Synthesis, X-ray structure, DFT and thermodynamic studies of mono- and binuclear palladium(II) complexes involving 1,4-bis(2-hydroxyethyl)piperazine, bio-relevant ligands and 4,4′-bipiperidine

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ABSTRACT

[Pd(BHEP)Cl2] (BHEP = 1,4-bis(2-hydroxyethyl)piperazine) was synthesized and characterized. The palladium center has a typical square-planar geometry with a tetrahedral distortion. The alcohol groups of the ligand do not participate in binding to Pd(II). The DFT/B3LYP method was used for geometric optimization of the ligand and the complex using the Gaussian 09 program and compared with experimental results. The stoichiometry and stability constants of the complexes formed between [Pd(BHEP)(H2O)2]2+ and some selected amino acids, peptides, and DNA constituents were investigated at 25 °C and 0.1 M ionic strength. The binuclear complex [(H2O)(BHEP)Pd(Bip)Pd(BHEP)(H2O)]4+ was detected, where Bip = 4,4′-bipiperidine. Inosine, uracil, and thymine interact with the binuclear complex via substitution of both coordinated water molecules. The potentiometric results were complimented by spectroscopic measurements. The concentration distribution diagrams of the various species formed were evaluated.
1. Introduction

Cisplatin (cis-diamminedichloroplatinum(II)) is one of the most active antitumor agents in clinical use [1]. Cisplatin is used worldwide in the treatment of testicular and ovarian cancer and is increasingly used against cervical, bladder, and head/neck tumors [2]. Following the example of cisplatin, thousands of platinum-containing compounds have been synthesized and evaluated as potential antitumor drugs [3–11], but only a few compounds reached clinical applications, viz. carboplatin or oxaliplatin [12–14]. The development of new platinum compounds continues in the hope to suppress side effects, overcome drug resistance during therapy and afford a broader range of applications. For this purpose, design of complexes and the study of relevant structure–activity relationships have been extended to new compounds having structural diversity.

Pd(II) and Pt(II) complexes have the same general structures and thermodynamic properties. However, the complexes formed by Pd(II) are five orders of magnitude more reactive than their platinum counterparts. Therefore, Pd(II) complexes are good models for the analogous Pt(II) complexes in solution. Recent work in our laboratories focused on complex-formation reactions of cis-(diamine)palladium(II) complexes with DNA, the major target in chemotherapy of tumors, and amino acids, peptides, and dicarboxylic acids and esters [15–21]. In this project, we have synthesized and characterized [Pd(BHEP)Cl2], where BHEP = 1,4-bis(2-hydroxyethyl)piperazine. BHEP was selected because: (1) the alcohol group of BHEP may undergo hydrogen bonding with DNA [22–24] and (2) the piperazine ring may undergo stacking interactions with the sugar group of DNA, again favoring interaction with DNA. The latter effect is similar to that reported for carboplatin, where the stacking interaction between the cyclobutane ring and the sugar group forms part of the increased antitumor activity [25]. Complex-formation equilibria between [Pd(BHEP)(H2O)2]2+ and biorelevant ligands, amino acids, peptides, and DNA constituents were investigated. Formation of the binuclear complex involving [Pd(BHEP)(H2O)2]2+ and 4,4′-bipiperidine linking two Pd(II) species was investigated.

2. Experimental

2.1. Materials

[Pd(BHEP)Cl2] was prepared by heating PdCl2 (0.177 g, 1.0 mmol) and KCl (0.149 g, 2.0 mmol) in minimum water to 70 °C under stirring. The clear solution of [PdCl4]2− was cooled to 25 °C, filtered and 1,4-bis(2-hydroxyethyl)piperazine (0.174 g, 1.0 mmol) was added to the stirred solution. The pH of the solution was adjusted to 2–3 by the addition of HCl. The solution was evaporated to a small volume (20 mL) under vacuum, after which an orange crystalline precipitate of [Pd(BHEP)Cl2] was formed on cooling. The precipitate was filtered off and washed with water; yield 92%. Anal. Calcld for C8H18N2O2PdCl2 (%) (MW = 351.55): C, 27.33; H, 5.17; N, 7.97. Found: C, 27.3; H, 5.3; N, 7.8%. Crystals suitable for X-ray crystallography were obtained by the recrystallization of [Pd(BHEP)Cl2] from hot acetonitrile/water, followed by slow evaporation during which orange crystals were obtained.

2.2. Apparatus and experimental measurements

Potentiometric titrations were performed with a Metrohm 686 titroprocessor equipped with a 665 Dosimat. The titroprocessor and electrode were calibrated with standard buffer solutions, prepared according to NBS specifications [26]. All titrations were carried out at 25.0 ± 0.1 °C in purified nitrogen. Elemental analysis was done by CHNS Automatic Analyzer, Vario EL III-Elementar, Germany. IR spectra were measured on a Shimadzu 8001-PC FT-IR spectrophotometer using KBr pellets. 1H- and 13C-NMR spectra were recorded on a Varian GEMINI 200 spectrometer at 200 MHz using d6-DMSO as solvent.

