Supporting Information
for
Structure-antiroliferative activity studies on L-proline- and homoproline-4-*N*-pyrrolydine-3-thiosemicarbazone hybrids and their nickel(II), palladium(II) and copper(II) complexes

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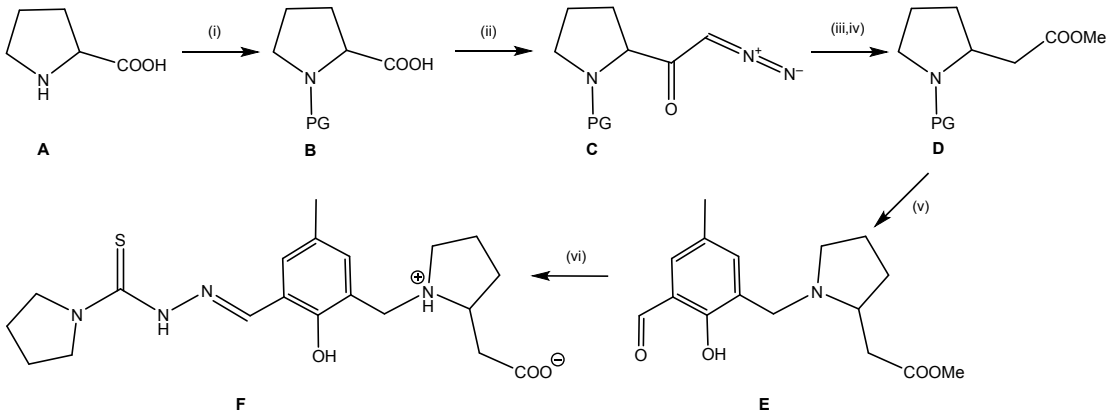

Reactions and conditions: (i) benzyl chloroformate (PG), NaOH, 30 min, 0 °C (88%); (ii) oxalyl chloride, 30 min, 0 °C, TMS-diazomethane, 6 h, 0 °C (80%); (iii) silver benzoate, triethylamine, dry MeOH, 1 h, r.t. (85%); (iv) H_2/Pd/C, dry MeOH, 1 h, r.t. (80%); (v) 3-(chloromethyl)-hydroxy-5-methylbenzaldehyde, triethylamine, 10 h, 60 °C, purification by column chromatography, 1.5:1 THF/CH_2Cl_2 (80%); (vi) 4-pyrrolidine-3-thiosemicarbazide, 1:1 EtOH/H_2O, 24 h, 80 °C, [(*S*/*R*)-H_2L^2, 43%].

Scheme S1. The synthesis of H_2L^2.

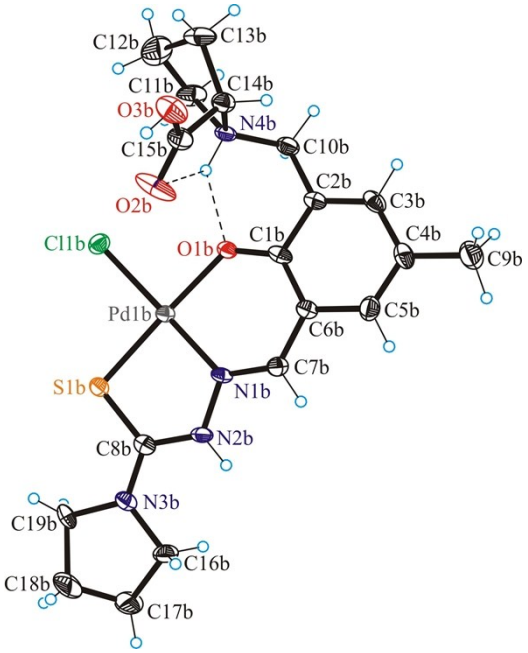

Figure S1. ORTEP view of another crystallographically independent complex cation in the asymmetric unit of [Pd(H_2L^1)Cl]Cl (**2**) with atom labeling scheme; thermal ellipsoids were drawn at 50% probability level. Selected bond distances (Å) and bond angles (deg): Pd1b−Cl1b 2.3109(12), Pd1b−O1b 2.010(3), Pd1b−N1b 1.972(4), Pd1b−S1b 2.2427(11), C1b−O1b 1.308(6), N1b−N2b 1.402(4), N2b−C8b 1.352(6), C8b−S1b 1.714(5), C8b−N3b 1.331(5); O1b−Pd1b−N1b 91.95(14), N1b−Pd1b−S1b 86.79(11). Parameters of intramolecular bifurcated hydrogen bonding interactions N4b−H···O1b and N4b−H···O2b are as follows: N4b···O1b 2.639(0) Å, N4b−H···O1b 133.3, N4b···O2b 2.696(0) Å, N4b−H···O2b 115.4°.

