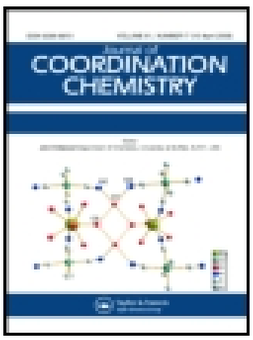

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# Synthesis, characterization, DFT calculations and antimicrobial activity of pentagonal-bipyramidal Zn(II) and Cd(II) complexes with 2,6-diacetylpyridine-bis(trimethylammoniumacetohydrazone)

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# Synthesis, characterization, DFT calculations and antimicrobial activity of pentagonal-bipyramidal Zn(II) and Cd(II) complexes with 2,6-diacetylpyridine-bis(trimethylammoniumacetohydrazone)

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Isothiocyanate complexes of Zn(II) and Cd(II) with the condensation product of 2,6-diacetylpyridine and trimethylammoniumacetohydrazide (Girard's T reagent) were synthesized, characterized, and their antimicrobial activities were evaluated. The structures of the complexes were determined by elemental analysis, IR and NMR spectroscopy. The crystal structure of the Zn(II) complex was also determined. Quantum-chemical calculations of the geometry and total energy of isomers of 2,6-diacetylpyridine-bis(trimethylammoniumacetohydrazone) were performed in vacuum and methanol solution, with the aim to explain conformational behavior and *E*/*Z* isomerism of this compound. DFT calculations of the molecular structures and the relative stabilities of linkage isomers of the Cd(II) complex showed that the isomer with N–Cd–N coordination of $SCN^-$ is the most stable. Complexes of Zn(II) and Cd(II) exhibited low-to-moderate activity against the tested microbial strains.

*Keywords:* Zn(II) and Cd(II) Complexes; 2,6-Diacetylpyridine dihydrazone; Crystal structure; DFT Calculations; Antimicrobial activity

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## 1. Introduction

Condensation products of 2,6-diacetylpyridine and different hydrazides are flexible ligand systems possessing at least five potential donors, *i.e.* pyridine nitrogen, two azomethine nitrogens and two carbonyl oxygens, which can be coordinated in non-, mono- and doubly-deprotonated form. Coordination of $N_3O_2$ donors allows formation of pentagonal-bipyramidal (PBPY-7) complexes with resonance stabilized nearly planar system in equatorial plane, consisting of four five-membered chelate rings and monodentate ligands in the apical positions [1, 2]. Pentagonal-bipyramidal (PBPY-7) geometry is not exclusive for complexes with 2,6-diacetylpyridine acylhydrazones and the extent of its deformation depends on conformational flexibility of ligand molecule, $d^n$ configuration of the metal ion and reaction conditions [2-6]. Coordination of 2,6-diacetylpyridine acylhydrazone ligands to Zn(II) may result in formation of both mono [7] and binuclear complexes [8, 9]. In binuclear Zn(II) complexes octahedral [8] and square-pyramidal geometry [9] around metal centers were reported. Mononuclear Cd(II) complexes with 2,6-diacetylpyridine acylhydrazones were obtained by chemical [10, 11] and electrochemical synthesis [12]. Electrochemical synthesis of Cd(II) complexes with 2,6-diacetylpyridine-bis(salicyloyhydrazone) in the presence of different electrolyte species resulted in formation of pentagonal-pyramidal and pentagonal-bipyramidal complexes with pentadentate $N_3O_2$ coordination of hydrazone ligand [12]. In chemical synthesis only pentagonal-bipyramidal complexes were obtained [10, 11]. Biological activity of Cd(II) complexes is scarcely explored, due to the known toxicity of this metal, which was designated as a human carcinogen by International Agency for Research on Cancer [13, 14]. Nevertheless, Cd(II) complexes with DNA binding ability, antibacterial and antitumor activities are reported [13, 15-18]. Among them Cd(II) complexes with Schiff base ligands containing pyridine moiety exhibited remarkable antitumor activity [19-22]. Contrary to cadmium, zinc is a bioelement with important role in the regulation of gene expression and activity of different enzymes [23]. Coordination of Zn(II) to Schiff base ligands in many cases results in enhanced antimicrobial and antitumor activity [24, 25]. Complexes of Zn(II) with hydrazones of formyl-, acetyl- or diacetylpyridine showed significant antitumor and antimicrobial activity [19, 26-28]. Girard's reagents (*N*-substituted glycine hydrazides) have been utilized for separation of carbonyl compounds from non-polar organic mixtures. Reaction of Girard's reagents with different mono- and dicarbonyl compounds

allowed synthesis of water soluble hydrazones, which form stable complexes with metal ions [29]. Main and Fritz synthesized 2,6-diacetylpyridine-bis(trimethylammoniumacetohydrazone) in the reaction of 2,6-diacetylpyridine and trimethylammoniumacetohydrazide (Girard's T reagent) and used it as an analytical reagent for chromatographic separation and quantitative determination of metal ions [30]. However, the authors did not provide elemental analysis and physicochemical characterization of this compound. Leovac *et al.* reported the synthesis of 2,6-diacetylpyridine-bis(trimethylammoniumacetohydrazone) in the form of its tetrahydrate salt and characterized it by elemental analysis, IR spectroscopy, conductometric measurements and TG/DSC analysis, but NMR spectroscopic data were not given [31]. This compound possesses a $N_3O_2$ set of donors and conformational flexibility which are common for 2,6-diacetylpyridine-acylhydrazone ligand types, but its unique characteristic is manifested in the presence of two positive charges in non-deprotonated form originating from two quaternary ammonium groups. Deprotonation of such a hydrazone could result in formation of monocationic or formally neutral zwitter-ionic ligand species. Recently, pentagonal-bipyramidal isothiocyanato Mn(II) [31], Co(II) and Ni(II) complexes [32] with condensation product of 2,6-diacetylpyridine and trimethylammoniumacetohydrazide (Girard's T reagent) were synthesized, structurally characterized and their antimicrobial activities were examined. As continuation of this study in the present work the synthesis, structural characterization and biological activity of Zn(II) and Cd(II) complexes with 2,6-diacetylpyridine-bis(trimethylammoniumacetohydrazone) were reported. Also, DFT calculations of the geometry and total energy of isomers of 2,6-diacetylpyridine-bis(trimethylammoniumacetohydrazone) were performed to explain conformational behavior and *E*/*Z* isomerism of this compound.