2.3. Potentiometric measurements

The acid dissociation constants of the ligands were determined by titrating 0.05 mmol samples of each with standard NaOH solutions. Ligands were converted into their protonated form with standard HNO3.
solutions. The aqua-aquocomplex $\text{[Pd(BHEP)(H}_2\text{O)}_2\text{]}^{2+}\text{(NO}_3\text{)}_2$ was prepared in solution by stirring the chloro-
plex with two equivalents of $\text{AgNO}_3$ overnight (with careful protection from light). The precipitated
$\text{AgCl}$ was removed by filtration and the filtrate made to the requested volume in a standard flask. The
acid dissociation constants of the coordinated water molecules in $\text{[Pd(BHEP)(H}_2\text{O)}_2\text{]}^{2+}$ were determined
by titrating 0.05 mmol of complex with standard 0.05 M NaOH solution. The formation constants of
the complexes were determined by titrating solution mixtures of $\text{[Pd(BHEP)(H}_2\text{O)}_2\text{]}^{2+}$ (0.05 mmol) and
and the ligand in the concentration ratio of 1:1 ($\text{Pd}$:ligand) for the amino acids and peptides and 1:2
for DNA constituents. The formation constant of the binuclear complex of $\text{[Pd(BHEP)(H}_2\text{O)}_2\text{]}^{2+}$ with
$4,4'$-bipiperidine ($\text{Bip}$) was determined by titrating a solution mixture of 0.06 mmol of $\text{[Pd(BHEP)(H}_2\text{O)}_2\text{]}^{2+}$
and 0.03 mmol Bip in a concentration ratio of 2:1 ($\text{Pd}$:Bip). The formation constants of the binuclear
DNA complexes were determined by titrating solution mixtures of 0.06 mmol of $\text{[Pd(BHEP)(H}_2\text{O)}_2\text{]}^{2+}$,
0.03 mmol of Bip, and 0.06 mmol of DNA constituent in the concentration ratio of 2:1:2 ($\text{Pd}$:Bip:DNA
constituent). The titrated solution mixtures each had a volume of 40 mL and the titrations were carried
out at 25 °C and 0.1 M ionic strength (adjusted with $\text{NaNO}_3$). A standard 0.05 M NaOH solution was
used as titrant. The pH meter readings were converted to hydrogen ion concentration by titrating a
standard $\text{HNO}_3$ solution (0.01 M), the ionic strength of which was adjusted to 0.1 M with $\text{NaNO}_3$, with
standard NaOH (0.05 M) at 25 °C. The pH values were plotted against $\text{p[H]}$ values. The relationship
$p\text{H} - \text{p[H]} = 0.05$ was observed.

The species formed were characterized by the general equilibrium,

$$p\text{M} + q\text{L} + rh \rightleftharpoons (\text{M})_p(\text{L})_q(\text{H})_r$$

(1)

for which the formation constants are given by,

$$\beta_{pqr} = \frac{[\text{(M)}_p(\text{L})_q(\text{H})_r]}{[\text{M}]^p[\text{L}]^q[\text{H}]^r}$$

(2)

where M, L, and H represent the $\text{[Pd(BHEP)(H}_2\text{O)}_2\text{]}^{2+}$ ion, ligand, and proton, respectively. For binuclear
complex with DNA constituent M, L and H stand for $((\text{Pd(BHEP)})_2(\text{Bip})(\text{H}_2\text{O})_2)^{4+}$, DNA constituent and
proton, respectively. The calculation were performed using MINIQUAD-75 [27]. The stoichiometry and
stability constants of the complexes formed were determined by trying various possible composition
models for the systems studied. The model selected was the one that gave the best statistical fit
and was chemically consistent with the magnitudes of various residuals, as described elsewhere [27].
Concentration distribution diagrams were obtained with the program SPECIES [28] under the experi-
mental condition used.

2.4. Spectrophotometric measurements

Spectrophotometric measurements of mono- and binuclear complexes were performed by recording
the UV-visible spectra of solution mixtures of $\text{[Pd(BHEP)(H}_2\text{O)}_2\text{]}^{2+}$, ligand and NaOH. Under these experi-
mental conditions and after neutralization of the hydrogen ions released, due to complex formation,
it is supposed that the complexes were completely formed. In each mixture, the volume was brought
to 10 mL by the addition of de-ionized water and the ionic strength was kept constant at 0.1 M $\text{NaNO}_3$.

2.5. Theoretical DFT calculations

Density functional theory was applied to calculate the optimized geometries using the Gaussian09
program [29]. The DFT/B3LYP method was used for geometry optimization. Full geometry optimization
was performed using B3LYP/6-31G (p,d) as a basis set to generate the optimized structure for the ligand
and using B3LYP/LANL2DZ for the complex.
3. Results and discussion

3.1. Characterization of the solid complex

3.1.1. IR, ¹H-NMR and ¹³C-NMR spectra

The analytical data indicated that the complex is of 1:1 stoichiometry and of formula [Pd(BHEP)Cl₂]. IR spectra of BHEP and [Pd(BHEP)Cl₂] were compared (figures S1 and S2 in the Supporting Information). The stretching vibration of the OH group of BHEP shows a broad band at 3151 cm⁻¹. This may be

| Crystal data | Data collection | Refinement |
|--------------|----------------|------------|
| Mol. formula | C₈H₁₈Cl₂N₂O₂Pd | CCD        |
| Formula wt. | 351.550        | Absorption correction: none |
| Crystal system | 2951 independent reflections | R(all) = 0.067 |
| Monoclinic | 2862 Measured reflections | R(gt) = 0.039 |
| Space group | P2₁/c          | wR(ref) = 0.104 |
| a = 10.7988(4) Å | θ(max) = 27.48° | wR(all) = 0.107 |
| b = 14.6677(5) Å | h = 0→13 | wR(gt) = 0.104 |
| c = 7.7230(2) Å | k = −18→0 | S(ref) = 1.470 |
| α = 90.00° | l = −9→9 | S(all) = 1.348 |
| β = 92.3188(11)° | h = 0→13 | S(gt) = 1.470 |
| γ = 90.00° | k = 0→18 | 1718 Reflections |
| V = 1222.27(7) Å³ | l = −9→9 | 136 Parameters |
| Z = 4 | | 0 Restraints |
| Dₐ = 1.910 Mg m⁻³ | | Only coordinates of H atoms refined |
| Mo Kα radiation | | Calculated weights σ |
| λ = 0.71073 | | Δ/σₘₐₓ = 0.012 |
| Cell parameters from S829 | | Δρₘₐₓ = 0.76 eÅ⁻¹ |
| θ = 2.910°→27.485° | | Δρₘᵣᵢ = −1.34 eÅ⁻¹ |
| μ = 1.94 mm⁻¹ | Extinction correction: none | |
| T = 298 K | Atomic scattering factors from Waasmaier and Kirfel, 1995 | |
| Needles | | |
| Brownish red | | |

Figure 1. ORTEP drawing of [Pd(BHEP)Cl₂] showing 50% probability ellipsoids and the atom-numbering scheme.
explained on the premise that the OH groups are involved in hydrogen bonding. This band is sharp in the complex and occurs at 3429 cm\(^{-1}\), indicating that the OH groups are not involved in coordination. The stretching vibration corresponding to \(\nu_{\text{Pd–N}}\) was assigned to the band at 472 cm\(^{-1}\) [30].

