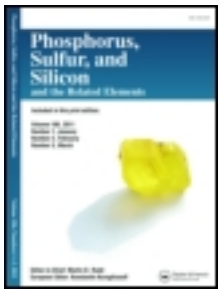

Phosphorus, Sulfur, and Silicon and the Related Elements

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Synthesis, characterisation, and antimicrobial activity of the ligand 3-methylpyrazole-4-carboxaldehyde thiosemicarbazone and its Pd(II) complex

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Synthesis, characterisation, and antimicrobial activity of the ligand 3-methylpyrazole-4-carboxaldehyde thiosemicarbazone and its Pd(II) complex

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Synthesis, characterisation, activity of Pd(II) complex

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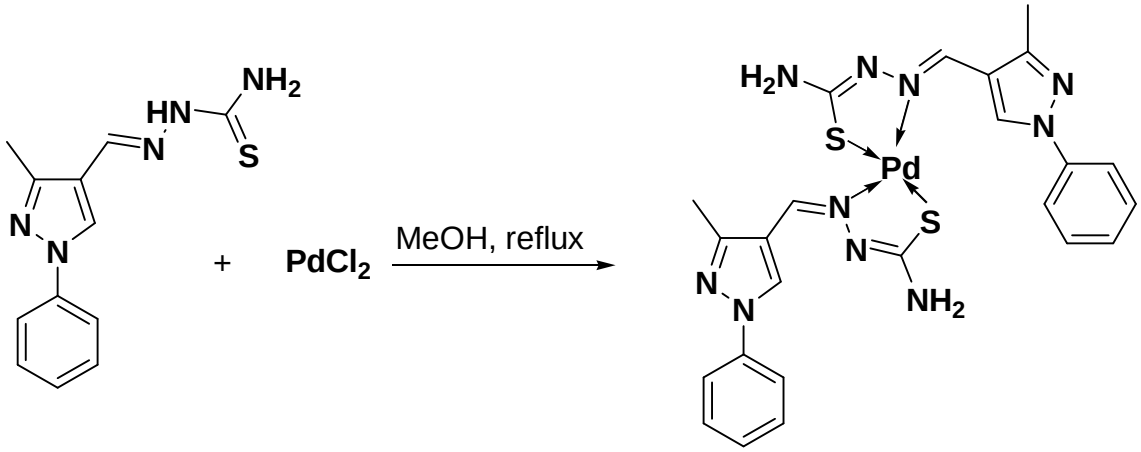
Synthesis, characterisation, and antimicrobial activity of the ligand 3-methylpyrazole-4-carboxaldehyde thiosemicarbazone and its Pd(II) complex

ABSTRACT



The synthesis and characterisation of a new palladium(II) complex [Pd(MePhPzTSC)$_2$] and its corresponding ligand 3-methylpyrazole-4-carboxaldehyde thiosemicarbazone (MePhPzTSC) are described. The bidentate ligand is coordinated to Pd(II) through the azomethine nitrogen atoms and sulfur in the form of thiol by deprotonation of the NH-C=S. The antimicrobial activity of these new compounds was evaluated against gram-negative (*Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus and Bacillus thuringiensis*) bacteria and two yeast strains (*Candida albicans* and *Saccharomyces cerevisiae*). Coordination of the ligand to the metallic ion showed improved antimicrobial activity compared to the free

ligand. For the gram-positive bacteria the antimicrobial activity of the complex was higher than that of the positive control used.

KEYWORDS

Thiosemicarbazone, palladium complexes, antimicrobial activity.

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INTRODUCTION

Thiosemicarbazones complexed with transition metals are of great chemical and biological interest because they exhibit diverse pharmacological actions, including antibacterial, antifungal, antitumor, antiviral, and antimalarial effects, among others.[1,2,3] It is also important to note that the pyrazole nucleus and its derivatives exhibit a wide range of biological activities, such as analgesic, anti-inflammatory, antipyretic, antibacterial, antifungal, and hypoglycaemic activities.[4,5]

Additionally, many Pd(II) complexes with ligands that have nitrogen and sulphur donor atoms, such as thiosemicarbazones, exhibit biological activity.[6] These findings have motivated the study of these types of compounds. This paper reports the synthesis and characterisation of a new pyrazolyl-thiosemicarbazone ligand and its Pd(II) complex (Figure 1). These compounds were characterised by spectroscopic techniques (infrared, FTIR and mono and two-dimensional nuclear magnetic resonance, NMR) and fast atom bombardment (FAB) mass spectrometry for the Pd(II) complex. The antimicrobial activity of the synthesised compounds was evaluated against gram-positive and gram-negative bacteria and two yeast strains.

[Insert Figure 1]

RESULTS AND DISCUSSION

MePhPzTSC Ligand

The ligand, 3-methylpyrazole-4-carboxaldehyde thiosemicarbazone (MePhPzTSC), was prepared from the condensation reaction between 3-methyl-1-phenylpyrazole-4-carboxaldehyde and thiosemicarbazide. The characteristic spectral bands of the product and the disappearance of the carbonyl band of the pyrazole precursor were observed in the IR spectrum (Figure S 1 Supplemental Materials). These observations confirmed the formation of the thiosemicarbazone compound. An absorption band was observed at 1209 cm^{-1} corresponding to the ν(C=S) elongation bound to the nitrogen atom, and another band was observed at 1097 cm^{-1} corresponding to the ν(C=S) elongation. In the 3000 to 4000 cm^{-1} region, the spectrum registered two bands at 3424 and 3248 cm^{-1}

that corresponded to the symmetric and asymmetric elongations, respectively, of the $\nu(NH_2)$ group. The band at 3144 cm^{-1} was assigned to the ν(NH) of the hydrazine group; a strong band observed at 1595 cm^{-1} was assigned to the ν(C=N) bond of the azomethine group, and a band at 1551 cm^{-1} corresponded to the $\nu(C=N)_{pz}$ of the pyrazole ring. The absence of a band between 2600 and 2800 cm^{-1}, corresponding to ν(SH), confirmed that the ligand exists as a thione tautomer in the solid state.[4, 7]