## 2. Experimental

### 2.1. *Materials and methods*

2,6-Diacetylpyridine (99%) and Girard's T reagent (99%) were obtained from Aldrich. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer using the ATR technique from 4000−400 $cm^{-1}$ $^1H$ (500 MHz), $^{13}C$ (125 MHz) and 2D NMR spectra were recorded on a Bruker Avance 500 spectrometer at room temperature using TMS as internal standard in $D_2O$ for ligand and in DMSO-$d^6$ for Zn(II) and Cd(II) complexes. Chemical shifts are expressed in ppm (δ)

values and coupling constants (*J*) in Hz. Elemental analyses (C, H and N) were performed by standard micro-methods using the ELEMENTARVario ELIII C.H.N.S.O analyzer.

### 2.2. *Synthesis*

**2.2.1. Synthesis of $H_2LCl_2·4H_2O$.** The white ligand **$H_2L$**$Cl_2·4H_2O$ was synthesized via the reaction of 2,6-diacetylpyridine and Girard's T reagent as described previously [31] and used in the synthesis of complexes without further purification. IR: 3394 (s), 3115 (m), 3071 (m), 3020 (m), 2969 (w), 2934 (w), 1709 (vs), 1630 (w), 1568 (w), 1489 (m), 1423 (m), 1366 (w), 1329 (w), 1281 (m), 1228 (m), 1153 (w), 1123 (w), 993 (w), 949 (w), 922 (w), 855 (w), 827 (w), 744 (w), 702 (w), 663 (w).

**2.2.2. Synthesis of $[ZnH_2L(NCS)_2][Zn(NCS)_4]$ (1).** **$H_2L$**$Cl_2·4H_2O$ (0.13 g, 0.25 mmol) was dissolved in acetonitrile (15 mL), then a solution of $ZnCl_2·2H_2O$ (0.04 g, 0.25 mmol) in acetonitrile (2 mL) and solid $NH_4SCN$ (0.08 g, 1.00 mmol) were added. The reaction mixture was stirred with heating for 3 h at 65 °C. Plate orange crystals suitable for X-ray analysis arose from reaction solution after three weeks. IR: 3470 (w), 3140 (w), 2924 (w), 2081 (vs, bs), 1690 (m), 1635 (w), 1550 (w), 1515 (w), 1466 (m), 1262 (w), 1199 (w), 964 (w), 917 (w), 813 (w). Elemental analysis calcd for $C_{25}H_{37}N_{13}O_4S_6Zn_2$: C 33.11 %, H 4.11 %, N 20.08 %, S 21.22 %; found: C 33.06 %, H 4.32 %, N 20.16 %, S 21.23 %.

**2.2.3. Synthesis of $[CdH_2L(NCS)_2][Cd(NCS)_4]$ (2).** $Cd(NO_3)_2·4H_2O$ (0.1 g, 0.25 mmol) and **$H_2L$**$Cl_2·4H_2O$ (0.13 g, 0.25 mmol) were dissolved in methanol (15 mL), and then solid $NH_4SCN$ (0.08 g, 1.00 mmol) was added. The reaction mixture was stirred with heating for 3 h at 65 °C, and after that at room temperature for 20 h. Yellow needle crystals arose from the reaction solution after two weeks. IR: 3625 (w), 3518 (m), 3406 (m), 3376 (m), 3191 (m), 3162 (m), 3032 (m), 2960 (m), 2082 (vs, bs), 1674 (s), 1633 (w), 1555 (s), 1481 (m), 1467 (m), 1414 (w), 1393 (w), 1373 (w), 1260 (w), 1210 (m), 1168 (w), 920 (w), 826 (w). Elemental analysis calcd for $C_{25}H_{37}N_{13}O_4S_6Cd_2$: C 30.00 %, H 3.73 %, N 18.19 %, S 19.22 %; found: C 30.43 %, H 4.03 %, N 18.20 %, S 19.52 %.

### 2.3. *Crystallographic structure determination*

Crystal data and refinement parameters of **1** are listed in table 1. The X-ray intensity data were collected at 150 K with an Agilent SuperNova dual source using an Atlas detector and equipped with mirror-monochromated Cu K$\alpha$ radiation ($\lambda$ = 1.54184 Å). The data were processed using CRYSALIS PRO [33]. The structure was solved by direct methods using SHELXS-97 [34] and refined with full-matrix least-squares based on $F^2$ using SHELXL-97 [34]. All non-hydrogen atoms were refined anisotropically. All hydrogens bonded to carbon were included in the model at geometrically calculated positions and refined using a riding model. The N-bound hydrogens and water hydrogens were located in a difference map and refined with distance restraints (DFIX) of N–H = 0.88 or O–H = 0.96 and with $U_{iso}$(H) = 1.2$U_{eq}$(N) or $U_{iso}$(H) = 1.5$U_{eq}$(O), respectively. Sulfur S5 is disordered over two orientations and was refined with the use of PART instruction. The occupancies of S5a and S5b were refined to a ratio 50% for both positions. The residual density peak (0.88 Å from Zn2) was unrefinable and therefore was not included in the model.

CCDC 1459323 contains the supplementary crystallographic data for **1**. These data can be obtained free of charge from the Cambridge Crystallographic Data Center *via* www.ccdc.cam.ac.uk/data_request/cif.