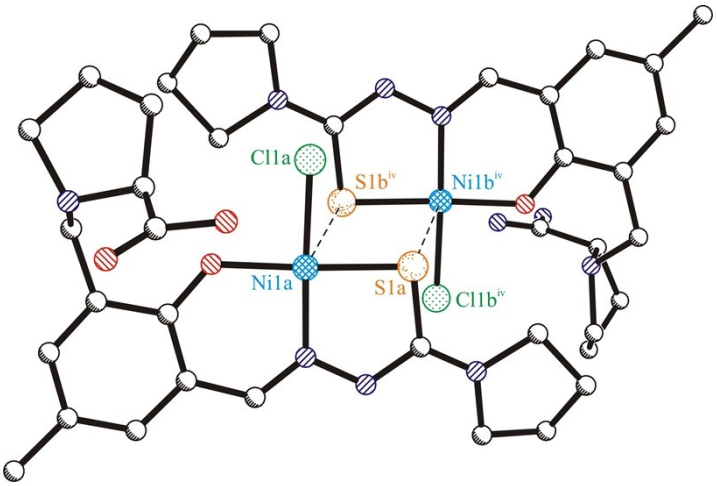

Figure S2. Fragment of crystal structure of **1** showing the intermolecular interactions Ni1a···S1b^{iv} (–*x*, *y* – 0.5, –*z* + 1) = 3.6868(15) Å and Ni1b^{iv}···S1a = 3.6969(15) Å. The interplanar separation between aromatic rings in the central pair is ca. 3.25 Å. The angles Cl1a–Ni1a–S1b^{iv} and Cl1b^{i}–Ni1b^{ii}–S1a are at 83.5 and 83.2°, respectively.

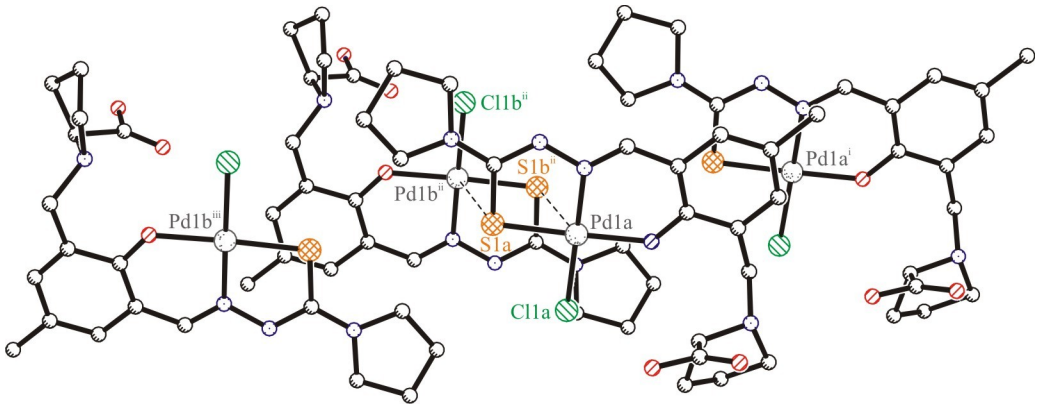

Figure S3. Fragment of the crystal structure of **2** showing the intermolecular interactions Pd1a···S1b^{ii} (x + 1, y, z) = 3.5879(17) Å and Pd1b^{ii}···S1a = 3.5967(16) Å. The interplanar separation between aromatic rings in the central pair is ca. 3.16 Å. The interplanar separation between aromatic rings in terminal pairs is ca. 3.66 Å (right) and 3.77 (left). The angles Cl1a–Pd1a–S1b^{ii} and Cl1b^{ii}–Pd1b^{ii}–S1a are at 84.2 and 83.2°, respectively. Atoms Pd1a^{i}, Pd1b^{ii} and Pd1b^{iii} were generated by symmetry transformations x – 1, y, z, x + 1, y, z and x + 2, y, z, respectively, of atoms Pd1a and Pd1b.

