Trans-dichlorobis(N-p-tolylpyridin-2-amine)palladium(II): Synthesis, structure, fluorescence features and DNA binding

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Reaction of N-p-tolylpyridin-2-amine (LMe) with H2PdCl4 in boiling acetonitrile and ethanol solvents mixture (1:1) affords trans-Pd(LMe)2Cl2 (I) in high yield. (I) is substantiated by spectral data, single crystal X-ray structure determination, etc. Both (LMe) and (I) absorb strongly in the UV region (λmax, nm (ε, 10^4 M⁻¹ cm⁻¹), DCM: (LMe), 310 (1.97), 276 (4.95); (I), 320 (4.36), 270 (6.98). (LMe) is brightly fluorescent due to intra-ligand charge transfer singlet excited state (λex = 332 nm, λem = 400 nm; Φ = 0.621 measured with respect to anthracene) as elucidated by DFT and TD DFT calculations. In (I), the luminescence of (LMe) is significantly (~ 97 %) quenched (Φ = 0.016) and the fluorescence parameters are red shifted (λex = 378 nm, λem = 430 nm). Binding of (I) to CT-DNA has been investigated by UV-vis spectrum confirming a significant interaction with the intrinsic binding constant as \( K_b = ~ 9.78 \times 10^5 \) M⁻¹ and CT-DNA-ethidium bromide fluorescence quenching experiment giving the apparent binding constant as \( K_{app} = 9.02 \times 10^5 \) M⁻¹.

Keywords: Density functional calculations, Fluorescence quenching, Palladium, DNA binding

Platinum and palladium amine complexes incorporating easily ionizable chloro ligand are bioactive. In the context of bio-activity, square planar heterocyclic N-donor complexes have drawn special attention. Herein, we have modeled a four-membered NN-chelate of palladium(II) or platinum(II) of type (A) with N-p-tolylpyridin-2-amine (LMe). Unfortunately, the reaction of (LMe) with H2PdCl4 does not afford the required four membered chelate. However, the reaction yields in good yield a pyridine coordinated product, trans-Pd((LMe)2)Cl2 (I) which quenches the fluorescence of the free (LMe) ligand and interacts strongly with DNA.

In this article, syntheses and spectra of (LMe) and (I) including the single crystal X-ray structure of (I) have been reported. Origin of absorptions of the free (LMe) ligand and (I) has been investigated by gas phase DFT and TD DFT calculations on (LMe) and (I). Interaction of DNA with (I) has been studied by UV-vis and fluorescence spectra in solution at room temperature.

Materials and Methods
Reagents or analytical grade materials were obtained from commercial suppliers and used without further purification. Spectroscopic grade solvents were used for spectroscopic measurements. The C, H, N contents of the compounds were obtained from Perkin-Elmer 2400 series II elemental analyzer. Infrared spectra of the samples were measured from 4000 to 400 cm⁻¹ as KBr pellets at room temperature on a Perkin-Elmer FT-IR spectrophotometer Spectrum RX 1. ¹H NMR spectral measurements were carried out on a Bruker DPX-600 MHz spectrometer with tetramethylsilane (TMS) as an internal reference. ESI mass spectra were recorded on a micro mass Q-TOF mass spectrometer. Electronic absorption spectra in solution at 298 K were measured on a Perkin-Elmer Lambda 25 spectrophotometer in the range 200-1100 nm. Emission spectra of CH2Cl2 solutions were recorded on a Perkin-Elmer LS 55 fluorescence spectrophotometer.
Synthesis of N-p-tolylpyridin-2-amine ($\text{L}^{\text{Me}}$)

To a mixture of 2-chloropyridine (1.0 ml, 10.6 mmole) and p-toluidine (1.15 gm, 10.6 mmole), a pinch of Na$_2$CO$_3$ was added and refluxed for 45 min (at 170 °C). The mixture was cooled at room temperature and the slurry was extracted with ether repeatedly (5 $\times$ 5 ml). Upon evaporation of the ether solution under vacuum, a brown yellow solid was collected. The solid mass was further recrystallized from methanol solution. Yield: 1.28 g (~ 66 % with respect to p-toluidine), m. pt: 88-90 °C. Anal. (%): Calcd for C$_{12}$H$_7$N$_2$ (184.24 g/mol): C, 78.23; H, 6.57; N, 15.21; Found: C, 78.11; H, 6.50; N, 15.10.