The ^{1}H NMR spectrum of the MePhPzTSC ligand (Figure S 2) showed eight signals corresponding to the expected protons for the proposed structure. The NH_2 group protons appeared at different chemical shifts because there is no free rotation around the C-N bond, due to its double-bond character.[4,8] The ^{13}C NMR spectrum (Figure S 3) displayed the nine expected signals for the synthesised compound, a signal at 13.2 ppm corresponded to the methyl group, and another signal at 117.3 ppm (C_q) represented the C-4 carbon of the pyrazole ring. The signals corresponding to the aromatic ring appeared at 117.9, 126.2, and 129.5 ppm for C_o, C_p, and C_m, respectively. The chemical shift at 128.3 ppm represented the C-5 carbon on the pyrazole ring, and the signal at 135.8 ppm corresponded to the azomethine group carbon (CH=N). Three signals were detected in the lower range: one at 139.0 ppm (C_q) corresponding to the C_i of the aromatic ring and another signal at 148.7 ppm (C_q) corresponding to the C-3 of the pyrazole ring. These two signals have lower electron densities caused by the inductive effect of the N-1 nitrogen atom for C_i and of the surplus N-2 π-character of the pyrazole for the C-3. The last signal was observed at 177.3 ppm (C_q) and corresponded to the

carbon thiocarbonyl group (C=S). A distortionless enhancement by polarisation transfer (DEPT) 135 experiment identified the quaternary carbons in the structure (C-3 and C-4 on the pyrazole ring, C_i on the aromatic ring, and the carbon from the C=S bond), and the bi-dimensional NMR experiments (heteronuclear single quantum correlation spectroscopy (HSQC, Figure S 4) and heteronuclear multiple-bond correlation spectroscopy (HMBC) confirmed the proposed structure.

[Pd(MePhPzTSC)$_2$] Complex

The IR spectrum of the [Pd(MePhPzTSC)$_2$] complex (Figure S 5) did not contain the band at 3143 cm^{-1} that corresponds to the ν(N-H) elongation of the hydrazine group on the free ligand, most likely because the group was deprotonated. The presence of a new band with low intensity at 1156 cm^{-1} was attributed to the ν(N-N) elongation, suggesting deprotonation of the hydrazine ν(N-H) group. The emergence of new bands at 400 cm^{-1} and 441 cm^{-1} corresponding to ν(Pd-S) and ν(Pd-N), respectively, indicated that the Pd(II) cation was coordinated to these donor atoms.[7,9,10] However, no bands were observed in the 305-335 cm^{-1} region, which is attributed to a ν(Pd-Cl) bond,[10] suggesting that the chloride ligand is not in the coordination sphere. Coordination of the ligand (MePhPzTSC) through its nitrogen and sulphur atoms to the metal ion was confirmed because the bands from the C=N bond of the azomethine group and the C=S bond were shifted compared to those observed in the spectrum of the [Pd(MePhPzTSC)$_2$] complex.

When comparing the chemical shifts in the ^{1}H NMR spectrum (Table 1) of the free ligand with the complex [Pd(MePhPzTSC)$_2$] in [DMSO-d$_6$] (Figure S 6), the corresponding proton from the NH-C=S group was observed to be absent, suggesting enolisation of the ligand and not supporting the proposed structure of the ligand in the tautomeric thione form. This result was also corroborated by the high-field shift of the amino group protons that overlap with the signal corresponding to H_p. This shift suggests a change in the electron density to this portion of the molecule (-CSNH$_2$) due to a change from a thione to a thiol in the thiocarbonyl group. The downfield shift of the azomethine proton displayed a $\Delta\delta$ = 0.07 ppm compared to that of the free ligand, suggesting the group has a lower spectroscopic electron density and is coordinated to the metal through the nitrogen atom.

(Table 1 goes here)

Assignments of the signals in the ^{13}C NMR spectrum are based on the chemical shifts and intensity patterns and coordination induced shift (CIS) of carbon signals in the complex, in comparison to those of the free ligand, $\Delta\delta$ = δ (complex) - δ (free ligand). The ^{13}C NMR spectrum of the [Pd(MePhPzTSC)$_2$] complex (Figure S 7, Table 2) displayed a signal at 172.2 ppm, which corresponds to the thiocarbonyl group, and when compared to the same signal from the free ligand (177.3 ppm), the high-field shift with a difference of 5.1 ppm ($\Delta\delta$) suggests a change from a thione to a thiol functional group.[11] This shift confirms that the sulphur atom in the thiol form of the ligand is

coordinated to the Pd(II) cation.[4,12] In addition, the signal corresponding to the carbon atom in the azomethine group in the Pd(II) complex was observed at 144.2 ppm. This chemical shift is downfield compared to the signal of the free ligand with a difference of 8.4 ppm ($\Delta\delta$). This shift suggests that the nitrogen on the azomethine group has a lower electron density, thereby confirming coordination of the nitrogen atom's free electron pair to the metal.[4]

(Table 2 goes here)

The positive ion FAB mass spectrum for the [Pd(MePhPzTSC)$_2$] complex (Figure S 8), showed two m/z peaks at 622.9272 and 644.9314 that correspond to M^+ and [M-H + Na]$^+$, respectively. This complex, [Pd(MePhPzTSC)$_2$], was prepared in a 1:1 ligand-metal molar ratio, however the complex obtained presented a 2:1 ligand-metal molar ratio. This is indicated by the FAB mass spectrum, as the peak of major abundance was observed at a m/z 622.9272, confirming the molecular mass of the compound. Considering this mass relation and the results obtained by ^{1}H y ^{13}C NMR spectra the purity and proposed structure of the Pd(II) complex was corroborated.

Antimicrobial activity assays

The MePhPzTSC ligand, $PdCl_2$ and the [Pd(MePhPzTSC)$_2$] complex did not exhibit antifungal activity in the range of concentrations tested, and the metal precursor did not

exhibit antimicrobial activity in the range tested for any of the organisms (Table S 1 Supplemental Materials).

For the antibacterial activity assays, the pyrazolyl-thiosemicarbazone ligand, MePhPzTSC, only registered growth inhibition for *B. thuringiensis* with a minimum inhibitory concentration of 965 μM (Table S1) and with an inhibition halo of 6 mm (Table S2).

