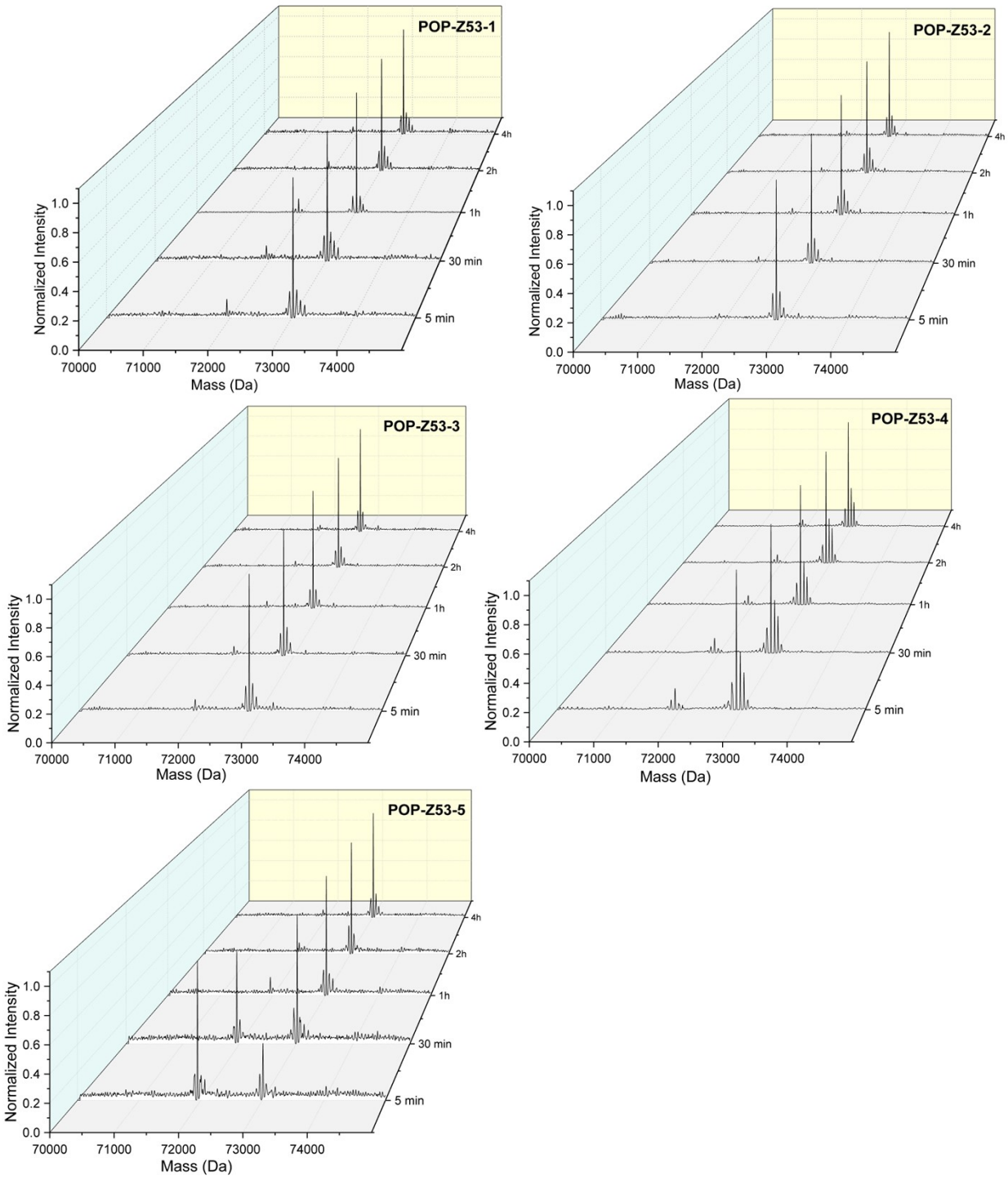


Figure S2. Time-course deconvoluted protein mass following bioconjugation between scaffold (POP-Z53) and different cofactors **1-5**.

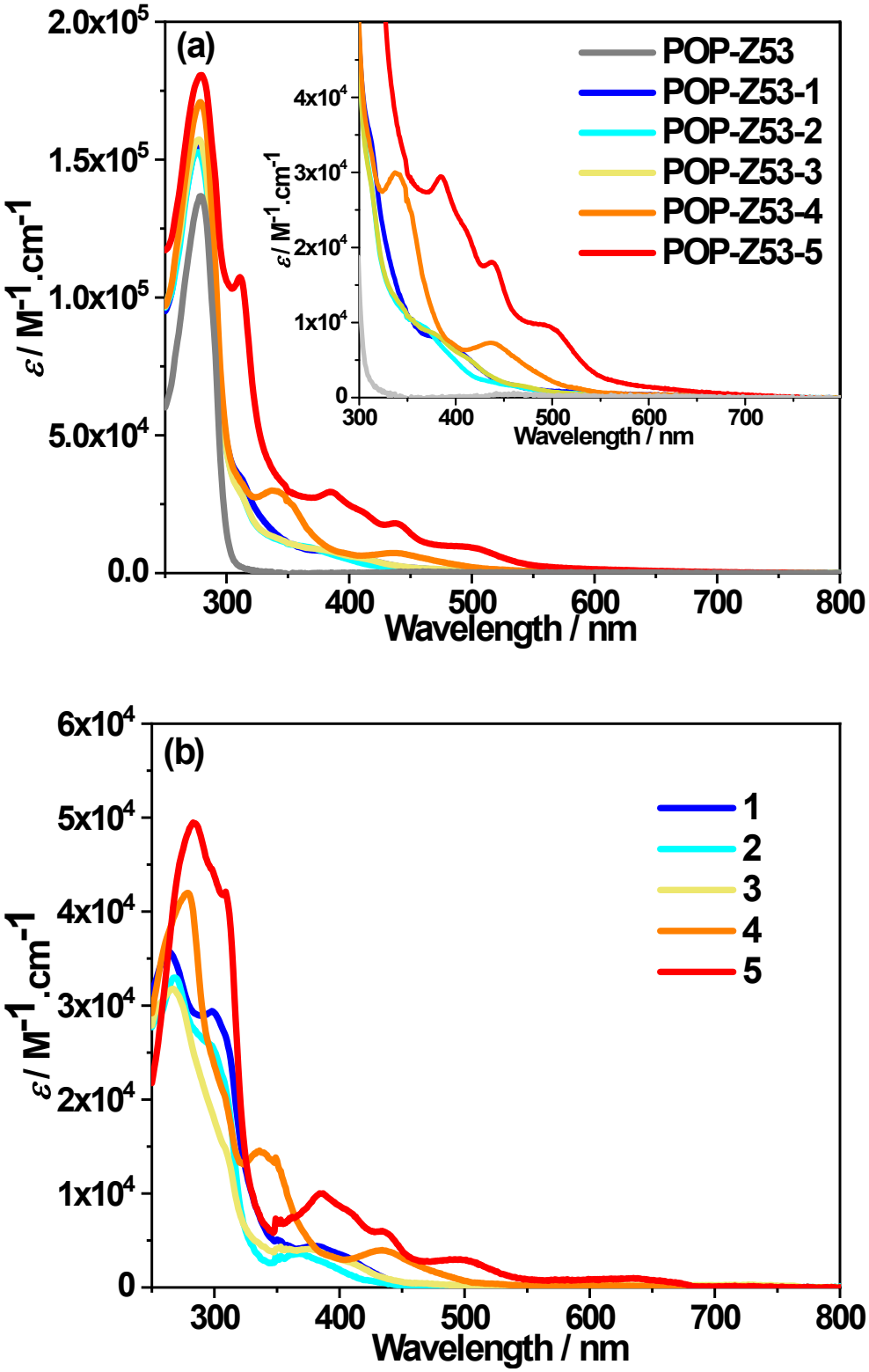


Figure S3. (a) UV-vis absorption spectra of POP-Z53 and **POP-Z53-1-5** in MQ H_2O and (b) **1-5** in 10% CH_3CN/MQ H_2O at room temperature.

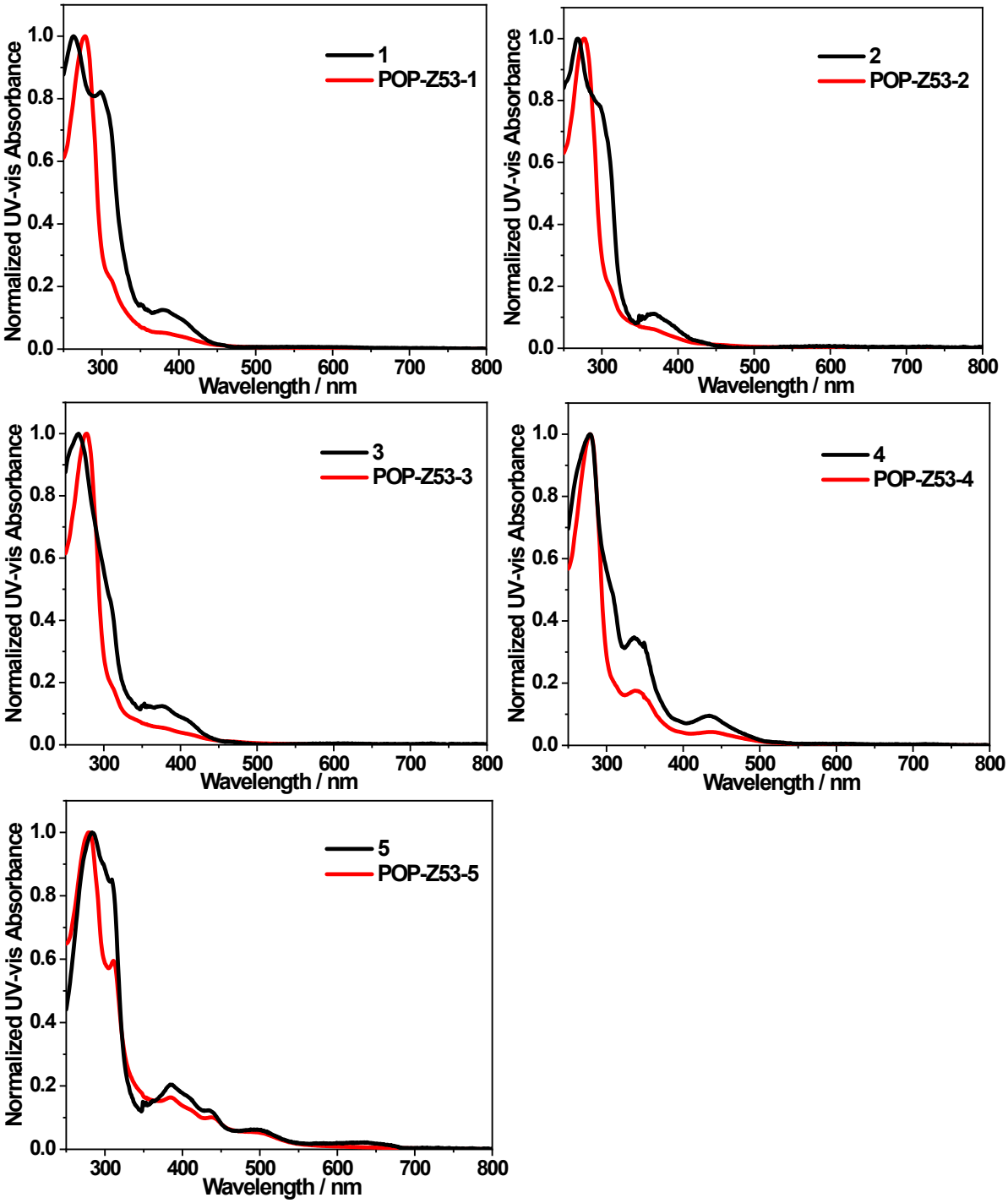


Figure S4. Normalized UV-vis absorption spectra of **1-5** (50 *u*M) in 10% CH_3CN/MQ H_2O and **POP-Z53-1-5** (50 *u*M) in MQ H_2O at room temperature.

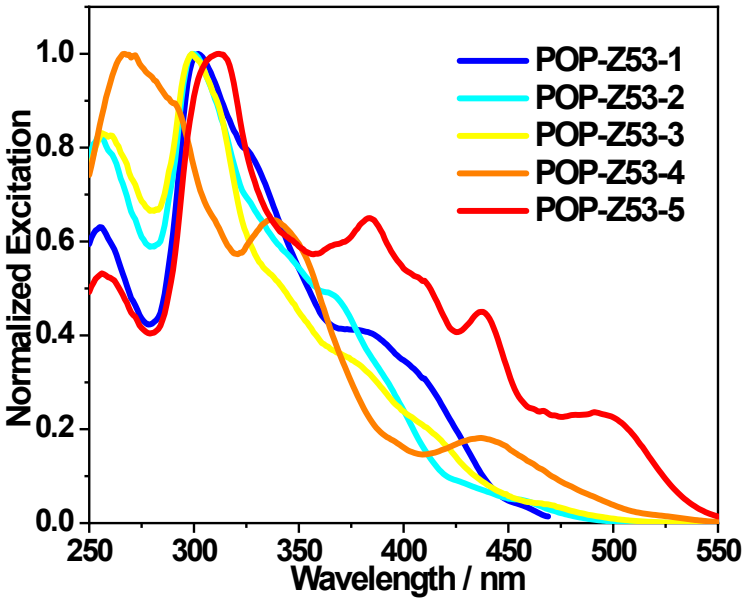


Figure S5. Normalized excitation spectra of **POP-Z53-1-5** (50 *u*M) in MQ H_2O at room temperature.

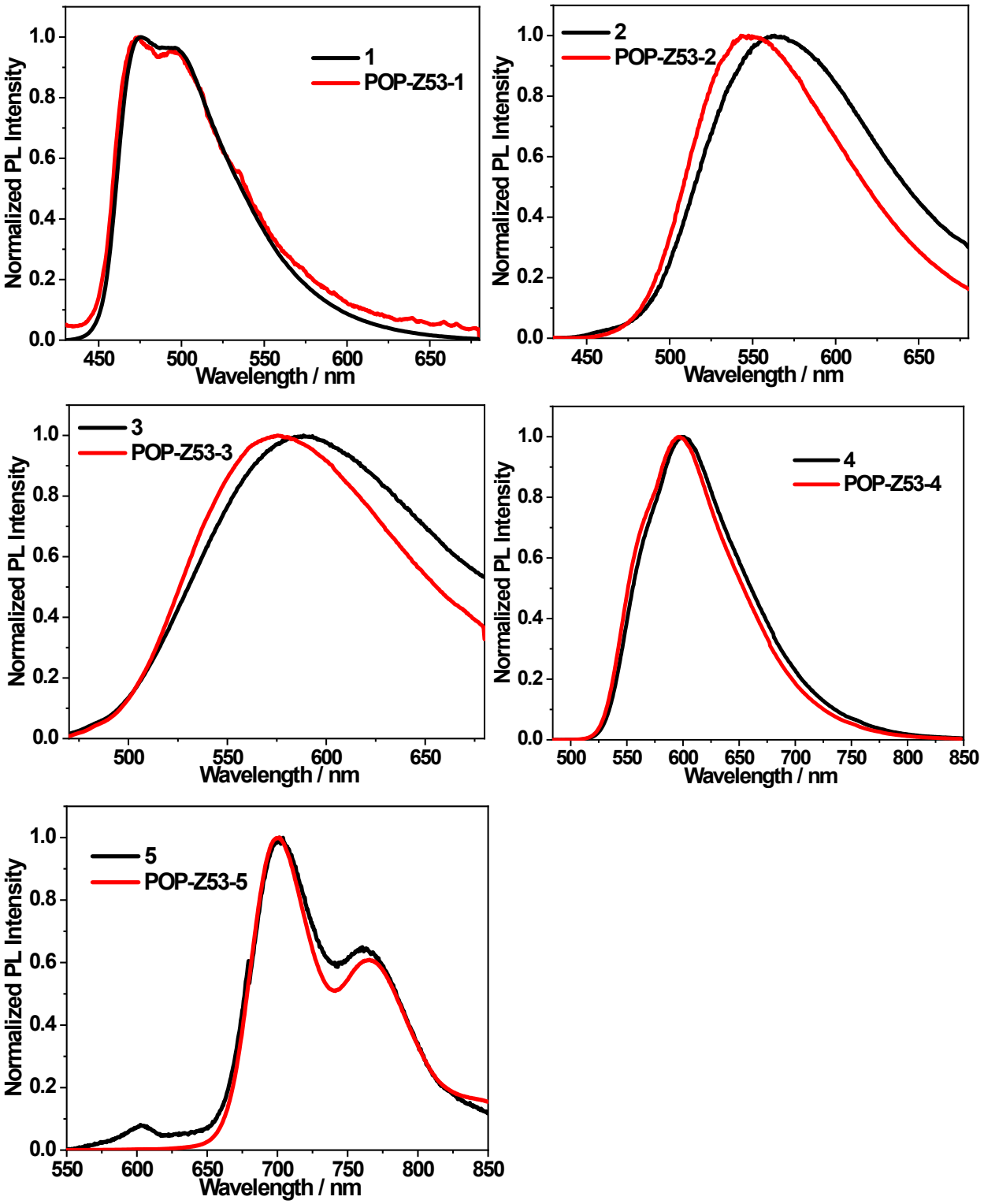


Figure S6. Normalized emission spectra of **1-5** (50 *u*M) in 10% CH_3CN/MQ H_2O and **POP-Z53-1-5** (50 *u*M) in MQ H_2O at room temperature.

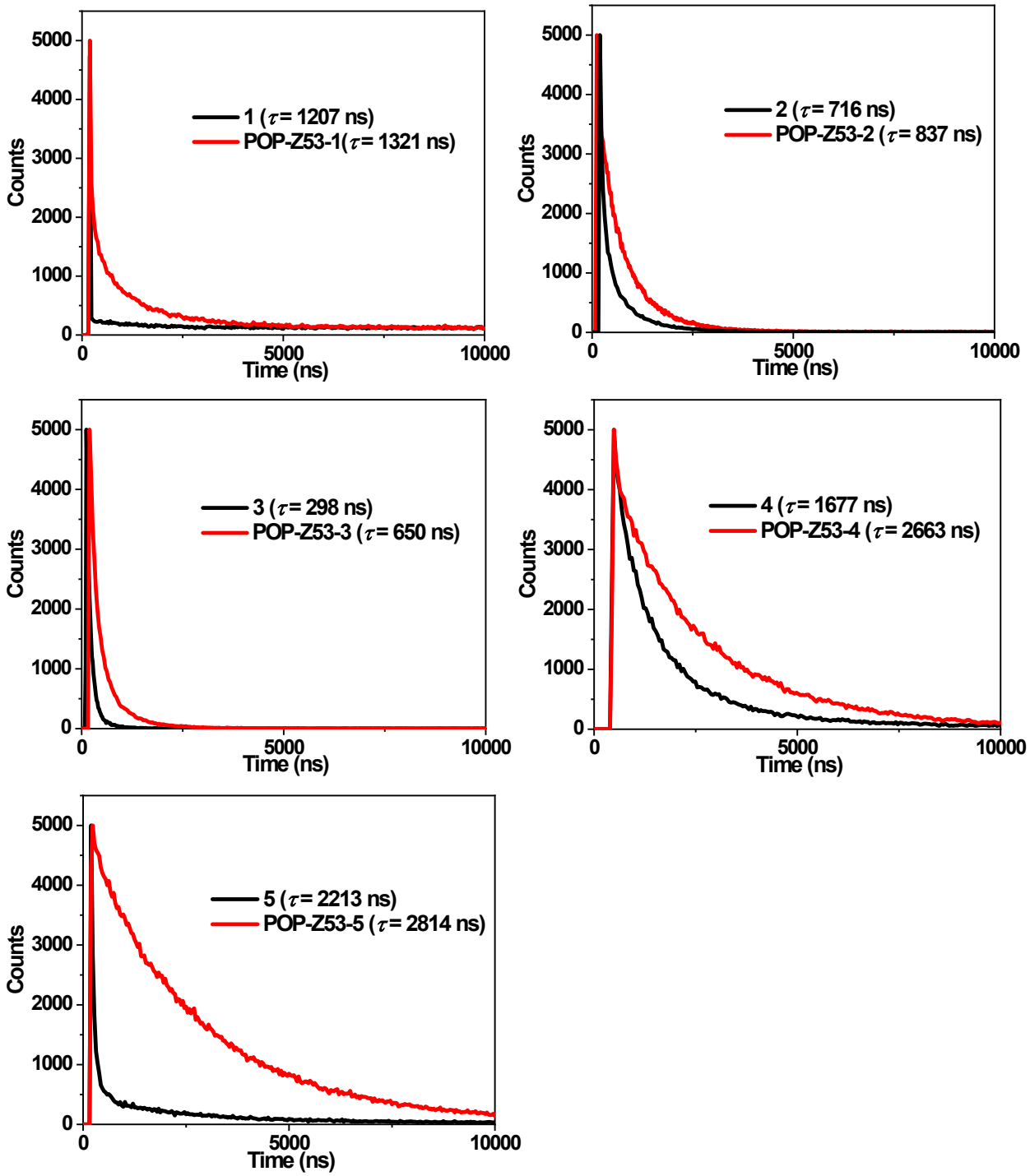


Figure S7. PL decay characteristics of **1-5** (50 *u*M) in 10% CH_3CN/MQ H_2O and **POP-Z53-1-5** (50 *u*M) in MQ H_2O at room temperature.

