Electronic Supplementary Material (ESI) for Analyst.
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Electronic Supplementary Information(ESI)

for

Azodye−Rhodamine-Based Fluorescent and Colorimetric Probe Specific for the Detection of Pd^{2+} in Aqueous Ethanolic Solution: Synthesis, XRD characterization, Computational Studies, and Imaging in Live Cells

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1. **^{1}H NMR spectrum of L (400 MHz, $CDCl_3$):**

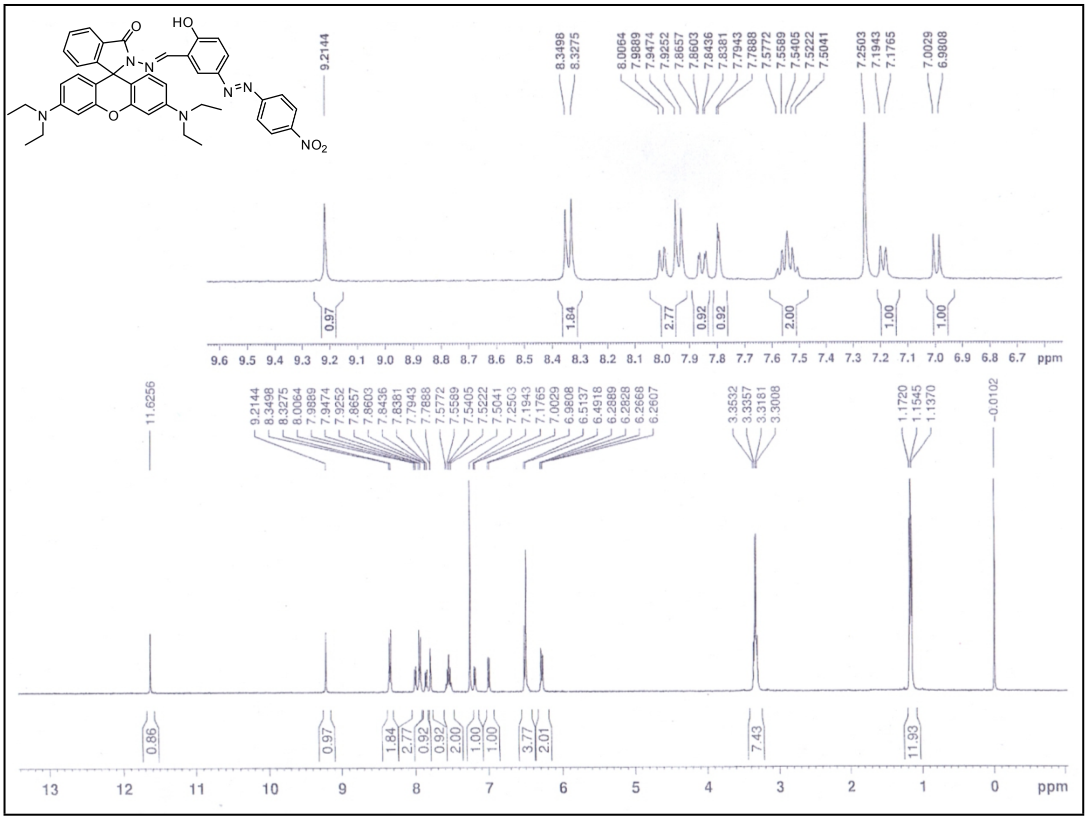

Figure S1: ^{1}H NMR spectrum of **L** in $CDCl_3$ solution.

2. ^{13}C NMR spectrum of L (400 MHz, CDCl$_3$):