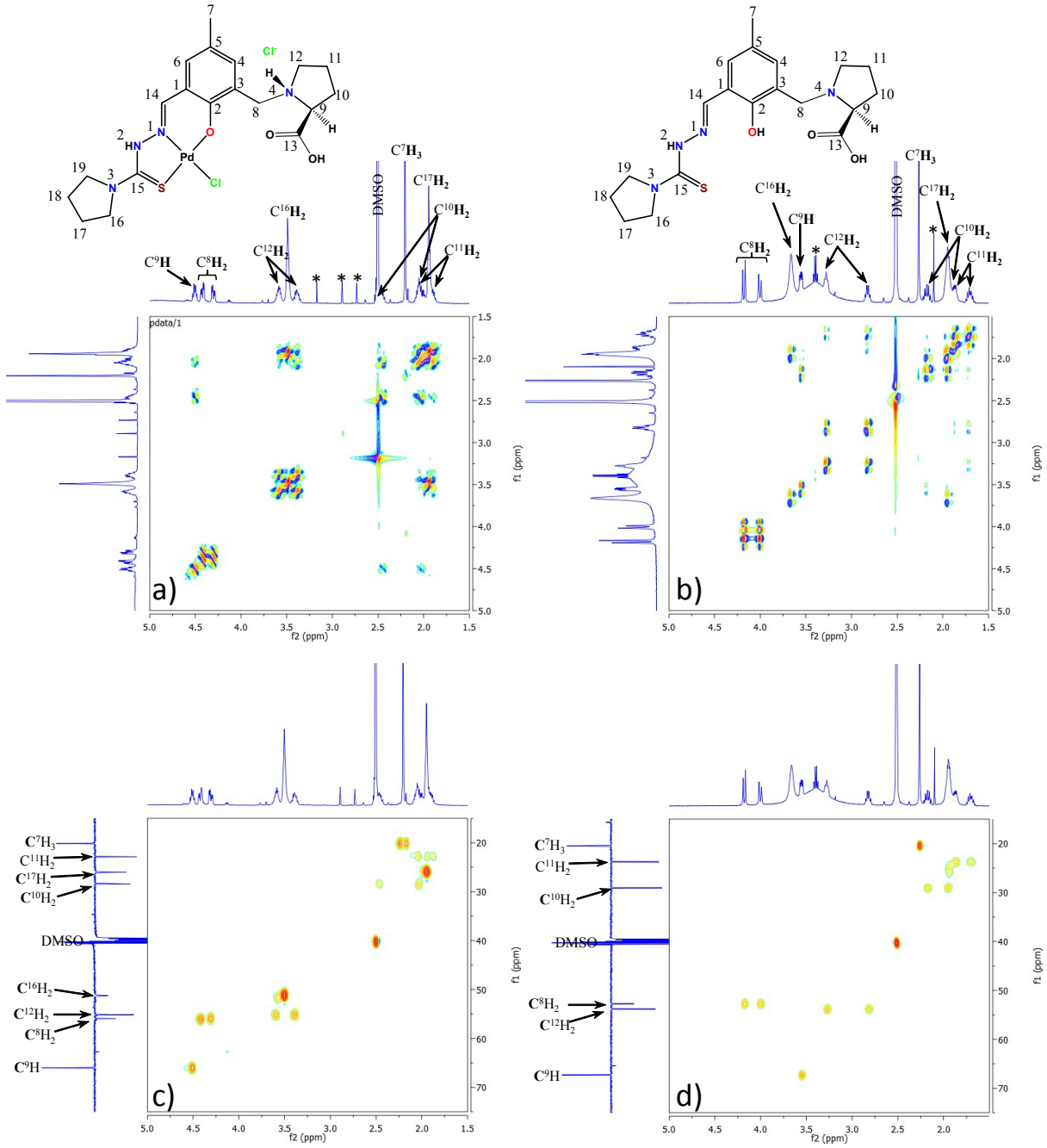

Figure S4. Fragment of COSY $^{1}H–^{1}H$ phase-sensitive experiment for **2** (a) and H_2L^1 (b); HSQC $^{1}H–^{13}C$ experiment for **2** (c) and H_2L^1 (d) in DMSO-d_6 at room temperature.

