

Supporting Information

The two isomers of a cyclometalated palladium sensitizer show different photodynamic properties in cancer cells

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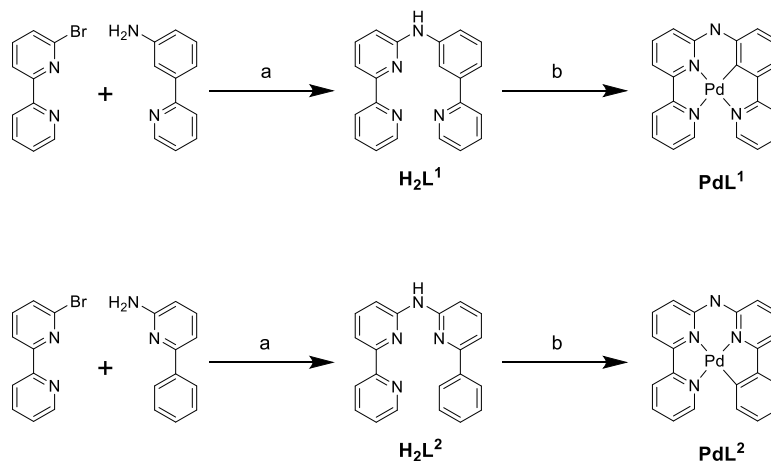
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General procedures

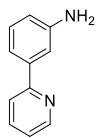
All syntheses were performed in a dinitrogen atmosphere. 2-bromopyridine, Pd(PPh₃)₄, 3-aminophenylboronic acid, 6-bromo-2,2'-bipyridine, Bis(dibenzylideneacetone)palladium(0) (Pd(dba)₂), (±)-2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene (racemic BINAP), 2-Amino-6-phenylpyridine and the other chemicals and materials, were purchased from Sigma-Aldrich. All reactants and solvents were used without further purification. All ¹H NMR, ¹³C attached-proton-test NMR (¹³C-APT NMR) were recorded on a Bruker DPX-300 or DMX-400 spectrometers. Chemical shifts are indicated in ppm relative to the residual solvent peak. Electrospray ionization mass spectra (ESI-MS) were recorded by using a MSQ Plus Spectrometer positive ionization mode. High resolution mass spectra (HRMS) of two palladium complexes were recorded on Waters XEVO-G2 XSQ-TOF) mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.0 kV, desolvation gas flow 900 L/hr, temperature 250 °C) with resolution R = 22000 (mass range m/z = 50-2000) and 200 pg/uL Leu-enkephalin (m/z = 556.2771) as a "lock mass". Uv-vis spectra were recorded on a Cary 50 spectrometer from Varian. The singlet oxygen spectra and phosphorescence quantum yield were measured on a special custom-built setup which was described previously.¹ The phosphorescence lifetime of complexes in methanol were measured by LifeSpec-II from Edinburgh Instruments, with excitation source of 375 nm pulsed diode lasers. The partition coefficient log P_{ow} was measured by ICP-OES using a Vista-MPX CCD Simultaneous ICP-OES. The DFT calculations were carried out using the Amsterdam Density Functional software (ADF2017) from SCM, the PBE0 functional, a triple zeta basis set (TZP), and COSMO to simulate the solvents effects in methanol. Human cancer cell line A549 (human lung carcinoma) and A431 (human skin carcinoma) were purchased from Sigma Aldrich, distributed by the European Collection of Cell Cultures (ECACC). Dulbecco's Modified Eagle Medium (DMEM, with and without phenol red, without glutamine), Glutamine-S (GM; 200 mg/ml), tris(hydroxymethyl)aminomethane (Tris base), trichloroacetic acid (TCA), glacial acetic acid, and sulfo-rhodamine B (SRB) were purchased from Sigma Aldrich. The measurements of complexes on photocytotoxicity were performed according to the literature.²

Synthesis and analytical data for ligands and palladium complexes



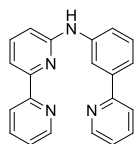
Scheme S1. (a) Toluene, BINAP, Pd(dba)₂, KO^t-Bu, 85 °C, N₂, 3 days; (b) CH₃COOH, N₂, 135 °C, 12 h.

Synthesis of 2-(3-Aminophenyl)pyridine.



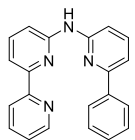
This compound was synthesized according to a modified literature procedure.³ To a suspension of 2-bromopyridine (0.78 mL, 8.16 mmol), 3-aminophenylboronic acid (1.05 g, 7.68 mmol), anhydrous K₂CO₃ (2.09 g, 15.35 mmol) and Pd(PPh₃)₄ (430.58 g, 0.38 mmol) in 1,2-dimethoxyethane (19 mL) under N₂, ethanol (6 mL) and water (13 mL) were added. The mixture was refluxed under N₂ at 95 °C for 24 h. It was allowed to cool and filtered, then water (100 mL) and EtOAc (100 mL) were added. The phases were separated and the aqueous phase was extracted with EtOAc (100 mL) for three times. The combined organic phases were dried over Na₂SO₄ and the solvent was rotary evaporated. The crude product obtained was purified by silica chromatography using pentane-EtOAc mixtures (2:1, R_f = 0.35) as eluent, to afford 1.01 g of the target compound (yield: 77%, 5.94 mmol). **ESI-MS** (cation): m/z calcd 171.1 (C₁₁H₁₀N₂ + H⁺), found 171.1. **¹H NMR** (300 MHz, CDCl₃): δ 8.69 (1 H, dt, J = 4.9, 1.4 Hz), 7.72 – 7.64 (2 H, m), 7.40 (1 H, t, J = 2.0 Hz), 7.36 (1 H, dt, J = 7.6, 1.4 Hz), 7.26 (1 H, s), 7.22 – 7.18 (1 H, m), 7.17 (0 H, d, J = 2.8 Hz), 6.73 (1 H, ddd, J = 7.9, 2.5, 1.1 Hz), 3.89 (2 H, s).

Synthesis of H_2L^1 (N-(3-(pyridin-2-yl)phenyl)-[2,2'-bipyridin]-6-amine).



A mixture of 6-bromo-2,2'-bipyridine (665 mg, 2.83 mmol), Pd(dba)₂ (29.6 mg, 0.515 mmol), racemic BINAP (61.4 mg, 0.098 mmol) and KOt-Bu (997 mg, 8.87 mmol) was partially dissolved in dry toluene (53 mL) under N₂ atmosphere.⁴ The mixture was stirred for 10 min, then 2-(3-Aminophenyl)pyridine (484 mg, 2.83 mmol) was added, followed by heating the reaction mixture to 85 °C. After 3 days stirring, the brown mixture was cooled down. Demi water (75.0 mL) was added and the mixture was stirred for 1 h. The mixture was then filtered, but no solids were obtained. The H₂O layer was separated from the toluene layer. Extracted the H₂O layer with EtOAc (100 mL) for three times and combined the toluene and EtOAc layers, followed by rotary evaporation of the solvents. The crude product obtained was purified by silica chromatography using pentane-EtOAc mixtures (2:1, R_f = 0.25) as eluent, to afford 67.1 mg of the target compound H_2L^1 (yield: 73%, 0.21 mmol). **ESI-MS** (cation): m/z calcd 325.1 (C₂₁H₁₆N₄ + H⁺), found 325.4. **¹H NMR** (300 MHz, DMSO-*d*₆): 9.40 (1 H, s), 9.03 (1 H, t, *J* = 2.0 Hz), 8.75 (1 H, ddd, *J* = 4.8, 1.8, 1.0 Hz), 8.68 (1 H, ddd, *J* = 4.7, 1.9, 0.9 Hz), 8.61 (1 H, dt, *J* = 8.0, 1.1 Hz), 8.01 – 7.97 (1 H, m), 7.96 (1 H, d, *J* = 1.4 Hz), 7.91 (1 H, td, *J* = 7.6, 1.8 Hz), 7.84 (1 H, dd, *J* = 7.5, 0.9 Hz), 7.74 (1 H, t, *J* = 7.8 Hz), 7.68 (1 H, ddd, *J* = 8.1, 2.3, 1.0 Hz), 7.60 (1 H, dt, *J* = 7.9, 1.2 Hz), 7.48 – 7.44 (1 H, m), 7.43 (1 H, d, *J* = 4.5 Hz), 7.41 – 7.35 (1 H, m), 6.93 (1 H, dd, *J* = 8.2, 0.9 Hz). **¹³C-APT NMR** (75 MHz, DMSO-*d*₆), δ 156.9, 156.2, 155.8, 153.6, 150.0, 149.6, 142.6, 139.6, 138.7, 137.6, 129.5, 124.3, 123.1, 121.0, 120.6, 119.0, 118.9, 116.6, 112.2, 111.6.

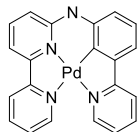
Synthesis of H_2L^2 (N-(6-phenylpyridin-2-yl)-[2,2'-bipyridin]-6-amine).