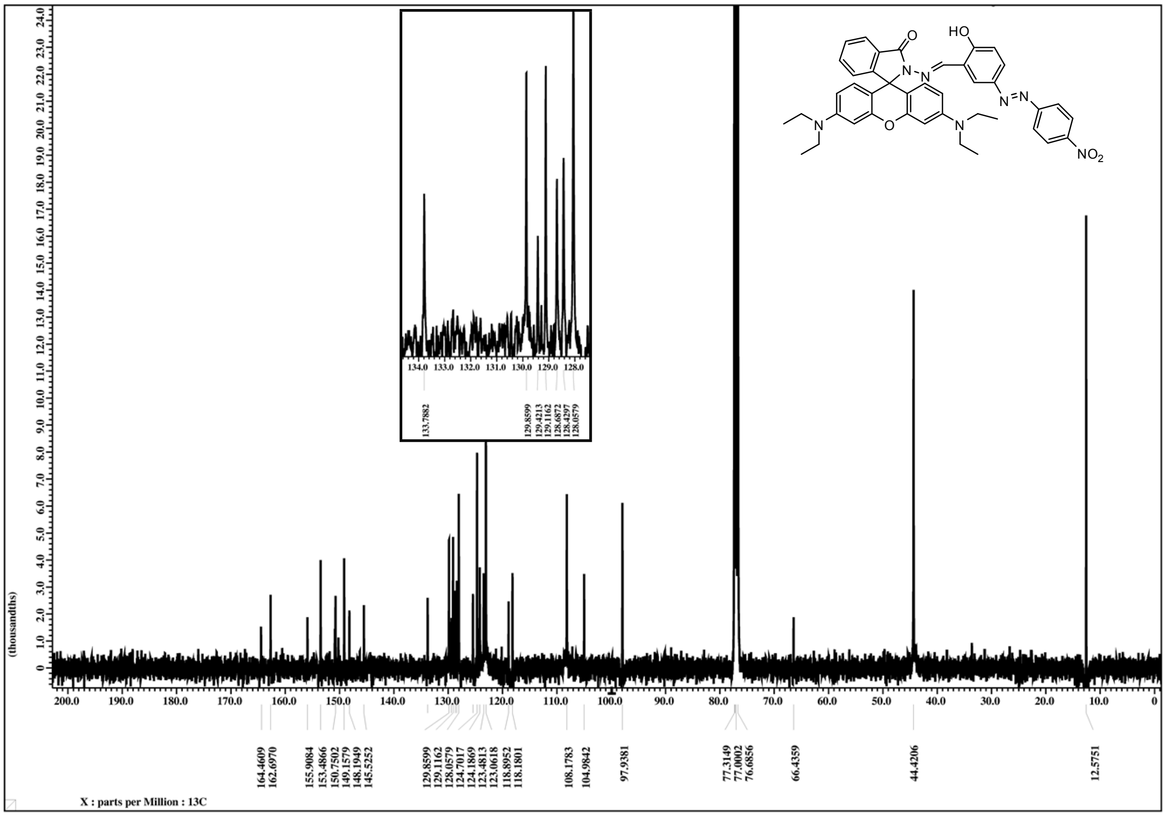

Figure S2: ^{13}C NMR spectrum of **L** in CDCl$_3$ solution.