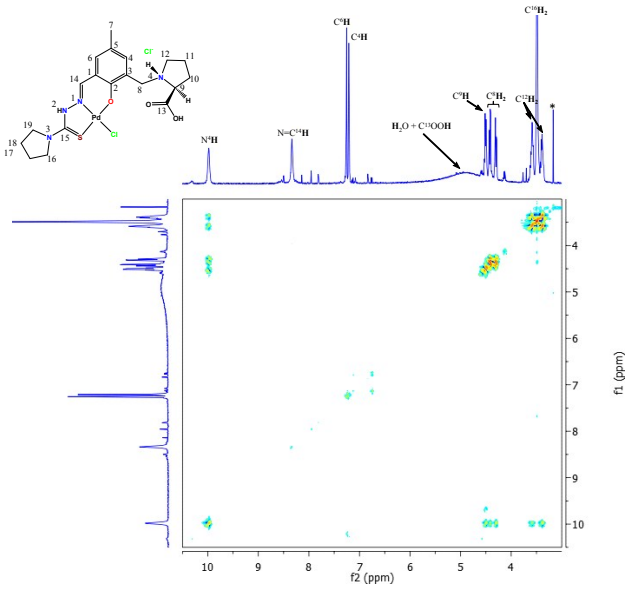

Figure S5. Fragment of COSY ^{1}H–^{1}H phase-sensitive experiment for **2**.

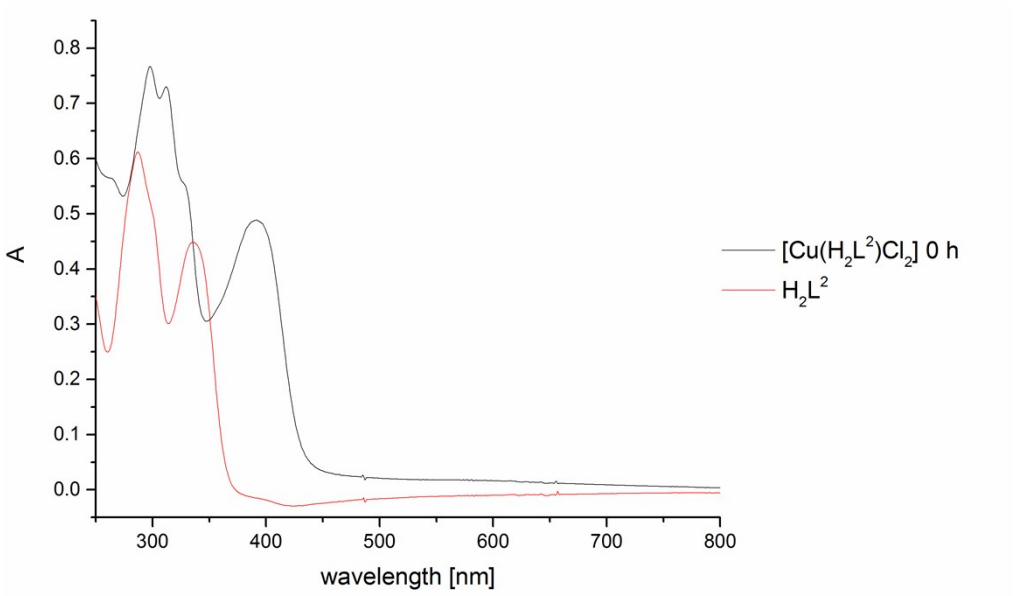

Figure S6. UV–vis spectra for H_2L^2 and **4** in aqueous solution with 1% dimethyl sulfoxide.