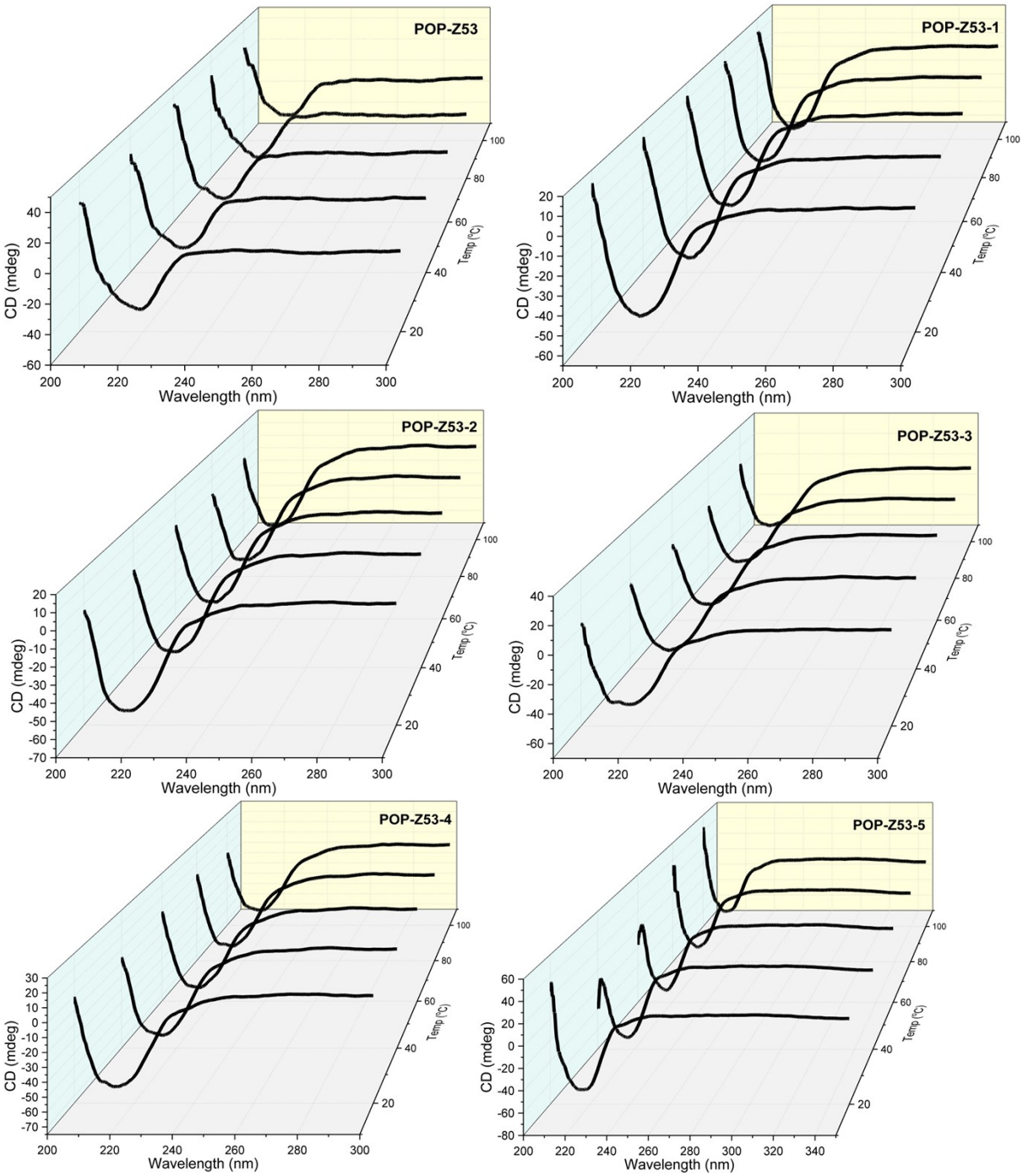


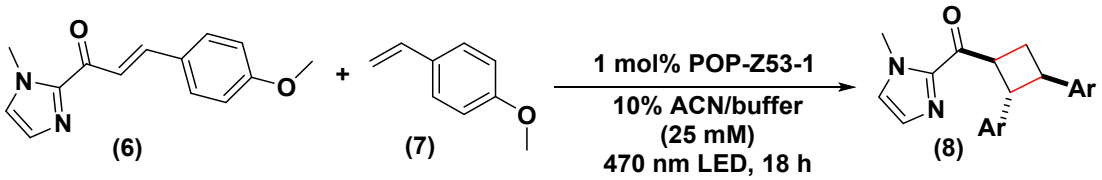
Figure S8. CD spectra of scaffold **POP-Z53** and **POP-Z53-1-5** in MQ H_2O (10 uM) were collected at different temperatures from 20 °C to 100 °C with 20 °C intervals.

	λ_{abs}/nm (log ε/$M^{-1}cm^{-1}$)	λ_{em} /nm (τ_{em}/ns)[a]	E_T (eV) [b]	Mw (Obs.)	Mw (Theor.)
1	263 (4.55), 299 (4.47), 380 (3.64)	473 (1207)	2.75	-	-
2	268 (4.52), 297 (4.41), 368 (3.55)	563 (716)	2.51	-	-
3	267 (4.50), 308 (4.18), 378 (3.61)	588 (298)	2.50	-	-
4	279 (4.62), 337 (4.16), 435 (3.59)	597 (1677)	2.32	-	-
5	284 (4.70), 308 (4.62), 385 (4.00), 435 (3.77), 496 (3.48)	702 (2213)	1.87	-	-
POP-Z53-**1**	277 (5.19), 312 (4.54), 380 (3.93)	476 (1321)	2.77	73011	73014
POP-Z53-**2**	277 (5.18), 310 (4.49), 368 (3.99)	548 (837)	2.56	72840	72842
POP-Z53-**3**	277 (5.19), 312 (4.46), 380 (3.94)	575 (650)	2.50	72803	72806
POP-Z53-**4**	279 (5.23), 308 (4.56), 339 (4.47), 436 (3.85)	600 (2663)	2.33	72909	72910
POP-Z53-**5**	279 (5.26), 311 (5.03), 385 (4.48), 437 (4.26), 503 (3.99)	700 (2814)	1.89	73005	73006

Table S1. Photophysical properties and deconvoluted mass data of Ir(III) ArMs. [a] Emission band maxima (λ_{em}) and lifetime (τ_{em}) in either deaerated aqueous solution (POP-Z53-**1-5**, 50 *u*M) or 10% ACN (**1-5**, 50 *u*M) at room temperature. [b] The triplet excited state energy is calculated from the short-wavelength range at 10% of the maximum intensity of the emission spectrum at room temperature.

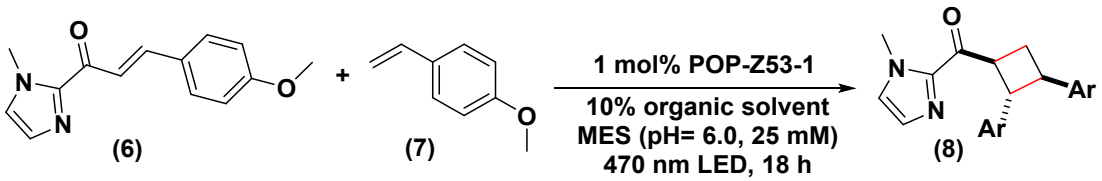
	K_m (uM)	V_{max}
POP-Z53	189 ± 9	2.33 ± 0.07
POP-Z53 + 10*inhibitor	144 ± 30	0.72 ± 0.12
POP-Z53 + 100*inhibitor	286 ± 61	0.46 ± 0.04
POP-Z53-**2**	448 ± 53	0.92 ± 0.06
POP-Z53-**2** + 10*inhibitor	353 ± 100	0.50 ± 0.06
POP-Z53-**2** + 100*inhibitor	434 ± 24	0.42 ± 0.02
POP-Z53-**5**	710 ± 209	1.07 ± 0.19
POP-Z53-**5** + 10*inhibitor	76 ± 11	0.22 ± 0.01
POP-Z53-**5** + 100*inhibitor	57 ± 6	0.14 ± 0.01

Table S2. Steady-state kinetic parameters for POP-Z53 and selected Ir(III) ArMs



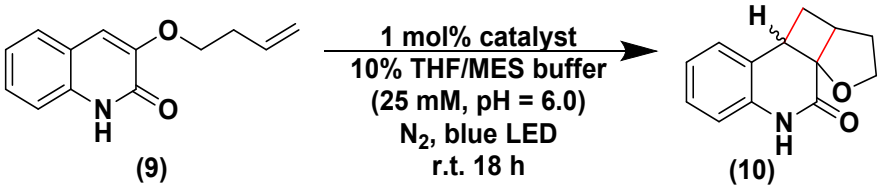
Entry	Buffer	[a]Pdt (%)	[a]d.r.
1	Water	34 ± 0	6.6 ± 0.6
2	MES (pH = 5.5)	30 ± 2	9.0 ± 0.5
3	MES (pH = 6.0)	36 ± 2	9.1 ± 0.2
4	MES (pH = 6.5)	32 ± 0	8.8 ± 0.5
5	Tris (pH = 7.4)	32 ± 0	8.3 ± 0.2
6	Tricine (pH = 8.5)	33 ± 0	6.1 ± 0.4
7	CHES (pH = 9.4)	34 ± 2	4.3 ± 0.2

Table S3. [a]Irradiated for 18 hours and performed in triplicate. Reactions were performed in triplicate (n=3) unless otherwise noted. Reaction conditions were as follows: 1 mM imidazole substrate **6**, 10 mM 4-methoxstyrene **7**, 10 uM catalyst, and 10% ACN (v/v) in a total of 100 µL. Reactions were performed under anaerobic conditions in the wetbox using glass vials. After irradiation, 100 µL of the internal standard (10 mM) 1,3,5-trimethoxybenzene (TMB) in ACN was added to the reactions. Samples were centrifuged to remove precipitation and then filtered through a 0.2 µm filter plate prior to analysis by achiral UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **8** and TMB.



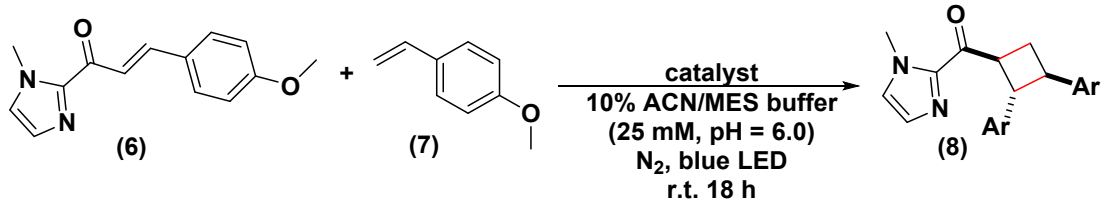
Entry	Solvent	[a]Pdt (%)	[a]d.r.
1	ACN	36 ± 2	9.1 ± 0.2
2	THF	38 ± 3	6.4 ± 0.3
3	DMSO	27 ± 2	5.2 ± 0.3
4	DMF	34 ± 1	5.9 ± 0.1
5	MeOH	19 ± 0	6.8 ± 0.2
6	Acetone	18 ± 4	7.8 ± 0.3
7	Dioxane	32 ± 4	7.4 ± 0.4

Table S4. [a]Irradiated for 18 hours and performed in triplicate. Reactions were performed in triplicate (n=3) unless otherwise noted. Reaction conditions were as follows: 1 mM imidazole substrate **6**, 10 mM 4-methoxstyrene **7**, 10 uM catalyst, and 10% organic solvent/MES buffer (pH = 6.0, 25 mM) (v/v) in a total of 100 µL. Reactions were performed under anaerobic conditions in the wetbox using glass vials. After irradiation, 100 µL of the internal standard (10 mM) 1,3,5-trimethoxybenzene (TMB) in ACN was added to the reactions. Samples were centrifuged to remove precipitation and then filtered through a 0.2 µm filter plate prior to analysis by achiral UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **8** and TMB.



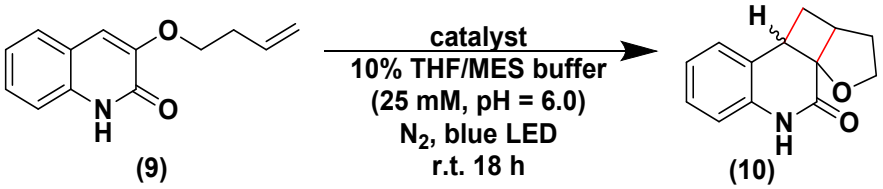
Cat.	Yield (%)[a]	Cat.	Yield (%)[a]
1	85 ± 4	POP-Z53-**1**	91 ± 2
2	60 ± 3	POP-Z53-**2**	64 ± 2
3	33 ± 4	POP-Z53-**3**	30 ± 2
4	10 ± 2	POP-Z53-**4**	10 ± 1
5	7 ± 0	POP-Z53-**5**	8 ± 0

Table S5. [a] Irradiated for 18 hours at room temperature and performed in triplicate. Reactions were performed in triplicate (n=3) unless otherwise noted. Reaction conditions were as follows: 1 mM 3-(3-buten-1-yloxy)-2(1H)-quinolinone **9**, 10 uM catalyst, and 10% THF/MES buffer (pH = 6.0, 25 mM) (v/v) in a total of 100 µL. Reactions were performed under anaerobic conditions in the wetbox using glass vials. After irradiation, 100 µL of the internal standard (10 mM) phenol in THF was added to the reactions. Samples were centrifuged to remove precipitation and then filtered through a 0.2 µm filter plate prior to analysis by achiral UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **9** and phenol.



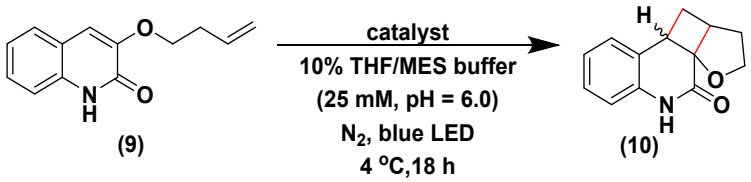
Cat.	Yield (%)	Cat.	Yield (%)
1 mol% **4**	94 ± 1	1 mol% POP-Z53-**4**	94 ± 2
0.5 mol% **4**	92 ± 5	0.5 mol% POP-Z53-**4**	91 ± 1
0.2 mol% **4**	84 ± 2	0.2 mol% POP-Z53-**4**	90 ± 3
0.1 mol% **4**	66 ± 3	0.1 mol% POP-Z53-**4**	89 ± 2

Table S6. Different loading of **1** and POP-Z53-**1** for intermolecular [2+2] photocycloaddition. [a]Irradiated for 18 hours and performed in triplicate. Reactions were performed in triplicate (n=3) unless otherwise noted. Reaction conditions were as follows: 1 mM imidazole substrate **6**, 10 mM 4-methoxstyrene **7**, 10 uM catalyst, and 10% organic solvent/MES buffer (pH = 6.0, 25 mM) (v/v) in a total of 100 µL. Reactions were performed under anaerobic conditions in the wetbox using glass vials. After irradiation, 100 µL of the internal standard (10 mM) 1,3,5-trimethoxybenzene (TMB) in ACN was added to the reactions. Samples were centrifuged to remove precipitation and then filtered through a 0.2 µm filter plate prior to analysis by achiral UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **8** and TMB.



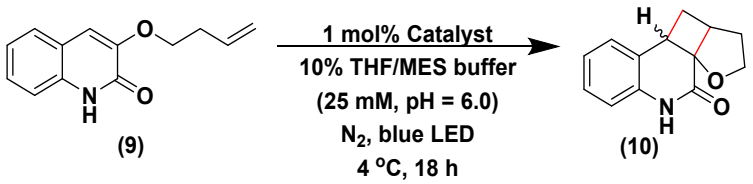
Cat.	Yield (%)	Cat.	Yield (%)
1 mol% **1**	85 ± 4	1 mol% POP-Z53-**1**	91 ± 2
0.5 mol% **1**	87 ± 1	0.5 mol% POP-Z53-**1**	89 ± 2
0.2 mol% **1**	24 ± 4	0.2 mol% POP-Z53-**1**	20 ± 2
0.1 mol% **1**	17 ± 2	0.1 mol% POP-Z53-**1**	18 ± 1

Table S7. Different loading of **1** and POP-Z53-**1** Intramolecular [2+2] photocycloaddition. Irradiated for 18 hours at room temperature and performed in triplicate unless otherwise noted. Reaction conditions were as follows: 1 mM 3-(3-buten-1-yloxy)-2(1H)-quinolinone **9**, 10 uM catalyst, and 10% THF/MES buffer (pH = 6.0, 25 mM) (v/v) in a total of 100 µL. Reactions were performed under anaerobic conditions in the wetbox using glass vials. After irradiation, 100 µL of the internal standard (10 mM) phenol in THF was added to the reactions. Samples were centrifuged to remove precipitation and then filtered through a 0.2 µm filter plate prior to analysis by achiral UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **9** and phenol.



Cat.	Yield (%)	e.e.	Cat.	Yield (%)	e.e.
1 mol% Δ-**2**	55 ± 1	-0.1 ± 0.1	1 mol% POP-Z53-Δ-**2**	79 ± 4	14.5 ± 0.5
0.5 mol% Δ-**2**	26 ± 3	0.1 ± 0.2	0.5 mol% POP-Z53-Δ-**2**	77 ± 2	13.4 ± 1.7
0.2 mol% Δ-**2**	17 ± 2	0 ± 0.2	0.2 mol% POP-Z53-Δ-**2**	47 ± 4	11.4 ± 1.2
0.1 mol% Δ-**2**	N.D.	N.D.	0.1 mol% POP-Z53-Δ-**2**	40 ± 2	12.3 ± 3.4

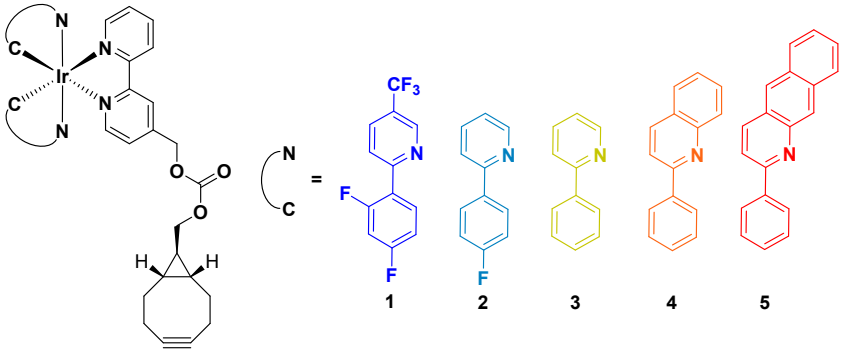
Table S8. Different loading of Δ-**2** and POP-Z53-Δ-**2** Intramolecular [2+2] photocycloaddition. Irradiated for 18 hours at 4 °C and performed in triplicate unless otherwise noted. Reaction conditions were as follows: 1 mM 3-(3-buten-1-yloxy)-2(1H)-quinolinone **9**, 10 uM catalyst, and 10% THF/MES buffer (pH = 6.0, 25 mM) (v/v) in a total of 100 µL. Reactions were performed under anaerobic conditions in the wetbox using glass vials. After irradiation, 100 µL of the internal standard (10 mM) phenol in THF was added to the reactions. Samples were centrifuged to remove precipitation and then filtered through a 0.2 µm filter plate prior to analysis by achiral UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **9** and phenol.



Cat.	Yield (%)	e.e.
POP-53-Δ-**2**	79 ± 4	14.5 ± 0.5
POP-99-Δ-**2**	60 ± 4	-2.5 ± 1
POP-326-Δ-**2**	51 ± 5	-4 ± 0.5
POP-338-Δ-**2**	47 ± 4	-2 ± 0.6

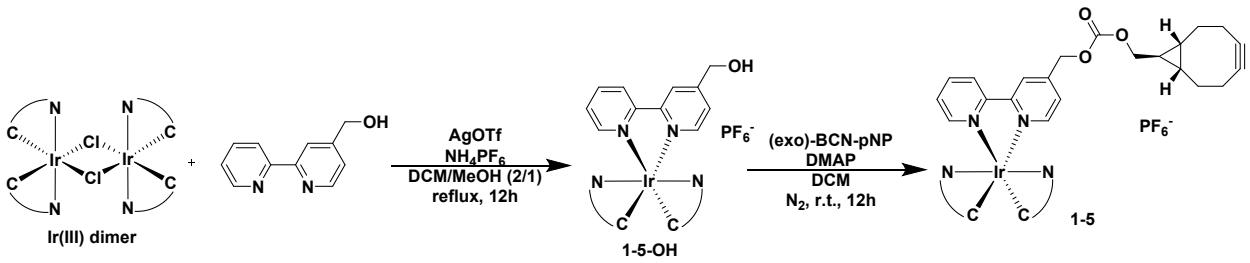
Table S9. Different active site mutations of POP scaffold. Irradiated for 18 hours at room temperature and performed in triplicate unless otherwise noted. Reaction conditions were as follows: 1 mM 3-(3-buten-1-yloxy)-2(1H)-quinolinone **9**, 10 uM catalyst, and 10% THF/MES buffer (pH = 6.0, 25 mM) (v/v) in a total of 100 µL. Reactions were performed under anaerobic conditions in the wetbox using glass vials. After irradiation, 100 µL of the internal standard (10 mM) phenol in THF was added to the reactions. Samples were centrifuged to remove precipitation and then filtered through a 0.2 µm filter plate prior to analysis by achiral UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **9** and phenol.