3. **TOF MS ES+ Mass Spectrum of L:**

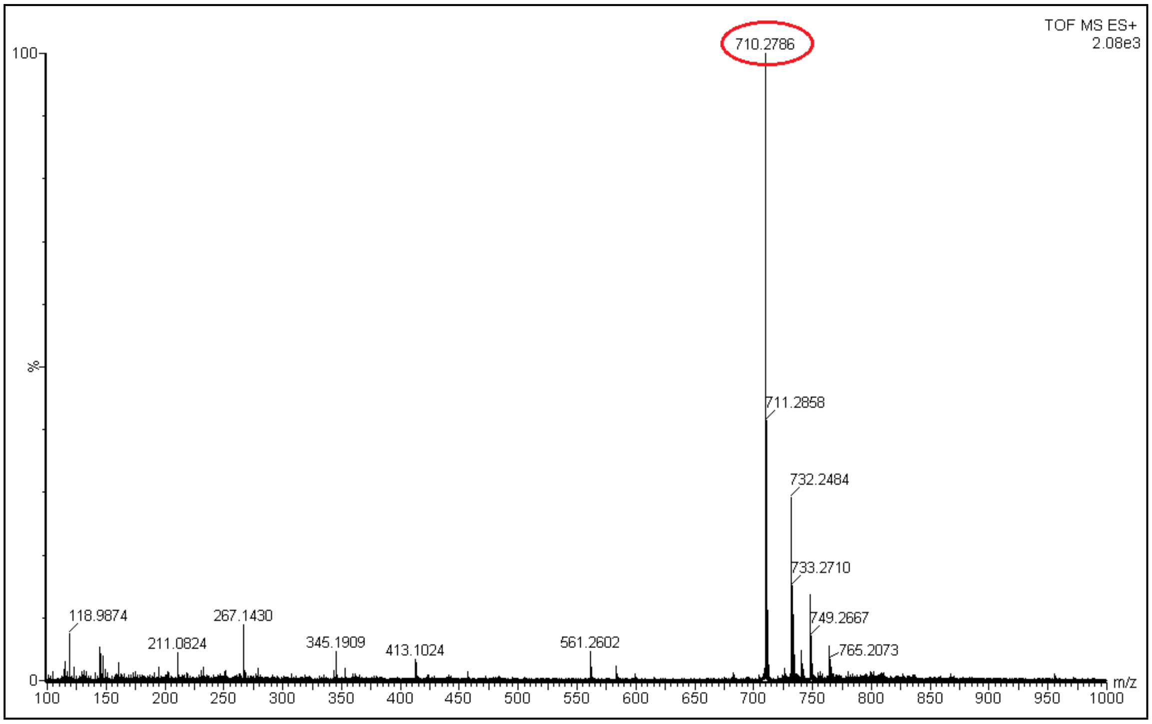

Figure S3: Mass spectrum of **L**.