IR (KBr, cm$^{-1}$): 328.06 for [(L+Cl)

Density functional theory calculations

All calculations reported in this article were done with the Gaussian 03W$^{14}$ programme package supported by GaussView 4.1. The DFT$^{15}$ and TD DFT$^{16}$

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Table 1—Crystallographic data of (1)

<table>
<thead>
<tr>
<th>Formula</th>
<th>C$_2$H$_2$Cl$_2$N$_2$Pd</th>
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<tbody>
<tr>
<td>Formula wt</td>
<td>545.77</td>
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<td>Crystal colour</td>
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<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P2(1)/c</td>
</tr>
<tr>
<td>a (Å)</td>
<td>12.104(3)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>9.222(3)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>11.529(3)</td>
</tr>
<tr>
<td>β (°)</td>
<td>114.864(12)</td>
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<tr>
<td>V (Å$^3$)</td>
<td>1167.5(6)</td>
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<tr>
<td>Z</td>
<td>4</td>
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<tr>
<td>μ (mm$^{-1}$)</td>
<td>2.085</td>
</tr>
<tr>
<td>T (K)</td>
<td>296(2)</td>
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<tr>
<td>Density$_{calc}$ (g cm$^{-3}$)</td>
<td>3.105</td>
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<tr>
<td>Uniq. reflections</td>
<td>2057</td>
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<tr>
<td>Ref (I&gt;$2σ(I)$)</td>
<td>1901</td>
</tr>
<tr>
<td>λ (Å)</td>
<td>0.71073</td>
</tr>
<tr>
<td>F(000)</td>
<td>1104</td>
</tr>
<tr>
<td>R$_1$ [I&gt;$2σ(I)$]</td>
<td>0.0243</td>
</tr>
<tr>
<td>GoF$_b$</td>
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</tr>
<tr>
<td>R$_1$ (all data)</td>
<td>0.0266</td>
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<tr>
<td>wR$_2$ [I&gt;$2σ(I)$]</td>
<td>0.0650</td>
</tr>
<tr>
<td>Parameters/restraints</td>
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<tr>
<td>Residual density (e Å$^{-3}$)</td>
<td>0.310</td>
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</table>

Obs. criterion: $I > 2σ(I)$. $^a$R₁ = Σ[I-Fo] / Σ[Fo].
$^b$GoF = [Σ(w(Fo²-Fc²)²)/(n-p)]$^{1/2}$.
$^c$wR$_2$ = [Σ(w(Fo²-Fc²)²)/Σ(wFo²)]$^{1/2}$.
where w = 1/[σ²(Fo²)+(aP)²+bP²]. P = (Fo²+Fc²)/3.

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X-ray crystallographic data collections and refinement of (I)

A yellow crystal of (I) was picked up with a nylon loop and was mounted on a Bruker Smart Apex diffractometer, equipped with graphite monochromator (Mo-K$_{α}$, $λ = 0.71073Å$). Final cell constants were obtained from least squares fits of all measured reflections. Intensities of data were corrected for absorption using intensities of redundant reflections. The structures were readily solved by direct methods and subsequent difference Fourier techniques. The crystallographic data have been listed in Table 1. The SHELXTL97$^{13a}$ software package was used for solution of the structure, while SHELXL97$^{13b}$ was used for the refinement. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed at the calculated positions and refined as riding atoms with isotropic displacement parameters.
calculations have been performed at the level of Becke three parameter hybrid functional with the non-local correlation functional of Lee-Yang-Parr (B3LYP)\(^\text{17}\). The gas phase geometries of \(\text{L}^\text{Me}\) and \(\text{I}\) have been optimized using Pulay’s Direct Inversion\(^\text{18}\) in the Iterative Subspace (DIIS) convergent SCF procedure\(^\text{19}\), ignoring symmetry. In all calculations of \(\text{I}\), a LANL2DZ basis set\(^\text{20,22}\) along with the corresponding effective core potential (ECP) was used for palladium metal atom. Valence double zeta with polarization and diffuse functional basis set, 6-31++G**\(^\text{23,24}\) for C, N, Cl and only valence double zeta 6-31G\(^\text{25}\) for H atoms have been used in all calculations. The sixty lowest singlet excitation energies on the optimized geometries of \(\text{L}^\text{Me}\) and \(\text{I}\) in gas phase have been calculated by TD DFT method.

### DNA binding

The DNA concentration per two nucleotide was determined by absorption spectroscopy using the molar absorption coefficient \((13200 \text{ M}^{-1}\text{cm}^{-1})\) at 260 nm. The buffer used in this study was prepared in doubly distilled water and the DNA solution was stored at 4 °C and used no more than 4 days. The interactions of \(\text{I}\) with DNA have been examined by UV-vis and fluorescence spectra. The DNA binding experiments were carried out in 10 mM phosphate buffer \(\text{pH } 7.0\) having 50 mM NaCl using solution of \(\text{I}\) in DMSO. An aliquot of solution of compound \(\text{I}\) in DMSO was added to buffer to record the spectra; the DMSO concentration never exceeds >7 % and this system was highly stable for at least half an hour. Absorption titration experiments were performed by varying the concentration of the DNA with the complex. All UV–vis spectra were recorded after equilibration for 20 mins.