### 2.4. *Computational details*

All quantum chemical calculations were performed using the DFT approach with the GAUSSIAN 03 program package [35] on Southern Federal University computing cluster. For quantum-chemical modeling of the **$H_2$L**$Cl_2$ isomers the hybrid exchange-correlation functional B3LYP [36] with Becke's exchange component [37] and the Lee–Yang–Parr correlation functional [38] were used in combination with the 6-311G(d) basis set. The influence of solvent was examined using the polarizable continuum model [39] (PCM) with methanol as solvent. Geometry optimization of linkage isomers of Cd(II) complex were carried out with B3LYP functional [36] using four different basis sets: CEP-31G, LANL2DZ and SDD basis sets for all atoms, and mixed one including SDD (with ECP) for cadmium atom and 6-311G(d) for the other atoms. For each fully optimized structure, frequencies of normal vibrations are calculated at same level of theory and no imaginary frequencies were found.

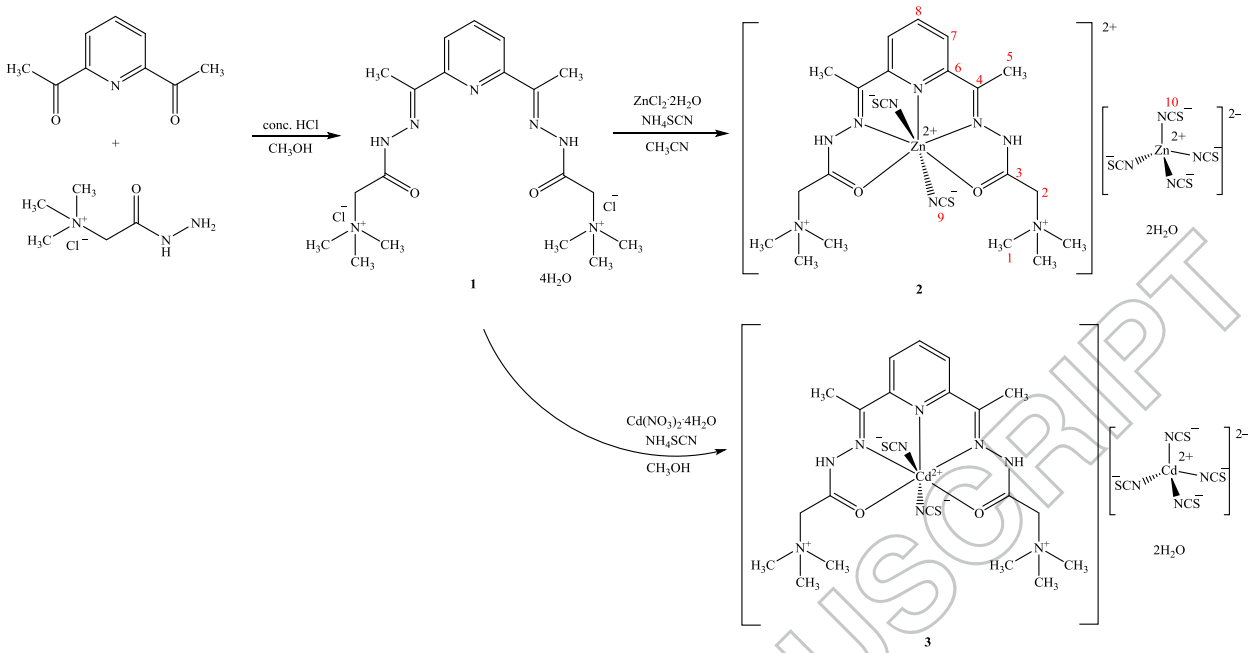
### 2.5. *Antimicrobial activity*

Antimicrobial activity was tested against ten laboratory control strains of microorganisms, *i.e.* Gram-positive bacteria: *Staphyloccocus aureus*, *Staphyloccocus epidermidis*, *Bacillus subtilis*, *Kocuria rhizophila* and *Enterococcus faecalis*; Gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella enterica*, and one strain of yeast *Candida albicans*. The broth microdilution method was performed according to Clinical and Laboratory Standards Institute guidelines [40]. The tested compounds were dissolved in distilled water and diluted with medium to the desired concentrations (ranging from 62.5–1000 μg/mL). All tests were performed in Müller-Hinton broth for the bacterial strains and in Sabouraud dextrose broth for *C. albicans*. In the tests, 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) (Aldrich Chemical Company Inc. USA) was added to the culture medium as a growth indicator of the tested microbial strains. TTC is a redox indicator used for differentiation between metabolically active and non-active cells. The colorless compound is enzymatically reduced to red 1,3,5-triphenylformazan by bacterial dehydrogenases, indicating bacterial metabolic activity (red color of the microtiter plate well). The final concentration of TTC after inoculation was 0.05%. After incubation for 24 h at 35 °C in aerobic conditions minimum inhibitory concentrations (MIC) were determined. The MIC is defined as the lowest concentration of the compound at which the microorganism does not demonstrate visible growth. All determinations were performed in triplicate and two positive growth controls were included. Each broth-microdilution test was repeated three times.

## 3. Results and discussion

### 3.1. *Synthesis*

Pentagonal-bipyramidal isothiocyanate complexes of Zn(II) (**1**) and Cd(II) (**2**) with condensation product of 2,6-diacetylpyridine and Girard's T reagent ($\mathbf{H_2L}Cl_2$) were prepared using direct method of synthesis in the reaction of the ligand, corresponding metal salt and $NH_4SCN$ in molar ratio 1:1:4 (scheme 1).




Scheme 1. Synthesis of ligand (**$H_2$LC$l_2$**), Zn(II) (**1**) and Cd(II) (**2**).