A mixture of 6-bromo-2,2'-bipyridine (665 mg, 2.83 mmol), Pd(dba)₂ (29.6 mg, 0.515 mmol), racemic BINAP (61.4 mg, 0.098 mmol) and KOt-Bu (997 mg, 8.87 mmol) was partially dissolved in 53 mL dry toluene under N₂ atmosphere.⁴ The mixture was stirred for 10 min, then 2-Amino-6-phenylpyridine (484 mg, 2.83 mmol) was added, followed by heating the reaction mixture to 85 °C. After 3 days stirring, the brown mixture was cooled down with an ice bath. Demi water (75.0 mL) was added and the mixture was stirred for 1 h. The mixture was filtered, but no solids were obtained. The H₂O layer was separated from the toluene layer. Extracted the H₂O layer with EtOAc (100 mL) for three times and combined the toluene and EtOAc layers, followed by evaporation of the solvents. The residues were dissolved in EtOAc and recrystallized in -20 °C, to get 596 mg of the target compound H_2L^2 (yield: 65%, 1.84 mmol). **ESI-MS** (cation): m/z calcd 325.1 (C₂₁H₁₆N₄ + H⁺), found 325.5. **¹H NMR** (400 MHz, DMSO-*d*₆) 9.86 (1 H, s), 8.69 (1 H, ddd, *J* = 4.7, 1.8, 0.9 Hz), 8.38 (1 H, dt, *J* = 7.9, 1.1 Hz), 8.13 – 8.07 (2 H, m), 7.99 (1 H, td, *J* = 7.7, 1.8 Hz), 7.95 – 7.79

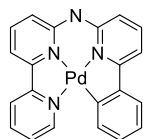
(5 H, m), 7.54 – 7.41 (5 H, m). δ ^{13}C -APT NMR (101 MHz, DMSO- d_6) δ 155.6, 154.4, 154.09, 154.0, 153.4, 149.3, 139.0, 138.8, 138.7, 137.3, 129.0, 128.8, 126.6, 124.1, 120.4, 112.8, 112.5, 112.4, 110.7.

Synthesis of PdL¹.



A mixture of H_2L^1 (36.1 mg, 0.11 mmol) and $\text{Pd}(\text{OAc})_2$ (25.2 mg, 0.11 mmol) in a glacial acetic acid was refluxed for 12 h at 135 °C under N_2 atmosphere to give a yellowish green solution. Then the solvent was rotary evaporated. The resulted yellow solid was washed with EtOAc, ether and dried in vacuum to get 43.3 mg of pure PdL^1 complex (yield: 92%, 0.10 mmol). **HRMS** (cation): m/z calcd 429.0326 ($\text{C}_{21}\text{H}_{14}\text{N}_4\text{Pd} + \text{H}^+$), found 429.0339. **^1H NMR** (400 MHz, Methanol- d_4) δ 8.34 (1 H, d, $J = 5.4$ Hz), 8.20 (1 H, d, $J = 8.1$ Hz), 8.14 (1 H, d, $J = 5.6$ Hz), 8.12 – 8.05 (1 H, m), 7.93 (1 H, td, $J = 7.9, 1.4$ Hz), 7.72 (2 H, dd, $J = 8.1, 6.1$ Hz), 7.58 (2 H, d, $J = 7.3$ Hz), 7.26 (1 H, t, $J = 6.5$ Hz), 7.15 (1 H, d, $J = 7.4$ Hz), 7.08 (1 H, t, $J = 7.6$ Hz), 7.02 (1 H, d, $J = 8.5$ Hz), 6.73 (1 H, d, $J = 7.7$ Hz). **^{13}C NMR** (101 MHz, Methanol- d_4) δ 165.9 (**Pd-C**), 155.6, 153.3, 149.7, 148.7, 147.6, 146.4, 141.1, 140.7, 138.7, 137.1, 134.6, 127.8, 127.3, 124.5, 124.0, 121.2, 119.9, 118.4, 117.6, 115.2. **Elemental analysis** calcd for $\text{PdL}^1 + 2\text{H}_2\text{O} + \text{CH}_3\text{COOH}$: C, 52.63; H, 4.23; N, 10.67; Found for $\text{PdL}^1 + 2\text{H}_2\text{O} + \text{CH}_3\text{COOH}$: C, 52.21; H, 4.42; N, 10.43.

Synthesis of PdL².



The synthesis of complex PdL^2 was similar to that of PdL^1 , except that ligand H_2L^2 was used. A mixture of H_2L^2 (32.4 mg, 0.1 mmol) and $\text{Pd}(\text{OAc})_2$ (22.4 mg, 0.1 mmol) in a glacial acetic acid was refluxed for 12 h at 135 °C under N_2 atmosphere to give a yellowish green solution. Then the solvent was rotary evaporated. The resulted yellow solid was washed with EtOAc, ether and dry in vacuum to get 40.7 mg of pure PdL^2 complex (yield: 95%, 0.095 mmol). The red rectangular single crystal of PdL^2 was obtained by slow evaporation of ether into a saturated methanol solution of PdL^2 . **HRMS** (cation): m/z calcd 429.0326 ($\text{C}_{21}\text{H}_{14}\text{N}_4\text{Pd} + \text{H}^+$), found 429.0337. **^1H NMR** (400 MHz, Methanol- d_4) δ 8.05 (1 H, d, $J = 5.6$ Hz), 7.80 – 7.65 (2 H, m), 7.53 (1 H, t, $J = 7.9$ Hz), 7.45 (1 H, t, $J = 7.8$ Hz), 7.25 (1 H, d, $J = 7.5$ Hz), 7.17 (1 H, t, $J = 6.3$ Hz), 6.99 (1 H, d, $J = 7.6$ Hz), 6.87 (1 H, d, $J = 7.6$ Hz), 6.80 (2 H, t, $J = 7.2$ Hz), 6.74 – 6.65 (1 H, m), 6.55 (1 H, d, $J = 8.2$ Hz), 6.49 (1 H, d, $J = 7.6$ Hz). **^{13}C NMR** (101 MHz, Methanol- d_4) δ 162.27 (**Pd-C**), 156.0, 154.3, 149.4, 148.8, 146.7, 145.7, 145.6, 139.1, 138.8, 138.8, 129.9, 128.4, 125.8, 124.8, 123.4, 122.8, 116.4, 114.9, 112.7, 111.2. **Elemental analysis** calcd for $\text{PdL}^2 + \text{H}_2\text{O} + \text{MeOH}$: C, 55.18; H, 4.21; N, 11.70; Found for $\text{PdL}^2 + \text{H}_2\text{O} + \text{MeOH}$: C 55.13; H 4.22; N 11.15.

^1H and ^{13}C -APT NMR of ligands and palladium complexes

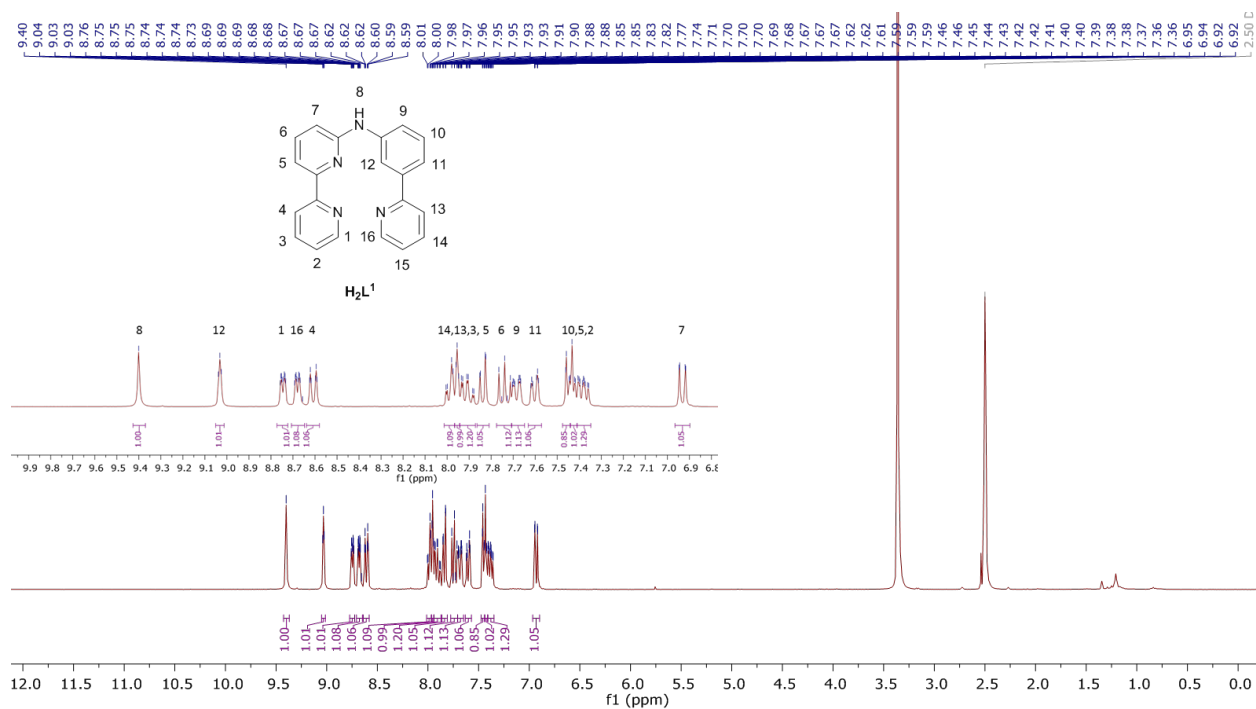


Fig. S1. ^1H NMR of ligand H_2L^1 in $\text{DMSO}-d_6$.