Synthetic Procedures



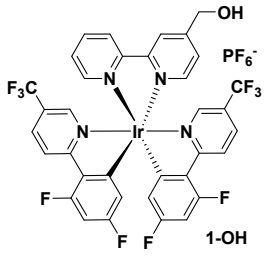
All solvents and reagents were obtained from Fisher Scientific Co. and used as received unless otherwise mentioned. Silica gel (60 Å, 230−400 mesh) and neutral alumina gel (activated, neutral, Brockmann I) for column chromatography were obtained from Silicycle Inc and Fisher Scientific Co., respectively. ((1R,8S,9R)-Bicyclo[6.1.0]non-4-yn-9-yl)methyl 4-nitrophenyl carbonate (exo-BCN-pNP) was purchased from Conju-Probe LLC. The commercially available cyclometalling ligands, namely 2-(2,4-difluorophenyl)-5-(trifluoromethyl)-pyridine, 2-(4-fluorophenyl)-pyridine, 2-phenylpyridine, and 2-phenylquinoline were purchased from TCI America, Inc.. [2,2'-bipyridine]-4-methanol (bpy-OH),[1] 2-phenyl-benzo[g]quinoline,[2] the corresponding Ir(III) dimers,[3,4,5,6,7] C-cinnamoyl imidazole (**6**),[8] and 3-(3-buten-1-yloxy)-2(1H)-quinolinone (**9**)[9] were

synthesized following the previously reported literature, respectively. ^{1}H NMR spectra were recorded at room temperature on a Varian I500 (500 MHz) or I400 (400 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Varian I500 (125 MHz) spectrometer with complete proton decoupling. ESI mass spectra were obtained using Agilent Technologies 1290/6135B quadrupole LC-MS.

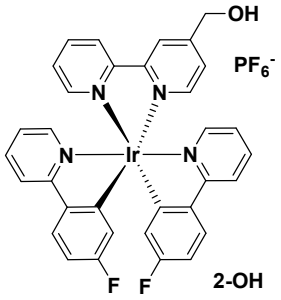


*Synthesis of racemic **1-5-OH***:

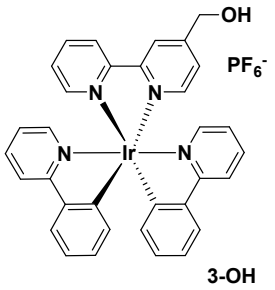
A mixture of corresponding Ir dimer (0.1 mmol), bpy-OH (0.2 mmol) and AgOTf (51.4 mg, 0.2 mmol) in DCM/MeOH (*v*/*v*: 2/1, 30 mL) was heated to reflux under N_2 atmosphere. After 12h, NH_4PF_6 (500 mg, 1 mmol) was added, and the obtained suspension was stirred for another 1h at room temperature. After removal of the solvent, the residue was purified by column chromatography on silica gel eluting with DCM/MeOH (100/1) to afford the target product.



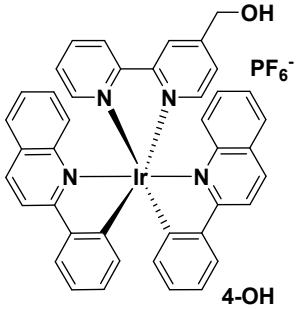
1-OH, yellow powder (114 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (d, J = 8.2 Hz, 1H), 8.71 (s, 1H), 8.48 (dt, J = 9.2, 4.8 Hz, 2H), 8.27 (td, J = 7.9, 1.6 Hz, 1H), 8.08 – 7.99 (m, 2H), 7.95 (dd, J = 5.5, 1.5 Hz, 1H), 7.85 (d, J = 5.7 Hz, 1H), 7.67 – 7.53 (m, 3H), 7.49 (d, J = 1.9 Hz, 1H), 6.72 – 6.58 (m, 2H), 5.64 (ddd, J = 10.3, 8.0, 2.3 Hz, 2H), 4.94 (s, 2H). ESI-MS: Calcd. $[C_{35}H_{20}F_{10}IrN_4O]^+$ for 895.11, found 895.12.



2-OH, yellow powder (106 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64 (d, J = 10.8 Hz, 2H), 8.11 (t, J = 7.9 Hz, 1H), 7.96 (d, J = 5.4 Hz, 1H), 7.85 (dd, J = 12.7, 7.2 Hz, 3H), 7.77 (q, J = 7.1 Hz, 2H), 7.70 (ddd, J = 8.4, 5.4, 2.6 Hz, 2H), 7.50 (d, J = 5.9 Hz, 1H), 7.47 – 7.36 (m, 3H), 7.11 (t, J = 6.8 Hz, 1H), 7.06 – 6.97 (m, 1H), 6.83 – 6.71 (m, 2H), 5.91 (td, J = 8.9, 2.6 Hz, 2H), 5.00 – 4.86 (m, 2H). ESI-MS: Calcd. $[C_{33}H_{24}F_2IrN_4O]^+$ for 723.15, found 723.16.

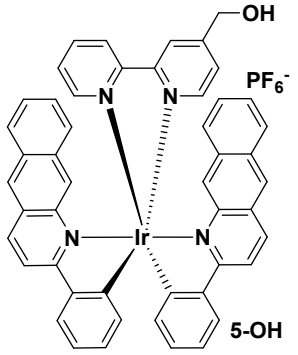


3-OH, yellow powder (98 mg, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 – 8.54 (m, 2H), 8.11 – 8.05 (m, 1H), 7.98 – 7.85 (m, 3H), 7.83 (d, J = 5.6 Hz, 1H), 7.75 (q, J = 7.2 Hz, 2H), 7.68 (d, J = 7.8 Hz, 2H), 7.54 (d, J = 5.7 Hz, 1H), 7.50 (d, J = 5.8 Hz, 1H), 7.41 – 7.33 (m, 2H), 7.10 (t, J = 6.3 Hz, 1H), 7.07 – 6.96 (m, 3H), 6.92 (ddd, J = 7.5, 6.2, 3.1 Hz, 2H), 6.30 (t, J = 7.0 Hz, 2H), 4.98 – 4.84 (m, 2H). ESI-MS: Calcd. $[C_{33}H_{26}IrN_4O]^+$ for 687.17, found 687.17.



4-OH, yellow powder (76 mg, 41%). ^{1}H NMR (500 MHz, Acetonitrile-d_3): δ 8.49 – 8.33 (m, 4H), 8.25 (ddd, J = 5.5, 1.6, 0.8 Hz, 1H), 8.22 – 8.11 (m, 4H), 8.11 – 8.06 (m, 1H), 7.96 (td, J = 7.9, 1.6 Hz, 1H), 7.87 (ddd, J = 8.1, 4.2, 1.5 Hz, 2H), 7.54 – 7.46 (m, 2H), 7.43 (ddd, J = 8.0, 6.9, 1.0 Hz,

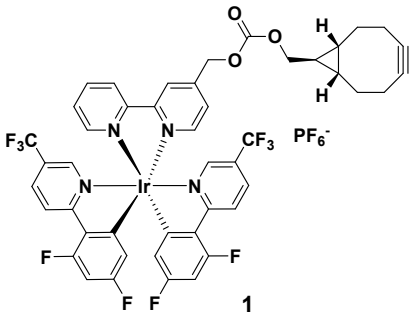
2H), 7.37 (ddd, J = 9.1, 5.0, 0.9 Hz, 2H), 7.21 (td, *J* = 7.6, 1.2 Hz, 2H), 7.08 (dddd, *J* = 8.7, 6.7, 5.0, 1.5 Hz, 2H), 6.85 (td, *J* = 7.4, 1.3 Hz, 2H), 6.57 (dt, *J* = 7.7, 1.4 Hz, 2H), 4.70 (d, *J* = 5.4 Hz, 2H). ESI-MS: Calcd. $[C_{41}H_{30}IrN_4O]^+$ for 787.20, found 787.20.



5-OH, red powder (146.6 mg, 71%). ^{1}H NMR (500 MHz, Acetonitrile-d_3): δ 8.52 (dd, *J* = 9.0, 4.9 Hz, 2H), 8.45 (d, *J* = 4.9 Hz, 2H), 8.42 – 8.40 (m, 1H), 8.36 – 8.30 (m, 3H), 8.21 (dt, *J* = 8.1, 1.5 Hz, 2H), 8.03 – 7.89 (m, 7H), 7.64 – 7.56 (m, 2H), 7.50 – 7.43 (m, 2H), 7.34 (tdd, *J* = 8.1, 6.7, 1.2 Hz, 2H), 7.19 (t, *J* = 7.5 Hz, 2H), 6.90 (dd, *J* = 8.6, 5.1 Hz, 2H), 6.80 (td, *J* = 7.4, 1.3 Hz, 2H), 6.66 (dt, *J* = 7.6, 1.7 Hz, 2H), 4.64 (s, 2H). ESI-MS: Calcd. $[C_{49}H_{34}IrN_4O]^+$ for 887.24, found 887.23.

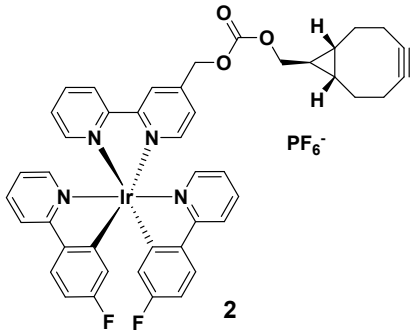
*Synthesis of **1-5***:

The corresponding **1-5-OH** (0.03 mmol), exo-BCN-pNP (10.4 mg, 0.033 mmol), and DMAP (36 mg, 0.3 mmol) were mixed in dry DCM (5 mL). The mixture was purged with nitrogen and stirred at r.t. in dark for 12h. After removal of the solvent, the residue was purified by column chromatography on neutral alumina gel eluting with DCM/MeOH (100/1) to afford the titled product.



1

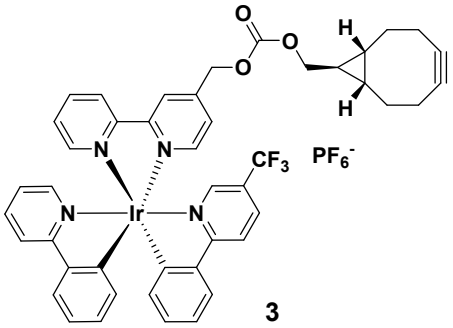
1, yellow powder (16.5 mg, 45%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.82 (d, J = 8.3 Hz, 1H), 8.71 (s, 1H), 8.49 (td, J = 8.6, 2.9 Hz, 2H), 8.30 (t, J = 8.0 Hz, 1H), 8.06 (d, J = 8.9 Hz, 2H), 7.96 (d, J = 5.5 Hz, 1H), 7.92 (d, J = 5.7 Hz, 1H), 7.63 (t, J = 6.6 Hz, 1H), 7.60 (d, J = 5.7 Hz, 1H), 7.57 (s, 1H), 7.50 (s, 1H), 6.71 – 6.60 (m, 2H), 5.63 (d, J = 7.8 Hz, 2H), 5.47 (s, 2H), 4.10 (d, J = 6.7 Hz, 2H), 2.41 (dd, J = 13.4, 3.1 Hz, 2H), 2.28 (t, J = 14.2 Hz, 2H), 2.18 – 2.12 (m, 2H), 1.39 (t, J = 11.4 Hz, 2H), 0.80 – 0.74 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.58, 155.34, 154.73, 150.36, 150.28, 144.98, 141.50, 136.90, 129.37, 126.69, 126.63, 124.28, 124.06, 123.90, 98.86, 73.50, 66.49, 33.25, 29.85, 23.24, 23.23, 23.16, 22.73, 21.62, 21.43. ESI-MS: Calcd. $[C_{46}H_{32}F_{10}IrN_4O_3]^+$ for 1071.19, found 1071.19.



2

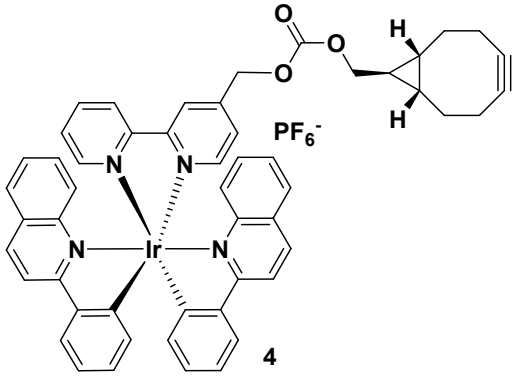
2, yellow powder (27 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.66 (d, J = 8.2 Hz, 1H), 8.56 (s, 1H), 8.20 – 8.09 (m, 1H), 7.95 (dd, J = 5.4, 1.6 Hz, 1H), 7.90 (d, J = 5.7 Hz, 1H), 7.85 (t, J = 6.9 Hz, 2H), 7.81 – 7.73 (m, 2H), 7.69 (ddd, J = 8.8, 5.5, 3.5 Hz, 2H), 7.51 (d, J = 5.7 Hz, 1H), 7.44 (dd, J = 11.8, 5.6 Hz, 3H), 7.13 – 7.07 (m, 1H), 7.07 – 7.00 (m, 1H), 6.78 (tt, J = 8.7, 2.3 Hz, 2H),

5.90 (dt, J = 9.2, 2.2 Hz, 2H), 5.43 (s, 2H), 4.09 (d, J = 6.8 Hz, 2H), 2.40 (dd, J = 13.3, 2.9 Hz, 2H), 2.31 – 2.23 (m, 2H), 2.19 – 2.11 (m, 2H), 1.37 (t, J = 11.5 Hz, 2H), 0.82 – 0.69 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.75, 166.52, 163.07, 155.91, 155.73, 154.88, 152.88, 150.27, 149.95, 149.04, 148.73, 140.13, 139.88, 139.75, 138.70, 138.66, 128.40, 126.87, 126.79, 126.02, 125.80, 123.82, 123.77, 123.59, 119.82, 119.72, 118.25, 118.11, 118.01, 110.47, 110.28, 110.25, 98.86, 73.38, 66.82, 33.27, 29.84, 23.24, 23.22, 21.44. ESI-MS: Calcd. $[C_{44}H_{36}F_2IrN_4O_3]^+$ for 899.24, found 899.23.



3

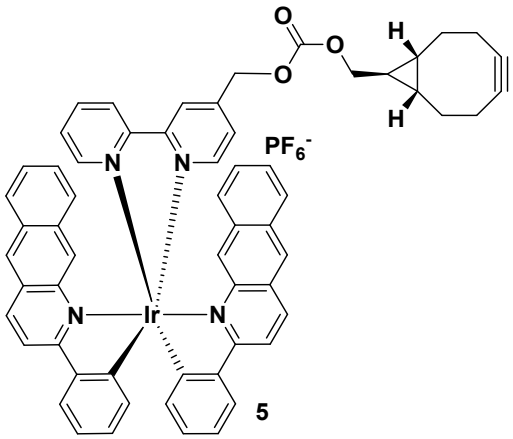
3, yellow powder (21 mg, 59%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.64 (d, J = 8.2 Hz, 1H), 8.54 (s, 1H), 8.16 – 8.07 (m, 1H), 7.99 – 7.83 (m, 4H), 7.75 (td, J = 7.6, 4.9 Hz, 2H), 7.71 – 7.62 (m, 2H), 7.55 (d, J = 5.9 Hz, 1H), 7.51 (d, J = 5.8 Hz, 1H), 7.41 (t, J = 7.0 Hz, 2H), 7.14 – 6.98 (m, 4H), 6.94 – 6.87 (m, 2H), 6.29 (d, J = 7.5 Hz, 2H), 5.42 (s, 2H), 4.09 (d, J = 6.6 Hz, 2H), 2.40 (d, J = 13.3 Hz, 2H), 2.27 (t, J = 14.2 Hz, 2H), 2.14 (d, J = 16.1 Hz, 2H), 1.36 (d, J = 11.2 Hz, 2H), 0.85 – 0.68 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.92, 156.00, 150.40, 149.47, 149.06, 143.69, 143.55, 139.77, 138.27, 131.93, 131.82, 131.02, 130.90, 128.25, 125.52, 124.87, 123.78, 123.55, 123.47, 122.83, 119.74, 119.63, 98.86, 73.36, 66.86, 33.26, 23.22, 21.44. ESI-MS: Calcd. $[C_{44}H_{38}IrN_4O_3]^+$ for 863.26, found 863.26.



4

4, orange powder (31 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (d, J = 8.2 Hz, 1H), 8.25 – 8.08 (m, 7H), 7.99 (dd, J = 8.1, 6.6 Hz, 3H), 7.71 (dd, J = 11.9, 8.1 Hz, 2H), 7.43 – 7.32 (m, 4H),

7.27 (s, 1H), 7.22 – 7.10 (m, 3H), 7.07 – 6.95 (m, 2H), 6.82 (t, J = 7.5 Hz, 2H), 6.61 – 6.46 (m, 2H), 5.32 (d, J = 17.9 Hz, 4H), 4.08 (d, J = 6.8 Hz, 2H), 2.40 (d, J = 13.4 Hz, 2H), 2.27 (t, J = 14.1 Hz, 2H), 2.15 (d, J = 16.0 Hz, 2H), 1.41 – 1.35 (m, 2H), 0.79 – 0.72 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.80, 155.30, 150.75, 149.41, 147.46, 147.35, 145.49, 145.44, 140.04, 139.93, 139.82, 134.74, 134.62, 131.42, 130.98, 129.09, 127.62, 127.52, 127.09, 126.89, 124.82, 124.64, 124.49, 123.07, 122.17, 117.29, 98.73, 73.24, 66.33, 33.13, 29.71, 23.11, 23.08, 21.31. ESI-MS: Calcd. $[C_{52}H_{42}IrN_4O_3]^+$ for 963.29, found 963.30.



5

5, red powder (31 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (dd, J = 15.2, 9.0 Hz, 2H), 8.35 – 8.28 (m, 4H), 8.19 – 8.13 (m, 3H), 8.08 – 7.99 (m, 4H), 7.92 – 7.88 (m, 2H), 7.82 (s, 1H), 7.71 (s, 1H), 7.61 – 7.53 (m, 2H), 7.46 – 7.40 (m, 2H), 7.38 – 7.30 (m, 2H), 7.19 – 7.12 (m, 2H), 6.85 – 6.75 (m, 4H), 6.68 – 6.62 (m, 1H), 6.60 (d, J = 7.7 Hz, 1H), 5.33 – 5.22 (m, 2H), 4.06 (d, J = 6.8 Hz, 2H), 2.36 (d, J = 13.6 Hz, 2H), 2.23 (t, J = 14.4 Hz, 2H), 2.14 – 2.07 (m, 2H), 1.32 (d, J = 16.3 Hz, 2H), 0.68 – 0.76 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.29, 171.25, 155.39, 154.68, 152.60, 149.71, 148.14, 148.01, 145.88, 145.83, 143.39, 143.18, 140.77, 140.67, 140.14, 134.89, 134.77, 134.63, 134.57, 131.67, 131.53, 131.48, 128.85, 128.42, 128.10, 128.07, 128.00, 127.94, 127.45, 127.25, 125.49, 125.41, 125.14, 124.89, 123.25, 123.14, 122.97, 122.04, 117.02, 98.83, 73.38, 66.36, 33.23, 29.85, 23.23, 23.19, 21.41. ESI-MS: Calcd. $[C_{60}H_{46}IrN_4O_3]^+$ for 1063.32, found 1063.32.