The [Pd(MePhPzTSC)$_2$] complex registered activity for both gram-positive bacteria examined (Tables S 1 and S 2). A minimum inhibitory concentration (MIC) of 322 μM was observed for *S. aureus* with an inhibition halo of 14 mm; the results suggest that the antimicrobial activity occurs due to synergy between the metal and the ligand because individually they showed no effect (Table S2). This behaviour can be explained by the Overtone's concept of cell permeability[13,14] and Tweedy's chelation theory.[15] According to the Overtone's concept, the lipid membrane surrounding the cell only favours the passage of lipid-soluble materials; therefore, the lipid is an important factor that controls antimicrobial activity. According to Tweedy's theory, the polarity of the Pd(II) cation is reduced by the overlap of the ligand orbitals and the partial exchange of the metal ion's positive charge to the donor atoms of the ligand, thereby increasing π-electron delocalisation on the chelate ring and improving the lipophilicity of the complex. This increased lipophilicity promotes penetration of the complex through the lipid membranes and blocks the metal-binding sites on the enzymes of the microorganisms.

This effect possibly disturbs the process of cell respiration and therefore blocks the synthesis of the proteins that limit the organism's growth.[15] For *B. thuringiensis*, the [Pd(MePhPzTSC)$_2$] complex registered a MIC of 483 μM (Table S 1 Supplemental Materials) and an inhibition halo of 8 mm (Table S 2). As observed with *S. aureus*, an increase in the antibacterial activity of the complex compared to that of the free ligand was observed with *B. thuringiensis* (Table S 2). Again this shows the synergetic effect that the ligand and the metal show when assembled as the Pd(II) complex. One could expect a higher antimicrobial activity effect by the complex compared to the free ligand, since the complex contains two ligand molecules in its structure. However, the coordination between the ligand and the metal introduces different chemical characteristics to the novel Pd(II) complex which not necessarily have additive antimicrobial activity of the free ligand.

The results for antimicrobial activity showed that the synthesised compounds have lower inhibition halos than the antibiotic positive controls (Table S 2). However, considering the concentrations used for the antimicrobial controls in the biological assays, it can be seen that the synthesised Pd(II) complex has a high growth inhibitory effect on the Gram-positive bacteria evaluated. For example, for *S. aureus* a concentration of 322 μM of the [Pd(MePhPzTSC)$_2$] complex originated a 14 mm zone of inhibition, while for the same bacteria the positive antimicrobial control used (cephalothin) showed a 34 mm zone of inhibition but at a 310 times higher concentration (100'000 μM). The same situation is observed for *B. thuringiensis* with an

inhibition zone of 8 mm for the complex at 483 μM concentration, while the control showed a 14 mm inhibition zone but at a 207 times the concentration (100'000 μM) of the positive inhibition control cephalothin. It is interesting to notice that the free ligand, at the concentration used (965 μM), had only effect against *B. thuringiensis* and that this inhibition was also higher (>100 times) than the positive antimicrobial control used.

These antimicrobial assays show an interesting potential of the newly synthesised [Pd(MePhPzTSC)$_2$] complex to be used as an antimicrobial compound directed specifically to Gram-positive bacteria..Nevertheless, further studies should be carried out to confirm the antimicrobial ability of the complex against other Gram-positive bacteria. This complex should also be used in biological assays to observe its effect against parasitic organisms and tumor cells, since similar Pd(II) compounds have shown promising effects against these type of cells.[1,2,16]

EXPERIMENTAL

Determination of the synthesised compounds' melting points was performed with a Melt-Temp II melting point meter (Laboratory Devices). The IR spectra were captured from 4000 to 250 cm^{-1} using an FTIR-8400/8900 (Shimadzu) and a Nicolet iS10 Spectrometer (Thermo Fisher Scientific). The samples were prepared in KBr tablets. The ^{1}H and ^{13}C NMR and two-dimensional spectra were determined on a 400 MHz Bruker Ultrashield™ NMR spectrophotometer using DMSO-d_6 as the deuterated solvent. The high-resolution mass spectrum with an EBE configuration was captured on

an AUTOSPEC-Q spectrometer. Positive ion FAB was used as the ionisation technique, and thioglycerol was the ionisation matrix.

The following organisms were used for the antimicrobial assays: i) gram-positive bacteria: *Staphylococcus aureus* ATCC 29213 and *Bacillus thuringiensis* sp., ii) gram-negative bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* sp., and iii) yeast strains: *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 38626.

Synthesis of the MePhPzTSC Ligand

The ligand, MePhPzTSC, was prepared by combining a 1:1 molar ratio of 3-methyl-1-phenylpyrazole-4-carboxaldehyde (1 g, 5 mmol) and thiosemicarbazide (0.49 g, 5 mmol) in ethanol (20 mL) and refluxed for 4 h. Several drops of acetic acid were added to the mixture as a catalyst. The white precipitate was filtered and washed three times with ethanol to remove traces of the aldehyde precursor and was dried with anhydrous calcium chloride. A 72% percent yield was obtained. The ligand is stable at room temperature (19±1 °C) and is soluble in DMSO.

MePhPzTSC: Mp 200-204 ^{o}C, yield 72%. IR (KBr)/cm^{-1} 3423-3247 (NH_2 asym and sym), 3143 (NH hydrazine), 1590 (C=N azomethine), 1551 (C=N pyrazole), 1209 (NHC=S), 1096 (C=S). ^{1}H NMR (DMSO-d_6) δ 2.38 (s, 3H, CH_3), 7.29 (t, 1H, J=7.5 Hz, H_p), 7.46-7.50 (t, 3H, H-NH_2, y, H_m), 7.78 (d, 2H, J=8.03Hz, H_o), 8.07 (s, 1H, CH=N),

8.17 (brs, 1H, H-NH_2), 8.85 (s, 1H, H-5pz), 11.33 ppm (s, 1H, NH-C=S). ^{13}C NMR (DMSO-d_6) δ 13.2 (CH_3), 117.3 (C-4 pz), 117.9 (C_o), 126.2 (C_p), 128.3 (C-5 pz), 129.5 (C_m), 135.8 (CH=N), 139.0 (C_i), 148.7 (C-3 pz), 177.3 (C=S).

Synthesis of the [Pd(MePhPzTSC)$_2$] Complex

The complex, [Pd(MePhPzTSC)$_2$] (Graphical Abstract), was prepared in a 1:1 metal-ligand molar ratio by adding a hot solution of MePhPzTSC (50 mg, 0.19 mmol) and methanol (10 mL) to a refluxing solution of $PdCl_2$ (35 mg, 0.20 mmol) in methanol (10 mL). The mixture was refluxed for 24 h. Fifty-four milligrams of an orange solid were obtained and washed several times with methanol to remove excess Pd. The solid was dried with anhydrous calcium chloride, and the percent yield of the synthesised complex was 45%. The complex is stable at room temperature (19±1 °C) and is soluble in DMSO.