The 1H-NMR spectrum of BHEP shows a singlet at 4.33 ppm for two OH protons. It also shows three triplets at 2.47, 2.37 (for \(\alpha\) and \(\beta\) CH\(_2\) groups), and 2.34 ppm for the four CH\(_2\) groups in the ring (figures S3 and S4 in the Supporting Information). The spectrum of the complex shows a downfield shift due to complex formation to 4.75, 3.70, 2.77, and 2.60 ppm, respectively. The 13C-NMR spectrum for the complex shows signals at 62.12, 56.95, and 56.08 ppm for CH\(_2\) \(\alpha\) and \(\beta\) to the OH groups and for CH\(_2\) groups in the ring, respectively (figures S5 and S6 in the Supporting Information).

### 3.1.2. Single-crystal X-ray crystallography

Crystals suitable for X-ray crystallography were obtained by the recrystallization of \([\text{Pd(BHEP)}\text{Cl}_2]\) from hot acetonitrile/water followed by slow evaporation. The three-dimensional structure of the complex was investigated. Geometric information is tabulated below. All diagrams and calculations were performed using maXus (Bruker Nonius, Delft & MacScience, Japan). The crystal and structure refinement data are given in table 1.

The crystal structure consists of monomeric \([\text{Pd(BHEP)}\text{Cl}_2]\) molecules as shown in figure 1. The compound crystallizes in the space group \(P2_1/c\), \(a = 10.7988(4)\) Å, \(b = 14.6677(5)\) Å, \(c = 7.7230(2)\) Å, \(\alpha = 90.00^\circ\), \(\beta = 92.3188(11)^\circ\), \(\gamma = 90.00^\circ\), \(V = 1222.27(7)\) Å\(^3\), \(Z = 4\) (i.e. four molecules per unit cell, figure S7 in the Supporting Information). The palladium center has a typical square-planar geometry with a tetrahedral distortion. The environment consists of two chlorides in the cis-positions and one ligand coordinated via piperazine nitrogens. Selected bond lengths and angles are given in tables 2 and 3.

The ligand is a bidentate chelate forming a five-membered metallocyclic ring with a distorted twist boat conformation. The bond distance Pd1–N4 is 2.068(2) Å, slightly shorter than that of Pd1–N7, viz.
2.078(2) Å. The Pd–Cl bond lengths (2.309(3) Å and 2.306(3) Å) can also be regarded as normal compared to distances found in the literature (2.220–2.361 Å) [31, 32]. The value of the N–Pd–N bite angle is 73.54(3)° and deviates from the square-planar geometry by 16.46°. This is probably due to steric strain of the piperazine ring. Moreover, the Cl–Pd–Cl angle is 90.57(3)°, showing a small deviation from square-planar geometry.

**Figure 2.** Optimized structure of ligand (upper) by DFT using the B3LYP/6-31G(d,p) functional and [Pd(BHEP)Cl₂] (lower) by DFT using the B3LYP/LANL2DZ functional.

**Table 4.** Calculated energies and dipole moments of ligand and [Pd(BHEP)Cl₂] at B3LYP.

| Compound       | $E^a$     | HOMO$^b$   | LUMO$^c$   | $\Delta E^d$ | Dipole moment$^e$ |
|---------------|-----------|------------|------------|---------------|------------------|
| BHEP          | $-575.62$ | $-5.6055$  | $1.9741$   | $7.5796$      | $1.8755$         |
| [Pd(BHEP)Cl₂] | $-732.27$ | $-6.0262$  | $-2.3447$  | $3.6815$      | $10.1803$        |

$^aE$: the total energy (a.u.).  
$^b$HOMO: highest occupied molecular orbital (eV).  
$^c$LUMO: lowest unoccupied molecular orbital (eV).  
$^d\Delta E$: $E_{\text{LUMO}} - E_{\text{HOMO}}$ (eV).  
$^e$Dipole: dipole moment calculate (Debye).

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3.1.3. Molecular modeling studies

Density functional theory (DFT) calculations [33] have been carried out to investigate the equilibrium geometry of the ligand and [Pd(BHEP)Cl2] using Gaussian 09 [29] at the B3LYP/6-31G (d,p) level of theory for the ligand and at the B3LYP/LANL2DZ level of theory for [Pd(BHEP)Cl2]. The most stable conformer was fully optimized and is represented in figure 2.

Figure 2 shows the chair structure of the piperazine ring as the most stable configuration. This configuration was transformed to the boat structure in the complex. This was clear from the N–N distance calculated to be 2.861 Å in the free ligand compared to 2.525 Å in the complex. The N–C bond lengths from modeling data (1.505–1.512 Å) are larger in the complex than those of the free ligand (1.457–1.465 Å). Similarly, the bond lengths of C–C in the piperazine ring (C3–C4, C5–C6) of the complex (1.533 Å) are longer than those of the free ligand (1.528 Å). This is probably due to the involvement of piperazine N in complexation with palladium and stretching of the adjacent bonds in the ring compared to the free ligand. The C3–N1–C5 and C4–N2–C6 bond angles in the piperazine ring decrease upon complex formation from 111.2° and 109.9° to 108.0° (tables in the Supporting Information).

The computed total energy, the highest occupied molecular orbital (HOMO) energies, the lowest unoccupied molecular orbital (LUMO) energies, and the dipole moment for the ligand and [Pd(BHEP)Cl2] were calculated (table 4).

A comparison of the bond lengths and angles of the optimized structure of the complex in the gas phase, with that obtained experimentally from the X-ray structure, shows good agreement (tables S1 and S2 in the Supporting Information). The bond lengths of the X-ray structure are shorter than in the gas phase, since the X-ray data are for the condensed solid phase.

