Coordination of Thiosemicarbazones and Bis(thiosemicarbazones) to Bismuth(III) as a Strategy for the Design of Metal-Based Antibacterial Agents

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Complexes $[Bi(2Fo4Ph)Cl_2]$ (**1**), $[Bi(2Ac4Ph)Cl_2]$ (**2**), $[Bi(2Bz4Ph)Cl_2]$ (**3**), $[Bi(H_2Gy3DH)Cl_3]$ (**4**), $[Bi(H_2Gy4Et)(OH)_2Cl]$ (**5**), and $[Bi(H_2Gy4Ph)Cl_3]$ (**6**) were prepared with pyridine-2-carbaldehyde 4-phenylthiosemicarbazone (H2Fo4Ph), 1-(pyridin-2-yl)ethanone 4-phenylthiosemicarbazone (H2Ac4Ph), phenyl(pyridin-2-yl)methanone 4-phenylthiosemicarbazone (H2Bz4Ph), as well as with glyoxaldehyde bis(thiosemicarbazone) (H_2Gy4DH) and its 4-Et (H_2Gy4Et) and 4-Ph (H_2Gy4Ph) derivatives. The complexes exhibited antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. Coordination to Bi^{III} proved to be an effective strategy to increase the antibacterial activity of the thiosemicarbazones and bis(thiosemicarbazones).

1. Introduction. – Bacterial infections are major causes of morbidity and mortality in hospitals around the world. Nosocomial *Staphylococcus aureus* infections alone results in 12,000 patient deaths per year [1]. Furthermore, the emergence of resistant bacteria has contributed to shortening lifecycles of antimicrobial agents [2]. Paradoxically, as the problems accompanying the emergence of resistance to existing drugs increase, there has been a decline in the discovery and development of new antibacterial agents [3].

Bismuth is known to possess good antibacterial activity [4]. Bismuth subsalicylate (*Pepto-Bismol*®), colloidal bismuth subcitrate (*De-Nol*®), and ranitidine bismuth citrate (*Tritec*® and *Pylorid*®) are used worldwide to treat various gastrointestinal diseases which are related to the infection by *Helicobacter pylori* [5]. In addition to the currently used Bi pharmaceuticals, the development of new Bi-based compounds may provide some promising antimicrobial agents. Bismuth-thiols, for example, have shown activity against *Gram*-positive and *Gram*-negative bacteria. The thiol component functions as a lipophilic carrier that promotes Bi uptake into bacteria, thus enhancing the effects of Bi up to 1000-fold [6][7]. These properties indicate that bismuth-thiols could make excellent antimicrobials [8].

Thiosemicarbazones are thiol/thione compounds which present wide pharmacological applications as antitumor, antiviral, and antimicrobial agents [9]. Coordination to Sn, Cu, and Ga improved their antimicrobial activity [10–13]. Thus, the formation of Bi complexes with thiosemicarbazones could result in more potent compounds, as previously demonstrated [14][15].

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In the present work, Bi^{III} complexes with pyridine-2-carbaldehyde 4-phenylthiosemicarbazone (H2Fo4Ph), 1-(pyridin-2-yl)ethanone 4-phenylthiosemicarbazone (H2Ac4Ph), phenyl(pyridin-2-yl)methanone 4-phenylthiosemicarbazone (H2Bz4Ph), as well as with glyoxaldehyde (=ethanediol) bis(thiosemicarbazone) (H_2Gy4DH), and its 4-ethyl (H_2Gy4Et) and 4-phenyl (H_2Gy4Ph) derivatives (*Fig. 1*) were prepared, and assayed against *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* bacterial strains.

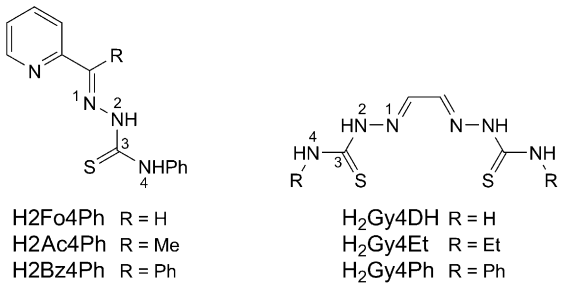

Fig. 1. *Generic representation for* a) *pyridin-2-yl-derived 4-phenylthiosemicarbazones and* b) *glyoxaldehyde bis(thiosemicarbazones)*

2. Results and Discussion. – 2.1. *Formation of the Bi^{III} Complexes.* Microanalyses and molar conductivity data are compatible with the formation of [Bi(2Fo4Ph)Cl_2] (**1**), [Bi(2Ac4Ph)Cl_2] (**2**), [Bi(2Bz4Ph)Cl_2] (**3**), in which an anionic thiosemicarbazone is attached to Bi^{III} together with two chlorides, and with the formation of [Bi(H_2Gy4DH)Cl_3] (**4**), [Bi(H_2Gy4Et)$(OH)_2$Cl] (**5**), and [Bi(H_2Gy4Ph)Cl_3] (**6**). In **4** and **6**, a neutral bis(thiosemicarbazone) is coordinated to Bi^{III} together with three Cl^- ions, whereas in **5** a neutral bis(thiosemicarbazone) is attached to the metal center together with one Cl^- and two HO^- ligands.