### 3.2. *IR spectra*

IR spectroscopy indicates symmetric coordination of **$H_2$LC$l_2$** in **1** and **2**. The observed bathochromic shift of ν(C=O) band from 1709 $cm^{-1}$ in the spectrum of **$H_2$LC$l_2$** to 1690 $cm^{-1}$ in the spectrum of **1** and 1674 $cm^{-1}$ in the spectrum of **2** originates from coordination of carbonyl oxygen. The coordination of azomethine nitrogens results in shift of ν(C=N) from 1630 $cm^{-1}$ in the spectrum of **$H_2$LC$l_2$** to 1635 $cm^{-1}$ and 1633 $cm^{-1}$ in the spectra of **1** and **2**, respectively [31]. In IR spectra of **1** and **2** strong bands at 2081 $cm^{-1}$ and 2082 $cm^{-1}$, respectively, originate from coordinated $SCN^-$ [31, 41].

### 3.3. *NMR spectra*

NMR spectra of hydrazone **$H_2$LC$l_2$** indicate the presence of more than one isomeric form in solution. Our attempts to separate isomers of **$H_2$LC$l_2$** using thin layer chromatography (TLC) were unsuccessful, due to its extreme polarity and thermal equilibrium between isomeric forms. To identify the most stable isomers of **$H_2$LC$l_2$** and explain the observed NMR results, DFT calculations of geometry and total energy of **$H_2$LC$l_2$** isomers were performed (Section 3.5). According to the quantum-chemical calculations the most stable isomers in methanol are hydrazone tautomer **b** with *E*,*E*-configuration of azomethine bond and hydrazone tautomer **e**

with *E*,*Z*-configuration of azomethine bond. In *E*,*Z*-isomers formation of hydrogen bonds hinder rotation of ligand chain with *Z* configuration, making hydrogens from C5 methyl group non equivalent, as confirmed from COSY spectrum. Also, in *E*,*Z*-isomers steric hindrance between methyl groups of azomethine and $-CH_2N^{(+)}(CH_3)_3$ moiety prevents free rotation around C2–C3 bond in the ligand with *Z* configuration and makes hydrogens H1 non equivalent. The presence of NH signal in the $^1$H NMR spectra of Zn(II) (**1**) and Cd(II) (**2**) complexes at 11.42 and 11.43 ppm, respectively, indicates that the ligand is coordinated in its dicationic form (table 2). In $^1$H NMR spectra of both Zn(II) (**1**) and Cd(II) (**2**) complexes upfield shift of H5 indicates coordination of azomethine nitrogen. Coordination of ligand through carbonyl oxygen results in downfield shift of methylene (H2) signals in the $^1$H NMR spectra of both Zn(II) (**1**) and Cd(II) (**2**) complexes. From the $^{13}$C NMR spectra (table 3) in both complexes the coordination sites are pyridine nitrogen, two azomethine nitrogens and two carbonyl oxygens. The coordination of carbonyl oxygen results in downfield shift of C3 signal and upfield shift of methylene carbon (C2) signal. Due to coordination via pyridine, the signals of carbons from pyridine ring (C8 and C7) are shifted upfield. The coordination of azomethine nitrogen results in upfield shift of azomethine C4 and methyl C5. Complexes **1** and **2** are not stable in solution. Appearance of two series of signals (ratio 3.4 : 1 in the spectrum of **1** and 4.5 : 1 in the spectrum of **2**) in their $^1$H NMR spectra in DMSO-d$^6$ solution indicates substitution of thiocyanate ligands with solvent.

### 3.4. *X-ray analysis of 1*

The crystals suitable for X-ray analysis were obtained by slow evaporation of solvent at room temperature. Compound **1** is ionic consisting of cationic and anionic species and two uncoordinated water molecules are included in the structure per one formula unit (figure 1). Selected bond lengths and angles of **1** are given in table 4. The cationic part possesses the pentadentate **$H_2$L** coordinated to zinc(II), thus forming four fused five-membered chelate rings. Two additional $SCN^-$ ligands fulfill the coordination number seven forming a distorted pentagonal-bipyramidal coordination geometry. The anionic part is tetrahedral $[Zn(NCS)_4]^{2-}$. The crystal structure of **1** is isomorphous with the structure of cobalt(II) complex $[Co\mathbf{H_2L}(NCS)_2][Co(NCS)_4]$ [32]. The crystal space group is the same and the cell dimensions are almost identical. In the solid state of **1** the cations are connected by N–H···Shydrogen bonds

into infinite chains. Additional N–H···Oand O–H···Ohydrogen-bonds exist between the cation and non-coordinated water molecules (figure S1, table S1).

### 3.5. *Theoretical calculations*

The isomerism of $\mathbf{H_2L}Cl_2$ includes geometric isomers related to the azomethine group (*E*,*Z*-isomers), keto-enolic tautomers (hydrazone and mono- and doubly enolized α-oxyazine) and rotational isomers (*syn* and *anti*). In hydrazone ligands, the configuration of azomethine group and keto-enol tautomery determines coordination behavior of ligand, so evidence in the stability of different ligand isomers can be useful in planning of synthesis and explanation of ligand coordination properties. In order to determine the most stable isomers of $\mathbf{H_2L}Cl_2$, quantum chemical calculations of the geometry and total energy of its possible isomeric forms were performed in vacuum (table S2). For the most stable isomers in vacuum (figure 2), calculations of the geometry and total energy were performed in methanol (figure 3) as solvent. The most stable isomers in vacuum are hydrazone tautomers **e** ($\Delta E$ = 0.0 kcal/mol) and **b** ($\Delta E$ = 0.6 kcal/mol) with close to planar structure (except quaternary ammonium groups) favoring propagation of π-conjugation. Formation of intramolecular hydrogen-bonds in isomer **e** allowed by *Z* configuration of azomethine bond contributes to stabilization of its structure, compensating additional steric repulsion of methyl group and pyridine ring hydrogen arising in **e** isomer. The enolization of the hydrazine moiety is significantly unfavorable as it is seen from the comparison of **e** and **f** tautomers. Also destabilized are the rotamers **a** and **d** in which azomethine bond nitrogen and carbonyl oxygen are on the same side relative to the NH-C(O) bond due to repulsion of their electron lone pairs. All non-planar structures (**c**, **g** and **h**) are not favorable due to steric repulsion and lower π-conjugation. Isomer **h** with symmetric conformations of the "arms" of the *bis*-hydrazone and two hydrogen bonds similar to the **e** isomer appears to be non-planar due to repulsion of the carbonyl oxygen and is 4.7 kcal/mol less stable than the latter.