3.2. Speciation studies

3.2.1. Hydrolysis of [Pd(BHEP)(H2O)2]2+

The acid dissociation constants of the ligands were determined under the same experimental conditions of ionic strength and temperature used to study the Pd(II) complexes. The hydrolysis of [Pd(BHEP)(H2O)2]2+ was investigated. Its acid/base chemistry was characterized by fitting the potentiometric data to various acid/base models. The best fit was found to be consistent with the formation of three species: 10-1, 10-2, and 20-1 as given in reaction (3). The dimeric species 20-2 was not detected. This may be explained on the premise that the steric crowding created by the alcohol groups attached to the piperazine nitrogens may generate some strain on the 20-2 species and consequently be energetically not favored.
The pKa₁ and pKa₂ values for \([\text{Pd}(\text{BHEP})(\text{H}_2\text{O})_2]^{2+}\) are 4.95 and 7.87, respectively. The equilibrium constant, \(\log K_{\text{dimer}}\), for the dimerization (3c) can be calculated by equation (4) and is 3.01.

\[
\log K_{\text{dimer}} = \log \beta_{20-1} - \log \beta_{10-1} = -1.94 - (-4.95) = 3.01
\]

The spectra of the hydrolyzed forms of \([\text{Pd}(\text{BHEP})(\text{H}_2\text{O})_2]^{2+}\) given in figure 3 show that the band at 36 nm corresponding to \([\text{Pd}(\text{BHEP})(\text{H}_2\text{O})_2]^{2+}\) undergoes a blue shift to 347 nm upon formation of \([\text{Pd}(\text{BHEP})\text{OH}]^{+}\) (10-1 species) by the addition of one equivalent of NaOH. This is due to ligand field splitting as a
result of deprotonation of a coordinated water. This band is further shifted to 338 nm by the addition of two equivalents of NaOH forming the dihydroxo species, [Pd(BHEP)(OH)2] (10-2 species).

The concentration distribution diagram for [Pd(BHEP)(H2O)2]2+ and its hydrolyzed species are shown in figure 4(A). The concentration of the monohydroxo species, 10-1, increases with increasing pH until it reaches a maximum concentration (90.32%) at pH 6.6. The dimeric species, 20-1, attains maximum concentration of ~29% at pH ~4.8. The monohydroxo species (10-1) is the main species in the pH range ~5–8, so it is the main species present under physiological conditions. A further increase in pH is accompanied by an increase in the dihydroxo species (10-2), which is the main species above pH ~8.

### 3.2.2. Amino acid complexes

Fitting of the potentiometric data for [Pd(BHEP)]-amino acid equilibrium indicated the formation of 1 : 1 complexes (table 5). Histidine is a tridentate ligand having amino, imidazole and carboxylate groups as binding sites. With [Pd(BHEP)(H2O)2]2+, only two of the three binding sites are involved in complex formation, hence histidine coordinates in either a glycine-like or a histamine-like mode. The stability constant of the histidine complex is in fair agreement with that of histamine and higher than those of

| MLH | OH⁻ | Log β | Valine |
|-----|-----|-------|--------|
| 10-1 | −4.95(0.02) | − | − |
| 10-2 | −12.82(0.03) | − | − |
| 20-1 | −1.94(0.04) | − | − |
| Log Kₛ | 3.01 | − | − |

| Proline | β-Phenylalanine | AlaIne |
| 011 | 9.51(0.01) | 9.12(0.01) | 9.71(0.01) |
| 012 | 11.82(0.02) | 11.01(0.03) | 12.17(0.02) |
| 110 | 8.91(0.03) | 8.34(0.03) | 9.08(0.05) |
| 011 | 10.11(0.02) | 9.69(0.01) | 9.15(0.01) |
| 012 | 13.75(0.03) | 15.75(0.02) | 15.30(0.02) |
| 013 | − | − | 17.00(0.06) |
| 110 | 8.63(0.04) | 10.05 (0.09) | 10.33(0.05) |
| 111 | 13.49(0.05) | 16.24(0.06) | 15.51(0.06) |
| pKₛ | 4.86 | 6.19 | 5.18 |

| Ethanolamine | Serine | Threonine |
| 011 | 9.46(0.01) | 9.14(0.01) | 9.06(0.01) |
| 012 | 11.40(0.02) | 11.03(0.02) | 10.03(0.02) |
| 110 | 5.92(0.02) | 8.70(0.02) | 8.18(0.02) |
| 120 | 9.38(0.03) | − | − |
| 11-1 | 0.07(0.09) | 0.46(0.05) | 0.33(0.03) |
| pKₛ | 5.85 | 8.24 | 7.85 |

| Ornithine | Lysine | Piperidine |
| 011 | 10.58(0.02) | 10.44(0.02) | 10.72(0.01) |
| 012 | 19.43(0.02) | 19.66(0.03) | − |
| 013 | 21.39(0.03) | 21.39(0.04) | − |
| 110 | 10.29(0.05) | 9.95(0.09) | 12.13(0.04) |
| 120 | − | − | 16.69(0.05) |
| 111 | 17.50(0.04) | 18.47(0.03) | 17.85(0.03) |
| pKₛ | 7.21 | 8.52 | − |

| S-Methylcysteine | Methionine |
| 011 | 8.65(0.02) | 9.12(0.02) |
| 012 | 10.61(0.02) | 11.39(0.03) |
| 110 | 8.54(0.03) | 8.23(0.04) |

aM, L, and H are the stoichiometric coefficients corresponding to Pd(BHEP), ligand, and H⁺, respectively; the coefficient −1 refers to a proton loss.
bLog β of Pd(BHEP)/ligand complexes. Standard deviations are given in parentheses; sum of square of residuals are less than SE-7.

The concentration distribution diagram for [Pd(BHEP)(H₂O)₂]²⁺ and its hydrolyzed species are shown in figure 4(A). The concentration of the monohydroxo species, 10-1, increases with increasing pH until it reaches a maximum concentration (90.32%) at pH 6.6. The dimeric species, 20-1, attains maximum concentration of ~29% at pH ~4.8. The monohydroxo species (10-1) is the main species in the pH range ~5–8, so it is the main species present under physiological conditions. A further increase in pH is accompanied by an increase in the dihydroxo species (10-2), which is the main species above pH ~8.