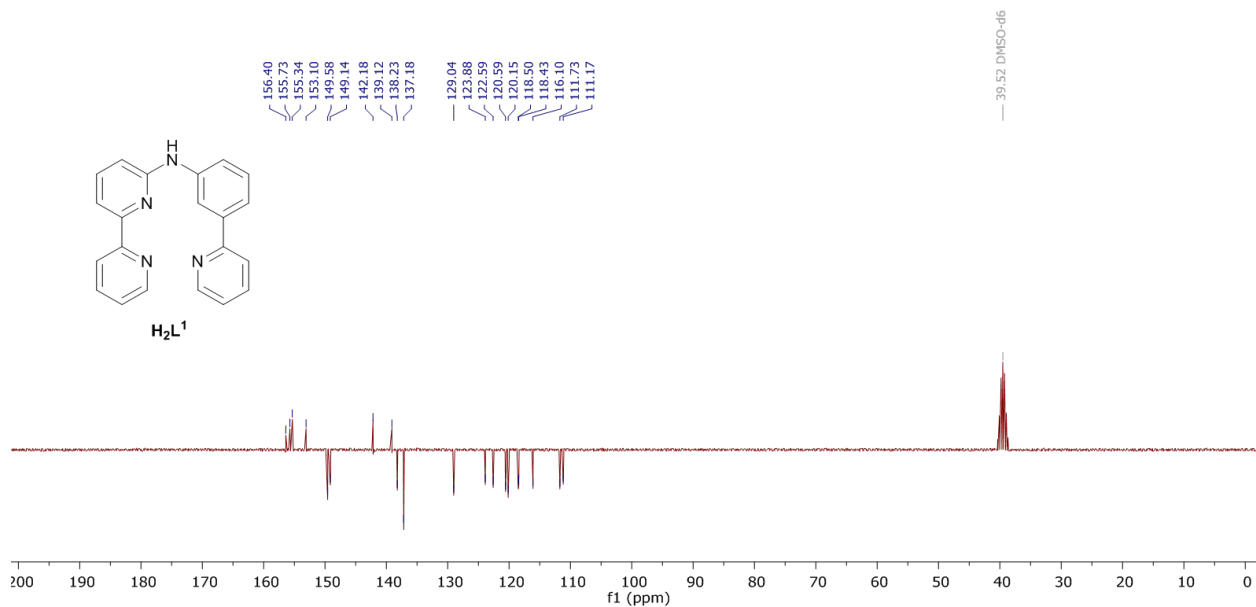
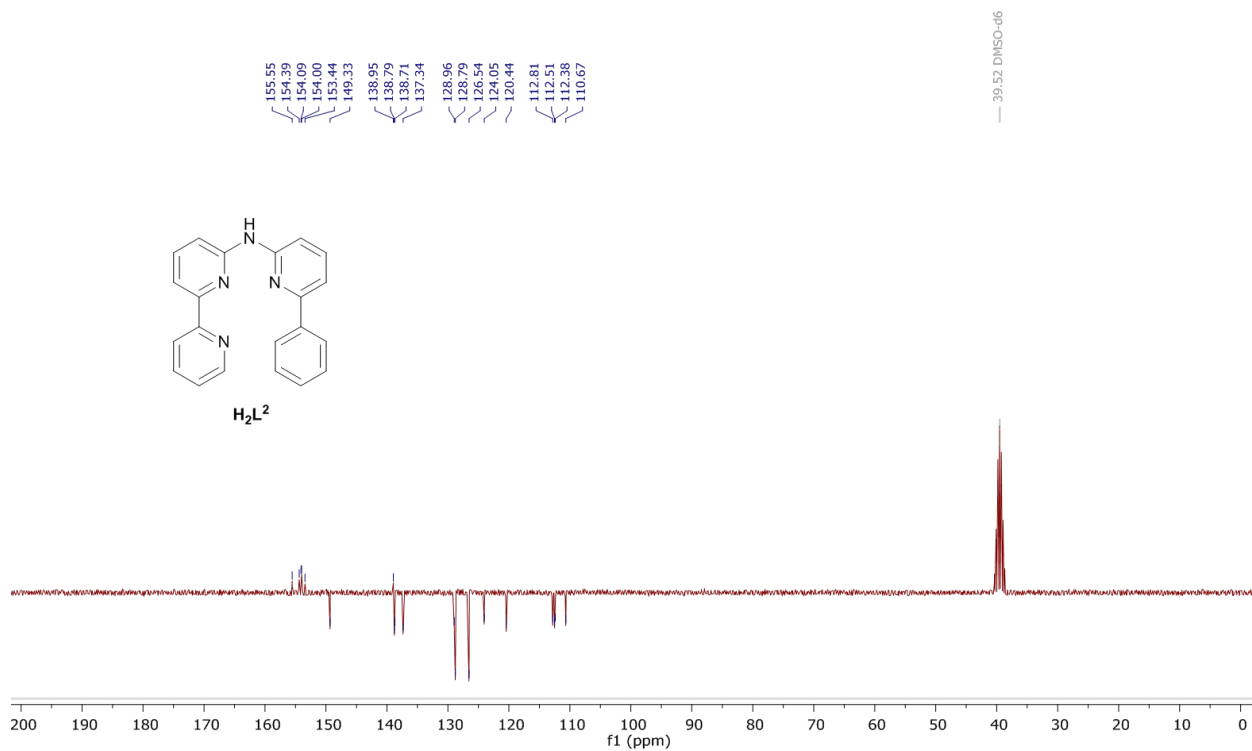
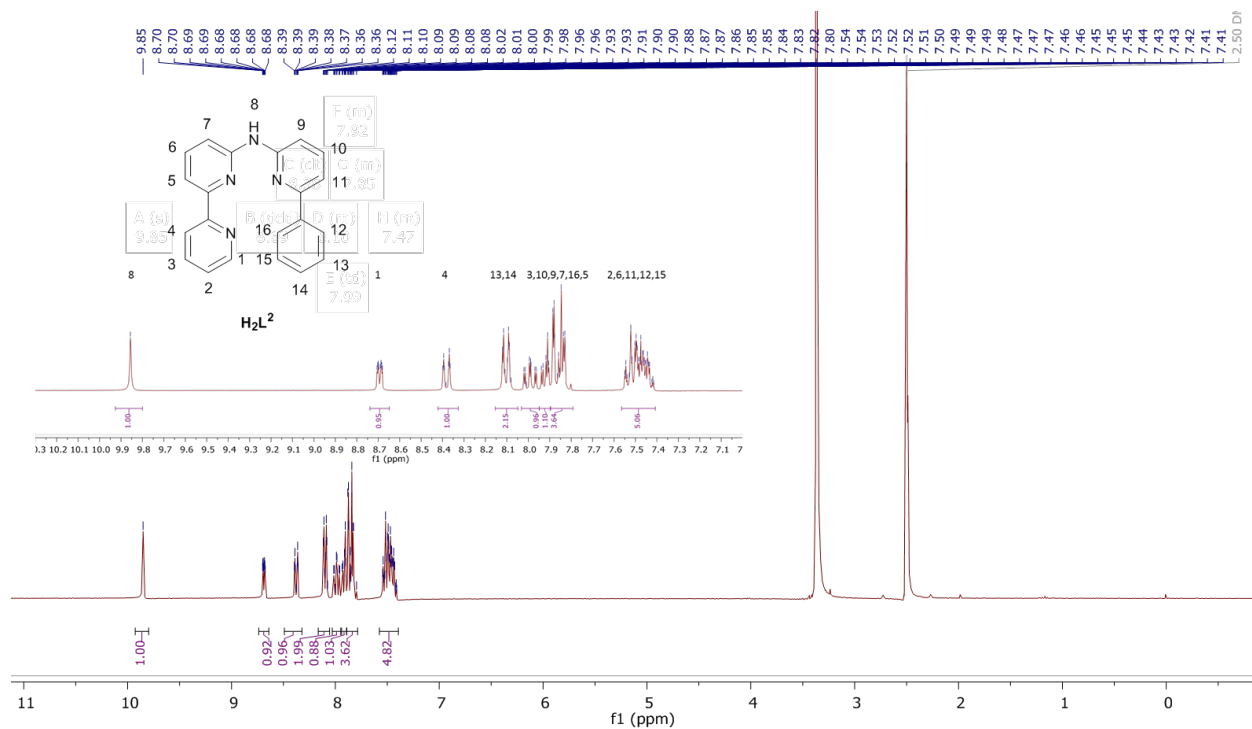


Fig. S2. ^{13}C -APT NMR of ligand H_2L^1 in $\text{DMSO}-d_6$.