In methanol, the most stable is hydrazone tautomer **b** with *E*,*E*-configuration of azomethine bond; hydrazone tautomer **e** with *E*,*Z*-configuration of azomethine bond is destabilized relative to it by 1.0 kcal/mol. Such inversion of the relative stability of the isomers can be explained by less contribution of the hydrogen-bond in polar media in stabilization of **e** isomer due to screening effect. Relative stability of other isomers in methanol is similar to that in vacuum, only enolic isomer **f** appears to be even more destabilized (13.6 kcal/mol in methanol

compared to 9.7 kcal/mol in vacuum). Hydrazone tautomer **b** displays conformational rearrangement around the C4–C6 and N–C3 bonds upon coordination to Zn(II) and Cd(II) (numbering of the atoms according to scheme 1) forming symmetric $N_3O_2$ coordination around the metal ion. Symmetric $N_3O_2$ coordination of $\mathbf{H_2L}Cl_2$ in mononuclear complexes is possible only in the case of isomer with *E*,*E*-configuration.

Since the X-ray determined structure of **2** was not obtained, calculations were performed to give insight into the coordination of thiocyanate in a pentagonal-bipyramidal complex cation. Ambidentate thiocyanate could be coordinated via nitrogen or sulfur so for the possible linkage isomers (figure 4) of **2** calculations of the geometry and total energy were carried out with B3LYP functional [36] using CEP-31G, LANL2DZ and SDD basis sets, as well as SDD (with ECP) for cadmium and 6-311G(d) for the other atoms (table 5). In all considered basis sets the relative stabilities are in the same order. The results showed that N–Cd–N coordination of $SCN^-$ (isomer **I**) is favored in all cases, the next in stability is isomer **III** with "mixed" coordination N-Cd-S and the most destabilized is isomer **II**. This finding is in contradiction with Pearson's acid-base concept within which Cd(II) as "soft" acid can be considered to interact more readily with thiocyanate as "soft" base than with "harder" isothiocyanate. Analysis of octahedral Cd(II) complexes containing two $NCS^-$ ligands in *trans*-position to each other in the Cambridge Structure Database (CSD) [42] shows that the majority of complexes (90 out of 94) are of type **I** and coordination of type **II** and **III** are rare (both count only 2 cases). The main reason for that can be the strong *trans*-influence of the sulfur "end" of the $NCS^-$ ligand resulting in weakening of the opposite coordination bond. This proposition can be supported by the fact that mixed complexes of type **III** are less destabilized compared to type **II**.

### 3.6. *Antimicrobial activity*

The antimicrobial activities are presented in table 6. Complexes **1** and **2** exhibited low to moderate activity against the tested microbial strains, while the activity of the ligand was negligible. The best antibacterial activity of **1** and **2** was observed against *B. subtilis*. The ligand, **1** and **2** were inactive against *C. albicans*. Coordination of the ligand to Zn(II) results in enhanced activity of **1** against *S. epidermidis*, *E. faecalis* and *B. subtilis* bacterial strains in comparison with free Zn(II). Lower activity of **1** in comparison with Zn(II) salt was observed against *K. pneumoniae*, while in the case of *K. rhizophila* and *E. coli* strains the activity of **1** and

$ZnCl_2 \cdot 2H_2O$ was the same. Complex **2** and $Cd(NO_3)_2 \cdot 4H_2O$ showed the same activity against *E. faecalis* and *B. subtilis*. The activity of **2** was higher than the activity of Cd(II) salt only against *S. epidermidis*, while for the rest of the examined microbial strains **2** showed lower activity than $Cd(NO_3)_2 \cdot 4H_2O$. Reduced toxicity of Cd(II) upon coordination is especially pronounced in the case of *C. albicans*. It is interesting that growth of this yeast is affected only by $Cd(NO_3)_2 \cdot 4H_2O$ salt whereas free ligand, **1** and **2**, and $ZnCl_2 \cdot 2H_2O$ do not show such activity. Both **1** and **2** exhibited better antimicrobial activity than pentagonal-bipyramidal isothiocyanato Ni(II), Co(II) and Mn(II) complexes with the same ligand [32]. All the complexes possess the same pentagonal-bipyramidal geometry with metal ions in +2 oxidation state, so the nature of metal ions and stability of complexes in water have influence on their biological activity. With the exception of Cd(II) complex, formation of pentagonal-bipyramidal isothiocyanate complexes results in synergistic effect, *i.e.* complexes showed better activity than free ligand, metal salts and $NH_4SCN$.

## 4. Conclusion

Ni(II) [32], Co(II) [32], Mn(II) [31], Zn(II) and Cd(II) ions with 2,6-diacetylpyridine bis(trimethylammoniumacetohydrazone) form complexes of PBPY-7 geometry with pentadentate ligand. The pentadentate ligand defines equatorial coordination plane, while PBPY-7 coordination geometry around metal atom is completed by two $SCN^-$ ions coordinated *via* N in apical positions. Complexes of Zn(II) and Cd(II) exhibited low-to-moderate activity against the tested microbial strains, while the activity of the ligand was negligible. In comparison with previously studied isothiocyanato Ni(II), Co(II) and Mn(II) complexes with 2,6-diacetylpyridine-bis(trimethylammoniumacetohydrazone) [14], the Zn(II) and Cd(II) complexes exhibited better antimicrobial activity. The literature contains many reports dealing with antimicrobial properties of Zn(II) and Cd(II) complexes [43-47]. Due to the extremely toxic nature of Cd(II), its complexes mainly showed higher antimicrobial activity than Zn(II) complexes with the same ligands [48-53]. At concentration $10^{-7}$–$10^{-5}$ mol/L, Zn(II) is necessary for normal microbial growth, but at millimolar concentration Zn(II) exhibits toxic effects [53]. In most of the cases, the coordination of ligands to Zn(II) enhanced their antimicrobial activity [44, 47-49]. Reported results [43-53] and the present work showed that stability, solubility, charge, geometry and nuclearity of Cd(II) and Zn(II) complexes determine their antimicrobial activity.