### 3.2.2. Amino acid complexes

Fitting of the potentiometric data for [Pd(BHEP)]-amino acid equilibrium indicated the formation of 1 : 1 complexes (table 5). Histidine is a tridentate ligand having amino, imidazole and carboxylate groups as binding sites. With [Pd(BHEP)(H₂O)₂]²⁺, only two of the three binding sites are involved in complex formation, hence histidine coordinates in either a glycine-like or a histamine-like mode. The stability constant of the histidine complex is in fair agreement with that of histamine and higher than those of
amino acids. This indicates that histidine interacts with the Pd\(^{II}\) complex in the same way as histamine coordinates. Analysis of the titration results for the ethanolamine complex reveals formation of 1:1 and 1:2 complexes. The stability constant of the 1:1 complex with ethanolamine has a lower value than those for the amino acids. This provides further support that the amino acids are bidentate ligands.

The stability constant values (log \(\beta_{110}\)) of simple amino acid complexes were compared. The stability constant of the complex with ornithine (log \(\beta_{110} = 10.29\)) is higher than those of the \(\alpha\)-amino acids. This may indicate that ornithine chelates through the two amino groups (N,N-donor set). However, in case of lysine, the stability constant of the complex (log \(\beta_{110} = 9.95\)) is a little bit higher than those of the \(\alpha\)-amino acids. This may indicate that lysine chelates through the \(\alpha\)-amino and carboxylate groups (N, O-donor set), i.e. low contribution of (N,N-donor set), which leads to formation of the less stable 8-membered chelate ring.

Methionine has three binding sites; the amino- and thioether and carboxylate groups. The stability constant value for the methionine complex (log \(\beta_{110} = 8.23\)) is lower than that of most simple amino acids. This may be explained on the basis that methionine coordinates by the amino and thioether groups. This is in accord with the high affinity of Pd\(^{II}\) toward the amino and sulfur sites [15].

Concentration distribution diagrams for glycine complexes (figure 4(B)) show the formation of the species 110 at low pH and predominates in the physiological pH range with a maximum concentration of \(\sim 83\%\). The monohydroxo species, 10-1, concentration does not exceed 16% and the dihydroxo-species, 10-2, predominates above pH 9.

The electronic spectra of [Pd(BHEP)(H\(_2\)O)\(_2\)]\(^{2+}\) in the presence and absence of glycine, taken as an example for amino acids and shown in figure 5, were compared. Upon complexation, the band of the [Pd(BHEP)(H\(_2\)O)\(_2\)]\(^{2+}\) complex at 360 nm shifted to 312 nm for [Pd(BHEP)glycine]\(^{+}\) (110 species). This shift is expected as a result of ligand-field splitting upon substitution of coordinated water by glycine.

### 3.2.3. Peptide complexes

In the case of peptide (HL) complexes, the potentiometric data were fitted on the basis of the formation of [Pd(BHEP)(L)]\(^{+}\) (110) and [Pd(BHEP)(LH\(_{-1}\))] (11-1). The former species is formed by coordination through the amino group and carbonyl oxygen. On increasing the pH, the coordination site can switch from the carbonyl oxygen to the amide nitrogen with release of the amide hydrogen, forming [Pd(BHEP)(LH\(_{-1}\))] . Such changes in coordination centers are now well documented [34, 35]. The pK\(_{H}\) of the coordinated amide group was calculated using equation (5) and is given in table 6. The pK\(_{H}\) for the glycaminamide complex is lower than for the other peptides. This signifies that the more bulky substituent group on the peptide may serve to hinder the structural change in going from protonated to deprotonated complexes. The asparagine complex has the highest stability constant, probably due to the presence

Figure 5. Electronic spectra of (A) \(8 \times 10^{-4} \text{M } [\text{Pd(BHEP)}(\text{H}_2\text{O})_2]^{2+}\); (B) \(8 \times 10^{-4} \text{M } [\text{Pd(BHEP)}(\text{H}_2\text{O})_2]^{2+}\) 8 \(\times 10^{-4} \text{M glycine and } 8 \times 10^{-4} \text{M NaOH}; (C) \(8 \times 10^{-4} \text{M glycine}.\)
of α-amino group that can coordinate first as glycine does. The α-amino group of asparagine is more basic than those of the other peptides, which results in an increase in stability constant of its complex.

The concentration distribution diagram of glycylglycine as a representative of peptide complexes, figure 4(C), indicates that the complex species (110) forms at low pH, predominates between pH 3.4–6.8, and the induced ionization of the peptide hydrogen of glycylglycine species (11-1) starts above pH ~5 and predominates between pH 6.8–10.4.

Spectral bands of \([\text{Pd(BHEP)}(\text{H}_2\text{O})_2]^{2+}\) and its glycylglycine complex are quite different in the position of the maximum wavelength (figure 6). The spectral band of \([\text{Pd(BHEP)}(\text{H}_2\text{O})_2]^{2+}\) appeared at 360 nm and is shifted to 325 nm upon formation of the \([\text{Pd(BHEP)}(\text{glycine})]^+\) complex (110 species). A further shift of the band to 313 nm during deprotonation and formation of \([\text{Pd(BHEP)}(\text{glycylglycineH}_-1)]^+\) is observed (11-1 species). The progressive shift toward lower wavelength in the absorption spectrum can be considered as evidence to support the potentiometric results for the induced ionization of amide upon complex-formation.