2.2. *Spectroscopic Characterization.* The vibrations attributed to $\tilde{\nu}$(C=N) at 1596–1575 cm^{-1} in the IR spectra of H2Fo4Ph, H2Ac4Ph, and H2Bz4Ph are shifted to 1601–1597 cm^{-1} in the spectra of complexes **1**–**3**, in agreement with coordination of the imine N-atom [16–18]. The $\tilde{\nu}$(CS) absorption observed at 788–774 cm^{-1} in the spectra of the free thiosemicarbazones is shifted to 759–747 cm^{-1} in the spectra of complexes **1**–**3**, indicating coordination of the S-atom. The 30–50-cm^{-1} shift is compatible with complexation of a thiolate S-atom [16–20]. The in-plane deformation mode of the pyridine ring at 600–584 cm^{-1} in the spectra of the thiosemicarbazones is shifted to 632–587 cm^{-1} in the spectra of **1**–**3**, suggesting coordination of the hetero-aromatic N-atom [16–20].

In the spectra of **1**–**3**, the absorptions at 512–431 and 422–404 cm^{-1} were attributed to the $\tilde{\nu}$(Bi–N_{imine}) and $\tilde{\nu}$(Bi–S) vibrations, respectively, and those at 321–200 cm^{-1} were assigned to $\tilde{\nu}$(Bi–N_{py}) vibrations [21–23]. Therefore, in these complexes the thiosemicarbazones are attached to the metal through the N_{py}–N–S chelating system. Besides, one absorption attributed to $\tilde{\nu}$(Bi–Cl) at 333–331 cm^{-1} was observed in the spectra of **1**–**3** [21].

The vibrations attributed to $\tilde{\nu}$(C=N) at 1596–1570 cm^{-1} in the IR spectra of bis(thiosemicarbazones) H_2Gy4DH, H_2Gy4Et, and H_2Gy4Ph are shifed to 1616–1595 cm^{-1} in the spectra of complexes **4**–**6**, in agreement with coordination of the imine N-atom [16–19]. The $\tilde{\nu}$(CS) absorption observed at 836–771 cm^{-1} in the spectra of the free bis(thiosemicarbazones) is shifted to 834–761 cm^{-1} in the spectra of **4**–**6**, indicating coordination of the S-atom. This shift is compatible with complexation of a thione S-atom [16–20].

In the spectra of complexes **4**–**6**, the absorptions at 331–285 and 273–248 cm^{-1} were attributed to the $\tilde{\nu}$(Bi–N_{imine}) and $\tilde{\nu}$(Bi–S) [24] vibrations, respectively. Therefore, in these complexes the bis(thiosemicarbazones) are attached to Bi^{III} through the N_2–S_2 chelating system. One absorption attributed to $\tilde{\nu}$(Bi–Cl) at 237–188 cm^{-1} was observed in the spectra of **4**–**6** [21]. One additional absorption at 440 cm^{-1} in the spectrum of **5** was assigned to $\tilde{\nu}$(Bi–O) [25].

The NMR spectra of thiosemicarbazones H2Fo4Ph and H2Ac4Ph, and their complexes **1** and **2**, respectively, were recorded in (D_4)MeOH, whereas the spectra for H2Bz4Ph and its complex **3** were recorded in (D_6)DMSO. These are the only solvents that dissolve both ligands and complexes. The ^{1}H resonances were assigned on the basis of chemical shifts and multiplicities. We could not obtain ^{13}C-NMR spectra of **1** and **2** due to their low solubility in (D_4)MeOH. For **3**, the C-atom type (C, CH) was determined by using distortionless enhancement by polarization transfer (DEPT-135) experiments. The assignments of the protonated C-atoms were accomplished by 2D hetero-nuclear multiple quantum coherence experiments (HMQC).

Only one signal was observed for each H-atom in the ^{1}H-NMR spectra of H2Fo4Ph and H2Ac4Ph, indicating the presence of only the (*E*)-isomer in solution [16]. The ^{1}H-NMR spectrum of H2Bz4Ph shows duplicated signals indicating the presence of the (*Z*)- and (*E*)-isomers (78 and 22%, resp.) in solution. In the first, H–N(2) is H-bonded to the heteroaromatic N-atom, while in the second H–N(2) is H-bonded to the solvent [16][17][21]. The signals of H–N(2) at 13.19 and 10.59 ppm were attributed to the (*Z*)- and (*E*)-isomers, respectively [16][17][21].