4. **TOF MS ES+ spectrum of L in the presence PdCl$_2$:**

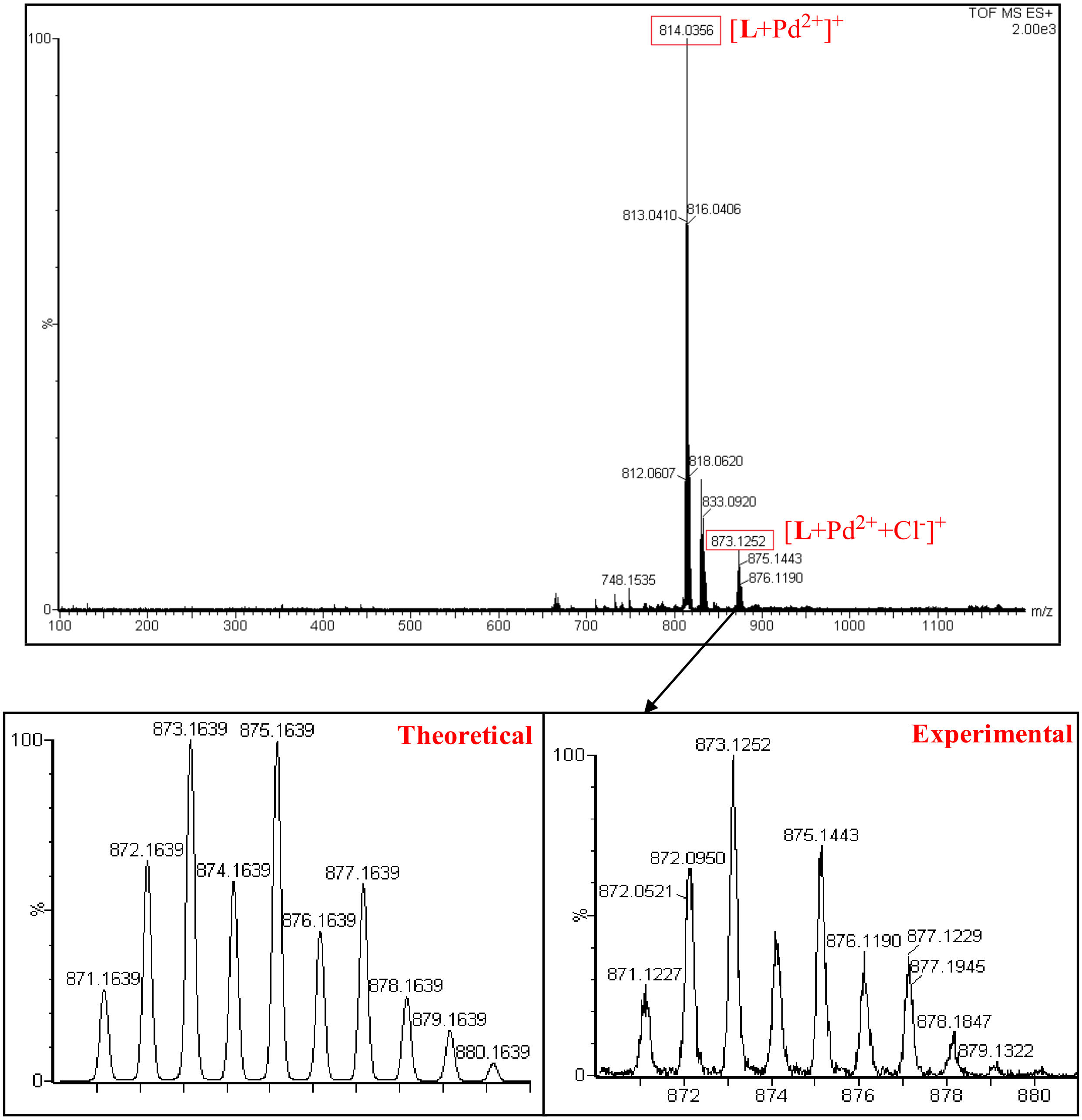

Figure S4: ESI Mass spectrum of **L**-Pd complex and (bottom left) theoretical simulation of **L**-Pd complex and (right) experimental spectrum of **L**-Pd complex.

5. **FTIR spectra for L and L-Pd^{2+}:**

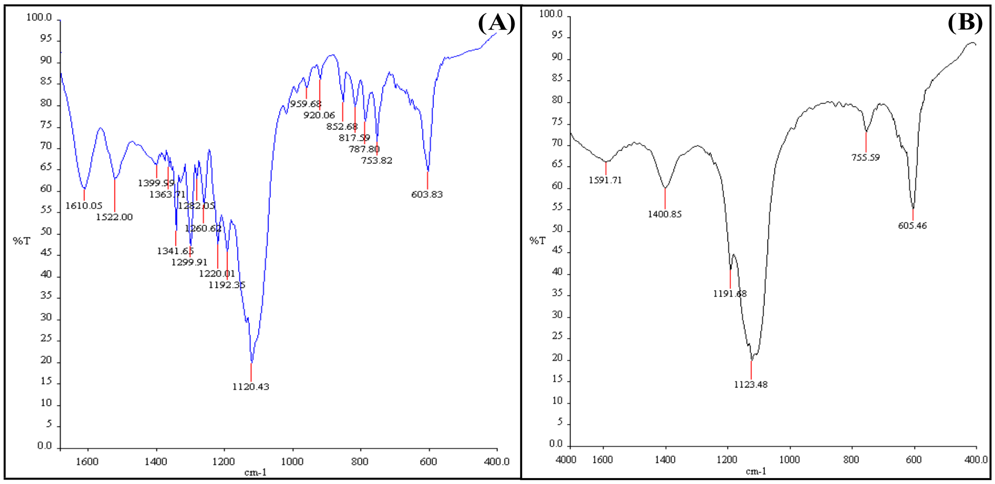

Figure S5. FTIR spectra of both (A) **L** and (B) **L**-Pd^{2+}.

6. **Job's plot for L-Pd^{2+}:**

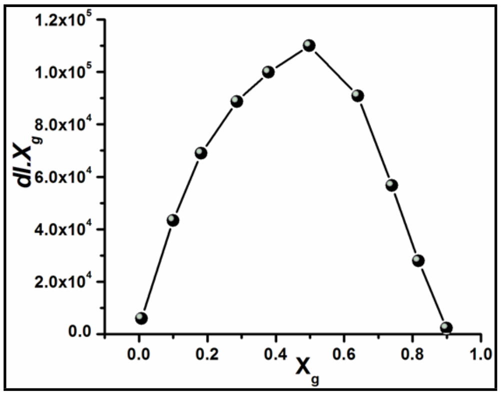

Figure S6. Fluorescence Job's plot for **L** with Pd^{2+} in EtOH–water (1: 1, v/v; 10 mM, HEPES buffer, pH = 7.4, ([H] = [G] = 4× 10^{-5} M).