### 3.2.4. Ethanolamine complexes

The potentiometric data for the Pd(II) complex with ethanolamine is best fitted considering the formation of the complex species (110) and (11-1). The formation of 11-1 occurs through induced ionization of the alcoholic OH group of ethanolamine. The \(pK_a\) of ionization of the coordinated OH group was calculated by equation (5) to be 5.85 for ethanolamine.

\[
pK_a = \log \beta_{110} - \log \beta_{11-1}
\]  

### Table 6. Formation constants for \([\text{Pd(BHEP)}(\text{H}_2\text{O})_2]^{2+}\) with peptides at 25 °C and 0.1 M ionic strength.

| MLH | Glycinamide | Glycylglycine | Asparagine |
|-----|-------------|---------------|------------|
| 011 | 7.88 (0.09) | 7.94 (0.01)  | 8.56 (0.01) |
| 012 | –           | 11.01 (0.01) | 10.79 (0.03) |
| 110 | 7.53 (0.06) | 7.79 (0.04)  | 8.22 (0.02) |
| 11-1| 2.62 (0.02) | 0.83 (0.04)  | 0.84 (0.02) |
| \(pK_a\)| 4.91        | 6.96          | 7.38       |

\(M, L,\) and \(H\) are the stoichiometric coefficients corresponding to Pd(BHEP), peptides, and \(H^+\), respectively; the coefficient \(-1\), refers to a proton loss.

\(\log \beta\) of Pd(BHEP)-peptide complexes. Standard deviations are given in parentheses; sum of square of residuals are less than 5E-7.

![Figure 6. Electronic spectra of (A) \(8 \times 10^{-4} \text{ M } [\text{Pd(BHEP)}(\text{H}_2\text{O})_2]^{2+}\); (B) \(8 \times 10^{-4} \text{ M } [\text{Pd(BHEP)}(\text{H}_2\text{O})_2]^{2+}\), \(8 \times 10^{-4} \text{ M } \text{glycylglycine (HL)}\) and \(8 \times 10^{-4} \text{ M } \text{NaOH}\); (C) \(8 \times 10^{-4} \text{ M } [\text{Pd(BHEP)}(\text{H}_2\text{O})_2]^{2+}\), \(8 \times 10^{-4} \text{ M } \text{glycylglycine (HL)}\) and \(1.6 \times 10^{-3} \text{ M } \text{NaOH}\); (D) \(2 \times 10^{-4} \text{ M } \text{glycylglycine (HL)}\).](image)
The log $\beta_{110}$ value for ethanolamine is lower than that for amino acids. This may be due to coordination of ethanolamine at low pH through the amino group, while in the case of serine the coordination is through amino and carboxylate groups. At higher pH, the hydroxyl group undergoes induced ionization and participates in complex-formation to form 11-1. The pK$_H$ value of the coordinated alcohol group in ethanolamine (5.85) is lower than that of serine (8.24). This is consistent with a reaction scheme where the alcohol group of ethanolamine is coordinated to Pd$^{II}$, whereas the alcohol group of serine is competing with the carboxylate group in binding to the Pd(BHEP)$_2^+$ ion. Due to the donation of the electron pair on the oxygen to the metal center, the OH bond is considerably weakened and, thus, the ionization of the proton occurs at a lower pH.

### 3.2.5. DNA complexes

Inosine and inosine-5$'$-monophosphate form 1:1 and 1:2 complexes in addition to the mono-protonated species of the 1:1 complex (table 7). The pK$_N$ value of the protonated inosine complex is 4.60. This value corresponds to N1H. The lowering of this value with respect to that of free inosine is due to acidification upon complex-formation [36, 37]. The pK$_N$ value of the protonated inosine-5$'$-monophosphate complex is 7.26, higher than that of the phosphate group (6.22). This may be explained on the basis that the phosphate OH is involved in H-bonding with the free alcohol groups of BHEP. DNA constituents such as the pyrimidines uracil, uridine and thymine have basic nitrogen donors (N3) as reflected from the high pK$_N$ values of pyrimidines. They form 1:1 and 1:2 complexes predominating above pH 8.5. The thymine complex is more stable than that of uridine, probably due to the high basicity of the N3 group of thymine resulting from the extra electron-donating methyl group. The 1:1 inosine complex formed with [Pd(BHEP)(H$_2$O)$_2$]$^{2+}$ (log $K = 7.95$) is higher than that of the 1,4-piperazine complex (log $K = 6.87$) [38]. This may be explained on the basis that the formed complex is stabilized by hydrogen bonding between the alcohol group of BHEP and inosine.

The spectra given in figure 7 show that the band at 360 nm corresponding to [Pd(BHEP)(H$_2$O)$_2$]$^{2+}$ (figure 7(A)) undergoes a blue shift to a band at 335 nm for [Pd(BHEP)(Inosine-5$'$-monophosphate-H)]$^+$, 110 (figure 7(B)). This band is further shifted to a shoulder at 320 nm for [Pd(BHEP)(inosine-5$'$-monophosphate-H)$_2$], species 120 (figure 7(C)). The band appears as a shoulder due to the large absorption of inosine-5$'$-monophosphate (figure 7(D)).
3.2.6. Formation equilibrium of the binuclear Pd(BHEP)$^{2+}$ complex with 4,4′-bipiperidine

The titration curve for a solution mixture of 4,4′-bipiperidine and [Pd(BHEP)(H$_2$O)$_2$]$^{2+}$ in the ratio 2:1 (figure 8) shows a sharp inflection at $a = 2$ ($a$ is the number of mole of base added per mole of 4,4′-bipiperidine), corresponding to the complete formation of the [(H$_2$O)(BHEP)Pd-(Bip)-Pd(BHEP)(H$_2$O)]$^{4+}$ complex with a formation constant log $\beta_{210} = 21.05$ (scheme 2, table 8). It is interesting to compare the stability constant of the 1:1 complex formed between 4,4′-bipiperidine and [Pd(BHEP)(H$_2$O)$_2$]$^{2+}$ with that formed from piperidine (taken as a model for 4,4′-bipiperidine) and [Pd(BHEP)(H$_2$O)$_2$]$^{2+}$. The formation constant of the former complex is log $\beta = 13.72$ (table 8), whereas that of the latter complex is log $\beta = 11.21$. These values are in fair agreement, if we consider the inductive effect of the piperidine ring of 4,4′-bipiperidine on the basicity of the other coordinated piperidine nitrogen. This supports the formation of the binuclear Pd(BHEP)$^{2+}$ complex with 4,4′-bipiperidine.