[Pd(MePhPzTSC)$_2$]: Decomposition temperature 208 ºC, yield 45%. IR (KBr)/cm^{-1} 3446-3308 (NH_2 asym and sym), 1619 (C=N azomethine), 1536 (C=N pyrazole), 1156 (N-N), 863 (C-S), 400 (Pd-S), 441 (Pd-N). Mass (m/z) 622.9272 (M^+ 100%), 644.9312 ([M-H + Na]$^+$ 23%). ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 7.34-7.44 (t, 3H, NH_2, y, H_p), 7.52 (t, 2H, *J*=7.58 Hz, H_m), 7.85 (d, 2H, *J*=7.57Hz, H_o), 8.14 (s, 1H, CH=N), 9.23 (s, 1H, H-5 pz). ^{13}C NMR (DMSO-d_6) δ 11.9 (CH_3), 114.2 (C-4 pz), 119.1 (C_o), 130.0(C_m), 127.4 (C_p), 132.7 (C-5 pz), 139.1 (C_i), 144.2 (CH=N), 152.2 (C-3 pz), 172.2 (C-S).

Antimicrobial activity assays

The antibacterial activity was analysed by the disk diffusion method in a Petri dish using TSA medium (Trypticase Soy Agar, Scharlau) for bacteria and PDA (Potato Dextrose Agar, Scharlau) for yeasts. The minimum inhibitory concentration (MIC in mcg/mL or ppm) of the studied compounds was estimated using the methodology of Bauer et al.[17] The compounds (20 μL each), in concentrations ranging between 50 and 500 ppm (282-2820 μM for $PdCl_2$, 193-1930 μM for MePhPzTSC, and 80-803 μM for [$Pd(MePhPzTSC)_2$]), were dissolved in DMSO, and placed in 7 mm-diameter wells, where the microorganisms under evaluation were grown (~10^8 UFC/mL) in their respective culture media. The microbial cultures were incubated at 35 °C for 24 h for the bacterial strains and at 20 °C for 72 h for the yeast strains. DMSO was used to prepare the compounds and as the negative control for all of the growth inhibition assays. Cephalothin (40 mg/mL) was used as the positive control for all bacterial strains, except ciprofloxacin (0.3%), which was used for *P. aeruginosa*. Clotrimazole (1%) was used as the positive control for the yeasts. The susceptibility of the microorganisms to each of the tested compounds was determined by observing the formation of an inhibition zone around each well on agar, which was measured in millimetres.

CONCLUSIONS

A new pyrazolyl-thiosemicarbazone ligand, MePhPzTSC, and its respective complex with Pd(II), [$Pd(MePhPzTSC)_2$], were synthesised and characterised. The ligand is a bidentate chelator and coordinated to the metal through the nitrogen and sulfur atoms in

its thiol form. The results obtained by various spectroscopic techniques confirmed the proposed structure of each compound. The synthesised compounds showed antimicrobial activity only against the gram-positive bacteria evaluated and this activity was higher than that of the antibiotic controls. Compared to the free ligand, the metal complex showed a better antimicrobial activity.

Supplemental Materials

Biological Activities

Table S 1: Minimum inhibitory concentration of $PdCl_2$, MePhPzTSC, and the $[Pd(MePhPzTSC)_2]$ complex in the examined microorganisms

	Minimum inhibitory concentration (μM) of the compound		
Microorganism	MePhPzTSC	$[Pd(MePhPzTSC)_2]$	$PdCl_2$
E. coli	> 1930	> 803	> 2820
K. pneumoniea	> 1930	> 803	> 2820
P. aeruginosa	> 1930	> 803	> 2820
S. aureus	> 1930	322	> 2820
B. thuringiensis	965	483	> 2820
C. albicans	> 1930	> 803	> 2820
S. cerevisiae	> 1930	> 803	> 2820

Table S 2: Diameter of the antimicrobial inhibition halo for $PdCl_2$, MePhPzTSC, the $[Pd(MePhPzTSC)_2]$ complex, and the antibiotic control in the examined microorganisms

	Inhibition halo (mm) over the growth of

Compound	Bacteria					Yeasts	
	EC	KP	PA	SA	BT	CA	SC
MePhPzTSC	0	0	0	0	6	0	0
[Pd(MePhPzTSC)$_2$]	0	0	0	14	8	0	0
$PdCl_2$	0	0	0	0	0	0	0
Control antibiotic	20	10	40	34	14	25	30

EC, *Escherichia coli*; *KP, Klebsiella pneumoniae*; PA, *Pseudomonas aeruginosa*; *SA, Staphylococcus aureus*; BT, *Bacillus thuringiensis*; CA, *Candida albicans*; *SC, Saccharomyces cerevisiae*.