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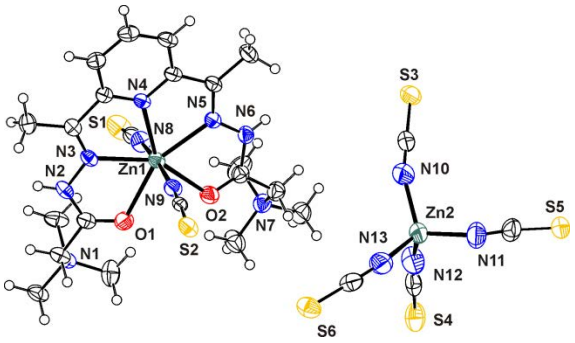


Figure 1. Graphical representation of **1**. Non-coordinated water molecules have been omitted for clarity.

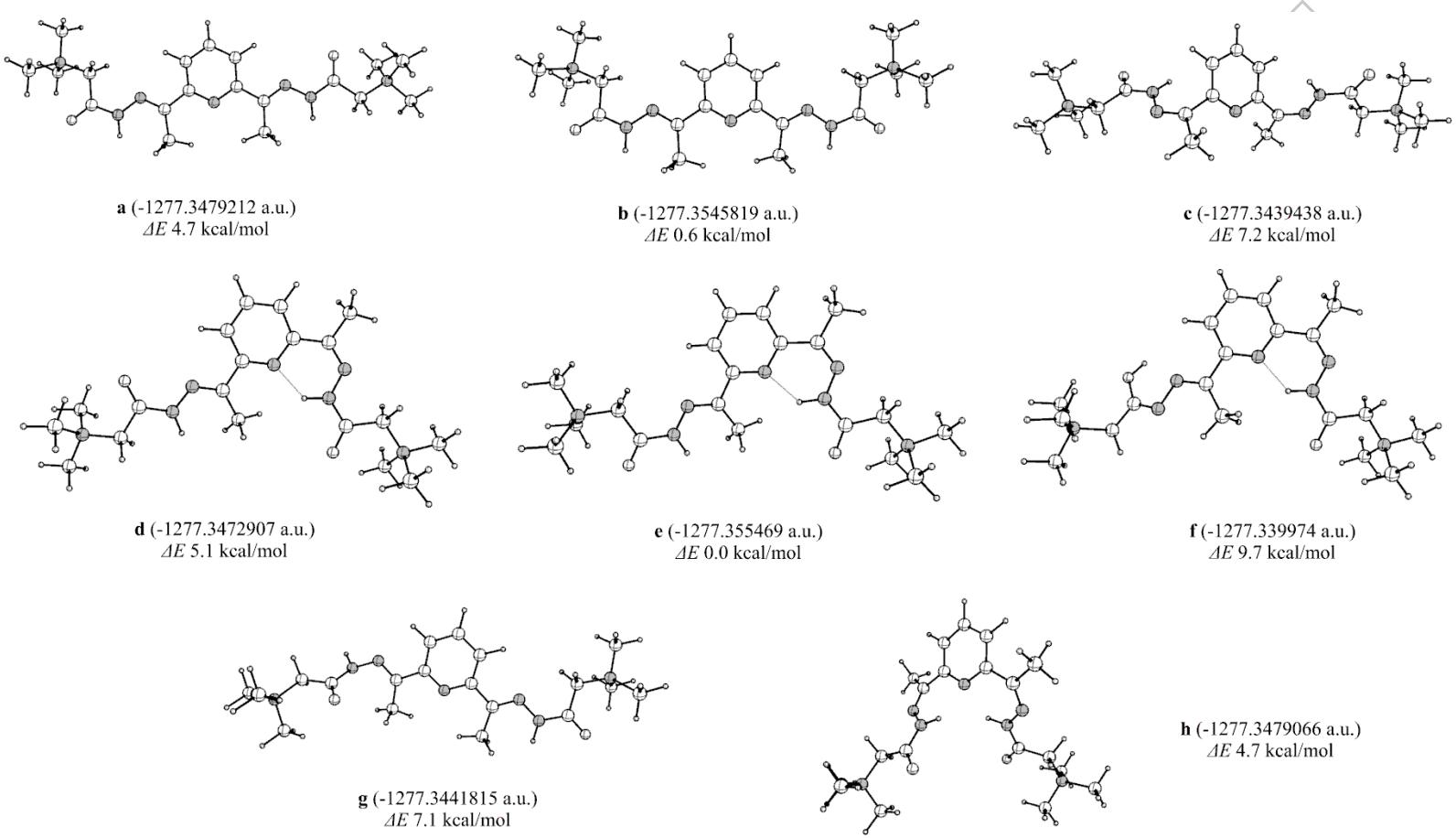


Figure 2. Total energies (a. u.) and relative stability *ΔE* (kcal/mol) of isomers of $\mathbf{H_2L}Cl_2$ in vacuum.