Beyond $a = 2$, the binuclear complex is subjected to hydrolysis. In this range, the titration data were fitted considering the formation of the hydrolyzed species with stoichiometric coefficients 10-1 and 10-2 as given in scheme 3. The pK$_{a}$ of the bridged complex [(H$_2$O)(BHEP)Pd(Bip)Pd(BHEP)(H$_2$O)]$^{4+}$ (6.67) is lower than that of the corresponding ethylenediamine bridged complex [(H$_2$O)(en)Pd(Bip)
Figure 9. Concentration distribution of various species as a function of pH in the Pd(BHEP)-bipiperidine system at a concentration of 1.5 and 0.75 mM for [Pd(BHEP)(H₂O)₂]²⁺ and bipiperidine, respectively.

Figure 10. The electronic spectra of (A) 8 × 10⁻⁴ M of [Pd(BHEP)(H₂O)₂]²⁺; (B) 8 × 10⁻⁴ M of [Pd(BHEP)(H₂O)₂]²⁺, 4 × 10⁻⁴ M of Bip and 8 × 10⁻⁶ M of NaOH; (C) 8 × 10⁻⁴ M of [Pd(BHEP)(H₂O)₂]²⁺, 8 × 10⁻⁴ M of Bip and 16 × 10⁻⁶ M of NaOH; (D) 4 × 10⁻⁴ M of Bip.

Figure 11. Concentration distribution of various species as a function of pH in the Pd(BHEP)-Bip-Pd(BHEP)-inosine system at a concentration of 0.75 and 1.5 mM for Pd(BHEP)-Bip-Pd(BHEP) and inosine, respectively.
Figure 12. The electronic spectra of (A) $8 \times 10^{-4}$ M of $[\text{Pd}(\text{BheP})(\text{H}_2\text{O})_2]^2+$, $4 \times 10^{-4}$ M of Bip, $8 \times 10^{-4}$ M of NaOH; (B) $8 \times 10^{-4}$ M of $[\text{Pd}(\text{BheP})(\text{H}_2\text{O})_2]^2+$, $4 \times 10^{-4}$ M of Bip, $4 \times 10^{-4}$ M of inosine and $12 \times 10^{-4}$ M of NaOH; (C) $8 \times 10^{-4}$ M of $[\text{Pd}(\text{BheP})(\text{H}_2\text{O})_2]^2+$, $4 \times 10^{-4}$ M of Bip, $8 \times 10^{-4}$ M of inosine and $16 \times 10^{-4}$ M of NaOH; (D) $8 \times 10^{-4}$ M of inosine.

Scheme 1. Structure of Pd(BHEP)Cl$_2$ complex.

Scheme 2. Complex-formation equilibrium of Pd(BHEP)-Bip complexes.
Table 8. Formation constants for mixed ligand complexes of [Pd(BHEP)(H₂O)₂]²⁺ with bipiperidine at 25 °C and 0.1 M ionic strength.

| System   | M | L | H | Log β | pKₐ |
|----------|---|---|---|-------|-----|
| Bipiperidine | 0 | 1 | 1 | 10.96(0.02) | 10.96 |
|           | 0 | 1 | 2 | 21.12(0.01) | 10.16 |
|           | 1 | 1 | 0 | 13.72(0.05)  |      |
|           | 2 | 1 | 0 | 21.05(0.06)  |      |

log β of Pd(BHEP)-Bipip. Standard deviations are given in parentheses; sum of square of residuals are less than 5e⁻⁷.

The pKₐ of the ligand or the protonated complex.

![Image of Scheme 3](image1)

Scheme 3. Acid-base equilibria of [(H₂O)(BHEP)Pd(Bip)Pd(BHEP)(H₂O)]⁴⁺.

![Image of Scheme 4](image2)

Scheme 4. Complex-formation equilibria of [(H₂O)(BHEP)-Pd-(Bip)-Pd(BHEP)(H₂O)]⁴⁺-inosine complex.
Pd(en)(H₂O)⁴⁺ (9.64), reported previously [39]. This is explained on the premise that the coordinated water in [(H₂O)(BHEP)Pd(Bip)Pd(BHEP)(H₂O)]⁴⁺ is involved in H-bonding with the alcohol groups of the coordinated BHEP ligand. Also, the pKₐ₁ and pKₐ₂ values of the bridged complex [(H₂O)(BHEP)Pd(Bip)Pd(BHEP)(H₂O)]⁴⁺ are nearly equal. This indicates that the two Pd(BHEP) units behave as two separate species, i.e., the Bip bridge does not allow the two Pd(BHEP) units to communicate electronically with each other. This can be explained on the basis of the absence of π-conjugation in the Bip bridge.

The speciation diagram for the Pd(BHEP)–bipiperidine system is given in figure 9. The binuclear complex, [(H₂O)(BHEP)Pd(Bip)Pd(BHEP)(H₂O)]⁴⁺ (210), starts to form at low pH and on increasing pH, its concentration increases and is the predominant species up to pH 8.6. It reaches a maximum concentration of 94% at pH 5.6.

Electronic absorption bands of [Pd(BHEP)(H₂O)₂]²⁺ and its 4,4′-bipiperidine complex are quite different in terms of the position of the maximum wavelength and molar absorptivity (figure 10). The spectrum of the [Pd(BHEP)(H₂O)₂]²⁺ complex (figure 10(A)) shows an absorption maximum at 360 nm. The spectrum obtained for [(H₂O)(BHEP)Pd(Bip)Pd(BHEP)(H₂O)]⁴⁺ (figure 10(B)) exhibits a band at 340 nm. This band is further shifted to 331 nm for the formation of [(Bip)Pd(BHEP)(OH₂)]²⁺ (figure 10(C)). An isosbestic point is observed at 326 nm revealing an equilibrium between mono- and binuclear complexes.