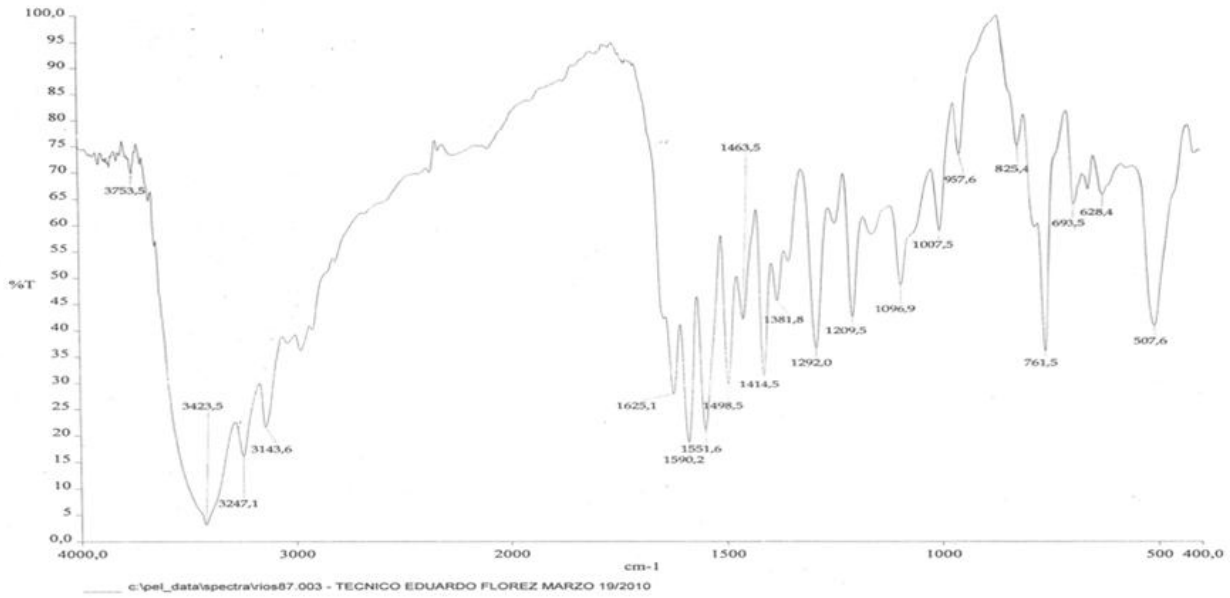

Figure S 1: IR spectrum of MePhPzTSC Ligand

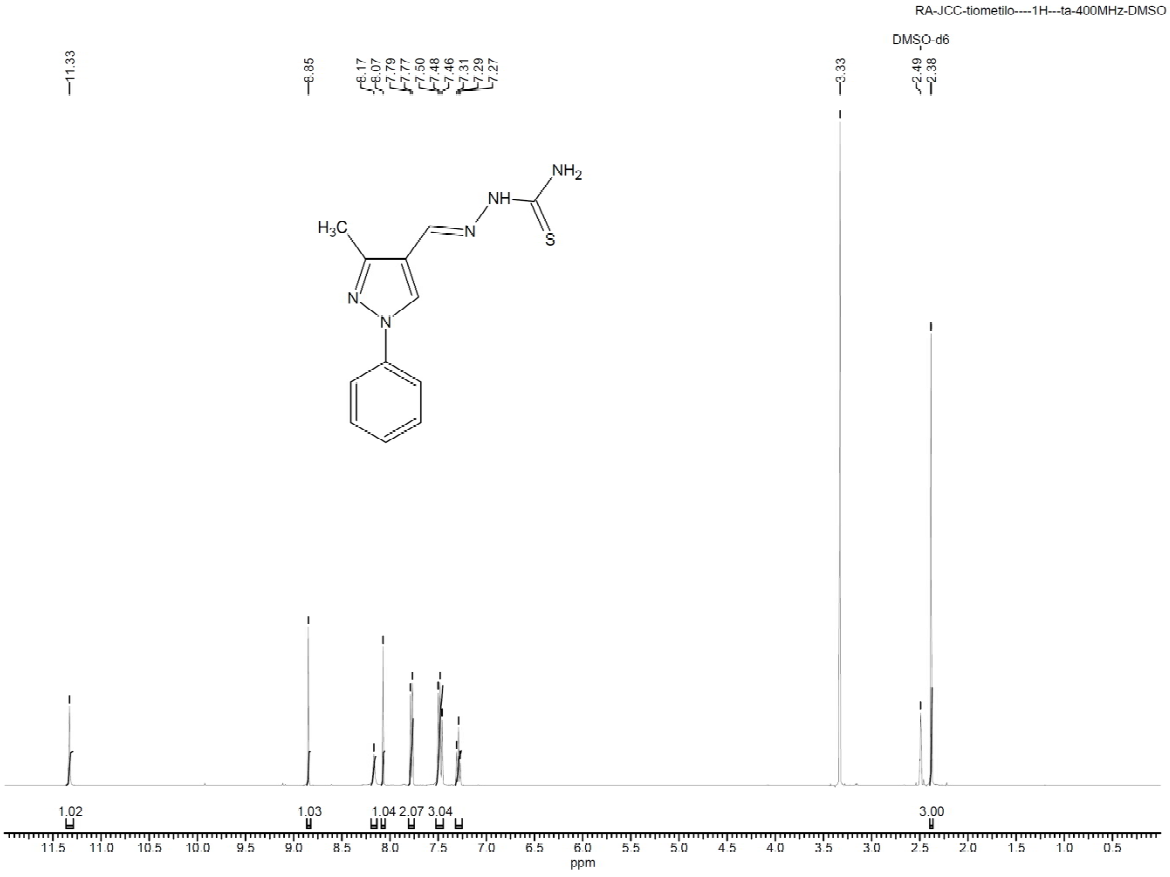

Figure S 2: ^{1}H NMR spectrum of the MePhPzTSC ligand