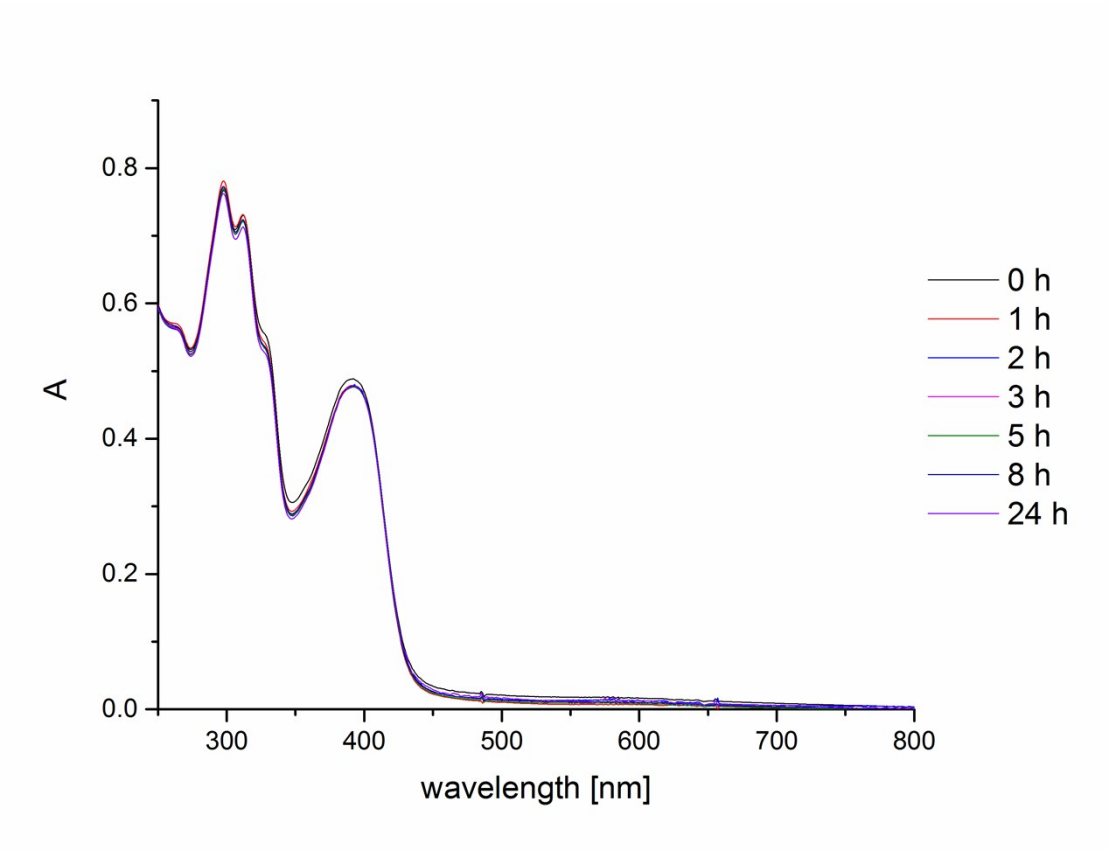

Figure S7. The optical spectra of **4** in water containing 1% dimethyl sulfoxide measured over 24 h.

Assay for antiproliferative effect in NIH/3T3 mouse embryonal fibroblast cells

The effect of increasing concentrations of the compounds on cell proliferation was tested in 96-well flat-bottomed microtiter plates. 5×10^3 NIH/3T3 mouse embryonal fibroblast cells in 100 µL of medium were added to each well, with the exception of the medium control wells. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% of bovine calf serum. The cells were seeded for 4 h then the compounds were diluted in a volume of 100 µL of medium and added to the cells. The culture plates were incubated at 37 °C for 72 h; at the end of the incubation period, 20 µL of MTT (thiazolyl blue tetrazolium bromide, Sigma) solution (from a stock solution of 5 mg/mL) were added to each well. After incubation at 37 °C for 4 h, 100 µL of sodium dodecyl sulfate (SDS) (Sigma) solution (10% in 0.01 M HCI) were added to each well and the plates were further incubated at 37 °C overnight. Cell growth was determined by measuring the optical density (OD) at 550/630 nm with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA). Inhibition of the cell growth was determined according to the formula:

$$IC_{50} = 100 - \left[\frac{OD\,sample - OD\,medium\,control}{OD\,cell\,control - OD\,medium\,control} \right] \times 100$$

Results are expressed in terms of IC_{50}, defined as the inhibitory dose that reduces the growth of the cells exposed to the tested compounds by 50%.