7. Calculations for detection limit:

The detection limit (LOD) of **L** for Pd^{2+} were determined from the following equation[1]:

DL = 3Sb1/S , Where Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

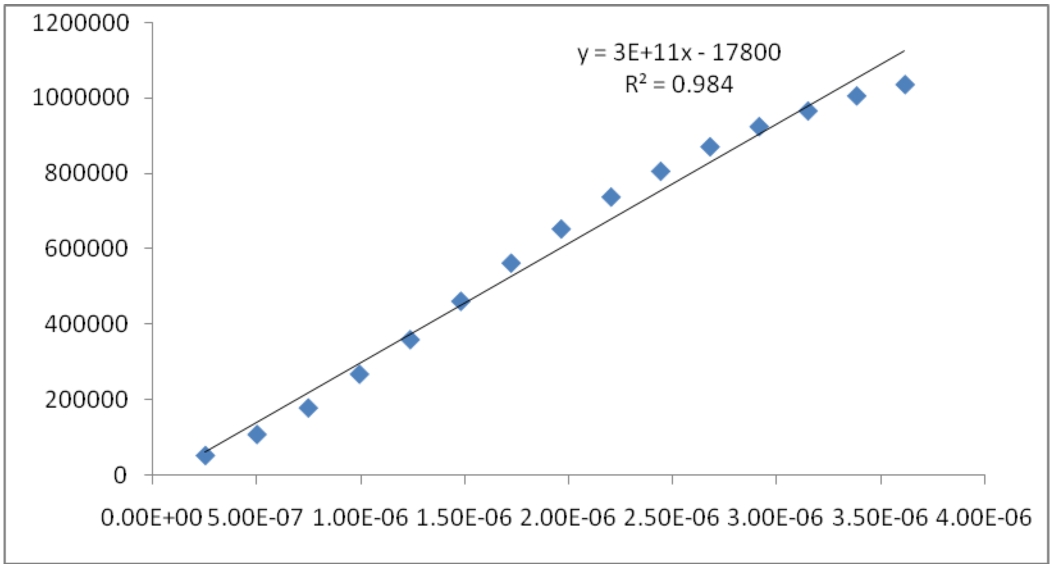

Figure S7: Calibration curve for fluorescence titration of **L** with Pd^{2+}.

From the graph we get slope = 3×10^{11}, and Sb1 value is 45043.81.

Thus using the formula we get the Detection Limit = 0.45μM= 4.5×10^{-7} M.

8. **UV-vis Bar Diagram of L in presence of various metal ions:**

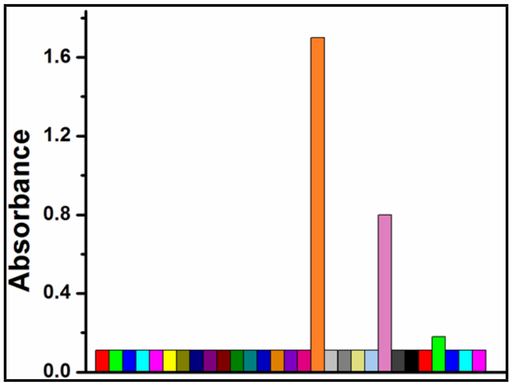

Figure S8. Change in the absorption spectrum of receptor **L** [c = 4× 10^{-5} M, EtOH / H_2O = 1 : 1, v/v, 10 mM HEPES buffer, pH = 7.4) with respective metal cations (c = 1× 10^{-4} M, left to right- **L**, Li^+, Na^+, K^+, Ca^{2+} , Mg^{2+}, Sr^{2+}, Ba^{2+}, Zr^{4+}, Hf^{4+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+},Ru^{3+}, Pd^{2+}, Pt^{2+}, Ag^+, Au^{3+}, Sn^{4+}, Pb^{2+}, Pd^0 and Al^{3+}).