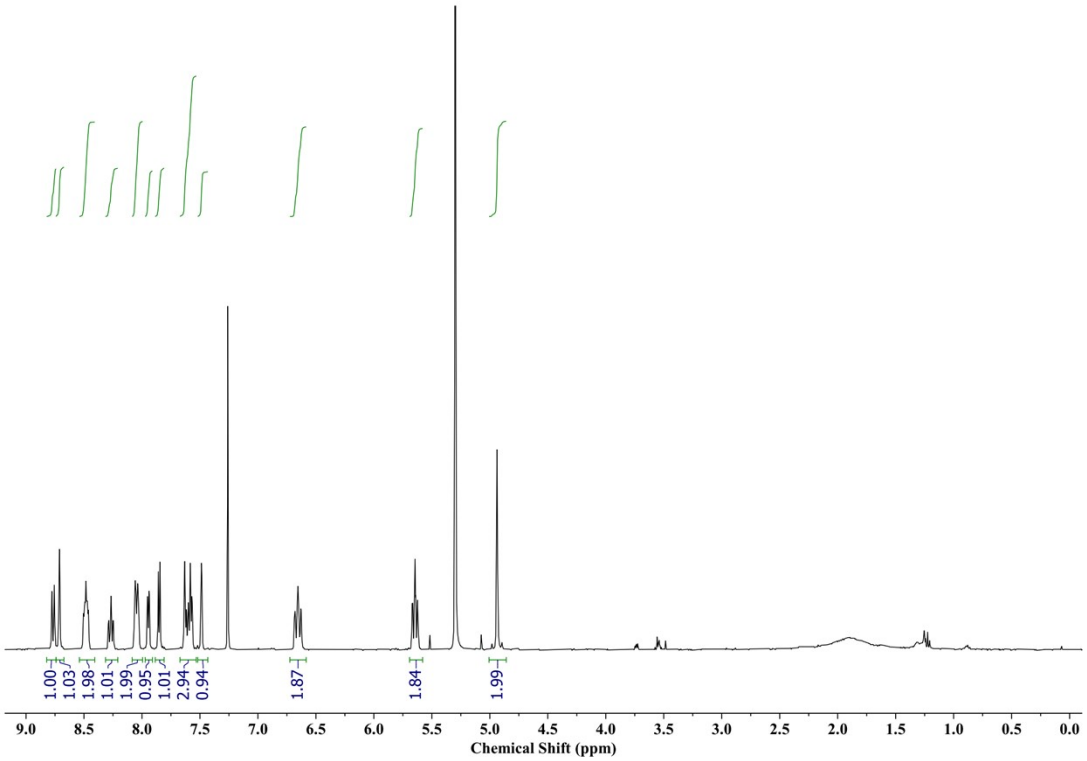


Figure S9. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) for **1-OH**.

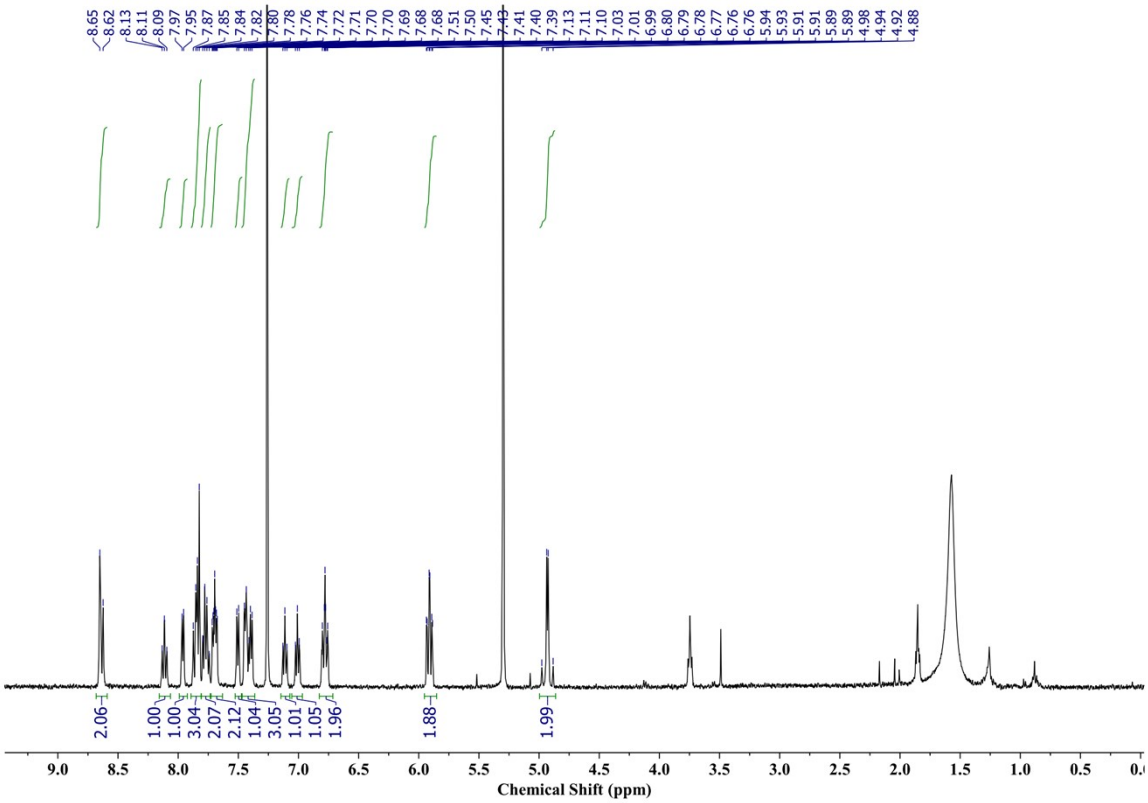


Figure S10. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) for **2-OH**.

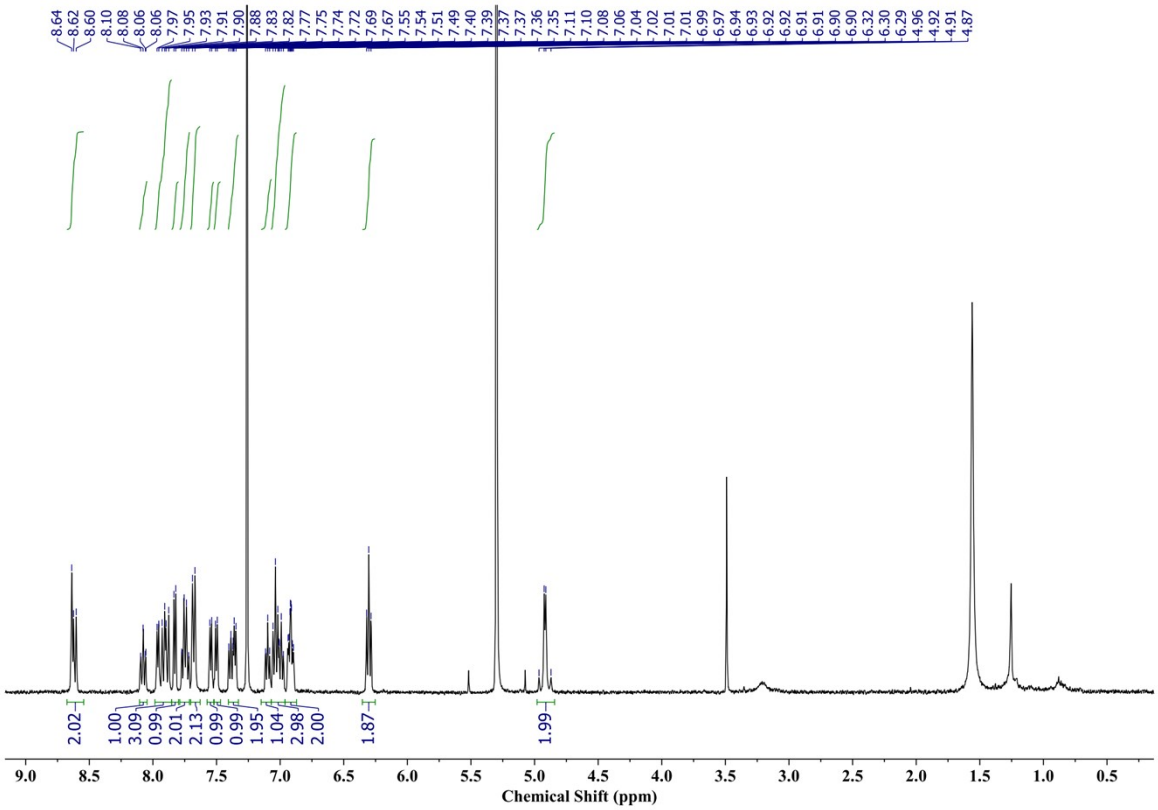


Figure S11. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) for **3-OH**.

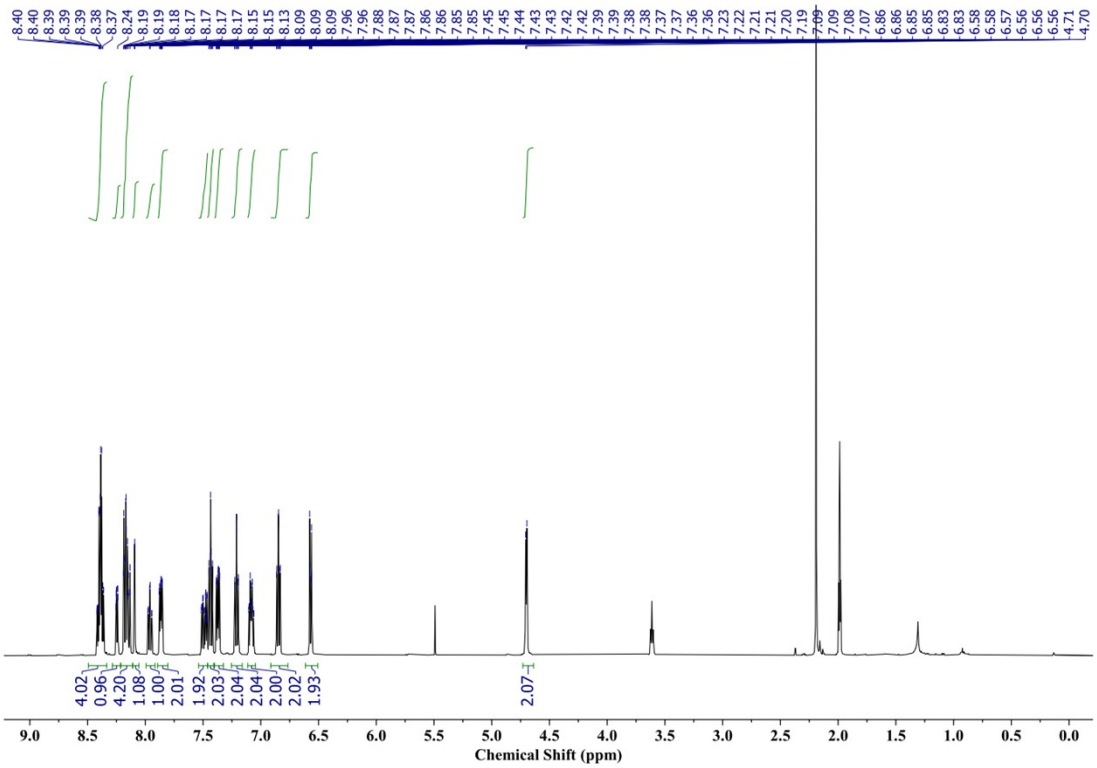


Figure S12. ^{1}H NMR spectrum (500 MHz, Acetonitrile-d_3) for **4-OH**.

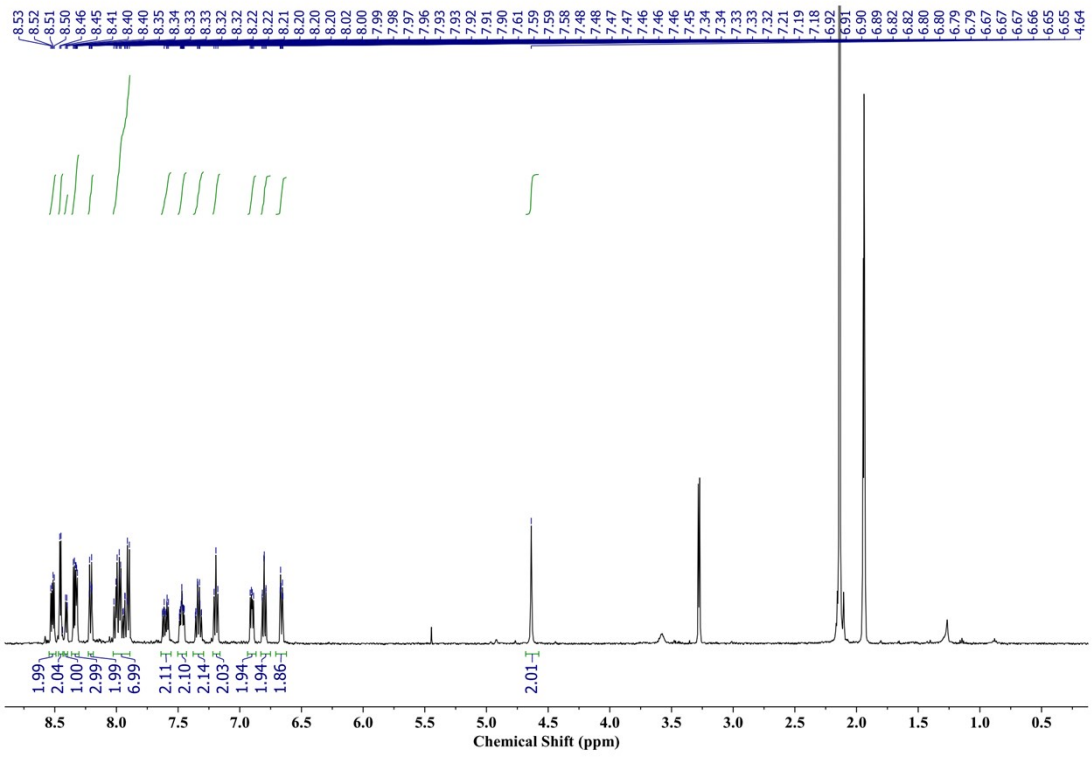


Figure S13. ^{1}H NMR spectrum (500 MHz, Acetonitrile-d_3) for **5-OH**.

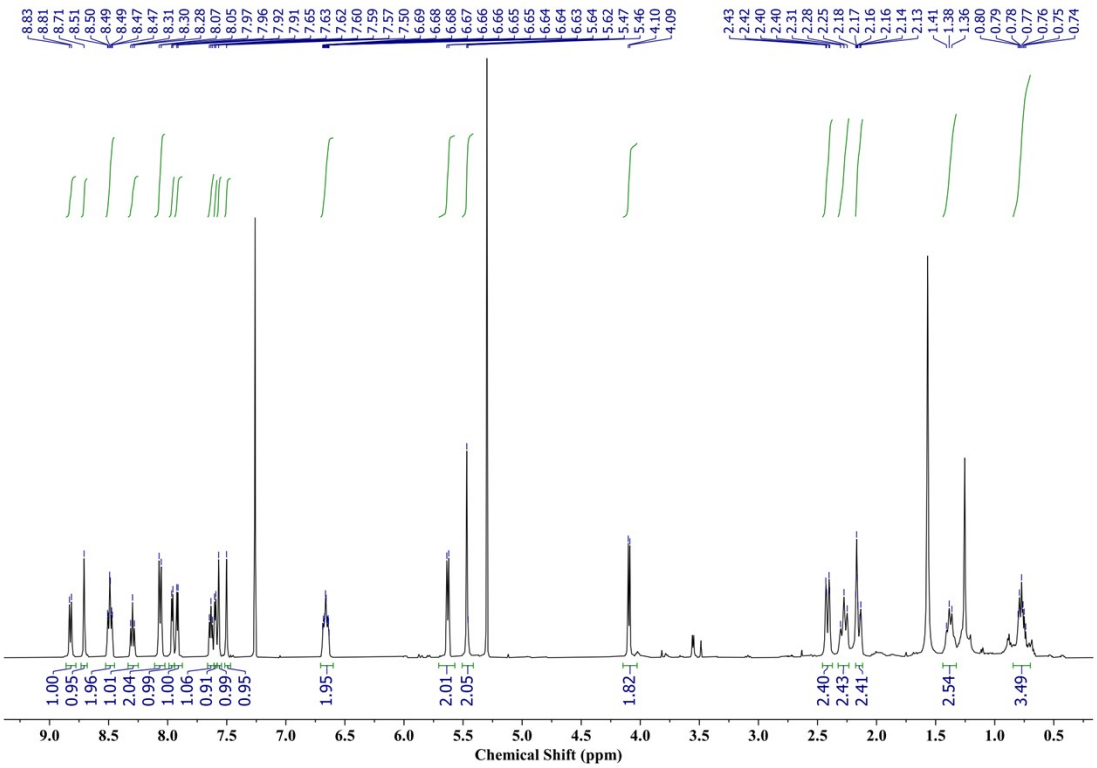


Figure S14. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) for **1**.

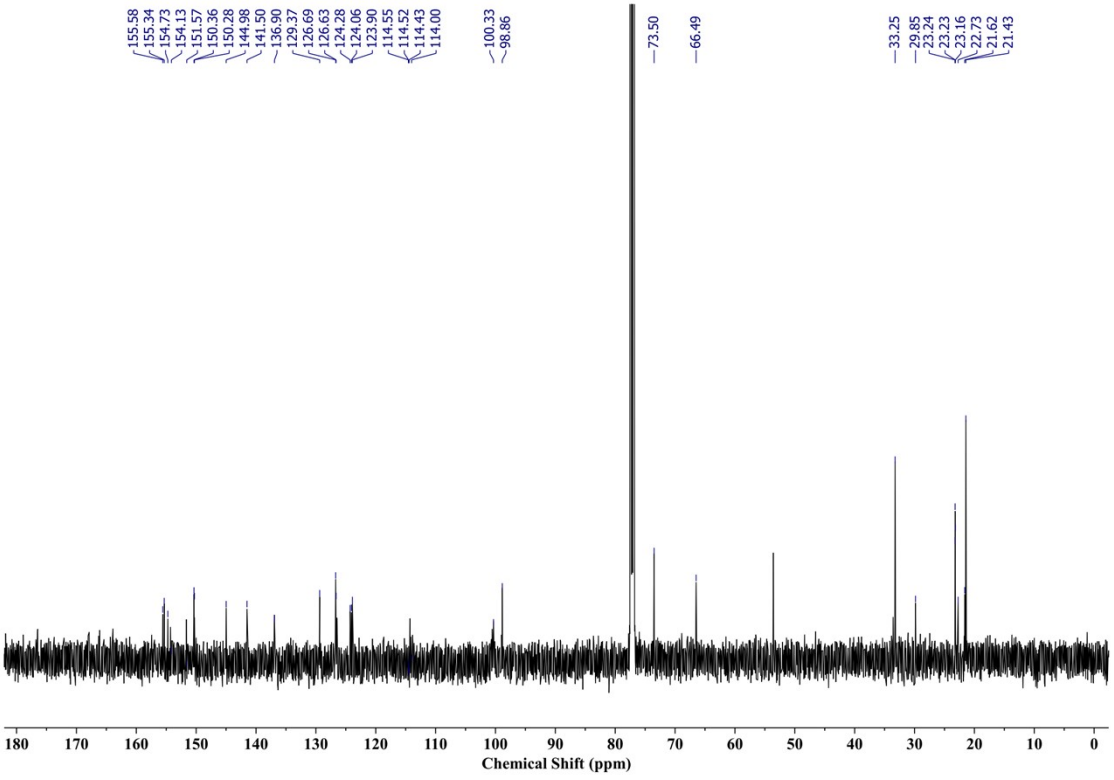


Figure S15. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) for **1**.

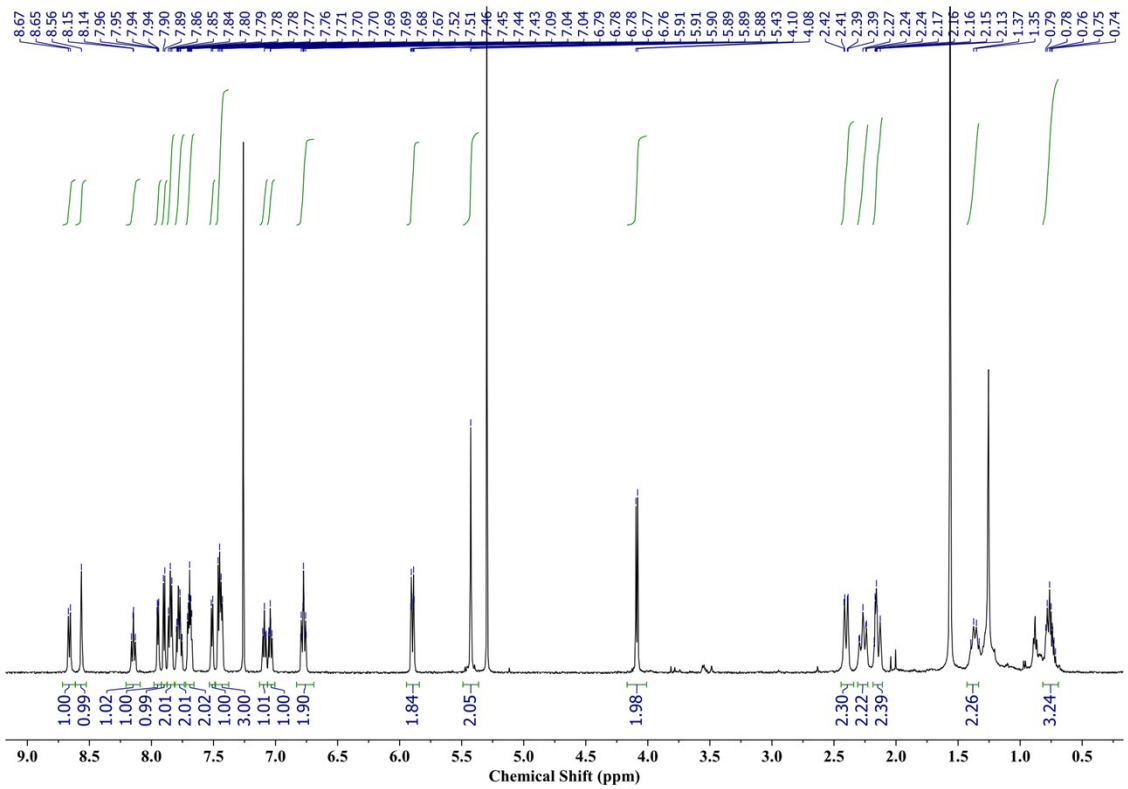


Figure S16. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) for **2**.