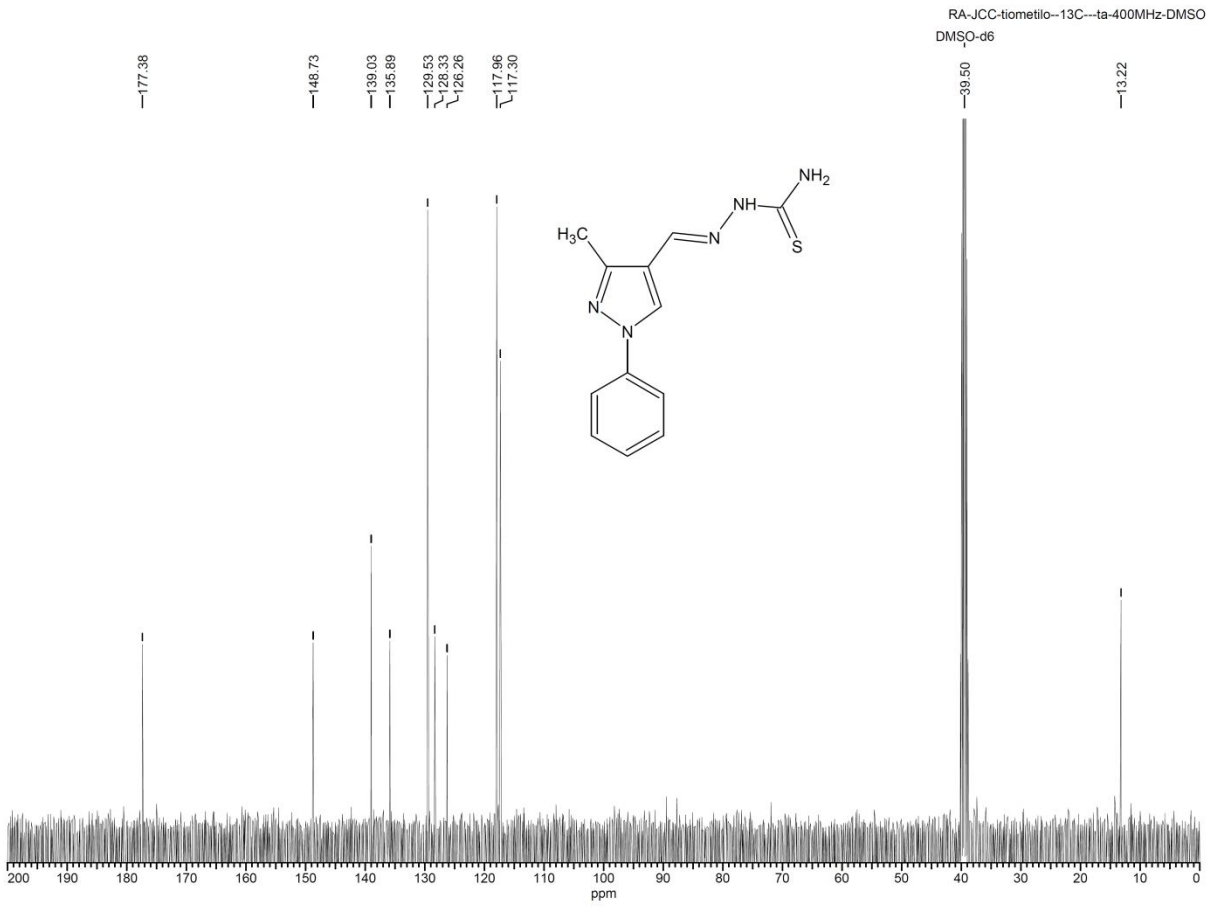

Figure S 3: ^{13}C NMR spectrum of the MePhPzTSC ligand

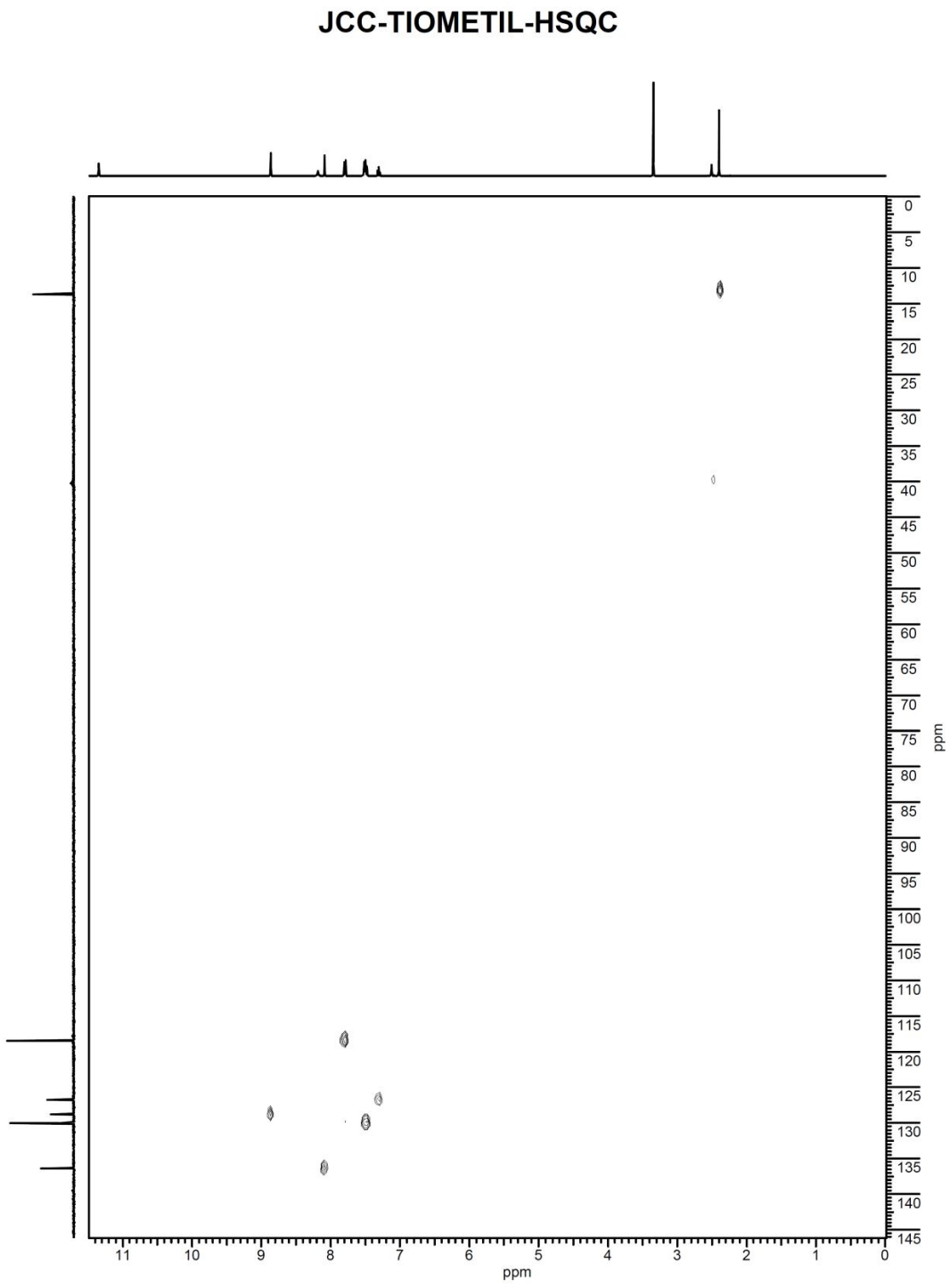

Figure S 4: HSQC Spectrum of MePhPzTSC ligand