9. Competitive experiments of L:

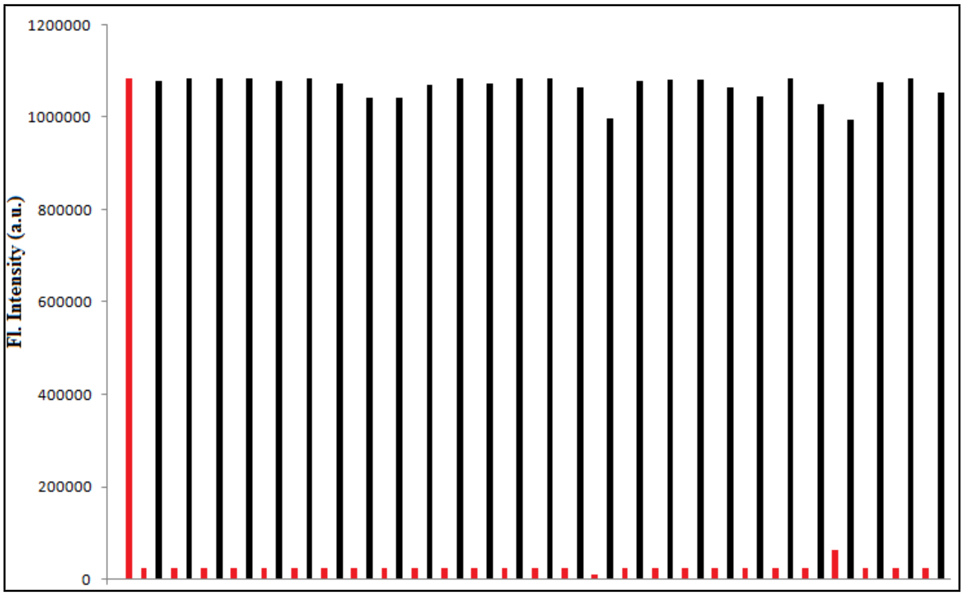

Figure S9. Fluorescence response of **L** (c= 4× 10^{-5} M) to 1 eqv. addition of Pd^{2+} (c= 1× 10^{-4} M) and 10 eqv. of other metal ions (c= 1× 10^{-4} M) [the red bar portion] and to the mixture of 10 eqv. of other metal ions with 1 eqv. addition of Pd^{2+} [the black bar portion]. [Metal ions from left to right: Pd^{2+}, Li^+, Na^+, K^+, Ca^{2+} , Mg^{2+}, Sr^{2+}, Ba^{2+}, Zr^{4+}, Hf^{4+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+},Ru^{3+}, Pt^{2+}, Ag^+, Au^{3+}, Sn^{4+}, Pb^{2+} , Pd^0, and Al^{3+}].

10. Visual and fluorescence photographic image of L in presence of different metal ions

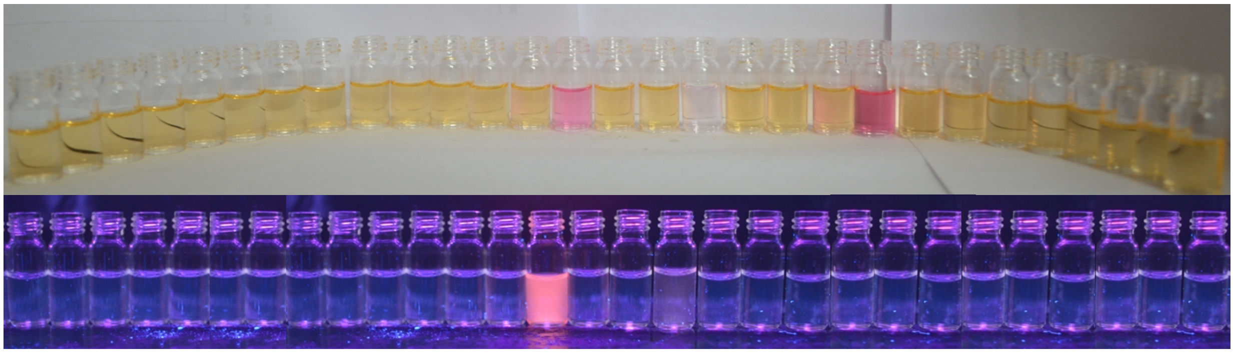

Figure S10. The Visible color (top) and fluorescence changes (bottom) of receptor **L** in EtOH–H_2O solution upon addition of various metal ions. [From left to right: **L** , Li^+, Na^+, K^+, Ca^{2+} , Mg^{2+}, Sr^{2+}, Ba^{2+}, Zr^{4+}, Hf^{4+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Pd^{2+}, Fe^{3+}, Co^{2+}, Sn^{4+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}, Ru^{3+}, Pt^{2+}, Ag^+, Au^{3+}, Pb^{2+} , Pd^0, and Al^{3+}].