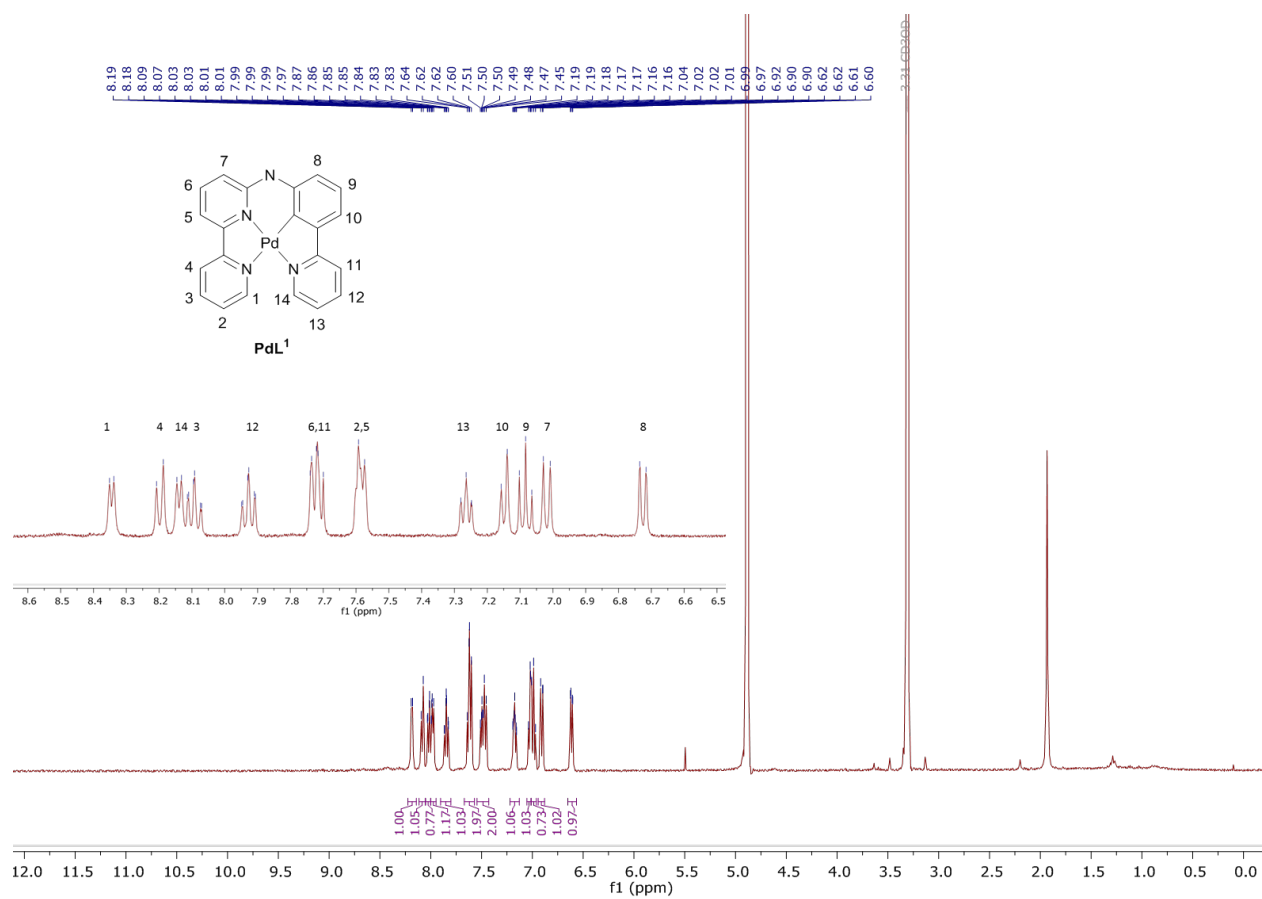


Fig. S5. ¹H NMR of PdL¹ in methanol-*d*₄.

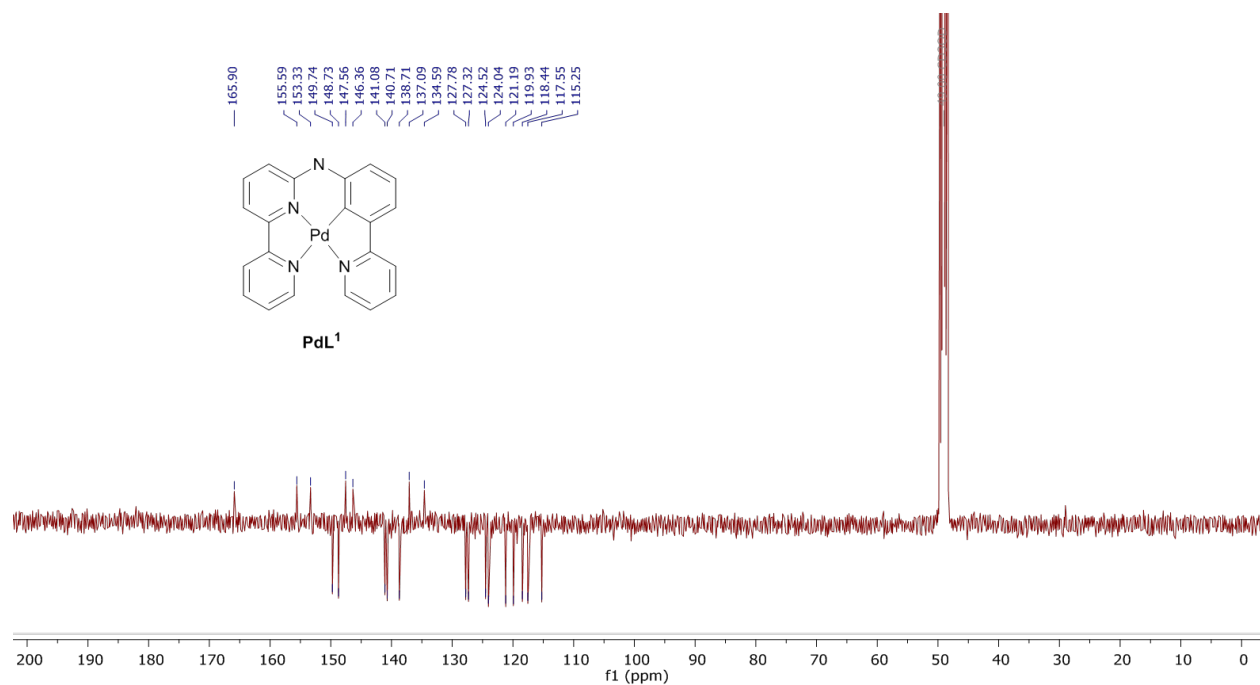


Fig. S6. ¹³C-APT NMR of PdL¹ in methanol-*d*₄.

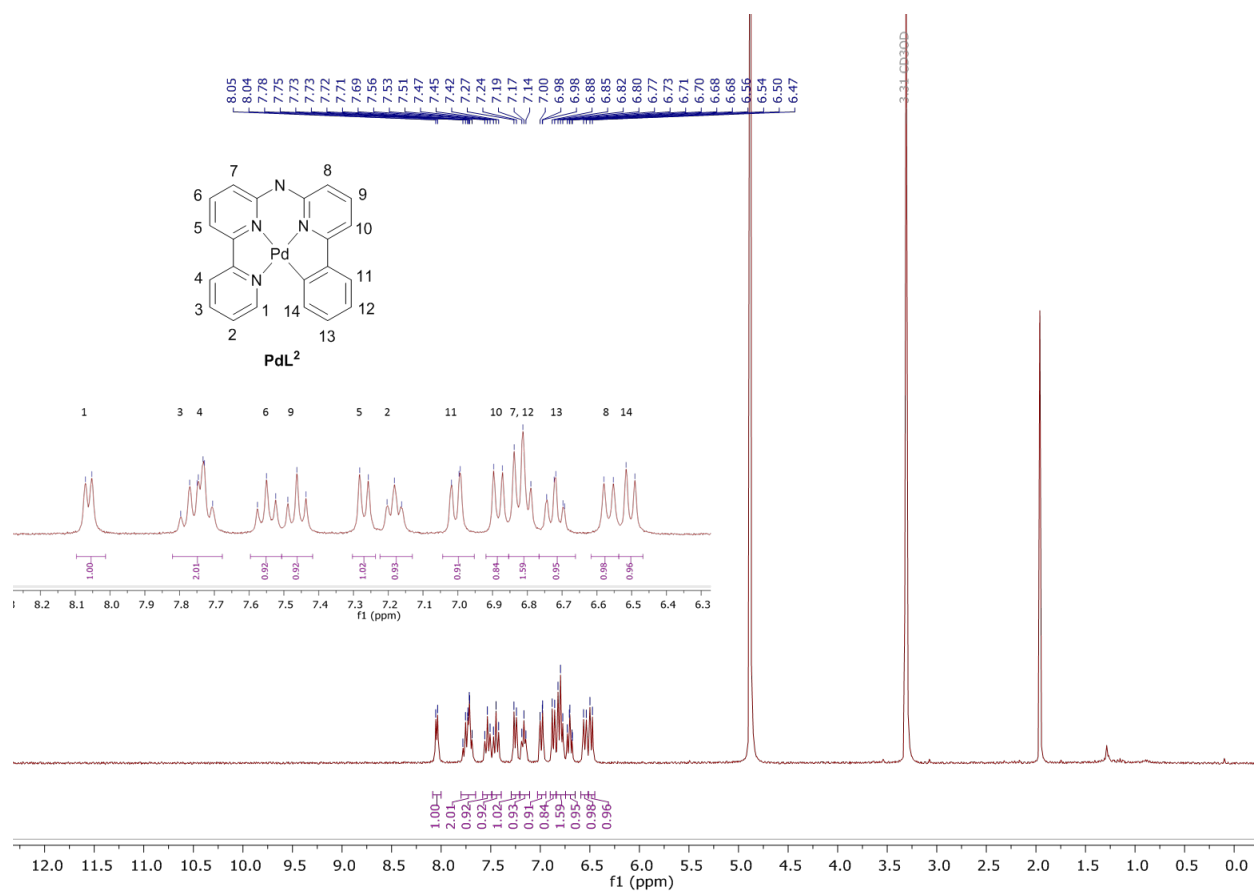


Fig. S7. ^1H NMR of PdL^2 in $\text{methanol-}d_4$.