The complex formation between [(H₂O)(BHEP)Pd(Bip)Pd(BHEP)(H₂O)]⁴⁺ and inosine, taken as an example for a DNA constituent, showed the formation of 1 : 1 and 1 : 2 complexes, as given in scheme 4. The stability constant of the DNA complexes is in the order thymine > uracil > uridine > inosine (table 9). This may be explained as a result of the difference in the basicity of the donor as reflected by the pKₐ values. It is interesting to compare the formation constant of the binuclear complex with DNA with those of Pd(II) complexes involving N,N-dimethylethylenediamine instead of BHEP [40]. The formation constant values of inosine complexes with BHEP are 6.62 and 10.55 for 1 : 1 and 1 : 2 complexes, respectively.

Table 9. Formation constants for mixed ligand complexes of [(H₂O)(BHEP)Pd(Bip)Pd-BHEP)(H₂O)]⁴⁺ with some DNA units at 25 °C and 0.1 M ionic strength.

| System                 | M L H⁺ | Log β⁰ | pKₐ c |
|------------------------|--------|--------|-------|
| [(H₂O)(BHEP)Pd(Bip)Pd(BHEP)(H₂O)]⁴⁺ | 1 0 −1 | −6.67(0.03) | 6.67   |
|                        | 1 0 −2 | −13.83(0.02) | 7.16   |
| Inosine                | 0 1 1  | 8.80(0.02)  | 8.80   |
|                        | 1 1 0  | 6.62(0.05)  |        |
|                        | 1 2 0  | 10.55(0.08) |        |
| Uridine                | 0 1 1  | 9.11(0.01)  | 9.18   |
|                        | 1 1 0  | 7.16(0.05)  |        |
|                        | 1 2 0  | 12.12(0.06) |        |
| Uracil                 | 0 1 1  | 9.18(0.01)  | 9.18   |
|                        | 1 1 0  | 7.16(0.05)  |        |
|                        | 1 2 0  | 12.12(0.06) |        |
| Thymine                | 0 1 1  | 9.65(0.01)  | 9.65   |
|                        | 1 1 0  | 7.98(0.04)  |        |
|                        | 1 2 0  | 13.45(0.05) |        |

*M, L, and H are the stoichiometric coefficients corresponding to Pd(BHEP)Bip, DNA units, and H⁺, respectively.

Log β of binuclear-DNA units. Standard deviations are given in parentheses; sum of square of residuals are less than 5e⁻⁷.

The pKₐ of the ligands or the aquo complexes.

The extra stability is probably due to the H-bonding between the alcohol groups of BHEP and the coordinated inosine. The same effect was observed for the mononuclear inosine complex.

The speciation diagram of the [(H₂O)Pd(BHEP)(Bip)Pd(BHEP)(H₂O)]⁴⁺-inosine complex is given in figure 11. The 1 : 1 complex dominates between pH 5.2 and 8.2 with a maximum concentration of 87% at pH 6.6. The 1 : 2 complex attains a maximum formation degree of 43% at pH 8.6. The hydrolyzed species are formed above pH 8.9. The DNA complex dominates in the physiological pH range such that the reaction of the binuclear complex with DNA is quite feasible.
The electronic absorption bands of Pd(BHEP)(H$_2$O)$_2$$_{2+}$ with 4,4′-bipiperidine and inosine are given in figure 12. The spectrum obtained for [(H$_2$O)(BHEP)Pd(Bip)Pd(BHEP)(H$_2$O)]$^{4+}$ (figure 12(A)) has a maximum at 360 nm. The spectra obtained for [(H$_2$O)(BHEP)Pd(Bip)Pd(BHEP)-(inosine)]$^{3+}$ (figure 12(B)) and [(inosine)(BHEP)Pd(Bip)Pd(BHEP)(inosine)]$^{2+}$ (figure 12(C)) exhibit bands at 339 and 329 nm, respectively. The spectral band shifts are taken as evidence for binuclear complex formation, supporting the potentiometric results. The isosbestic point at 307 nm is indicative for an equilibrium existing between the inosine binuclear complexes.

4. Conclusion

This paper reports the synthesis and structural characterization of a Pd$^{II}$-1,4-bis(2-hydroxyethyl)piperazine complex. The complex is formed through binding with piperazine nitrogens and formation of a five-membered chelate ring. The bond lengths and angles of the optimized structure of the complex in the gas phase show agreement with the data obtained experimentally by X-ray single-crystal analysis. The shape of the piperazine ring changed from chair in the free ligand to a boat structure in the complex. The alcohol groups do not participate in Pd(BHEP) complex formation and may interact with DNA, the major target in chemotherapy, via hydrogen bonding. [Pd(BHEP)(H$_2$O)$_2$]$_{2+}$ is hydrolyzed to give mono- and dihydroxo species in addition to the monohydroxo-bridged dimer. This is in line with a previous report [39]. Amino acids form 1:1 complexes. Peptides form 1:1 complexes in addition to the deprotonated species. Inosine forms 1:1 and 1:2 and the monoprotonated species of the 1:1 complex. The stability constant of the Pd(BHEP) complex with inosine is higher than that of the Pd-1,4-piperazine complex. The extra stability is probably due to hydrogen bonding between the alcohol groups of BHEP and inosine.

The binuclear complexes involving 1,4-bis(2-hydroxyethyl)piperazine DNA constituents and 4,4′-bipiperidine as linker were investigated. It is interesting to compare the results of the binuclear complexes in the present study with those previously reported for similar binuclear Pd(II) complexes involving N,N-dimethylethlenediamine [40] or ethylenediamine [39] instead of BHEP. The inosine complex of BHEP is more stable than that of N,N-dimethylethlenediamine or ethylenediamine. This reveals the role of H-bonding in the BHEP complex that favors the interaction with DNA, the major target in tumor therapy.

The binuclear complexes of the Pd(BHEP)-1,4-bipiperidine system are more stable than those of the Pd(MME)-1,4-bipiperidine system [41], where MME is methionine methyl ester and is bound to Pd$^{II}$ via N and S donors. This is explained on the premise that the labilization effect of the S donor in MME lowers the stability constant of the binuclear complexes.

Supplementary material

CCDC 1043577 contains the supplementary crystallographic data for the complex [Pd(BHEP)Cl$_2$].

Disclosure statement

No potential conflict of interest was reported by the authors.

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