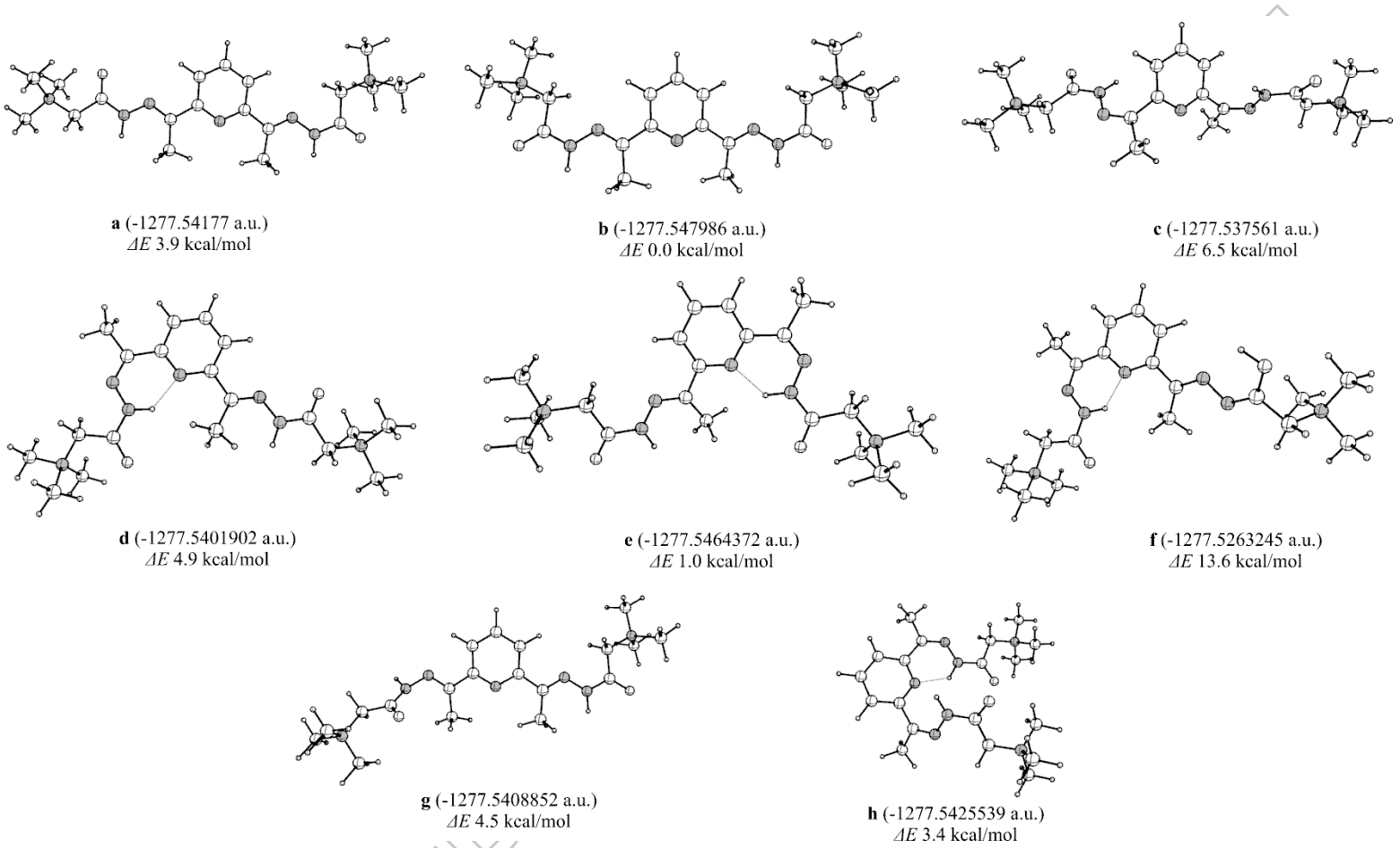


Figure 3. Total energies (a. u.) and relative stability *ΔE* (kcal/mol) of isomers of $\mathbf{H_2L}Cl_2$ in methanol.

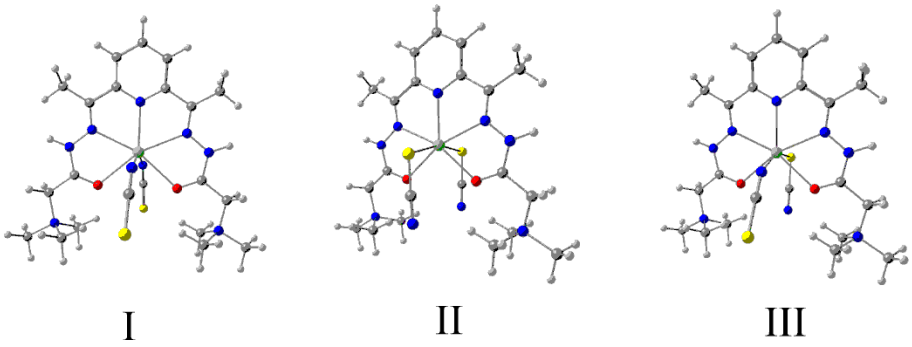


Figure 4. Optimized geometries (B3LYP/ LANL2DZ) of linkage isomers of **2**.

Table 1. Crystal data and structure refinement details for **1**.

| | |
|---|---|
| Formula | $C_{25}H_{37}N_{13}O_4S_6Zn_2$ |
| Fw (g mol$^{-1}$) | 906.78 |
| Crystal size (mm) | 0.30 × 0.18 × 0.05 |
| Crystal color | Orange |
| Radiation, wavelength (Å) | CuK$\alpha$, 1.54184 |
| Crystal system | Monoclinic |
| Space group | $P2_1/n$ |
| $a$ (Å) | 20.8497(3) |
| $b$ (Å) | 9.11020(10) |
| $c$ (Å) | 20.9695(2) |
| $\beta$ (º) | 90.7865(11) |
| $V$ (Å$^3$) | 3982.68(8) |
| $Z$ | 4 |
| Calcd density (g cm$^{-3}$) | 1.512 |
| $F$(000) | 1864 |
| No. of collected reflns. | 14986 |
| No. of independent reflns. | 7812 |
| $R_{int}$ | 0.0207 |
| No. of reflns. observed | 6860 |
| No. parameters | 487 |
| $R[I > 2\sigma(I)]^a$ | 0.0409 |
| $wR_2$ (all data)$^b$ | 0.1132 |
| *Goof* , $S^c$ | 1.030 |
| Max/min residual electron density (e Å$^{-3}$) | +1.09/–0.90 |

$^a$ $R = \sum||F_o| - |F_c||/\sum|F_o|$. $^b$ $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$.
$^c$ $S = \{\sum[(F_o^2 - F_c^2)^2]/(n/p)\}^{1/2}$ where $n$ is the number of reflections and $p$ is the total number of parameters refined.