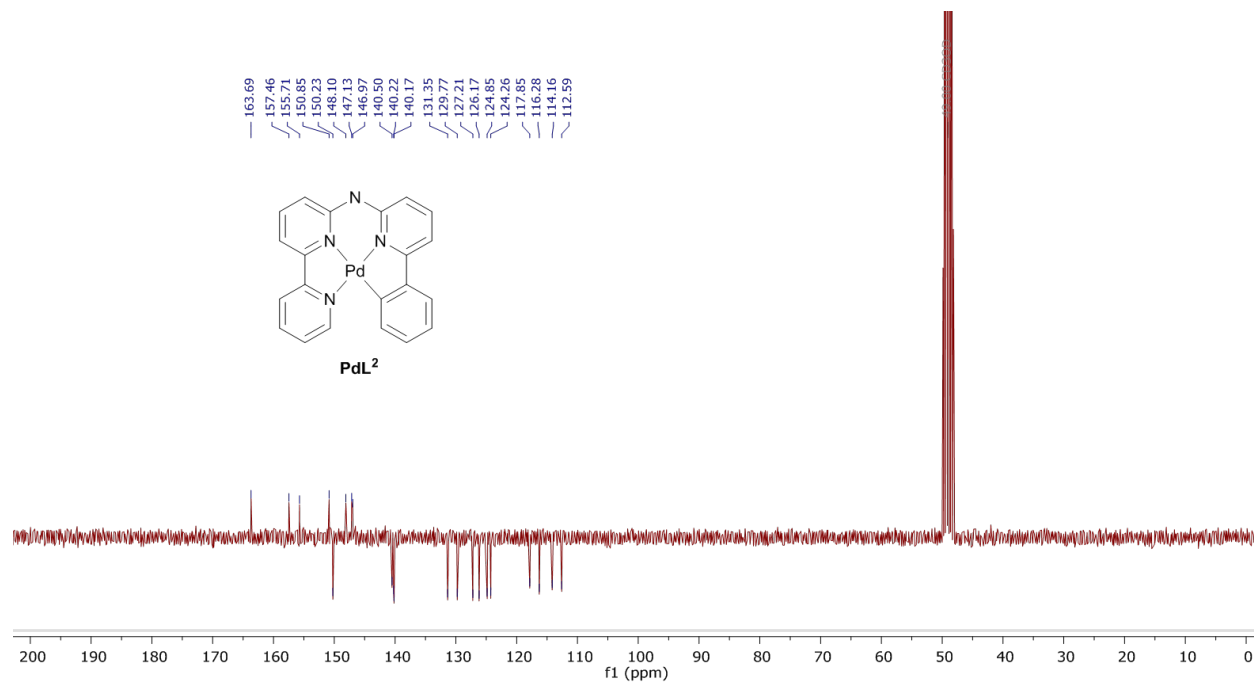


Fig. S8. ^{13}C -APT NMR of PdL^2 in $\text{methanol-}d_4$.

IR spectrum of ligands, palladium complexes, and reference complex [Fe(bbpya)(NCS)₂]

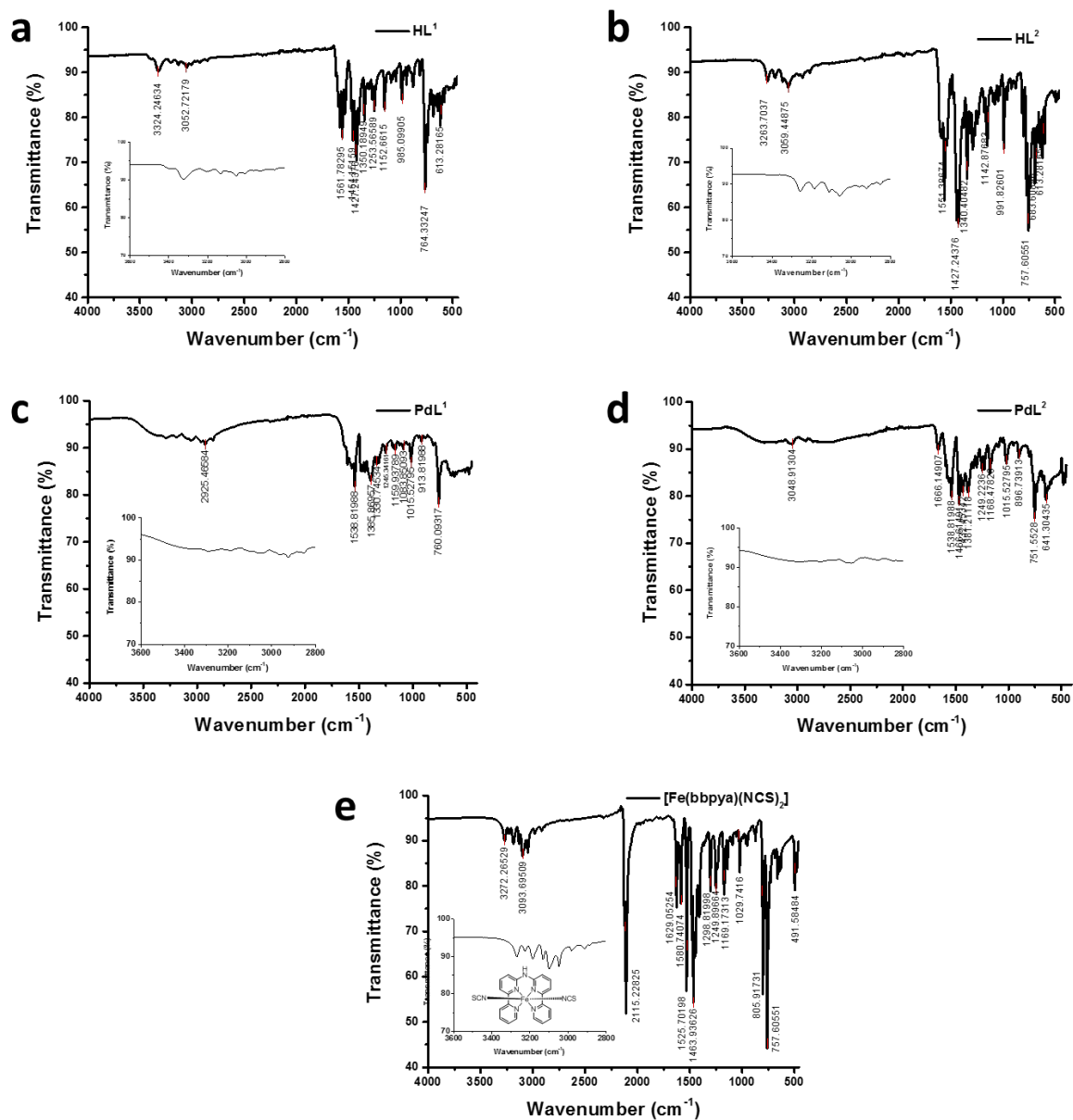


Fig S9. IR spectra of ligands HL¹ (a), HL² (b), palladium complexes PdL¹ (c), PdL² (d), and the reference complex [Fe(bbpya)(NCS)₂] (e). Insert show a zoom of the NH stretch region.

Single Crystal X-ray Crystallography

All reflection intensities were measured at 110(2) K using a SuperNova diffractometer (equipped with Atlas detector) with Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) under the program CrysAlisPro (Version CrysAlisPro 1.171.39.29c, Rigaku OD, 2017). The same program was used to refine the cell dimensions and for data reduction. The structure was solved with the program SHELXS-2014/7 (Sheldrick, 2015) and was refined on F^2 with SHELXL-2014/7⁵. Numerical absorption correction based on gaussian integration over a multifaceted crystal model was applied using CrysAlisPro. The temperature of the data collection was controlled using the system Cryojet (manufactured by Oxford Instruments). The H atoms were placed at calculated positions using the instructions AFIX 43 with isotropic displacement parameters having values 1.2 U_{eq} of the attached C atoms. The structure is disordered. The whole Pd complex is disordered over two orientations as both orientations may have the same space filling requirement. The occupancy factor of the major component of the disorder refines to 0.835(3).

Table S1. Crystallographic Data for **PdL²**

complex	PdL²
Crystal data	
Chemical formula	C ₂₁ H ₁₄ N ₄ Pd
M_r	428.76
Crystal system, space group	Monoclinic, $P2_1/n$
Temperature (K)	110
a, b, c (Å)	10.3734 (3), 7.2412 (2), 20.6547 (6)
β (°)	100.020 (3)
V (Å ³)	1527.83 (8)
Z	4
Radiation type	Mo $K\alpha$
μ (mm ⁻¹)	1.23
Crystal size (mm)	0.23 × 0.11 × 0.04

Data collection

Diffractionmeter SuperNova, Dual, Cu at zero, Atlas

Absorption correction Gaussian
CrysAlis PRO 1.171.39.29c (Rigaku Oxford Diffraction, 2017) Numerical absorption correction based on gaussian integration over a multifaceted crystal model Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.

T_{\min}, T_{\max} 0.625, 1.000

No. of measured,
independent and
observed [$I > 2\sigma(I)$]
reflections 20278, 3517, 3063

R_{int} 0.036

$(\sin \theta/\lambda)_{\text{max}}$ (\AA^{-1}) 0.650

Refinement

$R[F^2 > 2\sigma(F^2)], wR(F^2), S$ 0.025, 0.060, 1.07

No. of reflections 3517

No. of parameters 320

No. of restraints 581

H-atom treatment H-atom parameters constrained

$\Delta\rho_{\text{max}}, \Delta\rho_{\text{min}}$ (e \AA^{-3}) 0.88, -0.79

Computer programs: *CrysAlis PRO* 1.171.39.29c (Rigaku OD, 2017), *SHELXS2014/7* (Sheldrick, 2015), *SHELXL2014/7* (Sheldrick, 2015), *SHELXTL* v6.10 (Sheldrick, 2008).