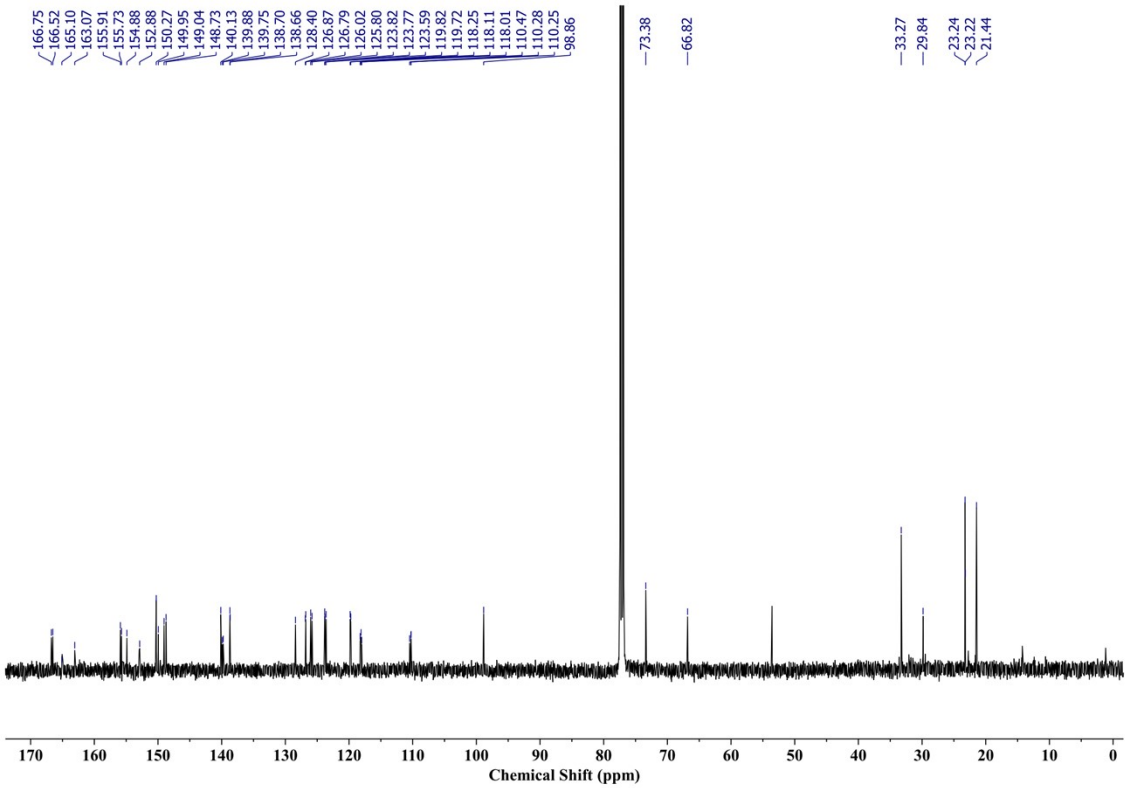


Figure S17. ^{1}H NMR spectrum (125 MHz, $CDCl_3$) for **2**.

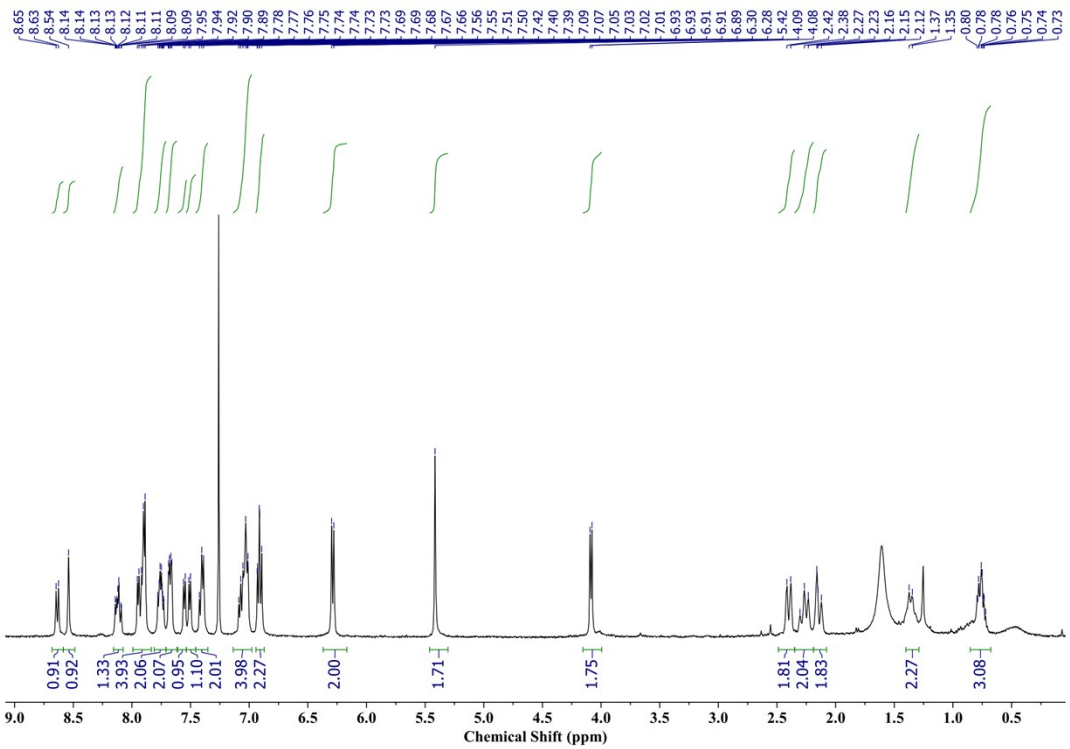


Figure S18. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) for **3**.

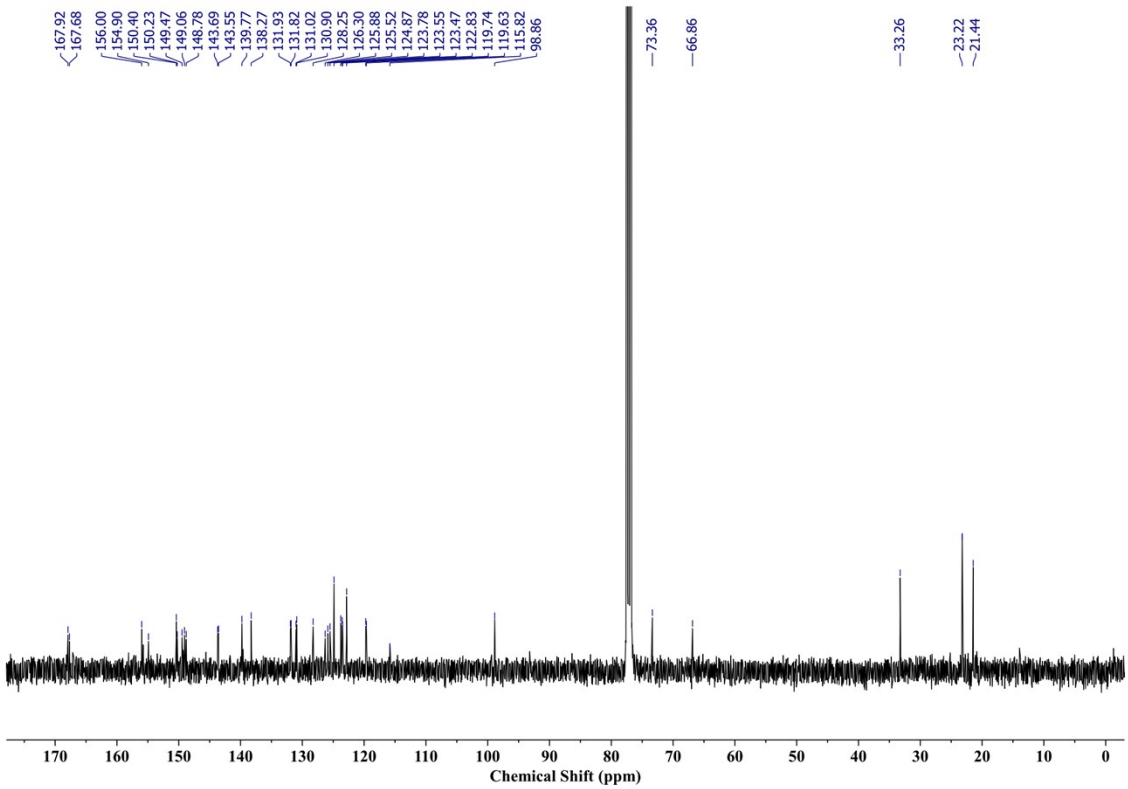


Figure S19. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) for **3**.

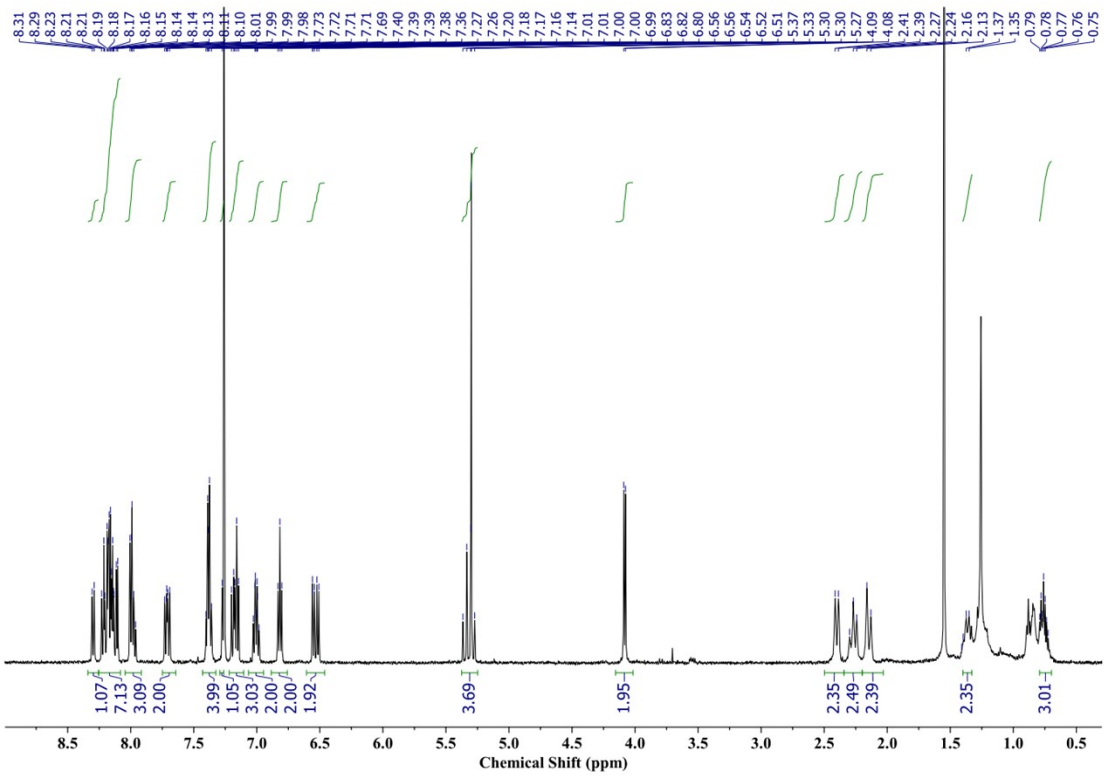


Figure S20. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) for **4**.

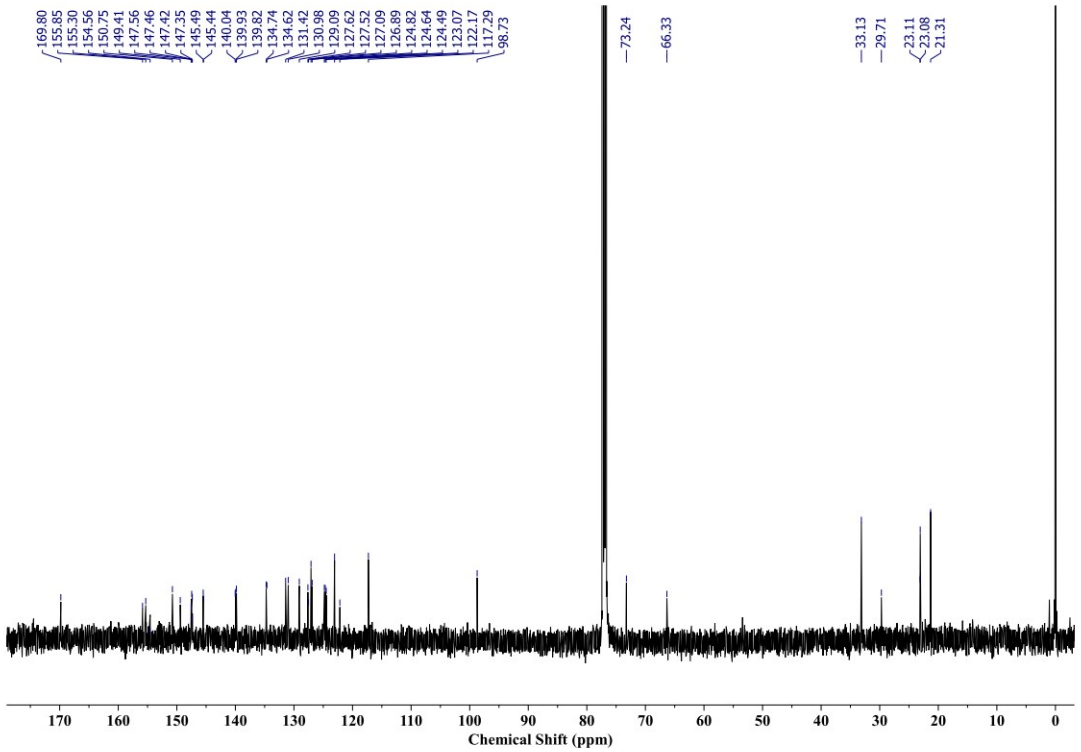


Figure S21. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) for **4**.

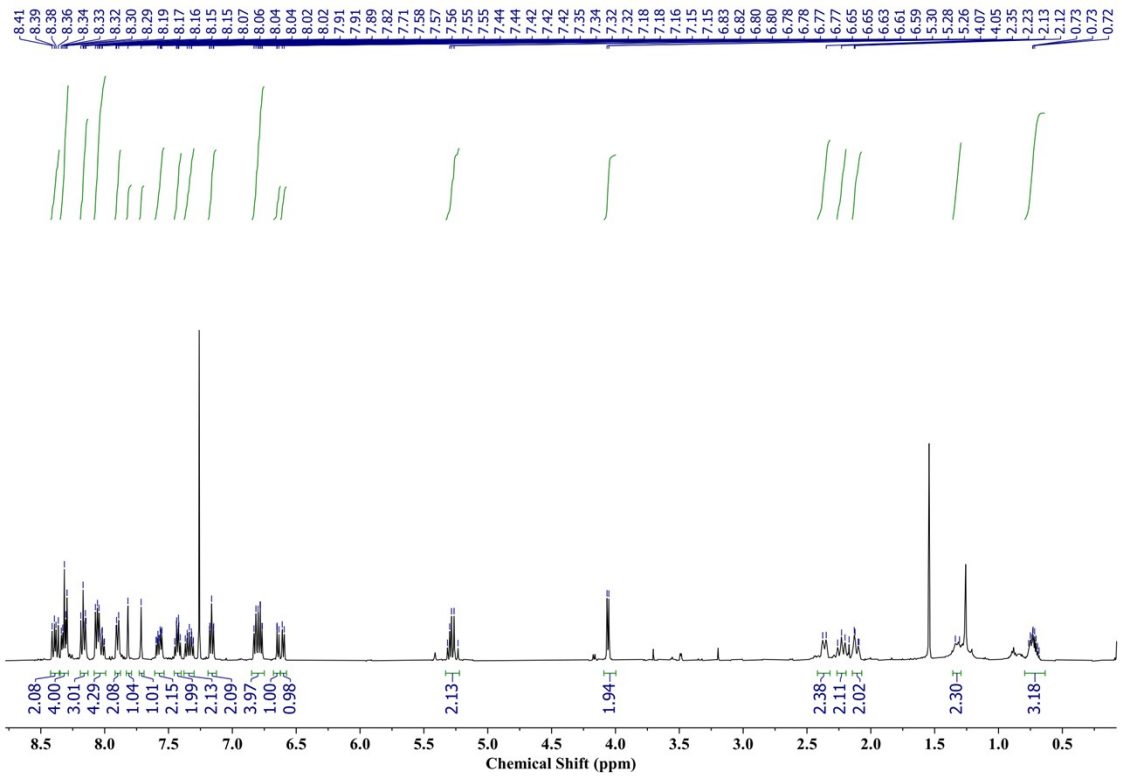


Figure S22. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) for **5**.

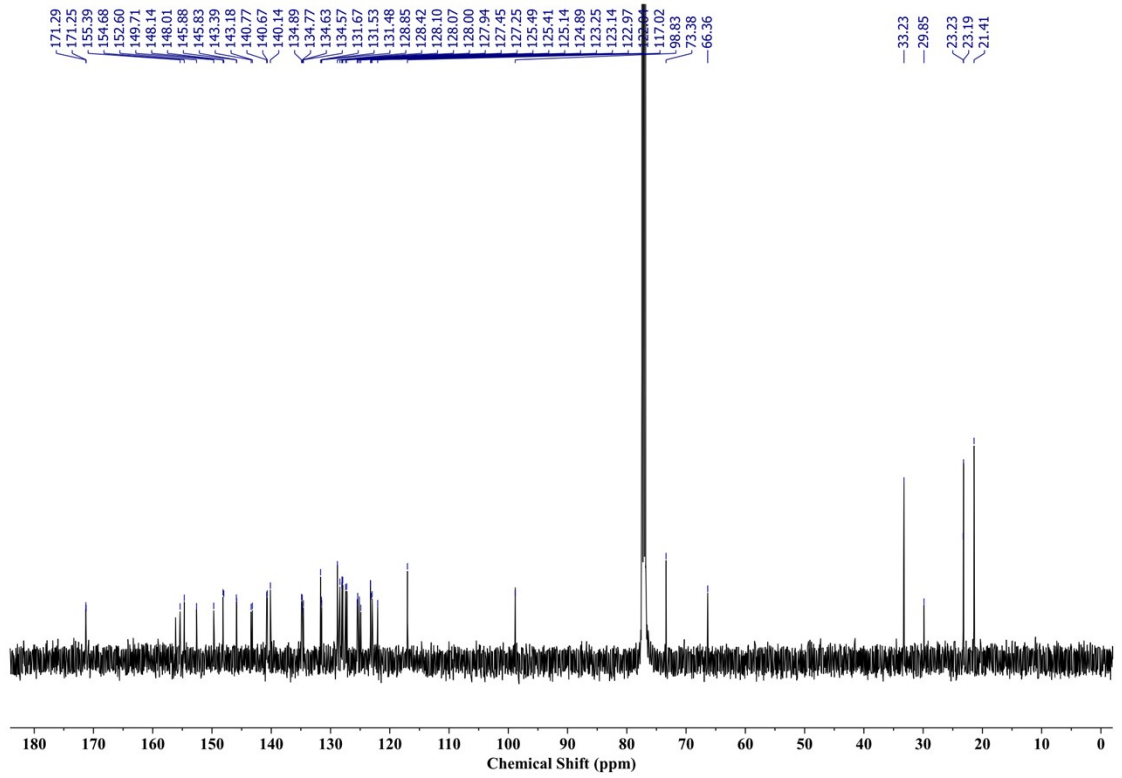
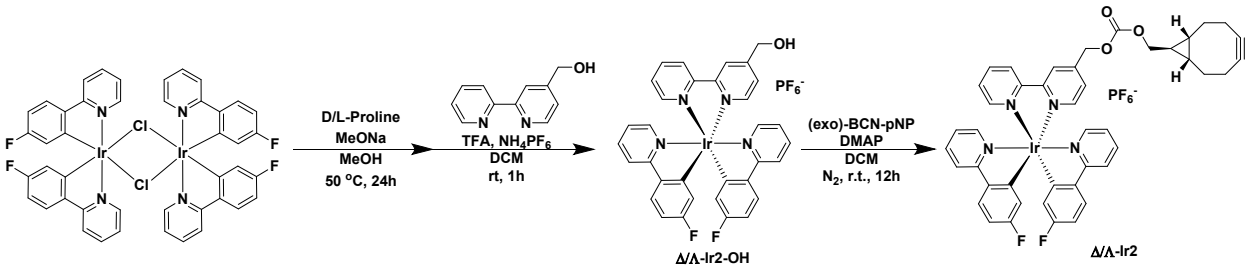


Figure S23. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) for **5**.