In complexes **1**–**3**, the signals of all H-atoms undergo significant shifts in relation to their positions in the free bases. In the spectra of **1** and **2**, only one signal was observed for each H-atom, suggesting the presence of only one isomer in solution. The crystal structure of [Bi(2Ac4Ph)(DMSO)Cl_2] (**2a**) reveals that the thiosemicarbazone adopts the (*E*)-configuration (see *Sect. 2.3*). In ^{1}H-NMR the spectrum of **3**, two signals were observed for each H-atom, suggesting the presence of (*Z*)- and (*E*)-isomers (64 and 36%, resp.). The absence of the H–N(2) signal in the spectrum of **3** indicates deprotonation with coordination of an anionic thiosemicarbazone. In the ^{13}C-NMR spectrum of complex **3**, two signals were observed for each C-atom, as well (see *Fig. 2*). Since significant shifts in the signals of C=N, C=S, and the pyridine C-atoms were verified, we can rule out the possibility of decomplexation in the solvent, and assume that (*Z*)- and (*E*)-isomers coexist in solution. In this case, the thiosemicarbazone is probably coordinated in a tridentate form through the N_{py}–N_{imine}–S chelating system in the (*E*)-isomer, and in a bidentate form through the N_{imine}–S system in the (*Z*)-isomer [16][17][21].

The ^{1}H- and ^{13}C-NMR spectra of bis(thiosemicarbazones) H_2Gy4DH, H_2Gy4Et and H_2Gy4Ph were recorded in (D_6)DMSO. The ^{1}H resonances were assigned on the

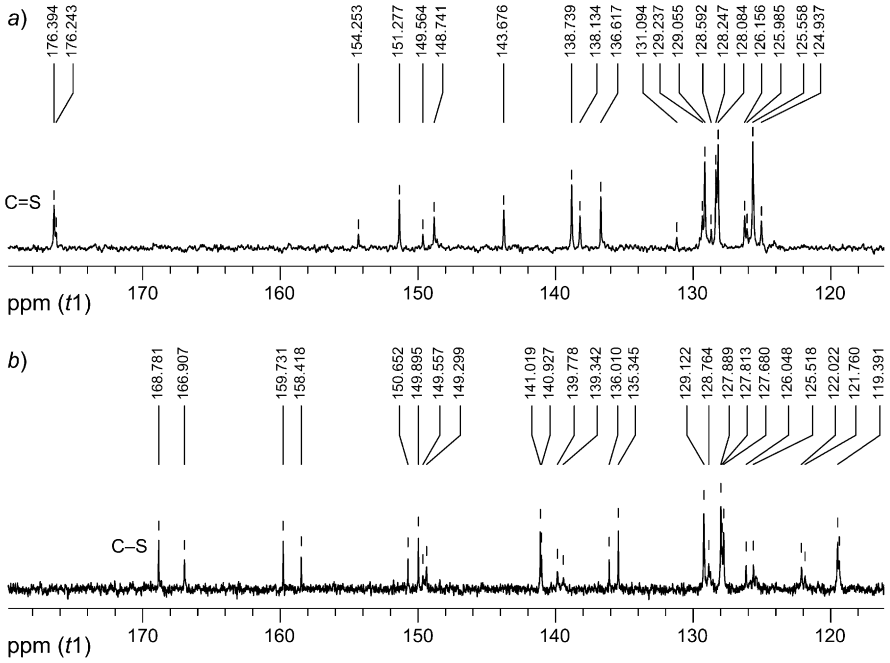

Fig. 2. *^{13}C-NMR Spectra of* a) *H2Bz4Ph and* b) *[Bi(2Bz4Ph)Cl$_2$]* (**3**) *in the 115–180-ppm range* ((D$_6$)DMSO)

basis of chemical shifts and multiplicities. The C-atom type (C, CH) was determined by using distortionless enhancement by polarization transfer (DEPT-135) experiments.

In the ^{1}H-NMR spectra of **4**–**6**, the signals of all H-atoms from the bis(thiosemicarbazones) were observed, indicating that they are coordinated to Bi^{III} as neutral ligands. These signals are not duplicated, indicating that the two 'arms' of the bis(thiosemicarbazones) are coordinated to the metal center in a similar way. However, the signals of the ligands do not undergo significant shifts in the ^{1}H- and ^{13}C-NMR spectra of **4**–**6** in relation to their position in the free bis(thiosemicarbazones).

To investigate if coordination did not affect the chemical shifts of the bis(thiosemicarbazone), or if decomplexation occurred, we recorded the ^{1}H-NMR spectra of complex **6** in (D$_7$)DMF and (D$_6$)acetone (data not shown). The signals of the bis(thiosemicarbazone) of **6** displayed minor changes regardless of the solvent used. Thus, probably in **6**, as well as in the other complexes, coordination to Bi^{III} did not affect the chemical shifts of the bis(thiosemicarbazones). Coordination of neutral ligands to Bi^{III} probably did not lead to large variations in electron density, which would account for the similarities between the spectra of the free bis(thiosemicarbazones) and those of their Bi^{III} complexes.

2.3. *X-Ray Crystallography.* Upon slow evaporation of **2** in acetone/DMSO 9:1 crystals of [dichlorido(*O*-dimethylsulfoxide)[1-(pyridin-2-yl)ethanone 4-phenylthiosemicarbazonato]bismuth(III)] ([Bi(2Ac4Ph)(DMSO)Cl$_2$]; **2a**) were formed. The molecular structure of **2a** with the atom numbering scheme is depicted in *Fig. 3*.