Stability of metal complexes

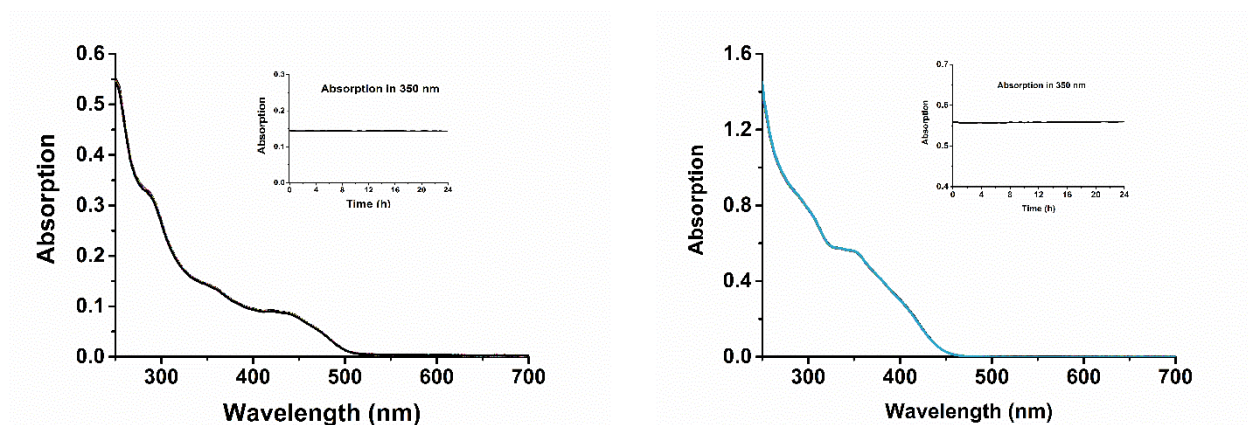


Fig. S10. Time-evolution of the absorption spectra of **PdL¹** (left) and **PdL²** (right) in PBS: DMSO (50 μm, 1:1) solution at 310 K for 24 hours. The spectra were measured every 30 min.

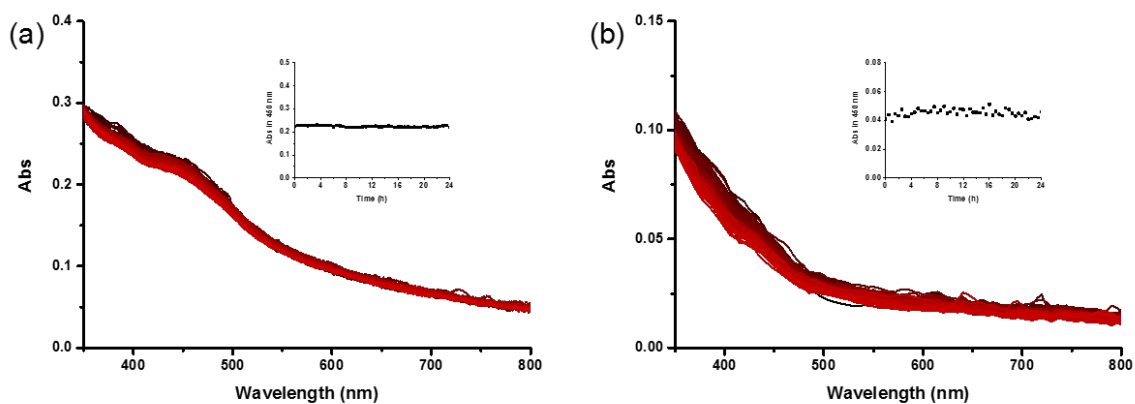


Fig. S11. Evolution of the absorption spectrum of complexes **PdL¹** (a) and **PdL²** (b) when incubated in optiMEM containing 2.5% FCS at 310 K for 24 hours. The spectra were measured every 30 min. Inset: time evolution of the absorbance at 450 nm. The color of spectra change from black (0 h) to red (24 h).

Partition coefficients (log P_{ow}) of palladium complexes

The partition coefficients of palladium complexes were determined by shake-flask method. Briefly, each palladium complexes was dissolved (0.8 mM) in octanol-saturated water and ultrasonicated for 1 h. After filtering with 0.2 μM membrane filters, aliquots of the stock solutions (0.2 mL) were transferred to 15 mL centrifuge tubes and diluted up to 6.0 mL. Then 6.0 mL of water-saturated octanol was added to one of the tubes and the mixture was shaken in a IKA Vibrax shaker for 24 h at 2200 pm. The mixture were then centrifuged for 20 min at 4300 rpm to separate the water phase. For each of the samples a 5.5 mL aliquot of the aqueous layer was taken using a syringe and 65% HNO₃ (vol) was added to it to give diluted samples with 5% HNO₃ final concentration. The palladium concentrations [Pd]_{aq} (in ppm) of the samples were determined by ICP-OES using a Vista-MPX CCD Simultaneous ICP-OES. Partition coefficients log P_{ow} were calculated using the equation below:

$$\log P_{ow} = \log ([Pd]_{oct}/[Pd]_{aq}) = \log ([Pd]_{total} - [Pd]_{aq}) / [Pd]_{aq}$$

where [Pd]_{total} is the concentration of palladium in the sample that was not extracted with octanol, and [Pd]_{aq} is the concentration of palladium in the aqueous layer after octanol addition, as a mean of three replicates.

Table S2. Octanol-water partition coefficients (log P_{ow}) of the two palladium complexes.

Complex	log P _{ow}
PdL ¹	-0.64
PdL ²	0.046

Cell culturing

Cells were thawed and at least passaged twice before starting photocytotoxicity experiments. Cells were cultured in DEMEM complete medium (Dulbecco's Modified Eagle Medium (DMEM) with phenol red, supplemented with 8.0% v/v fetal calf serum (FCS), 0.2% v/v penicillin/streptomycin (P/S), and 0.9% v/v Glutamine), under humidified normoxic conditions, 37 °C atmosphere, 21% O₂ and 7.0% CO₂ in 75 cm² flasks. They were sub-cultured upon reaching 70-80% confluence, approximately once per week. Cells were passaged never more than 8 weeks.

Cytotoxicity assay

The cell irradiation system consists of a Ditabis thermostat (980923001) fitted with two flat-bottom microplate thermoblocks (800010600) and a 96-LED array fitted to a standard 96-well plate. The 455 nm LED (FNL-U501B22WCSL), fans (40 mm, 24 V DC, 9714839), and power supply (EA-PS 2042-06B) were ordered from Farnell. Full description of the cell irradiation setup is given in Hopkins et al.² The cytotoxicity of **PdL¹** and **PdL²** were assayed in normoxic conditions (21 % O₂) against two human cancer cell lines (A549 and A431) according to a published protocol. Briefly, 24 h after seeding, the cells were treated with **PdL¹** and **PdL²** with a range of 6 different concentrations (0, 0.625, 3.125, 6.25, 12.5, 31.25, 62.5 μM, maximum 0.5% DMSO was used in each well). After 24 h incubation, one plate was irradiated with blue light (455 nm, 5 min, 10.5 mW cm⁻², 3.2 J cm⁻²) while the other was left in the dark. Cell viability was then assayed 96 h after seeding using standard sulforhodamine B (SRB) assay.⁶ Half maximal effective concentrations (EC₅₀) for cell growth inhibition were calculated by fitting the curves using a non-linear regression function (Equation S1) for the dose–response curves of treated vs. non-treated wells via Graphpad prism 5.

$$100 / (1 + 10^{\log_{10} EC_{50} - X} \times Hill\ Slope)$$

Equation S1

EC₅₀ curves and values of palladium complex in cancer cells

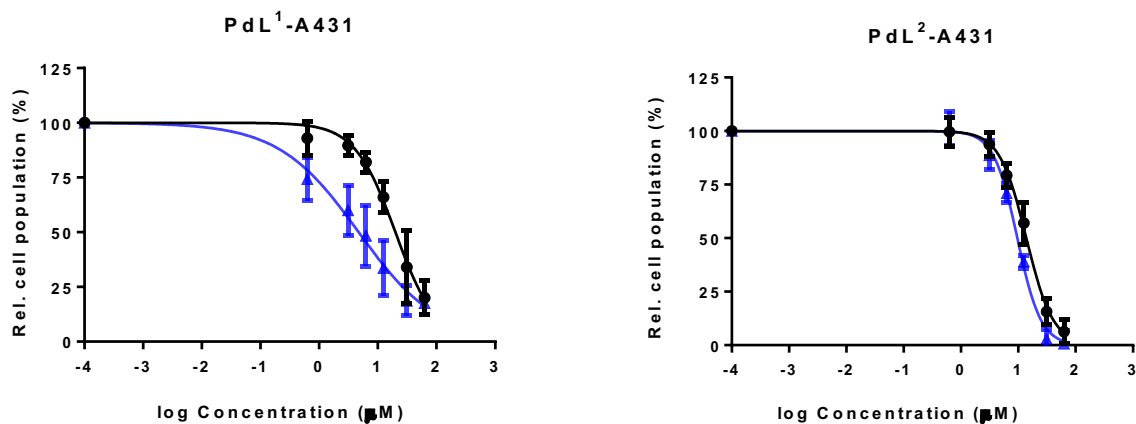


Fig. S12. Dose-response curves for A431 cells incubated with palladium complexes and irradiated 5 min with blue light (blue data points), or left in the dark (black data points). Photocytotoxicity assay outline: cells seeded at 5×10^3 cells/well at $t = 0$ h; treated with **PdL¹** or **PdL²** at $t = 24$ h; irradiated at $t = 48$ h, blue light (455 nm, 5 min, 10.5 mW cm^{-2} , 3.2 J cm^{-2}); SRB assay performed at $t = 96$ h. Incubation conditions: 37°C , 21% O_2 , 7% CO_2 .