Synthesis of Δ/Λ-**2**:



To a solution of iridium dimer (114.4 mg, 0.1 mmol) and D-Proline (or L-Proline) (34.5 mg, 0.3 mmol) in dry MeOH (10 mL) was added NaOMe (21.6 mg, 0.4 mmol). The obtained solution was heated to 50 °C for 24 h under N_2. After removal of volatiles, the residue was dissolved in dichloromethane (20 mL) and washed with water (3 × 20 mL). The organic layer was collected and concentrated under high vacuum to give a yellow solid as the crude product which was recrystallized by pure EtOH to afford the responding intermediate. Then, the obtained yellow solid and bpy-OH (37.2 mg, 0.2 mmol) were dissolved in DCM (5 mL) with TFA (45 μL, 0.6 mmol) under N_2 atmosphere. The mixture was stirred for 2 h and then washed with saturated NH_4PF_6 aqueous solution. The organic layer was collected and purified by column chromatography on silica gel eluting with DCM/MeOH (100/2) to afford **Δ-2-OH** (or **Λ-2-OH**).

Δ-2-OH, yellow powder (92 mg, 53%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (d, J = 6.8 Hz, 2H), 8.11 (td, J = 7.9, 1.6 Hz, 1H), 7.95 (dt, J = 5.3, 1.3 Hz, 1H), 7.85 (dd, J = 11.7, 7.3 Hz, 3H), 7.76 (tdd, J = 7.6, 5.8, 1.5 Hz, 2H), 7.69 (ddd, J = 8.3, 5.5, 2.5 Hz, 2H), 7.50 (dd, J = 5.7, 1.4 Hz, 1H), 7.47 – 7.35 (m, 3H), 7.11 (ddd, J = 7.3, 5.8, 1.5 Hz, 1H), 7.01 (ddd, J = 7.4, 5.8, 1.5 Hz, 1H), 6.77 (tdd, J = 8.7, 2.6, 1.4 Hz, 2H), 5.91 (td, J = 9.1, 2.5 Hz, 2H), 4.99 – 4.84 (m, 2H). ESI-MS: Calcd. $[C_{33}H_{24}F_2IrN_4O]^+$ for 723.15, found 723.15.

Λ-2-OH, yellow powder (106 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (d, J = 8.8 Hz, 2H), 8.11 (t, J = 7.9 Hz, 1H), 7.96 (d, J = 5.4 Hz, 1H), 7.85 (dd, J = 12.2, 7.4 Hz, 3H), 7.77 (q, J = 7.3 Hz, 2H), 7.70 (ddd, J = 8.4, 5.5, 2.6 Hz, 2H), 7.50 (d, J = 5.8 Hz, 1H), 7.42 (dd, J = 18.9, 6.1 Hz, 3H), 7.11 (t, J = 6.6 Hz, 1H), 7.01 (t, J = 6.6 Hz, 1H), 6.78 (t, J = 8.8 Hz, 2H), 5.91 (td, J = 9.0, 2.5 Hz, 2H), 4.98 – 4.85 (m, 2H). ESI-MS: Calcd. $[C_{33}H_{24}F_2IrN_4O]^+$ for 723.15, found 723.16.

Δ-2-OH (or **Λ-2-OH**) (26 mg, 0.03 mmol), exo-BCN-pNP (10.4 mg, 0.033 mmol), and DMAP (36 mg, 0.3 mmol) were mixed in dry DCM (5 mL). The mixture was purged with nitrogen and stirred at r.t. in dark for 12h. After removal of the solvent, the residue was purified by column

chromatography on neutral alumina gel eluting with DCM/MeOH (100/1) to afford the **Δ-2** (or **Λ-2**).

Δ-2, yellow powder (27 mg, 86 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.67 (d, J = 8.2 Hz, 1H), 8.58 (d, J = 1.4 Hz, 1H), 8.15 (td, J = 7.9, 1.6 Hz, 1H), 7.99 – 7.92 (m, 1H), 7.90 (d, J = 5.7 Hz, 1H), 7.85 (dd, J = 8.3, 5.2 Hz, 2H), 7.81 – 7.73 (m, 2H), 7.69 (ddd, J = 8.9, 5.4, 3.6 Hz, 2H), 7.51 (d, J = 5.8 Hz, 1H), 7.48 – 7.41 (m, 3H), 7.09 (ddd, J = 7.4, 5.8, 1.5 Hz, 1H), 7.04 (ddd, J = 7.3, 5.8, 1.5 Hz, 1H), 6.77 (tt, J = 8.7, 2.3 Hz, 2H), 5.90 (dt, J = 9.4, 2.0 Hz, 2H), 5.43 (s, 2H), 4.09 (d, J = 6.8 Hz, 2H), 2.40 (dd, J = 13.2, 2.9 Hz, 2H), 2.27 (td, J = 13.9, 12.7, 3.0 Hz, 2H), 2.18 – 2.10 (m, 2H), 1.35 – 1.29 (m, 2H), 0.81 – 0.71 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.75, 166.52, 165.10, 163.02, 155.90, 155.71, 154.87, 152.93, 150.28, 149.94, 149.01, 148.71, 140.13, 139.86, 139.75, 138.71, 138.67, 128.42, 126.87, 126.80, 126.02, 125.79, 123.81, 123.75, 123.59, 119.83, 119.72, 118.24, 118.14, 118.00, 110.47, 110.28, 98.86, 73.37, 66.80, 33.26, 29.85, 23.23, 21.44. ESI-MS: Calcd. $[C_{44}H_{36}F_2IrN_4O_3]^+$ for 899.24, found 899.25.

Λ-2, yellow powder (26 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.66 (d, J = 8.2 Hz, 1H), 8.57 (s, 1H), 8.15 (t, J = 7.8 Hz, 1H), 7.95 (d, J = 5.5 Hz, 1H), 7.90 (d, J = 5.7 Hz, 1H), 7.85 (t, J = 6.9 Hz, 2H), 7.78 (q, J = 7.1 Hz, 2H), 7.69 (dt, J = 8.8, 4.6 Hz, 2H), 7.51 (d, J = 5.9 Hz, 1H), 7.48 – 7.38 (m, 3H), 7.09 (t, J = 6.6 Hz, 1H), 7.04 (t, J = 6.6 Hz, 1H), 6.78 (t, J = 8.7 Hz, 2H), 5.90 (d, J = 9.2 Hz, 2H), 5.43 (s, 2H), 4.09 (d, J = 6.7 Hz, 2H), 2.40 (d, J = 13.3 Hz, 2H), 2.27 (t, J = 14.1 Hz, 2H), 2.14 (d, J = 16.0 Hz, 2H), 1.40 – 1.32 (m, 3H), 0.81 – 0.70 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.90, 155.74, 150.27, 149.96, 149.04, 148.73, 140.12, 128.39, 126.87, 126.80, 126.02, 123.59, 119.82, 119.72, 118.15, 110.26, 98.86, 73.39, 66.82, 33.27, 29.79, 23.24, 23.21, 21.44. ESI-MS: Calcd. $[C_{44}H_{36}F_2IrN_4O_3]^+$ for 899.24, found 899.23.

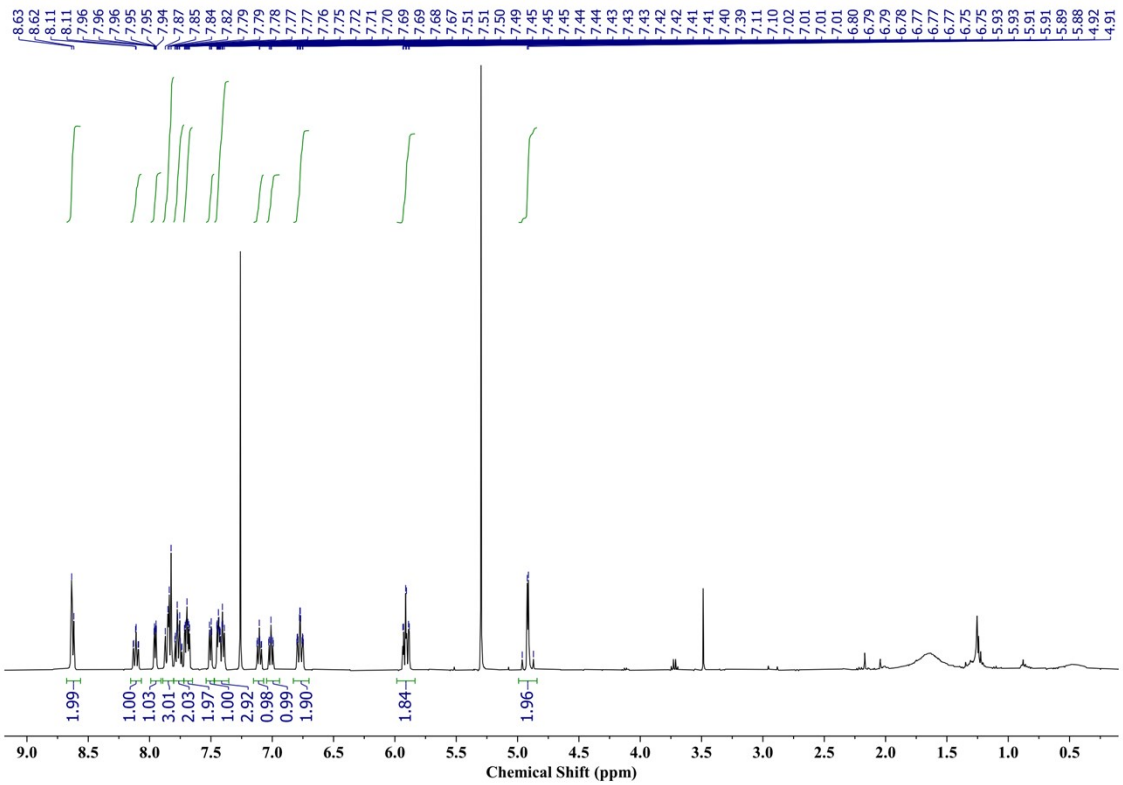


Figure S24. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) for **Δ-2-OH**.

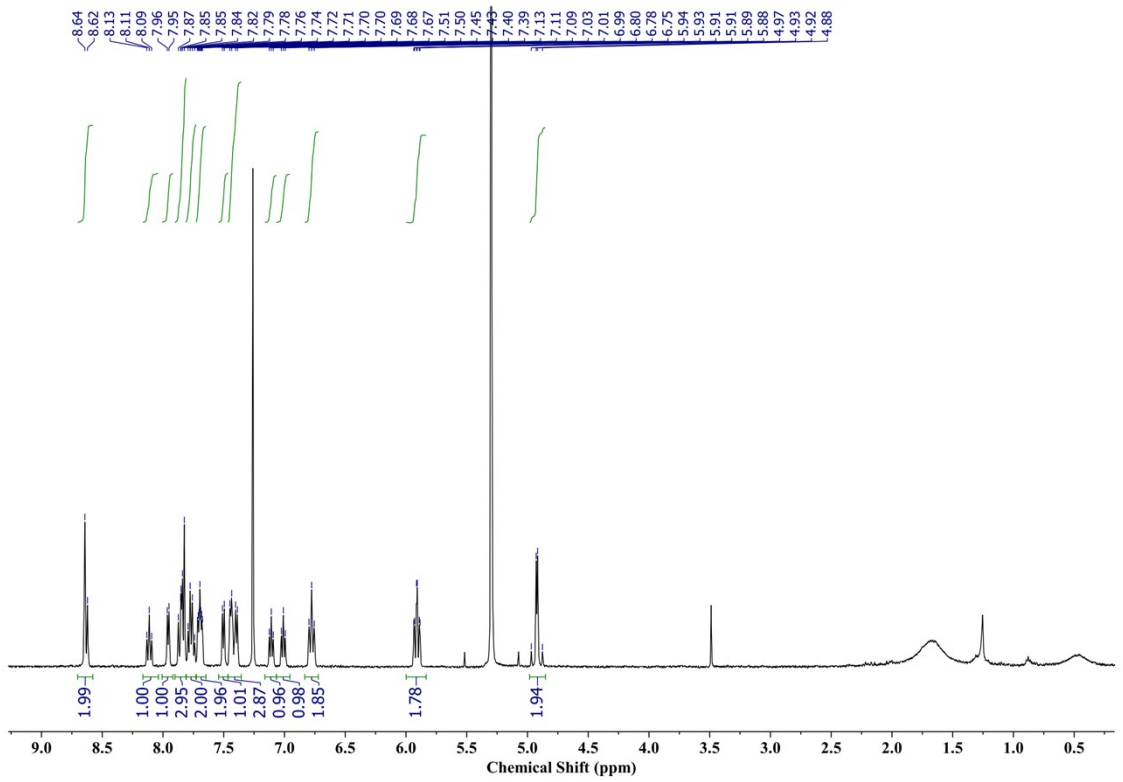


Figure S25. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) for **Λ-2-OH**.

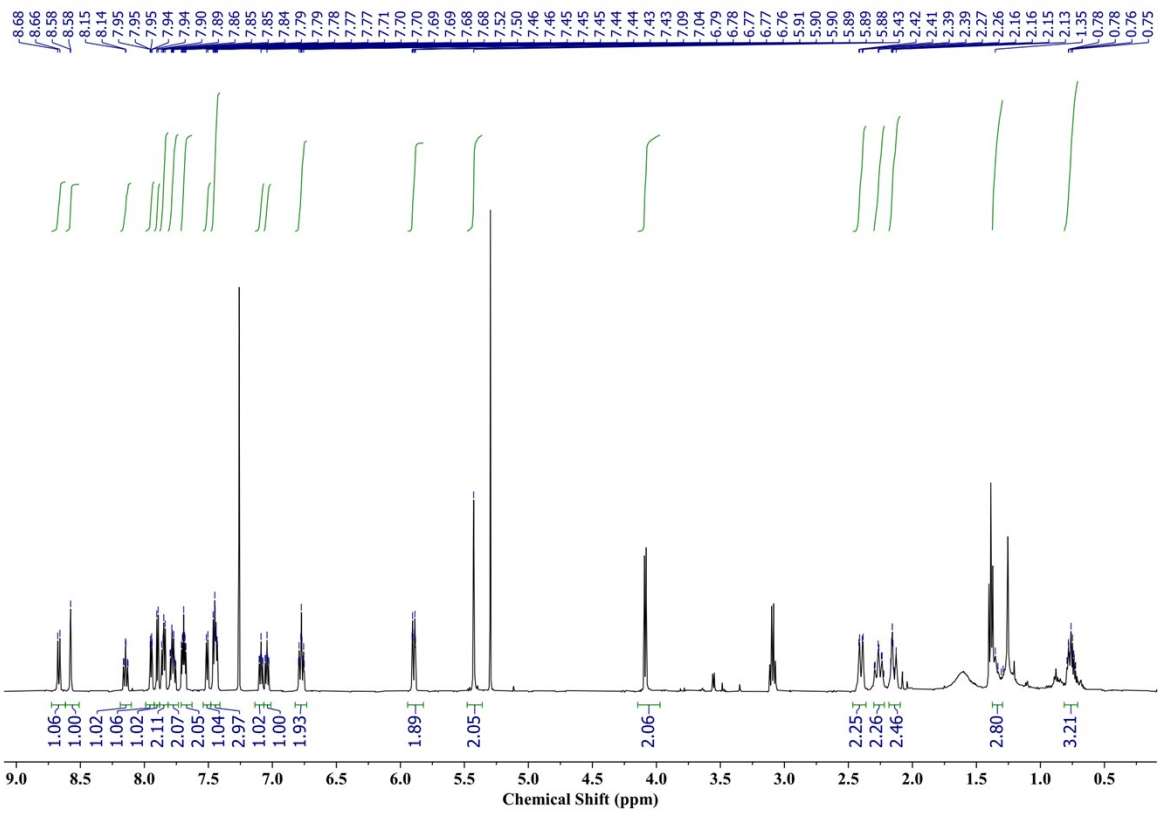


Figure S26. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) for **Δ-2**.

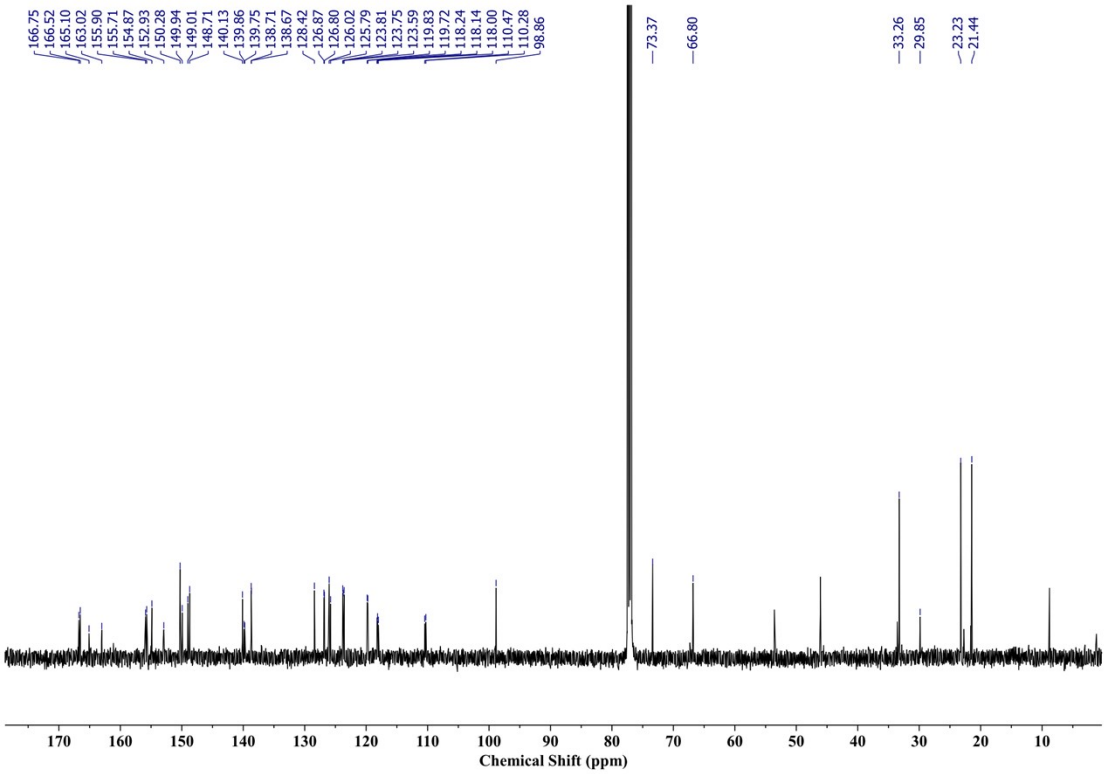


Figure S27. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) for **Δ-2**.

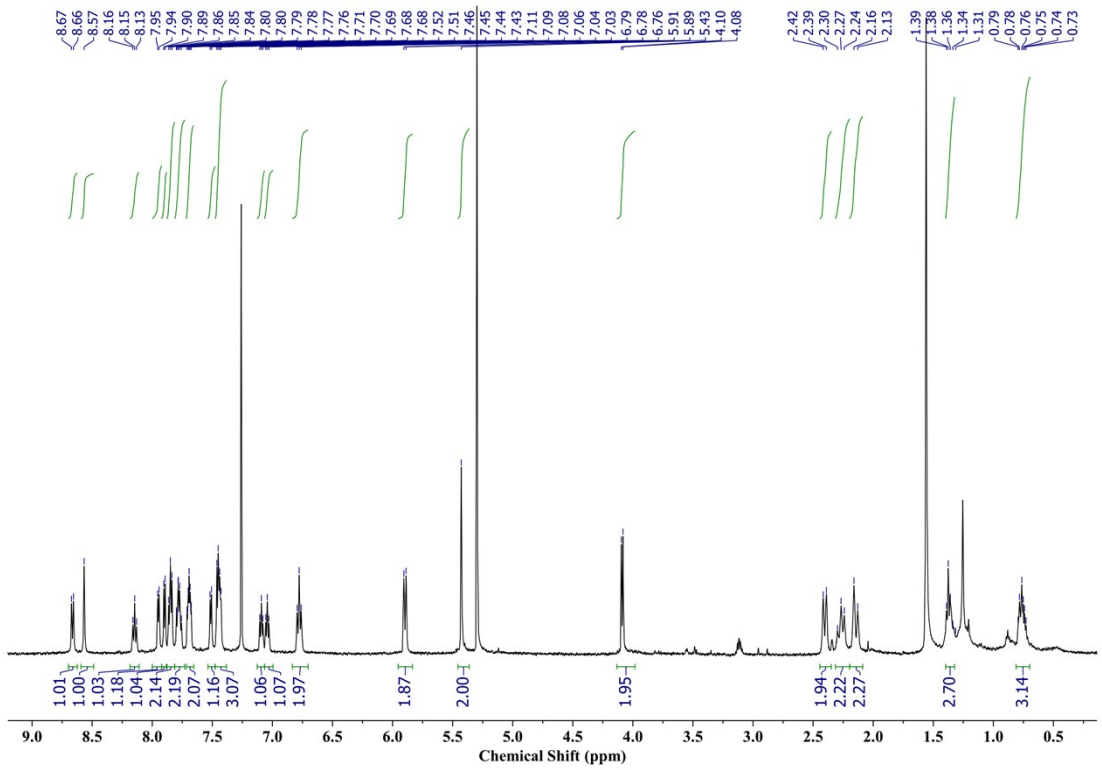


Figure S28. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) for **Λ-2**.