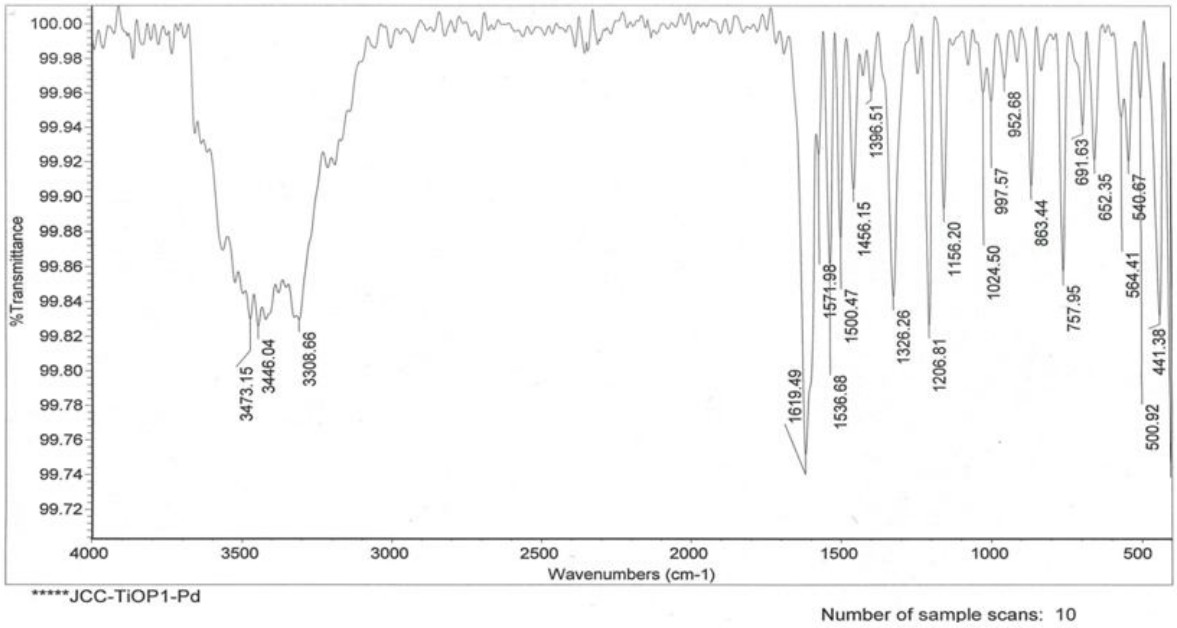

Figure S 5: IR spectrum of [Pd(MePhPzTSC)$_2$]

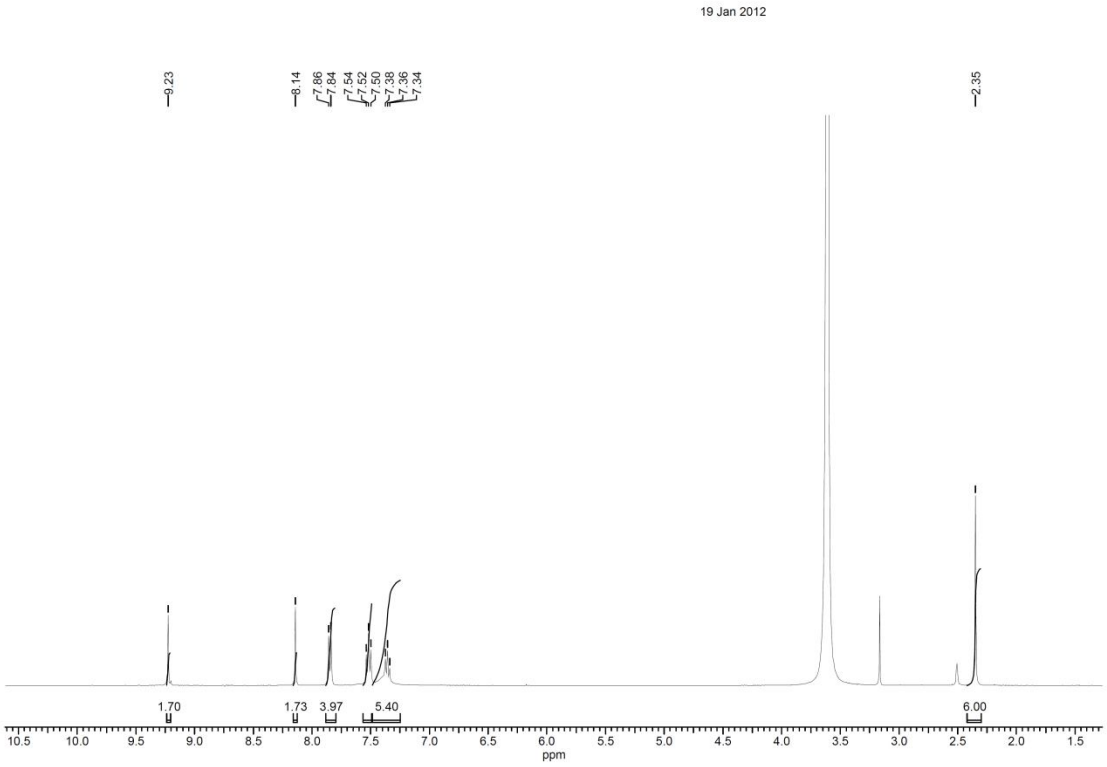

Figure S 6: ^{1}H NMR spectrum of [Pd(MePhPzTSC)$_2$]