### Ethidium bromide displacement

The relative binding of compound \(\text{I}\) to CT-DNA was studied by the fluorescence spectral method with ethidium bromide bound CT-DNA complex (DNA 6.48 \(\mu\text{M}\) and ethidium bromide 9.1 \(\mu\text{M}\)) in buffer as stated before with excitation at \(\lambda = 525\ \text{nm}\) and recording the emission spectra at \(\lambda = 600-650\ \text{nm}\). Increasing concentration of compound \(\text{I}\) was used so as to get a reduction of the emission spectra by 50 % on plotting compound concentration versus fluorescence intensity.

### Results and Discussion

#### Syntheses and characterization

The ligand \((\text{L}^\text{Me})\) has been synthesized in good yield by reacting \(p\)-toludine with 2-chloropyridine following a modified method\(^\text{26}\) of preparation of \(\text{L}^\text{II}\). Reaction of \((\text{L}^\text{Me})\) with \(\text{H}_2\text{PdCl}_4\) in boiling acetonitrile and ethanol solvent mixture for 0.5 h affords \(\text{I}\) in high yield. The N-H stretching frequency of the free ligand \((\text{L}^\text{Me})\) at 3221 cm\(^{-1}\) is blue shifted to 3276 cm\(^{-1}\) upon coordination to \(\text{Pd}^\text{II}\) ion in \(\text{I}\).

Both \((\text{L}^\text{Me})\) and \(\text{I}\) absorb strongly in the UV region. The absorption spectra in dichloromethane are shown in Fig. 1 and absorption parameters are listed in Table 2.

#### Molecular geometry

The \(\text{trans}\)-geometry in crystals has been confirmed by single crystal X-ray structure determination of \(\text{I}\). Selected bond parameters are summarized in Table 3 and an ORTEP plot is illustrated in Fig. 2(a).

![Fig. 1—Electronic spectra of (L\(^{Me}\)) (curve 1) and (I) (curve 2) in dichloromethane at 298 K.](image-url)
Two Cl atoms and two pyridine rings including the two amine N atoms form the square plane. The orientations of two (LMe) ligands in (1) are equivalent. The dihedral angle between the pendant phenyl ring and the square plane is 64°.

Fluorescence spectra

Emission spectra of dichloromethane solutions with the concentration in the order of 10⁻⁵ mol/L have been recorded at 298 K with λ ext at 332 and 378 nm (Table 2) respectively for (LMe) and (1). The excitation and emission spectra of (LMe) are displayed in Fig. 3. Figure 4 shows that both excitation and emission spectra of (1) in solution are structured. Quantum yield (Φ) calculations with respect to anthracene have established that the fluorescence feature of (LMe) (Φ = 0.621) is 97% quenched in the complex (1) (Φ = 0.016) as depicted in Fig. 5. To elucidate the origin of the excitations, DFT and TD DFT calculations were carried out on (LMe) and (1). Both the molecules in gas phase are optimized on...
theoretical coordinates. The optimized geometry of (1) is illustrated in Fig. 2(b). The calculated bond parameters are very similar to those obtained from the single crystal X-ray structure determination of (1). Excitation energies are calculated on the optimized geometries. The transition types of the lower energy absorption maxima are summarized in Table 4. Excitation energies of the transitions above 280 nm, with oscillator strengths greater than 0.05, have been tabulated. Analyses have shown that all the significant transitions in (LMe) and (1) are based on intra-ligand charge transfer (ILCT). Thus, these species are luminescent because of the excited \(^1\)ILCT state.

### DNA binding

The interaction of (1) with DNA examined by UV-vis is shown in Fig. 6. The interaction of the compound (1) with DNA as evident by the decrease in the intensity with the increase in the amount of DNA, showed a significant hypochromicity. This effect is suggestive of the strong interacting nature of the compound with DNA. The characteristic change is therefore indicative of either surface specific interaction or some form of minor intercalating. The intrinsic binding constant, \(K_b\), was determined from a plot of [DNA]/(\(\varepsilon_a - \varepsilon_f\)) versus [DNA] using Eq. (1) (ref. 27).

\[
\frac{[\text{DNA}]}{\varepsilon_a - \varepsilon_f} = \frac{[\text{DNA}]}{\varepsilon_b - \varepsilon_f} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \quad \ldots (1)
\]

where [DNA] is the concentration of DNA, and \(\varepsilon_a\), \(\varepsilon_f\) and \(\varepsilon_b\) are the extinction coefficients of the apparent, free, and bound metal complexes, respectively. \(K_b\) is determined from the ratio of the slope to the intercept of the plot of [DNA]/(\(\varepsilon_a - \varepsilon_f\)) versus [DNA]. The intrinsic binding constant, \(K_b\), obtained is approximately \(9.78 \times 10^5\) M\(^{-1}\).