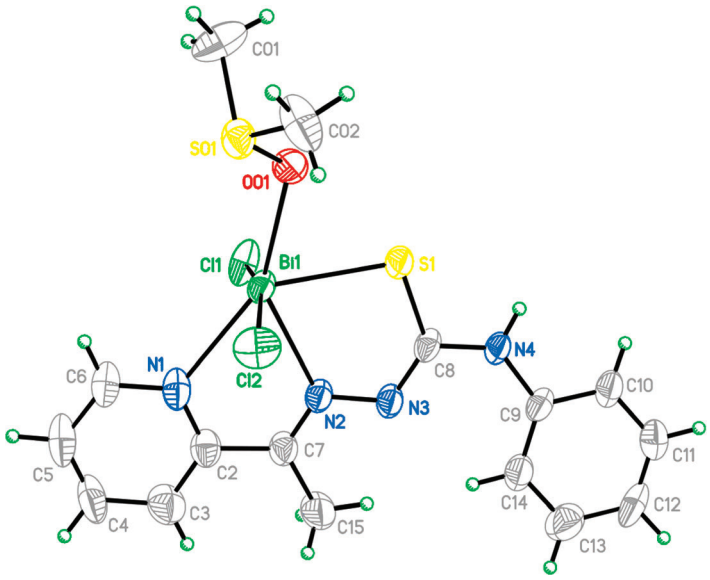

Fig. 3. *Asymmetric unit of [Bi(2Ac4Ph)(DMSO)Cl$_2$]* (**2a**) *showing the labelling scheme of the non-H-atoms and their displacement ellipsoids at the 50% probability level.* For comparison purposes in the crystal structure, the adopted atom numbering is in agreement with that used in the previous publications of our group.

Selected intramolecular bond lengths and angles for H2Ac4Ph [26] and [Bi(2Ac4Ph)(DMSO)Cl$_2$] (**2a**) are compiled in *Table 1*.

[Bi(2Ac4Ph)(DMSO)Cl$_2$] (**2a**) crystallizes as a neutral complex. In **2a**, an anionic thiosemicarbazone binds to the Bi^{III} center through the N_{py}–N_{imine}–S chelating system. A dimethyl sulfoxide (DMSO) is also coordinated to the metal through the O-atom, and two Cl^- ions complete the coordination sphere of Bi^{III}.

A Cl^- ligand from an adjacent molecule interacts weakly with the Bi^{III} center forming dimeric units (see *Fig. 4*). Due to the *Lewis* acidic character of Bi^{III}, additional intra- or intermolecular contacts are usually established in the presence of donor atoms, thus increasing coordination number [28]. Hence, in **2a** we consider that Bi^{III} is heptacoordinated. Furthermore, according to the 'semi bonding concept', the bonds at the Bi-atom might be generally described in terms of primary bonds (normal covalent bonds), and secondary bonds or interactions, with interatomic distances significantly longer than a covalent bond but shorter than the sum of the *Van der Waals* radii for the two elements concerned [28]. The behavior reported here was similar to that observed for other Bi^{III} complexes [29].

When comparing the Bi–Cl bonds (*Table 1*), we observe that the Bi1–Cl1 bond length (2.7674(18) Å) is longer than the Bi1–Cl2 length (2.671(2) Å), which suggests the presence of an interaction of Cl1 with another metal center. In fact, the Bi2$\cdots$Cl1 interaction occurs, resulting in a Cl1 bridge between two adjacent Bi centers. The bridge is asymmetric, since the Bi2$\cdots$Cl1 bond length is 3.128(2) Å. However, this distance is well within the limits of what may be considered a weakly bonding

Table 1. *Selected Bond Lengths* [Å] *and Angles* [°] *for 1-(Pyridin-2-yl)ethanone 4-Phenylthiosemicarbazone* (H2Ac4Ph) [26] *and [Bi(2Ac4Ph)(DMSO)Cl$_2$]* (**2a**)

Bonds	H2Ac4Ph	**2a**	Bonds	H2Ac4Ph	**2a**
S1–C8	1.677(2)	1.738(7)	N1–Bi1	–	2.570(6)
C2–C7	1.486(2)	1.486(9)	N2–Bi1	–	2.455(5)
N2–C7	1.284(3)	1.278(9)	S1–Bi1	–	2.6205(16)
N2–N3	1.376(2)	1.372(7)	Cl1–Bi1	–	2.7674(18)
N3–C8	1.358(3)	1.306(9)	Cl2–Bi1	–	2.671(2)
N4–C8	1.346(3)	1.359(8)	O01-Bi1	–	2.544(4)
N4–C9	1.421(3)	1.411(8)			
Angles	H2Ac4Ph	**2a**	Angles	H2Ac4Ph	**2a**
N1–C2–C7	116.2(2)	115.9(6)	N2–Bi1–S1	–	72.15(13)
C2–C7–N2	114.9(2)	119.5(6)	N2–Bi1–Cl1	–	84.28(13)
C7–N2–N3	118.8(2)	115.4(5)	N2–Bi1–Cl2	–	87.86(14)
N2–N3–C8	118.9(2)	116.6(5)	N2–Bi1–O01	–	140.77(17)
N3–C8–S1	119.7(1)	127.9(5)	S1–Bi1–Cl1	–	92.49(6)
N4–C8–N3	114.8(2)	118.9(6)	S1–Bi1–Cl2	–	98.35(7)
N4–C8–S1	125.5(2)	113.2(5)	S1–Bi1–O01	–	71.49(11)
C8–N4–C9	127.6(2)	130.7(6)	Cl1–Bi1–Cl2	–	163.99(7)
N1–Bi1–N2	–	65.02(18)	Cl1–Bi1–O01	–	111.46(13)
N1–Bi1–S1	–	137.04(14)	Cl2–Bi1–O01	–	83.28(13)
N1–Bi1–Cl1	–	80.37(14)	O01–Bi1–Cl1*i*[a])	–	83.0(2)
N1–Bi1–Cl2	–	83.67(14)	N1–Bi–Cl1*i*[a])	–	73.2(1)
N1–Bi1–O01	–	150.23(17)			