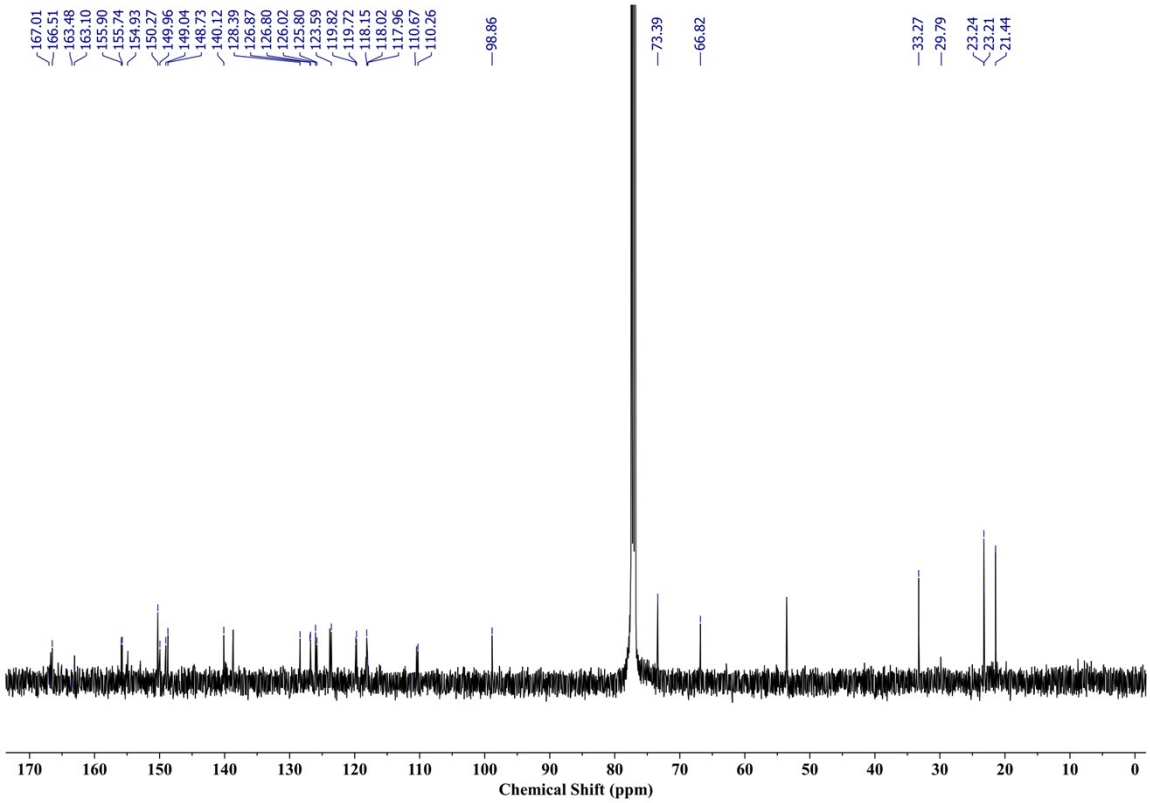


Figure S29. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) for **Λ-2**.

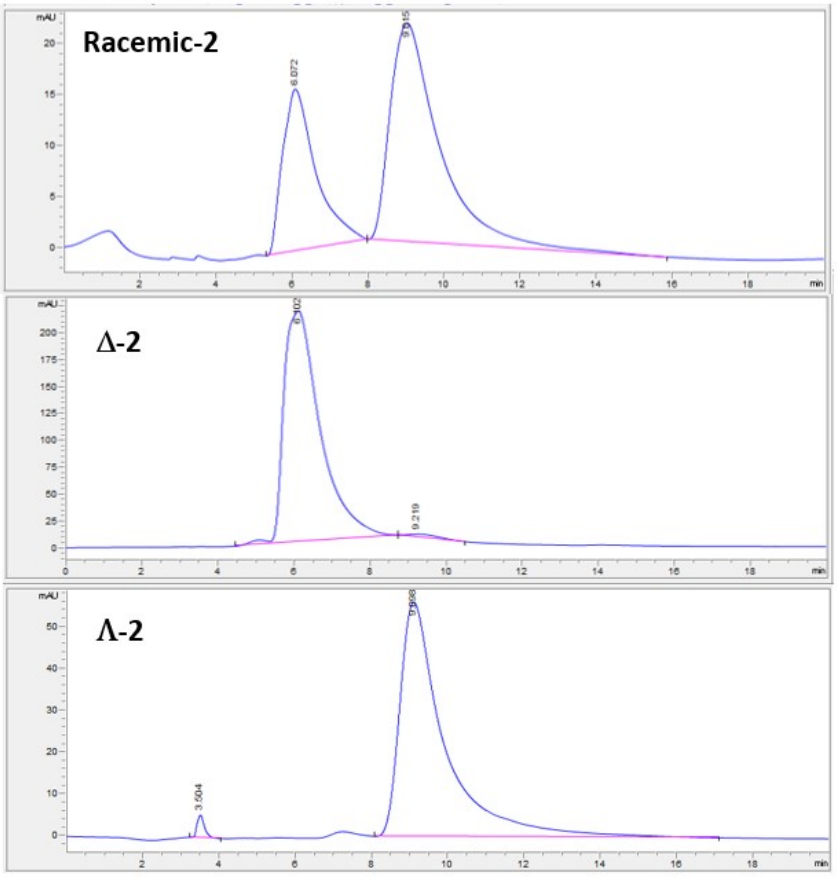
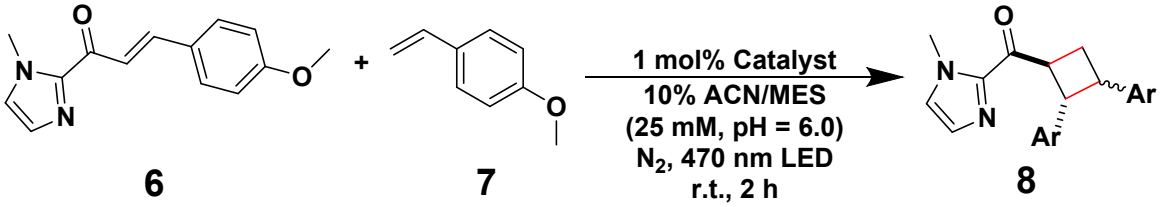


Figure S30. HPLC traces of racemic-**2**, Δ-**2**, and Λ-**2**. Conditions: Daicel Chiralpak AD-H column (250 × 4.6 mm), flow rate: 1.0 mL/min, UV absorption = 300 nm. Solvent A = n-hexane, solvent B = EtOH; linear gradient of 10 to 20% B in 20 min.

General Protocol for [2+2] Photocycloaddition of Substrates 6 and 7



6 **7** **8**

The [2+2] photocycloaddition reactions were carried out in a wetbox under an N_2 atmosphere. A 750 uL glass vial (8 * 30 mm) in custom sealable 96-well plate was charged with stock solutions of the C-cinnamoyl imidazole **6** (20 mM in ACN), 4-methoxystyrene **7** (200 mM in ACN), 50 mM MES (pH 6.0), and catalysts (POP-Z53-**1-5** in MQ H_2O, **1-5** in ACN). Typically, the 100 µL reaction mixture contains 1 mM **6**, 10 mM **7**, 25 mM MES (pH 6.0), 10 µM catalyst, and 10% ACN. Biocatalysis reactions were irradiated on the LED photoreactor for 2 hours in triplicates, and the collected results are averages (± standard deviation) of reactions. The reactions were quenched by adding 100 µL of 10 mM TMB in ACN. The obtained suspension was filtered by a

0.2 µm filter plate before being analyzed by UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **8** and TMB.

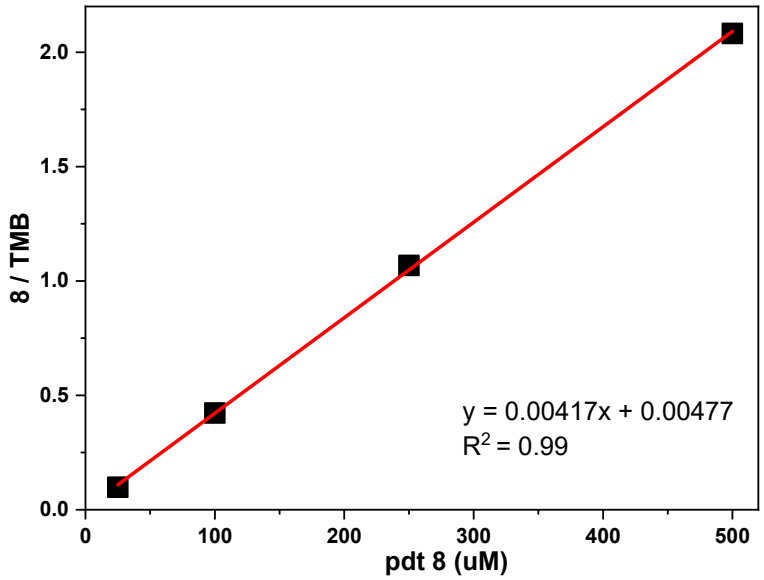


Figure S31. Calibration Curve of pdt **8** and TMB.

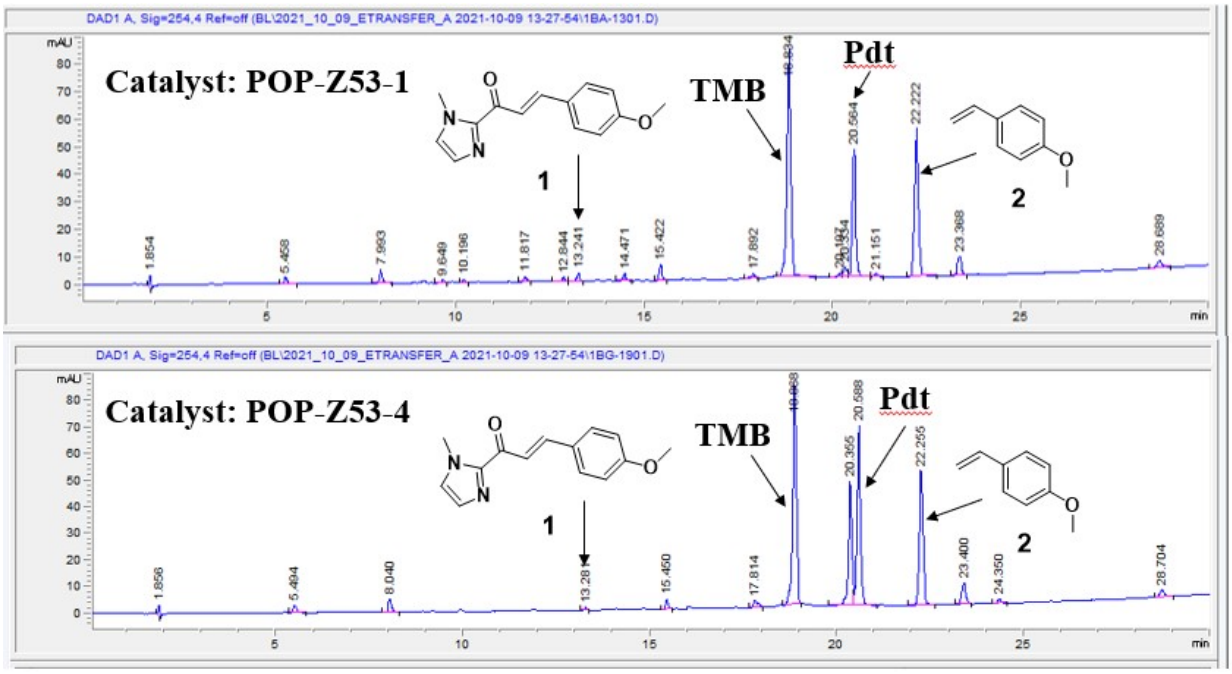
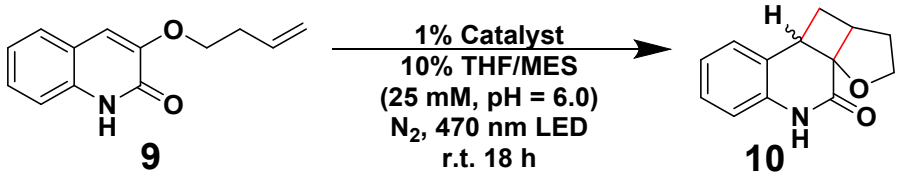


Figure S32. Representative UHPLC Traces for [2+2] Cycloaddition Reactions.
A 5 cm Eclipse Plus C18 column was applied for the UHPLC analysis. The mobile phase consisted of A: H_2O + 0.1% TFA, B: ACN + 0.1% TFA. Method: 1 minute at 10% B, 10% B to 90% B over 34 minutes, 5 minutes at 90% B, and a 3-minute post-time. A flow rate of 0.4 mL per minute was used. 5.0 µL of each sample was injected and elution of compounds was followed by monitoring absorbance at 254 nm.

General Protocol for Intramolecular [2+2] Photocycloaddition of Substrates 9



The intramolecular [2+2] photocycloaddition reactions were carried out in a wetbox under an N_2 atmosphere. A 750 uL glass vial (8 * 30 mm) in custom sealable 96-well plate was charged with stock solutions of the 3-(3-buten-1-yloxy)-2(1H)-quinolinone **9** (10 mM in THF), 50 mM MES (pH 6.0), and catalysts (POP-Z53-**1-5** in MQ H_2O, **1-5** in THF). Typically, the 100 µL reaction mixture contains 1 mM **6**, 25 mM MES (pH 6.0), 10 µM catalyst, and 10% THF. Biocatalysis reactions were irradiated on the LED photoreactor for 18 hours in triplicates, and the collected results are averages (± standard deviation) of reactions. The reactions were quenched by adding 100 µL of 10 mM phenol in THF. The obtained suspension was filtered by a 0.2 µm filter plate before being analyzed by UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **10** and phenol.

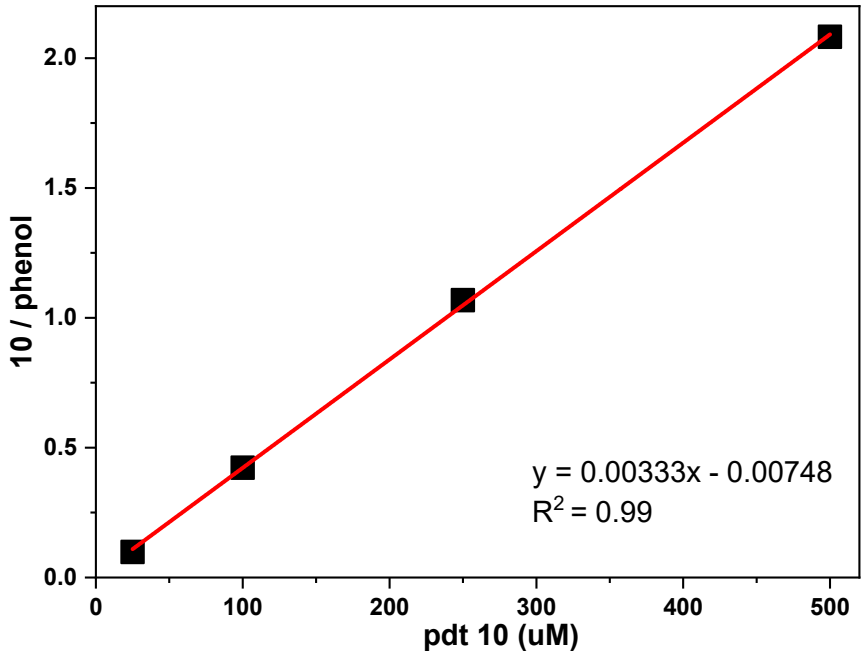


Figure S33. Calibration Curve of pdt **10** and Phenol.

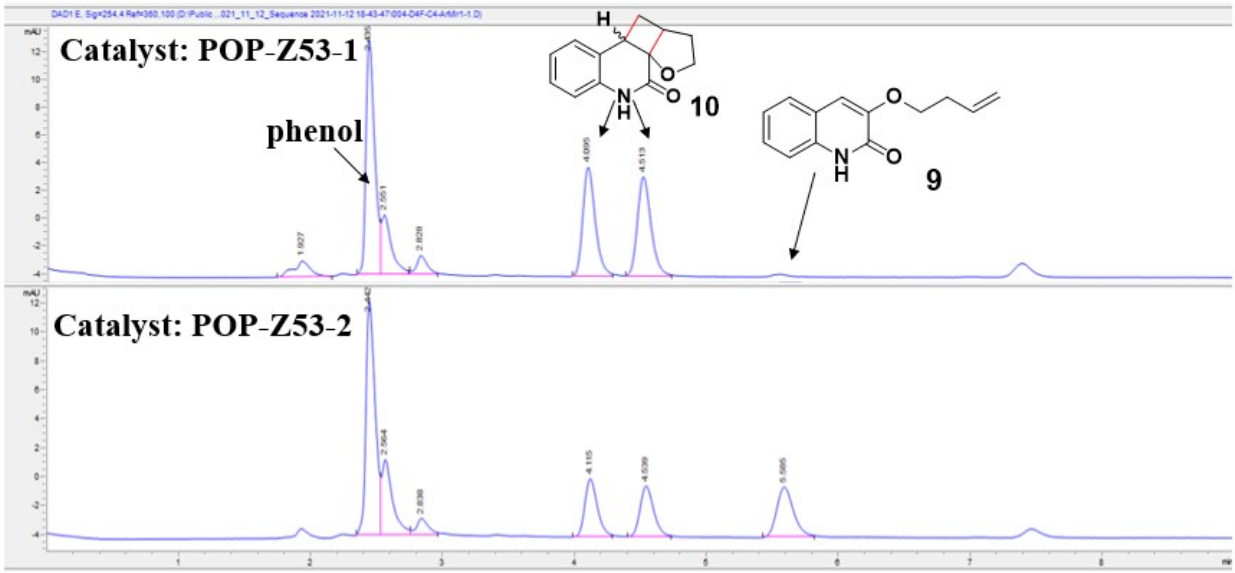
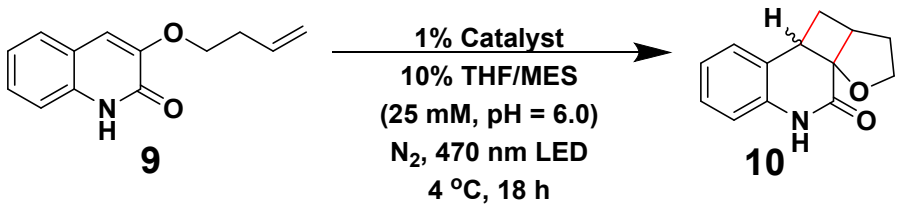
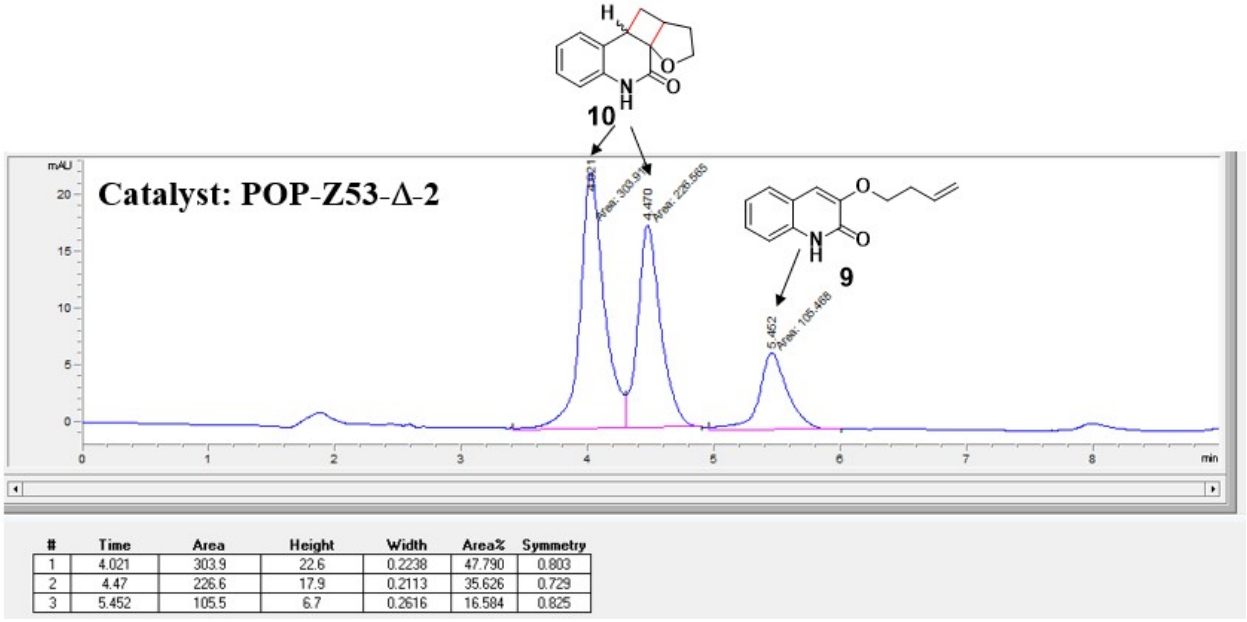


Figure S34. Representative UHPLC Traces for Intramolecular [2+2] Cycloaddition Reactions. A 15 cm Daicel CHIRALPAK IC-3 column was applied for the UHPLC analysis. The mobile phase consisted of A: NH_4HCO_3 (20 mM), B: ACN. Method: 8 minutes at 50% B, 0.5 minute at 100% B, and a 1-minute post-time. A flow rate of 1.0 mL per minute was used. 1 mL of each sample was injected, and elution of compounds was followed by monitoring absorbance at 254 nm.