Table 2. $^{1}$H NMR spectral data (chemical shift (ppm), multiplicity, number of hydrogens, coupling constant *J*) of **$H_2$L**$Cl_2$, **1** and **2**.

| Assignment | **$H_2$L**$Cl_2$ | **1** | **2** |
|---|---|---|---|
| H1 | 3.33 | 3.32 (s, 18H) | 3.37 (s, 18H) |
| H2 | 4.06 | 4.86 (s, 4H) | 4.88 (s, 4H) |
| H5 | 2.77 | 2.42 (s, 6H) | 2.42 (s, 6H) |
| H7 | 8.25 (m, 2H) | 8.14 (d, 2H, $^{3}J$ = 8 Hz) | 8.16 (d, 2H, $^{3}J$ = 8 Hz) |
| H8 | 8.05(m, 1H) | 7.98 (t, 1H, $^{3}J$ = 8 Hz) | 8.02 (t, 1H, $^{3}J$ = 8 Hz) |
| NH | $D_2O$ exchangeable | 11.42 (s, 2H) | 11.43 (s, 2H) |

Table 3. $^{13}C$ NMR spectral data (chemical shift in ppm) of **$H_2$L**$Cl_2$, **1** and **2**.

| Assignment | **$H_2$L**$Cl_2$ | **1** | **2** |
|---|---|---|---|
| C1 | 56.8 | 53.5 | 53.6 |
| C2 | 66.9 | 63.0 | 63.5 |
| C3 | 162.8 | 168.6 | 166.6 |
| C4 | 158.5 | 155.5 | 154.3 |
| C5 | 28.3 | 12.3 | 12.6 |
| C6 | 152.6 | 152.5 | 153.5 |
| C7 | 127.6 | 121.1 | 120.8 |
| C8 | 141.3 | 137.6 | 137.3 |
| C9 ($SCN^-$) | / | 146.8 | 137.4 |
| C10 ($SCN^-$) | / | 135.4 | 131.6 |

Table 4. Selected bond lengths (Å) and angles (°) for **1**.

| Bond | Bond length (Å) | Bond angle | (°) |
|---|---|---|---|
| Zn1–N3 | 2.236(2) | O1–Zn1–N3 | 69.40(7) |
| Zn1–N4 | 2.203(2) | O1–Zn1–N4 | 138.97(8) |
| Zn1–N5 | 2.214(2) | O1–Zn1–N5 | 150.18(8) |
| Zn1–N8 | 2.068(2) | O1–Zn1–N8 | 84.02(9) |
| Zn1–N9 | 2.062(2) | O1–Zn1–N9 | 88.13(8) |
| Zn1–O1 | 2.2846(19) | O1–Zn1–O2 | 79.26(7) |
| Zn1–O2 | 2.2418(19) | N8–Zn1–N9 | 170.80(9) |
| Zn2–N10 | 1.959(3) | N10–Zn2–N11 | 107.64(17) |
| Zn2–N11 | 1.943(4) | N10–Zn2–N12 | 107.82(14) |
| Zn2–N12 | 1.956(3) | N10–Zn2–N13 | 113.70(14) |
| Zn2–N13 | 1.949(3) | N11–Zn2–N13 | 105.32(15) |

Table 5. Relative stability $\Delta E$ (kcal/mol) of linkage isomers of **2** in vacuum.

| Relative energies in vacuum $\Delta$E kcal/mol | | | | |
|---|---|---|---|---|
| Isomer | CEP-31G | LanL2DZ | SDD | SDD/6-311G(d) |
| **I** | 0 | 0 | 0 | 0 |
| **II** | 9.4 | 11.5 | 10.8 | 4.4 |
| **III** | 4.3 | 5.4 | 5.1 | 1.9 |

Table 6. Antimicrobial activity of **$H_2$L**$Cl_2$ and its Zn(II) (**1**) and Cd(II) (**2**) complexes (MIC values are given in μg/mL).

| Microorganism | **$H_2$L**$Cl_2$ | **1** | **2** | $ZnCl_2 \cdot 2H_2O$ | $Cd(NO_3)_2 \cdot 4H_2O$ | Gentamicin | ceftriaxone | amphotericin B |
|---|---|---|---|---|---|---|---|---|
| *S. aureus* ATCC 6538 | >1000 | 1000 | >1000 | >1000 | 1000 | 2.0 | 0.5 | n.t. |
| *S. epidermidis* ATCC 12228 | >1000 | 500 | 500 | >1000 | 1000 | 1.0 | 1.0 | n.t. |
| *K. rhizophila* ATCC 9341 | >1000 | 250 | 250 | 250 | 62.5 | 0.5 | 1.5 | n.t. |
| *E. faecalis* ATCC 29212 | 1000 | 500 | 62.5 | 1000 | 62.5 | >5.0 | 2.0 | n.t. |
| *B. subtilis* ATCC 6633 | 500 | 125 | 62.5 | 250 | 62.5 | 3.0 | 1.5 | n.t. |
| *E. coli* ATCC 10536 | 1000 | 500 | 500 | 500 | 125 | 2.5 | 2.5 | n.t. |
| *K. pneumoniae* ATCC 13883 | 1000 | 1000 | 500 | 500 | 250 | 3.0 | 3.0 | n.t. |
| *P. aeruginosa* ATCC 9027 | >1000 | >1000 | >1000 | >1000 | 1000 | 2.5 | 3.5 | n.t. |
| *S. enterica* ATCC 6017 | >1000 | >1000 | >1000 | 1000 | 1000 | 1.5 | 2.5 | n.t. |
| *C. albicans* ATCC 10231 | >1000 | >1000 | 1000 | 1000 | 62.5 | n.t. | n.t. | 1.5 |

n.t. – not tested

## Graphical abstract