### Ethidium bromide displacement

The UV-vis spectral study on binding of compound (1) to DNA is indicative of minor intercalative in nature. However, the extensively studied DNA intercalative agent, ethidium bromide has been used as a probe to identify the unknown intercalative agent by displacement of bound ethidium bromide. Results of ethidium bromide displacement from CT-DNA-ethidium bromide complex by compound (1) (Fig. 7) illustrates the strong quenching activity. The ethidium bromide itself has negligible fluorescence and by binding to DNA as an intercalating agent the

<table>
<thead>
<tr>
<th>(\lambda) (nm) (f)</th>
<th>eV</th>
<th>Expt (\lambda) (nm)</th>
<th>Significant contributions</th>
<th>Transition type</th>
</tr>
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<tbody>
<tr>
<td>(L^\text{Me})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 (0.09)</td>
<td>4.14</td>
<td>310</td>
<td>HOMO -&gt; LUMO (80%) ILCT</td>
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<td>286 (0.31)</td>
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<td>HOMO -&gt; LUMO+1 (70%) ILCT</td>
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<tr>
<td>280 (0.07)</td>
<td>4.43</td>
<td>276</td>
<td>HOMO -&gt; LUMO+2 (79%) ILCT</td>
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<tr>
<td>341 (0.08)</td>
<td>3.64</td>
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<td>HOMO-8 -&gt; LUMO (30 %) ILCT</td>
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<tr>
<td>331 (0.14)</td>
<td>3.74</td>
<td>320</td>
<td>HOMO-7 -&gt; LUMO (50 %) ILCT</td>
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<tr>
<td>284 (0.62)</td>
<td>4.36</td>
<td>270</td>
<td>HOMO-1 -&gt; LUMO+4 (25 %) ILCT</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6—UV-vis spectra for titration of CT-DNA (0.3 \(\mu\)M \(\rightarrow\) 4.2 \(\mu\)M) with (1). [7.79 \(\mu\)M phosphate buffer: 10 mM pH 7.0 with 50 mM NaCl. Inset: Plots of [DNA]/(\(\varepsilon_a - \varepsilon_f\)) versus [DNA]].
fluorescence is enhanced several times and its displacement from intercalating site results in the marked reduction of fluorescence intensity or the change in structural orientation of CT-DNA-ethidium bromide complex. From the plot of intensity against complex concentration, the values of the apparent DNA binding constant (K<sub>app</sub>) were calculated using the equation:<sup>32</sup> K<sub>EB</sub>[EB] = K<sub>app</sub>[complex] in which the complex concentration is the value at a 50 % reduction of the fluorescence intensity of EB and K<sub>EB</sub> = 1.0 × 10<sup>7</sup> M<sup>-1</sup>. The K<sub>app</sub> value for compound (I) is 9.02 × 10<sup>5</sup> M<sup>-1</sup>.

Conclusions
Synthesis, single crystal X-ray structure, UV-vis, fluorescence spectra and their origins elucidated by DFT and TD DFT calculations of trans-dichlorobis(N-p-tolylpyridin-2-amine)palladium(II) (I) have been reported. The intrinsic binding of (I) to CT-DNA with K<sub>d</sub> = 9.78×10<sup>5</sup> M<sup>-1</sup> has been determined by UV-vis spectroscopy while the competitive binding of (I) to CT-DNA-ethidium bromide complex with apparent binding constant, K<sub>app</sub> = 9.02×10<sup>5</sup> M<sup>-1</sup> has been estimated by fluorescence spectroscopy. The results are promising and encourage us to model more such Pd(II) and Pt(II) complexes to substantiate the interactions with DNA in conjunction with cytotoxicity in details.

Supplementary Data
Crystallographic data of (I) have been deposited with the Cambridge Crystallographic Data Centre, under CCDC No. 825762. These data can be obtained free of charge from Crystal Structure Database via http://www.ccdc.cam.ac.uk.

Acknowledgement
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References
KUNDU et al.: SYNTHESIS & CHARACTERIZATION OF trans-DICHLOROBIS (N-p-TOLYPYRIDIN-2-AMINE)Pd(II)