[a]) Cl1*i* atoms generated by $1-x$, $1-y$, $1-z$ symmetry transformations. Data calculated by the Mercury proGram [27].

interaction between Bi^{III} and Cl, significantly longer than a covalent bond (2.49 Å) but shorter than the sum of their *Van der Waals* radii (4.2 Å) [28]. Cl1 and Cl2 occupy the axial positions of the Bi^{III} coordination sphere, with the Cl1–Bi1–Cl2 angle being 163.99(7)° (*Table 1*). The N1, N2, S1, Cl1, and O01 atoms occupy the equatorial position. The mean deviation from the plane formed by the N1–N2–S1–Bi1–Cl1–O01 atoms is 0.2511 Å, with major deviations involving the O01 (−0.4816 Å) and Cl1 (0.3802 Å) atoms.

The coordinated thiosemicarbazone is found in the (*E*,*Z*) conformation with respect to the C7=N2, and N3=C8 bonds, different from the (*E*,*E*)-conformation observed for H2Ac4Ph [26]. This conformation change upon coordination occurs frequently [16][21][30] and was accompanied by a variation in the N2–N3–C8–S1 torsion angle, from 172.7(1)° in H2Ac4Ph to −1.8(5)° in **2a**. As can be seen in *Table 1*, the most significant changes upon coordination involved the shortening of the N3–C8 bond length and increasing of the C8–S1 bond length, due to deprotonation at N3–H. The bond angles are more affected by changes in conformation and by the geometric restrictions due the formation of five-membered chelate rings (see *Table 1*).

In the supramolecular arrangement of this complex, the presence of intermolecular N4–H4A···Cl2i H-bonds (symmetry code: $i = x-1$, y, z; *Table 2*) results in a one-dimensional infinite chain along the (100) direction, as depicted in *Fig. 5*.

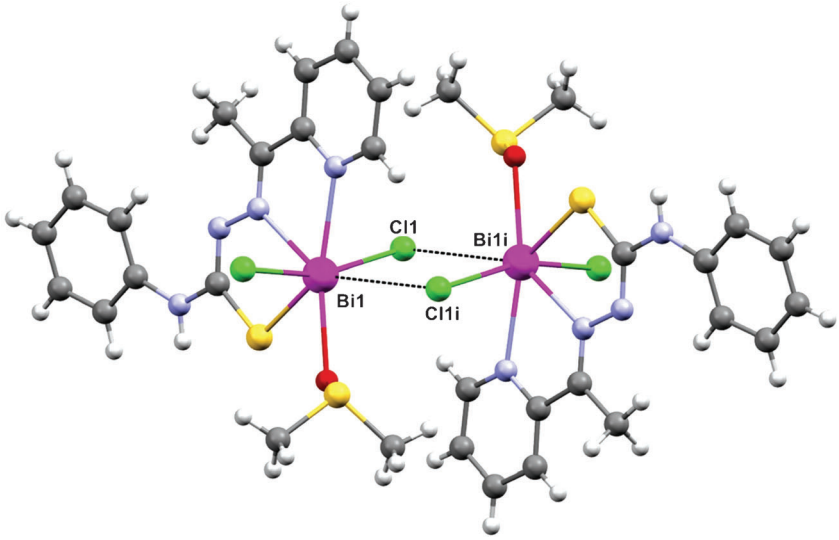

Fig. 4. *Perspective view of [Bi(2Ac4Ph)(DMSO)Cl$_2$]* (**2a**) *showing, in dashed lines, the interactions forming dimers*