11. Calculations for the Binding Constants Using Spectrophotometric Titration Data:

The association constant and stoichiometry for the formation of the respective complexes were evaluated using the Benesi−Hildebrand (B−H) plot (eq 1).[2]

$$1/(I - I_0) = 1/K(I_{max} - I_0)[M^{n+}] + 1/(I_{max} - I_0) \qquad (1)$$

where I_0, I_{max}, and I represent the emission intensity of free **L**, the maximum emission intensity observed in the presence of added metal ion at 579 nm for Pd^{2+} ($\lambda_{ext} = 561$ nm), and the emission intensity at a certain concentration of the metal ion added, respectively.

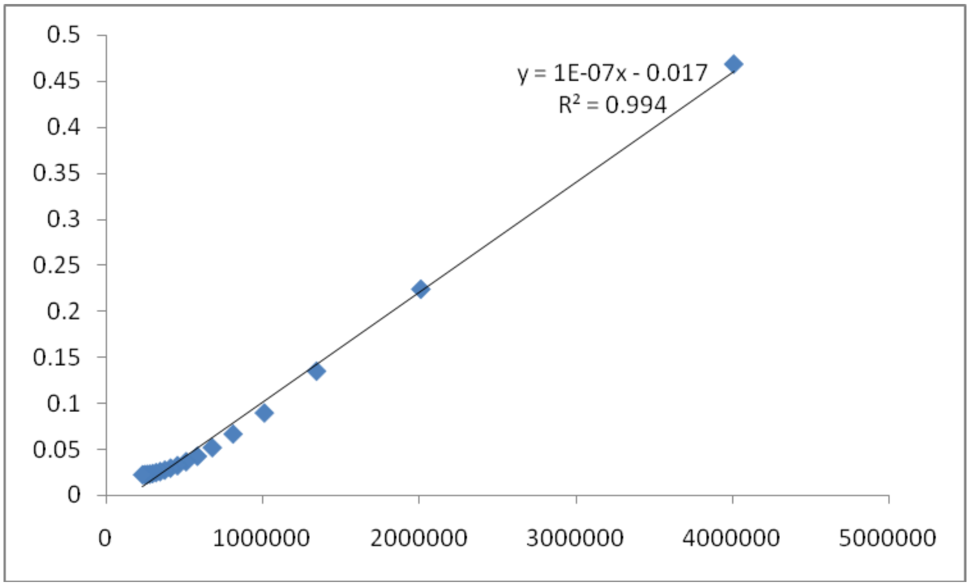

Figure S11: Bensei-Hildebrand plot obtained from the Fluorescence titration data of **L** (4 x 10^{-5} M) with Pd^{2+} (1x 10^{-4} M).

12. **Reversibility Study:**

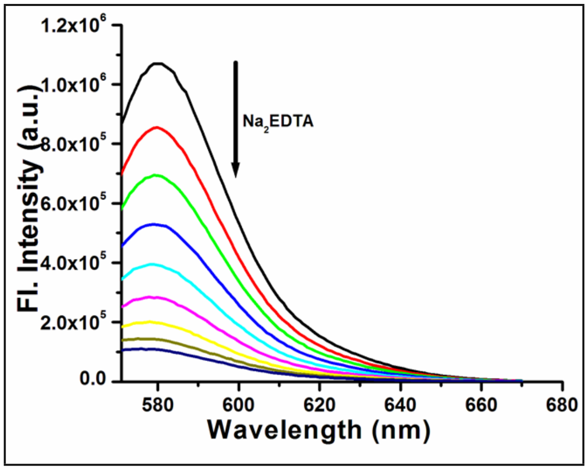

Figure S12: Change in emission spectra of **L**−Pd complex in EtOH–H_2O solution (EtOH : H_2O = 1: 1, v/v, 10 mM HEPES buffer, pH = 7.4) upon addition of Na_2EDTA.

13. **UV-vis spectra of 1 in presence of Pd^{2+} ions:**

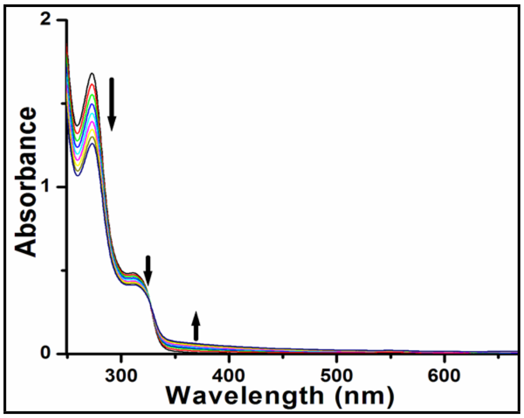

Figure S13: UV-Vis spectral changes of **1** ($c = 4\times 10^{-5}$ M) in EtOH/H_2O (1:1, v/v, 10 mM HEPES buffer, pH 7.4) solutions upon addition of Pd^{2+} ions.