Singlet oxygen generation and phosphorescence quantum yield

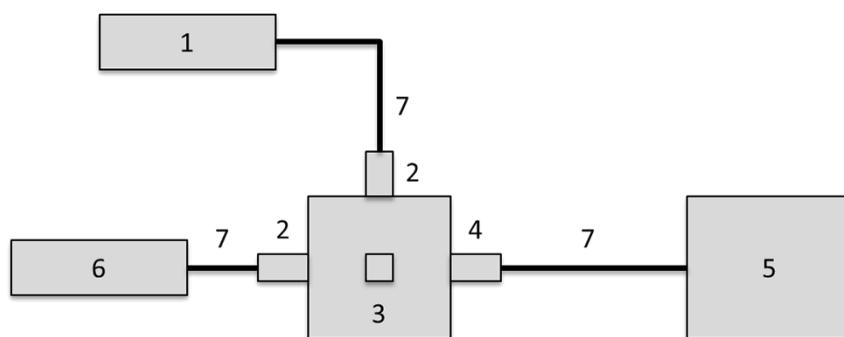
The quantum yields of singlet oxygen generation and phosphorescence were determined in a custom-built setup shown in Scheme S2. All optical parts were connected with optical fibres from Avantes, with a diameter of 200–600 μm . The sample, consisting of the compound in deuterated methanol (500 μL), was added to a 104F-OS semi-micro fluorescence cuvette from Hellma Analytics, and placed in a CUV-UV/VIS-TC temperature-controlled cuvette holder (Avantes). The sample was allowed to equilibrate at 298 K for 5 minutes. Emission spectroscopy was performed with a 450 nm fibre-coupled laser (LRD-0450, Laserglow), which was set to 50 mW at the cuvette (4 mm beam diameter; $0.4 \text{ W}\cdot\text{cm}^{-2}$) at a 90° angle with respect to the spectrometer. The excitation power was measured using a S310C thermal sensor connected to a PM100USB power meter (Thorlabs). The emission spectra were recorded using two separate spectrometers for the UV-Vis and NIR emission, i.e. from 300 nm to 1000 nm for the phosphorescence of the complex (Avantes 2048L StarLine spectrometer) and from 1000 nm to 1700 nm for the phosphorescence of singlet oxygen ($^1\Delta_g$) around 1275 nm (Avantes NIR256-1.7TEC spectrometer, detector set to -12°C). The infrared emission spectrum was acquired within 9 seconds, after which the laser was turned off directly. Similarly, the visible emission spectrum was acquired within 2 seconds. UV-Vis absorption spectra before and after emission spectroscopy were measured using an Avalight-DHc halogen-deuterium lamp (Avantes) as light source (turned off during emission spectroscopy) and the before mentioned UV-Vis spectrometer as detector, both connected to the cuvette holder at

a 180° angle. All spectra were recorded using Avasoft 8.5 software from Avantes and further processed using Microsoft Office Excel 2010 and Origin Pro 9.1 software.

The quantum yields of phosphorescence and singlet oxygen production was calculated using the relative method with [Ru(bpy)₃]Cl₂ as the standard ($\Phi_{\Delta} = 0.73$, $\Phi_p = 0.015$ in MeOD),^{7,8} according to Equation below:

$$\Phi_{\text{sam}} = \Phi_{\text{std}} \times \frac{A_{\text{std}}^{450}}{A_{\text{sam}}^{450}} \times \frac{E_{\text{sam}}}{E_{\text{std}}}$$

where Φ is the quantum yield, A^{450} is the absorbance at 450 nm (always kept below 0.1 for a 4 mm path length), E is the integrated emission peak of singlet oxygen at 1270 nm or the integrated phosphorescence emission peak between 520 and 950 nm, and *sam* and *std* denote the sample and standard, respectively.



Scheme S2. Setup used for Φ_{Δ} and Φ_p determination. (1) 450-nm CW laser light source, (2) collimating lens, (3) temperature-controlled cuvette holder, (4) double collimator, (5) UV-Vis (300-1000 nm) or NIR (1000-1700 nm) CCD spectrometer, (6) UV-Vis halogen-deuterium light source, and (7) optical fibers.

Photophysical data for PdL¹ and PdL²

Table S3. Photophysical data for PdL¹ and PdL²

Complex	λ_{abs} , nm ($\epsilon \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) ^a	λ_{em} (nm) ^b	ϕ_{p} ^b	ϕ_{Δ} ^b	Lifetime (ns) ^{a,c}	
					τ_1	τ_2
PdL ¹	251 (26.8), 286 (17.1), 422 (4.3)	539	0.0017	0.89	0.271±0.002 (97%)	6.4±0.3 (3%)
PdL ²	283 (16.7), 347 (11.7)	604	0.00084	0.38	0.333±0.005 (96%)	5.3±0.5 (4%)

^a Measurements were carried out in methanol.

^b Measurements were carried out at 450 nm excitation wavelength and in a solution of deuterated methanol in air atmosphere according to literature.⁹ The absorption of complexes at 450 nm were set below 0.1 to avoid the generation of excimer. A solution of [Ru(bpy)₃]Cl₂ (Tris(2,2'-bipyridyl)dichlororuthenium(II)) in deuterated methanol (photoluminescence quantum yield $\phi_{\text{p}} = 0.015$, singlet oxygen quantum yield $\phi_{\Delta} = 0.73$) was used as reference.¹⁰

^c Excitation source 375 nm. Biexponential model: $y = y_0 + A_1 \cdot \exp(-(x-x_0)/\tau_1) + A_2 \cdot \exp(-(x-x_0)/\tau_2)$

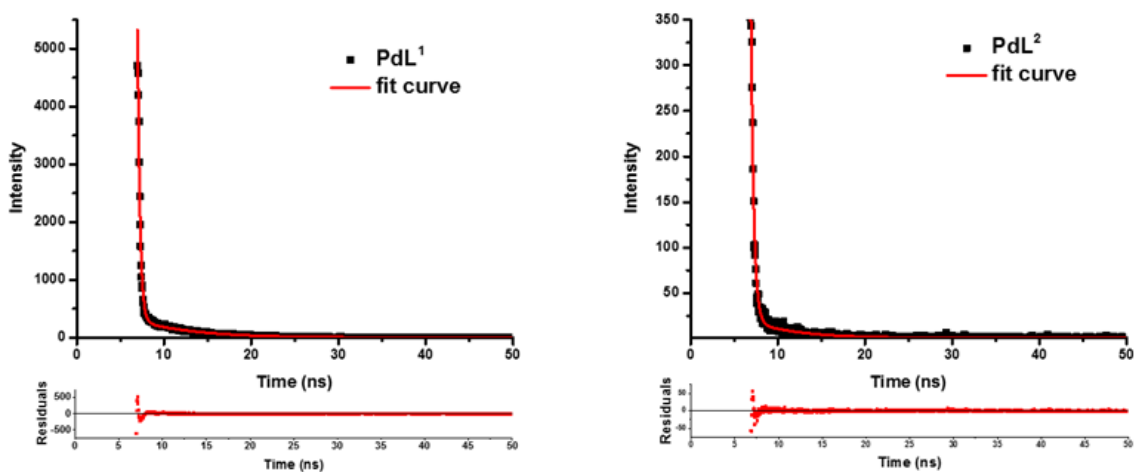


Fig. S13. The phosphorescence lifetime spectra and fit curve of palladium complexes in methanol. Fit equation: $y = y_0 + A_1 \cdot \exp(-(x-x_0)/\tau_1) + A_2 \cdot \exp(-(x-x_0)/\tau_2)$.

Calculated values and TDDFT-Calculated spectra of metal complexes

Both deprotonated and protonated isomers **PdL¹**, **PdL²**, **[Pd(HL¹)]⁺**, and **[Pd(HL²)]⁺**, were minimized by DFT at the PBE0/TZP level as implemented in the ADF2017 suite from SCM, using COSMO to simulate solvent effects (in methanol), no frozen core, and starting from the X-ray structure of **PdL²**. The x,y,z coordinates of the 4 minimized structures are given in Table S5 and S6, and the LUMO and HOMO energy levels in Table S4. The 20 first singlet electronic transitions were calculated by TDDFT also using ADF2017 and at the same level of theory, using the Davidson method.