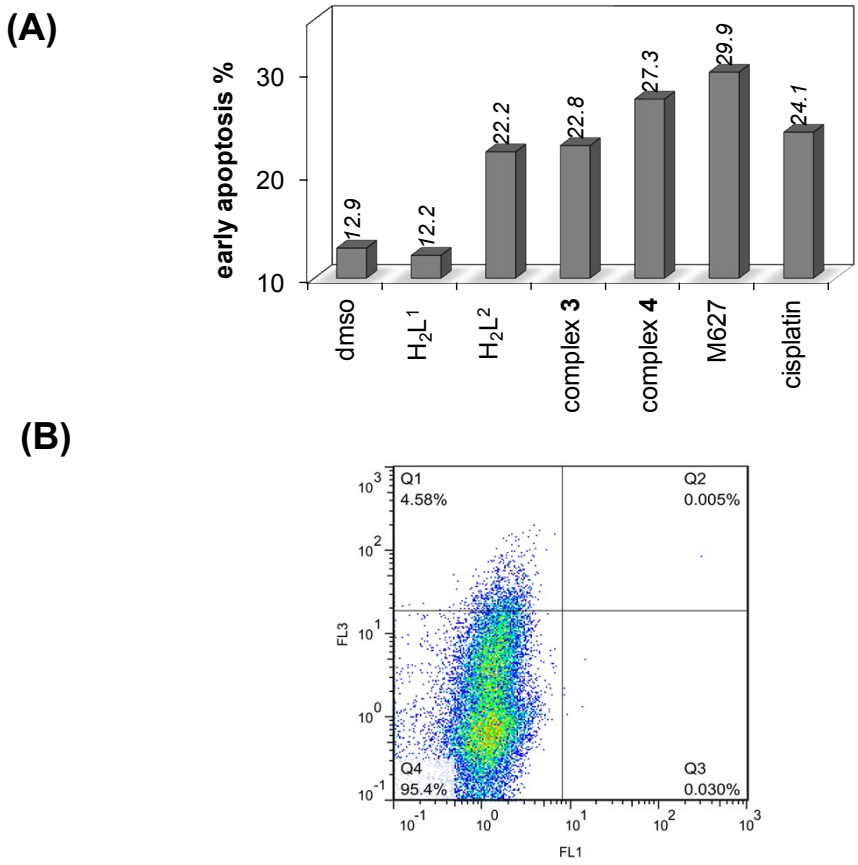

Figure S8. Apoptosis induction in A549 nonsmall lung carcinoma cells treated with 20 μM of compounds: (A) early apoptosis of the gated population shown for the tested compounds (1% DMSO was used for the test); (B) Vehicle-treatment control for the flow cytometric analysis of Annexin-V/PI staining used to analyse apoptosis in the A549 cells.

Table S1. Hydrogen bonding interactions in **1**.

D–H	H···A	D···A	∠D–H···A	H-bond
0.84	2.08	2.878(5)	159.0	O3A–H···Cl2B^{i}(x – 1, y, z + 1)
0.88	2.29	3.140(5)	161.3	N2A–H···Cl2A
1.00	2.22	2.739(7)	110.5	N4A–H···O2A
1.00	2.32	3.219(5)	149.2	N4A–H···Cl2B^{ii}(x, y, z + 1)
0.84	2.12	2.923(5)	161.3	O3B–H···Cl2A
0.88	2.34	3.169(5)	157.1	N2B–H···Cl2B^{iii}(x –1, y, z)
1.00	2.95	3.802(4)	143.2	N4B–H···Cl1B
1.00	1.90	2.661(5)	130.8	N4B–H···O1B
1.00	2.12	2.667	113.3	N4B–H···O2B

Table S2. Selected matrix elements for simulation of NMR spectra (see Figure 3 main text).

H_2L^1	Label	Value	N	Spin	Line width (Hz)
1(C8Ha)	A	4.177 ppm	1	1/2	3.3
	JA-B	13.00 Hz			
2(C8Hb)	B	4.005 ppm	1	1/2	5
	JB-A	13.00 Hz			
3(C9H)	C	3.554 ppm	1	1/2	3.6
	JC-E	4.50 Hz			
	JC-F	8.60 Hz			

$[Pd(H_2L^1)Cl]Cl$	Label	Value	N	Spin	Line width (Hz)
1 (C8Ha)	A	4.307 ppm	1	1/2	3.8
	JA-B	13.00 Hz			
	JA-D	5.00 Hz			
2 (C8Hb)	B	4.419 ppm	1	1/2	3.4
	JB-A	13.00 Hz			
	JB-D	5.00 Hz			
3(C9H)	C	4.5085 ppm	1	1/2	3.5
	JC-D	6.20 Hz			
	JC-E	7.00 Hz			
	JC-F	9.20 Hz			