14. DFT study:

The calculation has been carried out at B3LYP with 6-31G(d) basis expect for Pd, LANL2DZ with ECP basis set is used.

Species	E(HOMO)	E(LUMO)	ΔE(Hartree	ΔE(eV)	ΔE(kcal/mol)
L	-0.19782	-0.09829	0.09953	2.708370548	62.45602054
L-Pd^{2+}	-0.28057	-0.22138	0.05919	1.610654604	37.1422873

15. X-ray Crystallography study:

Single crystal suitable for X-ray analysis was performed on Bruker APEX II Duo CCD area-detector diffractometer using Mo Kα radiation (λ = 0.71073 Å). Data collection was performed using the *APEX2* software, whereas the cell refinement and data reduction were performed under the *SAINT* software. The crystal structure was solved by direct method and refined against F^2 by full-matrix least-squares refinement using *SHELXTL* package. The non-hydrogen atoms were refined anisotropically, whereby the O-bound and C-bound hydrogen atoms were located in difference fourier maps (O–H = 0.8999 Å) and positioned geometrically (C–H = 0.93 - 0.97 Å), respectively. One of the methyl groups is disordered over two positions with refined site-occupancies of 0.550(13) and 0.450(13). The same U_{ij} parameters were used for atom pairs C25/C26 and C28/C29. A rotating-group model was applied for the methyl groups. The final refinement converged well. Absorption correction was applied to the final crystal data by using the *SADABS* software. Crystallographic data for **L** has been deposited with the Cambridge Crystallographic Data Center No. CCDC **1013077.**

Table S1: Experimental details

Crystal data	
CCDC number	**1013077**
Emperical formula	$C_{41}H_{39}N_7O_5$
M_r	709.79
Crystal system, space group	Triclinic, *P*1
Temperature (K)	294
a, b, c (Å)	10.244 (1), 13.8307 (14), 14.5665 (14)
α, β, γ (°)	72.3415 (18), 70.1280 (18), 75.9246 (18)
V (Å^3)	1826.7 (3)
Z	2
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.09
Crystal size (mm)	0.62 × 0.27 × 0.11
Data collection	
Diffractometer	Bruker *SMART APEX* II DUO CCD area-detector diffractometer
Absorption correction	Multi-scan (*SADABS*; Bruker, 2009)
T_{min}, T_{max}	0.948, 0.991
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	21242, 6834, 4216
R_{int}	0.026

$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.609
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.087, 0.291, 1.09
No. of reflections	6834
No. of parameters	481
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.79, −0.58

Table S2: Hydrogen-bond geometry (Å, º)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O3—H1*O*3···N2	0.90	1.80	2.633 (5)	153
C11—H11*A*···O2^{i}	0.93	2.53	3.434 (5)	163
C21—H21A ···Cg1^{ii}	0.93	2.28	3.024(5)	137

Symmetry codes: (i) −*x*+2, −*y*+2, −*z* (ii) *x*, *y*, *z*.

* Cg1 is the centroid of O1/C6/C7/C12/C13 ring.

References:

1. (a) L. Long, D. Zhang, X. Li, J. Zhang, C. Zhang and L. Zhou, *Anal. Chim. Acta,* 2013, **775***,* 100–105; (b) M. Zhu, M. Yuan, X. Liu, J. Xu, J. Lv, C. Huang, H. Liu, Y. Li, S. Wang and D. Zhu, *Org. Lett.,* 2008, **10**, 1481-1484. (c) C. Kar, M. D. Adhikari, A. Ramesh and G. Das, *Inorg. Chem.,* 2013, **52**, 743−752.
2. (a) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703−2707; (b) C. Yang, L. Liu, T. -W. Mu and Q. -X. Guo, *Anal. Sci.*, 2000, **16**, 537−539; (c) Y. Shiraishi, S. Sumiya, Y. Kohno and T. Hirai, *J. Org. Chem.,* 2008, **73**, 8571−8574.