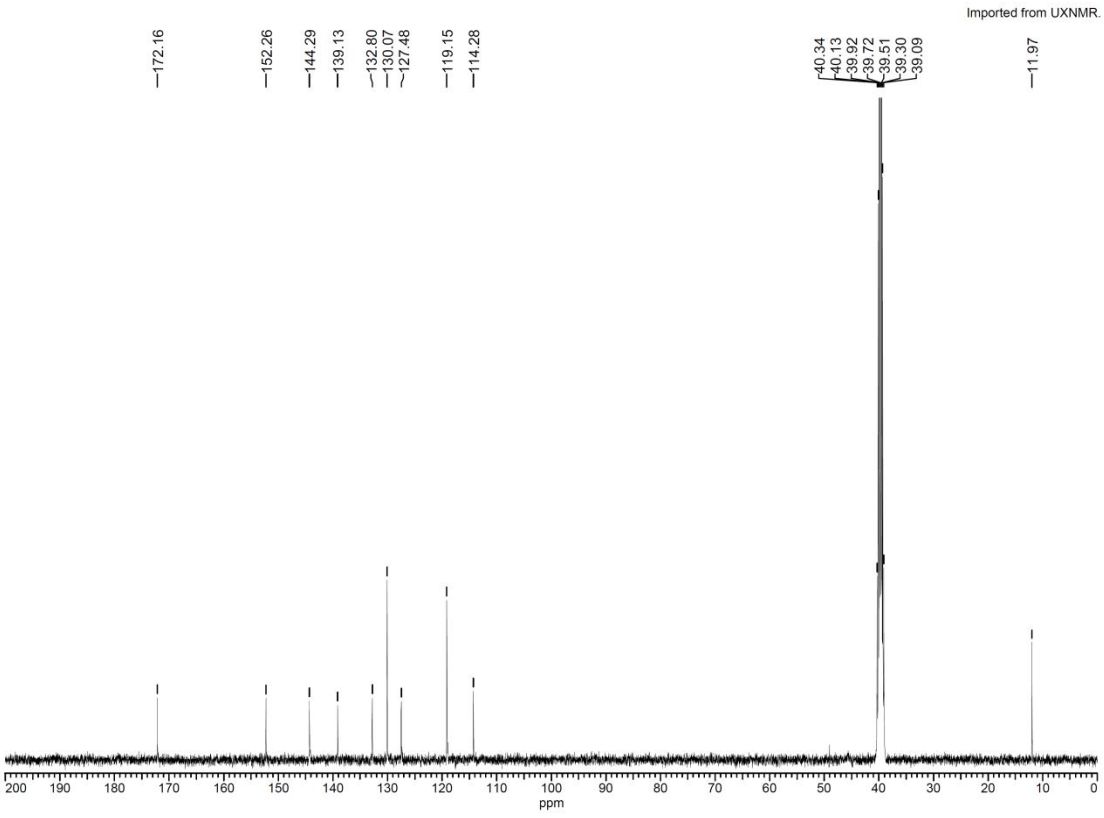

Figure S 7: ^{13}C NMR spectrum of the [Pd(MePhPzTSC)$_2$]

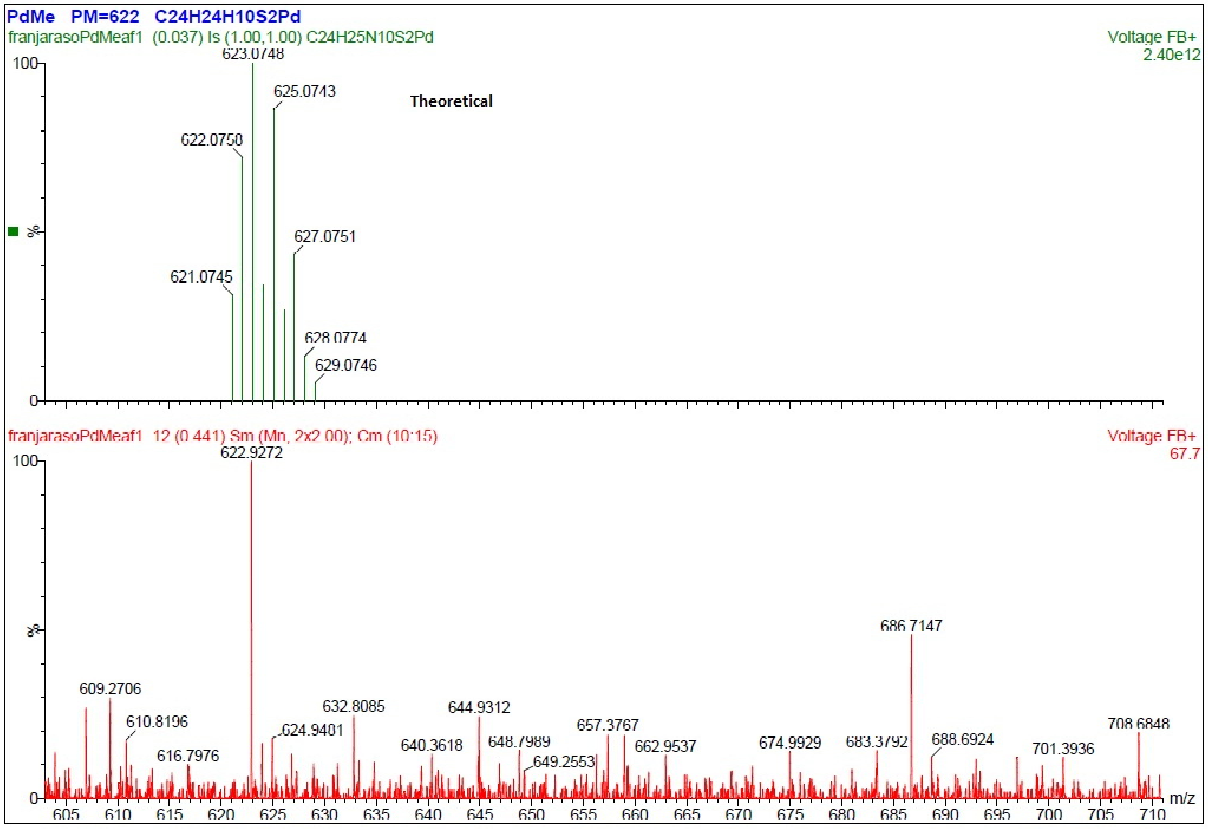

Figure S 8: Positive ion FAB mass spectrum for the [Pd(MePhPzTSC)$_2$]

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Table 1. Chemical shift of ^{1}H NMR (ppm) for MePhPzTSC ligand and [Pd(MePhPzTSC)$_2$] complex

Assignment	δ (ppm) Free ligand (MePhPzTSC)	δ (ppm) Complex [Pd(MePhPzTSC)$_2$]
-(CH_3)	2.38	2.35
CH=N	8.07	8.14
H-5	8.85	9.23
H_o	7.78	7.85
H_m	7.46-7.50	7.52
H_p	7.29	7.34-7.44
NH-C=S	11.33	11.33
N-H_2	8.17; 7.46-7.50	7.34-7.44

Table 2. Chemical shift of ^{13}C NMR (ppm) for MePhPzTSC ligand and [Pd(MePhPzTSC)$_2$] complex

Assignment	δ (ppm) Free ligand (MePhPzTSC)	δ (ppm) Complex [Pd(MePhPzTSC)$_2$]	Δδ = δ (complex-free ligand) (ppm)
-CH_3	13.2	11.9	1.3
C-3 pz	148.7	152.2	3.5
C-4 pz	117.3	114.2	3.1
C-5pz	128.3	132.7	4.4
CH=N	135.8	144.2	8.4
C=S	177.3	172.2	5.1
C_i	139.0	139.1	0.1
C_o	117.9	119.1	1.2
C_m	129.5	130.0	0.5
C_p	126.2	127.4	1.2

Figure 1