General Protocol for Intramolecular [2+2] Photocycloaddition of Substrates 9 at 4 °C



The intramolecular [2+2] photocycloaddition reactions were sealed in a wetbox under an N_2 atmosphere. A 750 uL glass vial (8 * 30 mm) in custom sealable 96-well plate was charged with stock solutions of the 3-(3-buten-1-yloxy)-2(1H)-quinolinone **9** (10 mM in THF), 50 mM MES (pH 6.0), and catalysts (POP-Z53-**1-5** in MQ H_2O, **1-5** in THF). Typically, the 100 µL reaction mixture contains 1 mM **6**, 25 mM MES (pH 6.0), 10 µM catalyst, and 10% THF. Biocatalysis reactions irradiated on the LED photoreactor for 18 hours at 4 °C in triplicates, and the collected results are averages (± standard deviation) of reactions. The reactions were quenched by adding 100 µL of 10 mM phenol in THF. The obtained suspension was filtered by a 0.2 µm filter plate before being analyzed by chiral UHPLC. Yields were determined by a calibration curve prepared using isolated authentic product **10** and phenol.



#	Time	Area	Height	Width	Area%	Symmetry
1	4.021	303.9	22.6	0.2238	47.790	0.803
2	4.47	226.6	17.9	0.2113	35.626	0.729
3	5.452	105.5	6.7	0.2616	16.584	0.825

Figure S35. Representative UHPLC Traces for Intramolecular [2+2] Cycloaddition Reactions at 4 °C.

A 15 cm Daicel CHIRALPAK IC-3 column was applied for the UHPLC analysis. The mobile phase consisted of A: NH_4HCO_3 (20 mM), B: ACN. Method: 8 minutes at 50% B, 0.5 minute at 100% B, and a 1-minute post-time. A flow rate of 1.0 mL per minute was used. 1 mL of each sample was injected, and elution of compounds was followed by monitoring absorbance at 254 nm.

Preparation of Protein Scaffolds and ArMs

General Materials and Methods

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Aqueous solutions were prepared using Milli-Q water. Protein variants and reagents used in this study were prepared previously as described in Zubi *et al* unless otherwise noted.[10] Protein concentrations were measured using the Pierce Coomassie (Bradford) Protein Assay Kit with a standard curve generated from standard BSA control samples and protein stocks were then flash frozen with liquid N_2 and stored at -80 °C until use. Lyophilization was performed using a LABCONCO benchtop FreeZone freeze dryer (2.5 L) according to the manufacturer's instructions. Gels were imaged with an Alpha Innotech AlphaImager EP. Intact protein mass spectrometry was performed using a Waters Synapt G2S HDMS using a C18 column. Protein samples were desalted using manufacturer specifications before MS analysis. UV-Vis spectroscopy was performed using a Cary 5000 UV-Vis-NIR spectrophotometer at room temperature after blanking with a solution containing just water using 10 mm pathlength quartz cuvettes. Circular dichroism (CD) spectra were obtained at room temperature on a JASCO J-1500 CD Spectrometer using 10 mm pathlength quartz cuvettes. UHPLC analysis was performed on an Agilent 1290 Infinity UHPLC. Excitation/emission spectra and luminescence lifetime measurements were performed in a low volume cuvette (Hellma Analytics High Precision Cell; 3x3 mm light path; 9,65 centre; Art. No. 105251005965-40) and data was acquired using a FLS1000 Spectrofluorometer (Edinburgh Instruments) with a 450 Xenon lamp or EPL-450 laser for excitation and a Hamamatsu R13456 PMT for detection.

Protein Expression, Purification, and Bioconjugation

Protein was expressed and purified as described previously.[10]

SPAAC bioconjugation of Ir(III) cofactors was performed by adding 120 µL of the cofactor stock solution (830 µM) in ACN to the scaffold solution (480 µL of 75 µM protein in 50 mM Tris, pH 7.4) while shaking at 4 °C, 750 rpm. The mixture was incubated at 4 °C, 750 rpm overnight (12-16) hours using a Thermo Scientific™ Thermal Mixer (with Blocks), at which point the shaking was stopped and 100 µL of a 50% suspension of N_3-agarose resin solution was added to scavenge the free cofactor. The mixture was agitated by end-over-end rotation at 4°C overnight (12-16 hours). After the resin purification was complete, the resin was pelleted by centrifugation at 5,000 rpm for 10 minutes and the supernatant was removed with a pipette. The resin was washed with 500 µL of 50 mM Tris (pH 7.4) and inverted several times. The resin was pelleted by centrifugation at 5,000 rpm for 10 minutes and the supernatant was removed with a pipette. The pooled supernatant fractions containing the ArMs were centrifuged for an additional 10 minutes at 5,000 rpm. The supernatant was removed by a pipette (~1 mL) and then concentrated to 50 µL. Diafiltration was performed using MQ H_2O. The obtained Ir(III) ArMs were flash frozen using liquid N_2, lyophilized, and stored at -80 °C until further use. When samples were ready for further experiments, they were transferred into an inert wetbox and redissolved in MQ H_2O.

Time course SPAAC was performed in a similar manner with aliquots of protein being removed at specified times for analysis by ESI MS.

Physical Characterization of ArMs

Protein ESI MS

Intact protein mass spectrometry was used as described previously to analyze the extent of bioconjugation and performed using a Waters Synapt G2S HDMS using a C18 column.[10] Diluted samples (5 μM) were first desalted using Zeba desalting columns and filtered through 0.2 μm nylon syringe filters A 10-minute LC method (A: H_2O with 0.1% formic acid, B = acetonitrile with 0.1% formic acid) with a linear gradient from 95% A to 1% A over 6 minutes followed by a 4-minute flush at 95% A was used with the mass spectrometer recording between 400-2000 Da (protein retention time ~ 4.38 mins). Deconvolution of the mass spectrum was performed using a 700-900 M/Z window with a deconvoluted mass range of 70-75 kDa.

UV-Vis Spectroscopy

Ir(III) ArMs samples were diluted to 50 μM with MQ H_2O and free cofactor samples were diluted to 50 μM in 10% ACN/MQ H_2O. UV-vis spectra were collected at room temperature using a Cary 5000 UV-Vis-NIR spectrophotometer. A spectrum of a blank solution was collected first and then subtracted from all sample spectra. Scanning from 800-250 nm (1 nm step) was performed in a 10 mm pathlength quartz cuvette.

Circular Dichroism Spectroscopy

Circular dichroism (CD) spectra were obtained at room temperature on a JASCO J-1500 CD Spectrometer. CD spectra of ArMs (10 μM) were collected in MQ H_2O. 5 accumulations from 350-200 nm were performed in a 10 mm pathlength quartz cuvette and the following parameters were utilized: 1.0 nm bandwidth, 100 nm/min scan rate, and 0.1 nm data pitch.

Temperature-dependent CD measurement was performed in a similar manner with aliquots of protein from 20 ^{o}C to 100 ^{o}C with 20 ^{o}C intervals.

Steady-State Luminescence Measurements

After preparing ArMs for analysis under anaerobic conditions, the samples were transferred to a low volume cuvette (Hellma Analytics High Precision Cell; 3x3 mm light path; 9,65 centre; Art. No. 105251005965-40). The sample was protected from light, tightly capped with a PFTE stopper, and wrapped with PTFE tape to minimize exposure to air once the sample was removed from the wetbox. Luminescence excitation and emission scans were recorded on a FLS1000 Spectrofluorometer (Edinburgh Instruments). The FLS1000 utilizes a 450 Xenon lamp for excitation and a Hamamatsu R13456 PMT for detection. Spectra are corrected for lamp intensity using a silicone reference detector and the emission spectral response was corrected for intensity and wavelength from calibrated lamps. The corresponding ^{1}MLCT absorption bands chosen as the excitation wavelength with 2 nm bandwidth were used for emission spectra. Excitation wavelengths of 550-250 nm (2 nm bandwidth) and corresponding emission peaks (2 nm bandwidth) were used for excitation spectra. 1 nm step sizes were applied. A total of two repeat scans were performed. Steady-state luminescence measurements were performed for Ir(III) ArMs and cofactors at 50 μL.

Luminescence Lifetime Measurements

Time-correlated single photon counting (TCSPC) luminescence lifetimes were recorded on the FLS1000 using an Edinburgh Instruments EPL-450 laser, with a max signal no greater than 5% of the repetition rate to avoid pulse-pileup. Monoexponential tail-fit analysis on a time range of 700 – 3000 ns was performed with the Fluoracle software to determine luminescence lifetimes. Samples were excited with the EPL-450 laser at their corresponding emission wavelength (8 nm bandwidth) collected unless otherwise noted. Measurements were performed with 50 μM samples. After preparing ArMs for analysis under anaerobic conditions, the samples were transferred to a low volume cuvette (Hellma Analytics High Precision Cell; 3x3 mm light path; 9,65 centre; Art. No. 105251005965-40). The sample was protected from light, tightly capped with a PFTE stopper, and wrapped with PTFE tape to minimize exposure to air once the sample was removed from the wetbox.

Steady-State Kinetic Assays

The hydrolysis of benzyloxycarbonyl-alanyl-prolyl-p-nitroanilide (Z-Ala-Pro-pNA) was monitored by spectrophotometry at 85 °C.[11] The initial rate of reaction was determined by measuring the production of the yellow product, *p*-nitroaniline (*p*NA) which has a calculated molar extinction coefficient of 7126 $M^{-1}cm^{-1}$ at 410 nm. 900 μL of master mix solution containing buffer (33 mM HEPES, pH 7.4 and 889 mM NaCl), and corresponding enzyme was added to a quartz cuvette and incubated at 85 °C in the spectrophotometer for 2 minutes. To initiate the reaction, 1-10 μL of the substrate Z-Ala-Pro-pNA (10 mM in DMSO), 0-10 μL (S)-1-Boc-2-cyanopyrrolidine (100 mM or 1 M in DMSO), and 0-99 μL of DMSO (final concentration of DMSO was 10% v/v) were added to the cuvette and mixed well. The final concentrations of components in the reaction were as follows: 30 mM HEPES (pH 7.4), 800 mM NaCl, 10-30 nM enzyme, 0.01-1.00 mM Z-Ala-Pro-pNA, either 0.1-10 mM or 1-100 mM (S)-1-Boc-2-cyanopyrrolidine, and 10% (v/v) DMSO. The reaction was monitored with absorbance measurements (at 410 nm) every 6 seconds for 1 minute. Initial rates were determined by converting absorbance values to concentrations using the molar extinction coefficient in Excel. Initial rates were plotted versus substrate concentration in Origin Pro and non-linear fitting of the data with the Michaelis-Menten equation was performed. All data were collected in triplicate and the standard deviation is represented by error bars.

Custom Photoreactor

General Information

The custom photoreactor was built by using 470 nm blue LEDs (VAOL-5GSBY4). The schematics for the basic circuitry on the boards (Figure S29), board layout (Figure S30), board layout with dimensions (Figure S31), and custom sealable 96-well plates for photoreactions (Figure S32) are described here.

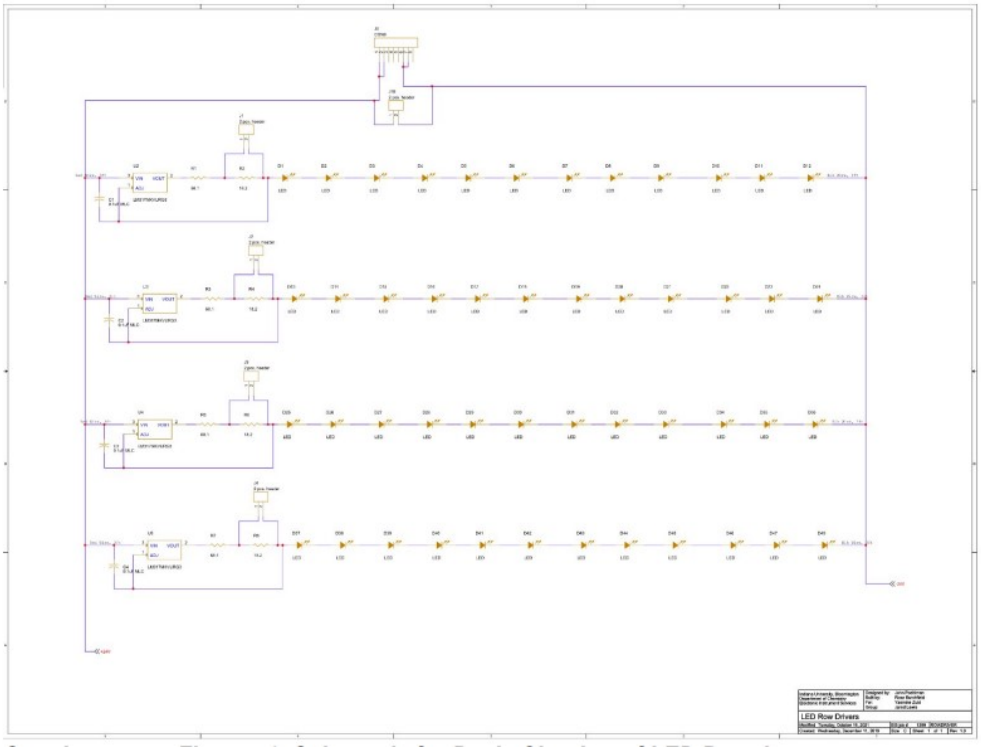


Figure S36. Schematic for basic circuitry of LED boards.

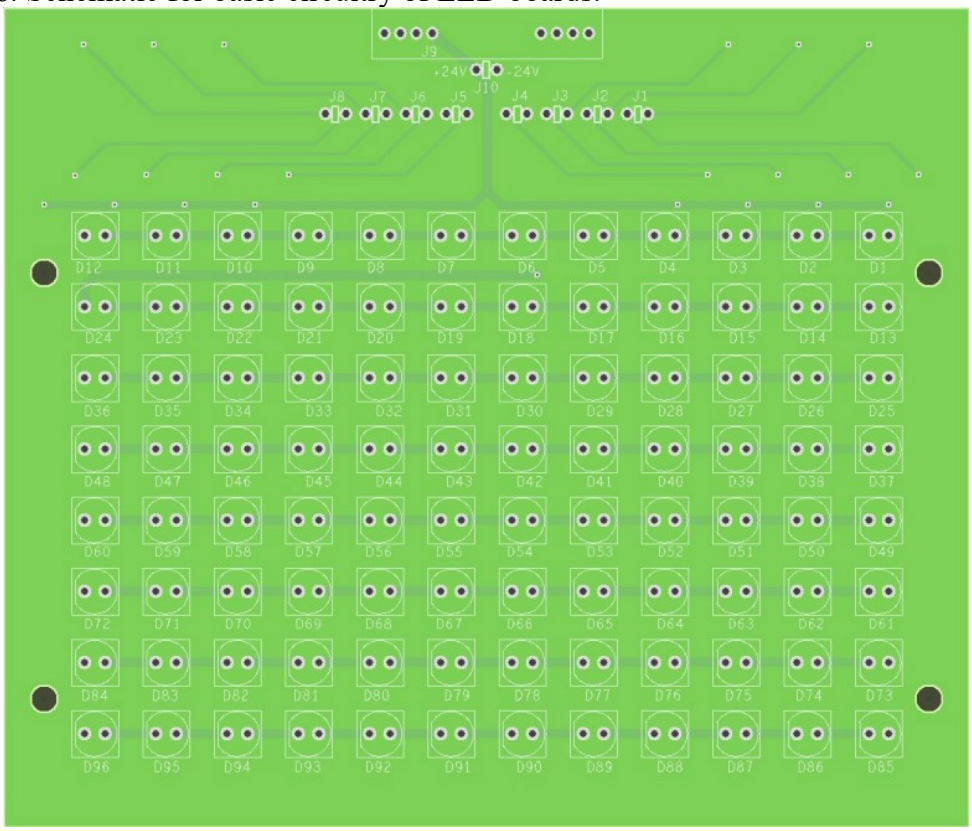
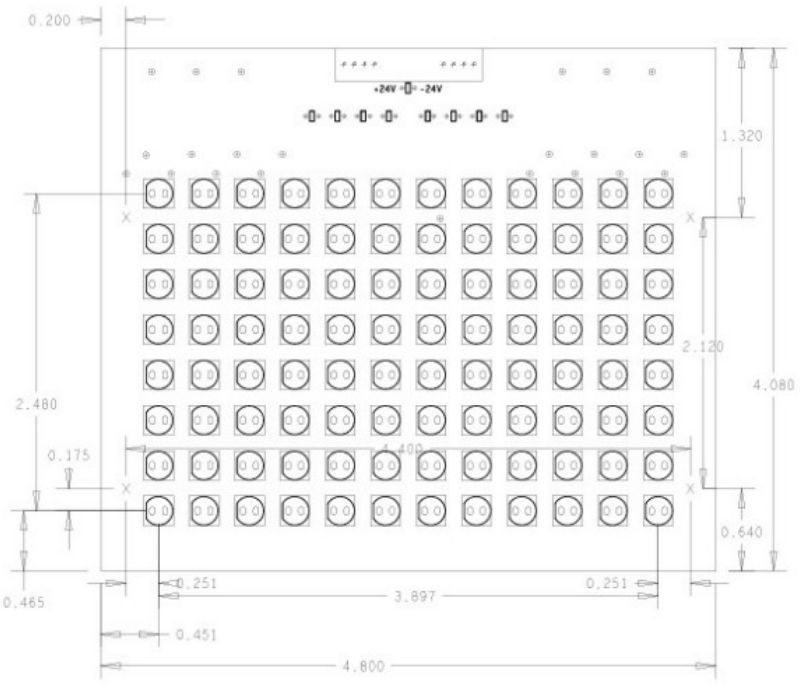


Figure S37. Board layout of blue LEDs



DRILL CHART: TOP to BOTTOM			
ALL UNITS ARE IN INCHES			
FIGURE	FINISHED_SIZE	PLATED	QTY
⊕	0.015	PLATED	23
o	0.035	PLATED	192
•	0.038	PLATED	18
◦	0.038	PLATED	8
x	0.125	NON-PLATED	4
		TOTAL HOLES:	245

Figure S38. LED Board Layout with Dimensions.

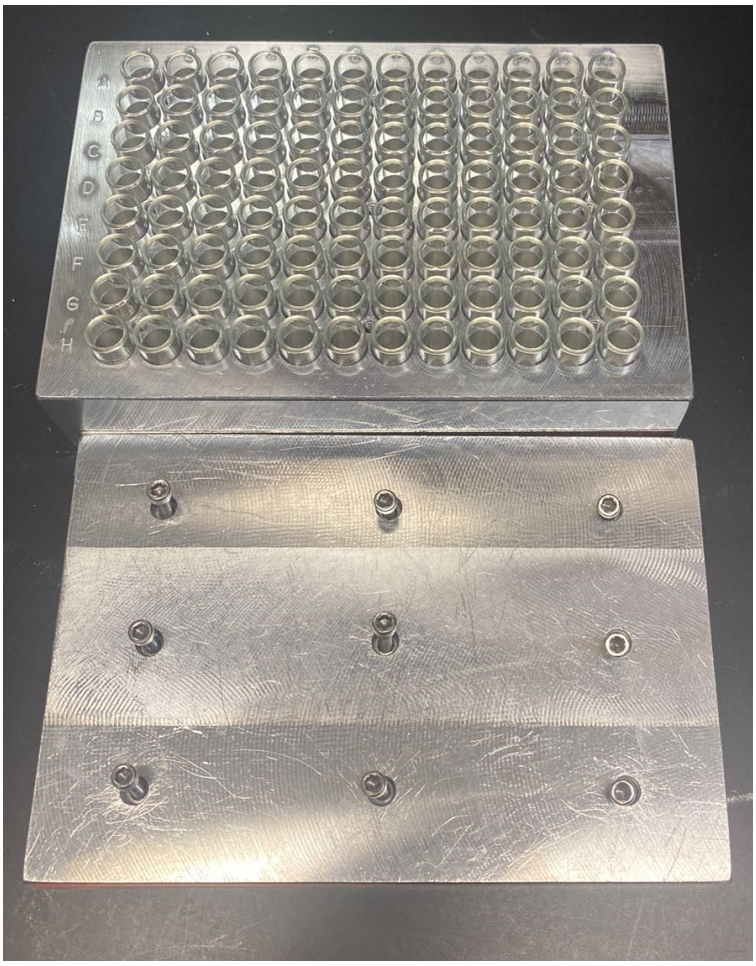


Figure S39. Custom sealable 96-well plates for photoreactions.

References

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(2) G. Zhang, H. Zhang, Y. Gao, R. Tao, L. Xin, J. Yi, F. Li, W. Liu, J. Qiao, *Organometallics* **2014**, *33*, 61–68.
(3) J. He, Z.Q. Bai, P.-F. Yuan, L.Z. Wu, Q. Liu, *ACS Catal*. **2021**, *11*, 446–455.
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