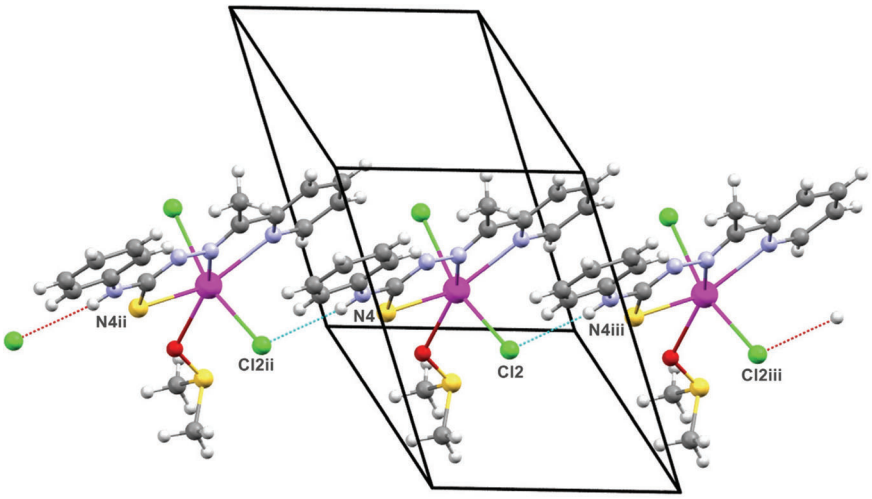

Fig. 5. *Molecular packing of [Bi(2Ac4Ph)(DMSO)Cl$_2$]* (**2a**) *showing the one-dimensional infinite chain along the (100) direction*. The H-bonds are indicated by dashed lines.

Table 2. *H-Bond Distances* [Å] *and Angle* [°] *for [Bi(2Ac4Ph)(DMSO)Cl$_2$]* (**2a**)

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	∠(D–H···A)
N4–H4A···Cl2*i*[a])	0.86	2.75	3.592(6)	165.6

[a]) Symmetry transformations used to generate equivalent atoms: $i = x-1, y, z$.

2.4. *Antibacterial Activity. Table 3* contains the minimum inhibitory concentrations (*MIC*s) against the growth of *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* for the thiosemicarbazones, bis(thiosemicarbazones), and their Bi^{III} complexes, together with values obtained for the drugs used as positive controls. $BiCl_3$ was not tested due to its low solubility.

Table 3. *Minimum Inhibitory Concentrations* (*MIC*s) *against* Staphylococcus aureus, S. epidermidis, Enterococcus faecalis, *and* Pseudomonas aeruginosa *for H2Fo4Ph, H2Ac4Ph, H2Bz4Ph, H_2Gy4DH, H_2Gy4Et, and H_2Gy4Ph, and Their Bi^{III} Complexes* **1**–**6**, *Respectively, Tetracycline Hydrochloride, and Ciprofloxacin*

Compound	*MIC* [μmol l^{-1}]			
	S. aureus	*S. epidermidis*	*E. faecalis*	*P. aeruginosa*
H2Fo4Ph	190.0	>386.2	>401.8	397.0
1	6.1	98.8	23.8	99.9
H2Ac4Ph	370.0	179.4	384.7	45.0
2	5.7	91.9	22.8	96.0
H2Bz4Ph	75.9	>302.9	>308.9	76.0
3	5.5	82.6	40.1	93.2
H_2Gy4DH	>509.1	>504.21	>509.10	>244.8
4	>100.1	>196.30	190.52	>94.3
H_2Gy4Et	>205.5	>384.40	>384.05	>201.6
5	11.1	>181.88	193.01	>92.8
H_2Gy4Ph	>144.5	>274.92	286.14	>144.5
6	2.4	>101.22	17.86	>72.9
Tetracycline hydrochloride	0.3	–	–	29
Ciprofloxacin	–	0.6	1.2	–

In general, the bis(thiosemicarbazones) exhibited lower antibacterial activities than the mono-thiosemicarbazones. Upon coordination to Bi^{III}, the antibacterial activities of both thiosemicarbazones and bis(thiosemicarbazones) increased against the *Gram*-positive bacteria. The increase in activity upon coordination was more pronounced against *S. aureus*. In fact complexes **1**–**3** proved to be 15 to 64 times more potent against *S. aureus* than the free ligands. However, in the case of *Gram*-negative bacteria (*P. aeruginosa*), coordination resulted in improved activity only in complex **1**.

Complexes **4**–**6** were more active than the free bis(thiosemicarbazones) against the *Gram*-positive strains. Although the free bis(thiosemicarbazones) showed lower activities than the mono-thiosemicarbazones, complex **6** was the most effective compound against *S. aureus* (*MIC* 2.4 μmol l^{-1}).

4. Conclusions. – Coordination to Bi^{III} proved to be an efficient strategy to increase the antibacterial activity of the studied thiosemicarbazones and bis(thiosemicarbazones), and to increase the solubility and bioavailability of Bi. The ligands could act as metal carriers or activity could be attributed to the complex *per se*. In the case of the antibacterial activity of complexes **1**–**3**, a synergistic effect involving the thiosemicarbazone and the metal also could take place.

Experimental Part

General. All common chemicals were purchased from *Aldrich* and used without further purification. The thiosemicarbazones were prepared according to standard procedures [16][31]. IR Spectra: *Perkin Elmer FT-IR Spectrum GX* spectrometer; KBr pellets (4000–400 cm^{-1}) and nujol mulls between CsI plates (400–200 cm^{-1}). NMR Spectra: *Bruker DPX-200 Avance* (200 MHz) spectrometer; (D_6)DMSO, (D_4)MeOH, or (D_7)DMF as solvents and TMS as internal reference. Partial elemental analyses: *Perkin Elmer CHN 2400* analyzer. An *YSI* model *31* conductivity bridge was employed for molar-conductivity measurements.