Table S4. HOMO and LUMO energies, Energy gap (ΔE) of **PdL¹** and **PdL²**

Complex	LUMO/eV	HOMO/eV	ΔE /eV
PdL¹	-2.048	-5.258	3.21
PdL²	-2.114	-5.644	3.53

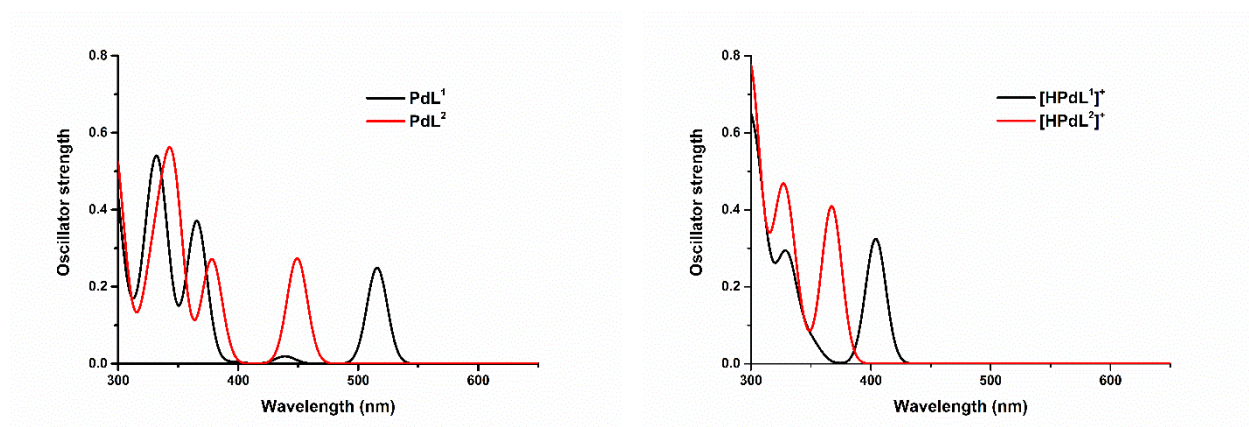


Fig. S14. TDDFT-calculated spectra for **PdL¹**, **PdL²** (left) and **[PdHL¹]⁺**, **[PdHL²]⁺** (right) at the TZP/COSMO level in methanol. The theoretical curves were calculated and plotted in ADF with Gaussian Fixed Oscillator Strengths. Scaling factor = 1.0, peak width = 30 nm.

Table S5. Cartesian coordinates for optimized geometries of **PdL¹** and **PdL²**.

PdL¹	x	y	z	PdL²	x	y	z
Pd	1.137643	3.615392	3.350828	Pd	1.128663	3.612644	3.367378
N	3.15572	4.153465	3.700072	C	3.083275	4.060677	3.699578
C	1.09046	5.493197	2.761197	N	1.096799	5.537853	2.759836
C	1.659889	0.77165	4.728443	C	1.660986	0.88833	4.788252
H	2.637876	1.169066	4.960959	H	2.613048	1.330304	5.040862
C	1.318841	-0.49985	5.142175	C	1.345791	-0.3861	5.217513
H	2.034532	-1.10227	5.68641	H	2.063558	-0.94891	5.799843
C	0.046343	-0.96365	4.843879	C	0.104141	-0.90423	4.887483
H	-0.26915	-1.95487	5.147712	H	-0.18356	-1.90068	5.201012
C	-0.82519	-0.13789	4.162015	C	-0.77624	-0.12218	4.163829
H	-1.8252	-0.48242	3.942829	H	-1.75927	-0.49886	3.919568
C	-0.41257	1.1366	3.773461	C	-0.39933	1.156309	3.766113
C	-1.30898	2.078572	3.074771	C	-1.31257	2.060515	3.042805
C	-2.57316	1.701863	2.6954	C	-2.55809	1.67547	2.601907
H	-2.93587	0.701522	2.876711	H	-2.92243	0.668971	2.747711
C	-3.39705	2.646107	2.058527	C	-3.34949	2.629865	1.948712
H	-4.39844	2.369854	1.747275	H	-4.33499	2.356713	1.588917
C	-2.92839	3.901628	1.843344	C	-2.88022	3.899753	1.768361
H	-3.53237	4.660417	1.362021	H	-3.47201	4.657769	1.271111
C	-1.6038	4.286312	2.238754	C	-1.582	4.265422	2.227006
C	-0.05669	6.112535	2.239546	C	-0.0293	6.128072	2.275787
C	0.047477	7.489996	1.9269	C	0.030752	7.521334	1.991426
H	-0.83286	7.980127	1.522831	H	-0.86669	7.988589	1.606976
C	1.214095	8.2029	2.120562	C	1.178772	8.232283	2.199221
H	1.247597	9.257719	1.869053	H	1.208029	9.294288	1.981226
C	2.346364	7.577266	2.631438	C	2.319669	7.589039	2.689942
H	3.259551	8.14294	2.774179	H	3.236525	8.137918	2.85459
C	2.277124	6.222072	2.948758	C	2.250252	6.241305	2.960709
C	3.41476	5.462444	3.462376	C	3.362549	5.424188	3.453059

C	4.686822	5.984231	3.68057	C	4.649781	5.935138	3.635244
H	4.878532	7.032668	3.496016	H	4.856586	6.983948	3.452541
C	5.701476	5.157503	4.124794	C	5.682262	5.103499	4.039133
H	6.69414	5.556865	4.297392	H	6.679514	5.505119	4.179856
C	5.433035	3.81313	4.333921	C	5.433318	3.751899	4.242905
H	6.196978	3.12212	4.665531	H	6.24048	3.087472	4.533976
C	4.14789	3.359703	4.107163	C	4.148132	3.244029	4.069597
H	3.908815	2.315267	4.246502	H	4.009002	2.178885	4.214491
N	-1.27699	5.541805	1.976759	N	-1.20775	5.53583	2.010508
N	-0.82179	3.335511	2.844845	N	-0.84587	3.317565	2.851486
N	0.832395	1.571672	4.052002	N	0.828901	1.641497	4.065899

Table S6. Cartesian coordinates for optimized geometries of $[\text{PdHL}^1]^+$ and $[\text{PdHL}^2]^+$.

$[\text{PdHL}^1]^+$	x	y	z	$[\text{PdHL}^2]^+$	x	y	z
Pd	1.12933	3.626128	3.363518	Pd	1.03899	3.630136	3.336455
N	3.14384	4.156755	3.692859	C	3.002852	4.028285	3.604625
C	1.101142	5.506642	2.774109	N	1.06163	5.521018	2.800544
C	1.645714	0.810118	4.769012	C	1.722927	1.109526	4.838272
H	2.607656	1.226333	5.031571	H	2.527535	1.532548	5.115776
C	1.315213	-0.46924	5.172368	C	1.479025	-0.18482	5.274022
H	2.026521	-1.05701	5.737415	H	2.111078	-0.63766	5.819688
C	0.063751	-0.95865	4.837184	C	0.313102	-0.79836	4.899305
H	-0.24006	-1.95644	5.130037	H	0.133744	-1.69608	5.153443
C	-0.80675	-0.14799	4.133328	C	-0.58737	-0.09028	4.154462
H	-1.79374	-0.51008	3.88603	H	-1.42419	-0.47455	3.926503
C	-0.40624	1.132311	3.762519	C	-0.27139	1.194629	3.731684
C	-1.30878	2.060044	3.055779	C	-1.21776	2.053152	3.017426
C	-2.56852	1.682087	2.653801	C	-2.44365	1.622158	2.52163
H	-2.92539	0.676941	2.81783	H	-2.69455	0.707179	2.569483
C	-3.39047	2.619851	2.025864	C	-3.30149	2.575777	1.950016

H	-4.38552	2.341159	1.701012	H	-4.15657	2.315353	1.631039
C	-2.93352	3.889985	1.828704	C	-2.89512	3.896195	1.853226
H	-3.54518	4.644498	1.35019	H	-3.47189	4.556011	1.485571
C	-1.62921	4.227472	2.248968	C	-1.61973	4.237386	2.308216
C	-0.01726	6.14645	2.258614	C	0.017626	6.181351	2.295673
C	0.049147	7.509476	1.933573	C	0.10258	7.527371	1.911028
H	-0.82795	8.00686	1.531374	H	-0.64191	7.970782	1.522811
C	1.221276	8.217053	2.123609	C	1.294583	8.189525	2.10966
H	1.255914	9.269865	1.868669	H	1.380701	9.097331	1.844321
C	2.350132	7.588377	2.633147	C	2.380242	7.529152	2.701628
H	3.263637	8.154172	2.771029	H	3.190263	7.992803	2.879865
C	2.286047	6.235562	2.955634	C	2.248757	6.188948	3.021845
C	3.418677	5.463804	3.461037	C	3.323068	5.338132	3.547003
C	4.696442	5.969707	3.668188	C	4.594055	5.79808	3.844581
H	4.897681	7.017042	3.487728	H	4.786088	6.728245	3.804106
C	5.705745	5.125575	4.093516	C	5.565592	4.913392	4.192818
H	6.705222	5.510561	4.257367	H	6.420408	5.222082	4.469048
C	5.422934	3.783306	4.291724	C	5.294339	3.576878	4.141336
H	6.182349	3.079159	4.604811	H	5.975374	2.937036	4.312249
C	4.129635	3.345501	4.078186	C	4.002833	3.171452	3.836879
H	3.880763	2.302705	4.208556	H	3.818964	2.239376	3.789737
N	-1.22857	5.50484	2.026842	N	-1.20815	5.554476	2.133152
H	-1.93519	6.082046	1.596617	H	-1.8496	6.093355	1.867487
N	0.822703	1.591995	4.069839	N	0.889773	1.793613	4.056455

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