Syntheses of pyridine-2-carbaldehyde 4-phenylthiosemicarbazone ((2*E*)-*N*-phenyl-2-(pyridin-2-ylmethylidene)hydrazinecarbothioamide; H2Fo4Ph), 1-(pyridin-2-yl)ethanone 4-phenylthiosemicarbazone (=(2*E*)-*N*-phenyl-2-[1-(pyridin-2-yl)ethylidene]hydrazinecarbothioamide; H2Ac4Ph), phenyl-(pyridin-2-yl)methanone 4-phenylthiosemicarbazone (=(2*E*)-*N*-phenyl-2-[phenyl(pyridin-2-yl)methylidene]hydrazinecarbothioamide; H2Bz4Ph), glyoxaldehyde bis(thiosemicarbazone) (=(2*E*,2′*E*)-2,2′-(1*E*,2*E*)-ethane-1,2-diylidenedihydrazinecarbothioamide; H_2Gy4DH), glyoxaldehyde bis(4-ethylthiosemicarbazone) (=(2*E*,2′*E*)-2,2′-(1*E*,2*E*)-ethane-1,2-diylidenebis(*N*-ethylhydrazinecarbothioamide); H_2Gy4Et), and glyoxaldehyde bis(4-phenylthiosemicarbazone) (=(2*E*,2′*E*)-2,2′-(1*E*,2*E*)-ethane-1,2-diylidenebis(*N*-phenylhydrazinecarbothioamide); H_2Gy4Ph). All thiosemicarbazones [32] and bis(thiosemicarbazones) [33] were prepared as previously described.

Bismuth(III) Complexes with H2Fo4Ph, H2Ac4Ph, and H2Bz4Ph. The Bi^{III} complexes were obtained by mixing an EtOH soln. (20 ml) of the desired thiosemicarbazone (1 mmol) with $BiCl_3$ in a 1:1 ligand-to-metal molar ratio at r.t. with stirring for 3.5 h. The resulting solids were filtered off, washed with EtOH, followed by Et_2O, and dried *in vacuo*.

Dichlorido(pyridine-2-carbaldehyde 4-phenylthiosemicarbazonato)bismuth(III) ([Bi(2Fo4Ph)Cl_2]; **1**). Yellow solid. Yield 78%. M.p. 238.6–240.5°. IR (KBr): 1601*s* (C=N), 747*m* (C=S), 632*w* (py). IR (CsI/nujol): 512*m* (Bi–N_{imine}), 421*m* (Bi–S), 200*w* (Bi–N_{py}), 333*m* (Bi–Cl). ^{1}H-NMR (200 MHz, (D_4)MeOH; main signals): 8.70 (*d*, H–C(6), py); 8.21 (*d*, H–C(3), py); 8.15–7.90 (*m*, H–C(4) of py, CH=N); 7.67 (*t*, H–C(5), py). Λ_M=15.75 Ω^{-1} cm^2 mol^{-1} in DMF. Anal. calc. for $C_{13}H_{11}BiCl_2N_4S$ (535.20): C 29.17, H 2.07, N 10.47; found: C 29.79, H 1.46, N 9.75. FW: 536.21 g mol^{-1}.

Dichlorido[1-(pyridin-2-yl)ethanone 4-phenylthiosemicarbazonato]bismuth(III) ([Bi(2Ac4Ph)Cl_2]; **2**). Yellow solid. Yield 92%. M.p. 253° (dec.). IR (KBr): 1594*s* (C=N), 759*m* (CS), 601*w* (py). IR (CsI/nujol): 510*m* (Bi–N_{imine}), 422*m* (Bi–S), 202*w* (Bi–N_{py}), 332*m* (Bi–Cl). ^{1}H-NMR (200 MHz, (D_4)MeOH; main signals): 8.99 (*d*, *J*=5.2, H–C(6), py); 8.35–8.20 (*m*, H–C(3), H–C(4), py); 7.79 (*t*, H–C(5), py); 2.81 (*s*, Me). Λ_M=16.63 Ω^{-1} cm^2 mol^{-1} in DMF. Anal. calc. for $C_{14}H_{13}BiCl_2N_4S$ (549.23): C 30.62, H 2.39, N 10.20; found: C 30.13, H 2.64, N 10.50. FW: 549.23 g mol^{-1}.

Dichlorido[phenyl(pyridin-2-yl)methanone 4-phenylthiosemicarbazonato]bismuth(III)] ([Bi(2Bz4Ph)Cl_2]; **3**). Yellow solid. Yield 83%. M.p. 300° (dec.). IR (KBr): 1542*s* (C=N), 755*m* (CS), 587*w* (py). IR (CsI/nujol): 431*w* (Bi–N_{imine}), 404*m* (Bi–S), 231 (Bi–Npy), 331*w* (Bi–Cl). ^{1}H-NMR (200 MHz, (D_6)DMSO): 9.79 (*s*, H–N(4), (*Z*)); 9.53 (*s*, H–N(4), (*E*)); 9.29 (*d*, *J*=4.6, H–C(6) (py), (*Z*)); 9.22 (*d*, *J*=4.6, H–C(6) (py), (*E*)); 8.06 (*t*, *J*=7.6, H–C(4) (py), (*E*,*Z*)); 7.68 (*d*, *J*=8.0, H–C(3) (py), (*Z*)); 7.24 (*t*, *J*=7.0, H–C(5) (py), (*E*,*Z*)); 6.90 (*d*, *J*=8.0, H–C(3) (py), (*E*)). ^{13}C-NMR (200 MHz, (D_6)DMSO; main signals): 168.8 (C(3), (*Z*)); 166.9 (C(3), (*E*)); 159.7 (C(2) (py), (*Z*)); 158.4 (C(2) (py), (*E*)); 150.6 (C=N, (*E*)); 149.9 (C=N, (*Z*)); 149.6 (C(6) (py), (*Z*)); 149.3 (C(6) (py), (*E*)); 141.0 (C(1) (Ph), (*Z*)); 140.9 (C(1) (Ph), (*E*)); 139.8 (C(4) (py), (*Z*)); 139.3 (C(4) (py), (*E*)); 136.0 (C(1) of Ph–C=N, (*Z*)); 135.3 (C(1) of *Ph*–C=N, (*E*)); 127.9 (C(3) (py), (*Z*)); 126.5 (C(5) (py), (*E*)); 125.5 (C(5) (py), (*Z*)); 125.3 (C(3) (py), (*E*)). Λ_M=11.07 $\Omega^{-1}$$cm^2$ mol^{-1} in DMF. Anal. calc. for $C_{19}H_{15}BiCl_2N_4S$ (611.30): C 37.33, H 2.47, N 9.17; found: C 37.56, H 2.20, N 8.27. FW: 611.30 g·mol^{-1}.

Syntheses of Bismuth(III) Complexes with Glyoxaldehyde Bis(thiosemicarbazone) (H_2Gy4DH), *Glyoxaldehyde Bis(4-ethylthiosemicarbazone)* (H_2Gy4Et), *and Glyoxaldehyde Bis(4-phenylthiosemicarbazone)* (H_2Gy4Ph). The Bi^{III} complexes were obtained by mixing a MeOH soln. (10 ml) of the desired bis(thiosemicarbazone) (1 mmol) with $BiCl_3$ in a 1:1 ligand-to-metal molar ratio at reflux for 4 h with stirring. The resulting solids were filtered off, washed with MeOH, followed by acetone, and dried *in vacuo*.

Trichlorido[glyoxaldehyde bis(thiosemicarbazonato)]bismuth(III) ([$Bi(H_2Gy4DH)Cl_3$]; **4**). Yellow solid. Yield 79%. M.p. 224.6° (dec.). IR (KBr): 3369*m*, 3258*m*, 3174*m* (N–H), 1595*s* (C=N), 834*m* (C=S). IR (CsI/nujol): 271*w* (Bi–S), 331*w* (Bi–N), 204*w* (Bi–Cl). ^{1}H-NMR (200 MHz, (D_6)DMSO): 7.70 (*s*, 2 CH=N); 11.68 (*s*, 2 H–N(2)); 8.31 (*s*, H–N(4)); 7.88 (*s*, H–N(4)). ^{13}C-NMR (200 MHz, (D_6)DMSO): 178.0 (C(3)); 140.5 (C=N). $\Lambda_M = 8.04\ \Omega^{-1}\ cm^2\ mol^{-1}$ in DMF. Anal. calc. for $C_4H_8BiCl_3N_6S_2$ (519.62): C 9.25, H 1.55, N 16.17; found: C 10.05, H 1.51, N 16.31%. FW: 519.62 g mol^{-1}.

[Chlorido(dihydroxido)[glyoxaldehyde bis(4-ethylthiosemicarbazonato)]bismuth(III) ([$Bi(H_2Gy4Et)(OH)_2Cl$]; **5**). Yellow solid. Yield 83%. M.p. 218.7–219.6°. IR (KBr): 3370*m*, 3154*m* (N–H), 1616*s* (C=N), 750*m* (C=S). IR (CsI/nujol): 248*w* (Bi–S), 296*w* (Bi–N), 237*w* (Bi–Cl), 440*w* (Bi–O). ^{1}H-NMR (200 MHz, (D_6)DMSO): 11.70 (*s*, 2 H–N(2)); 8.52 (*t*, $J = 5.7$, 2 H–N(4)); 7.72 (*s*, 2 CH=N); 3.53 (*qd*, $J = 13.5$, 6.6, 2 CH_2); 1.10 (*t*, $J = 7.1$, 2 Me). ^{13}C-NMR (200 MHz, (D_6)DMSO): 176.4 (C=S)); 140.0 (C=N); 38.3 (CH_2); 14.3 (Me). $\Lambda_M = 5.93\ \Omega^{-1}\ cm^2\ mol^{-1}$ in DMF. Anal. calc. for $C_8H_{18}BiClN_6O_2S_2$ (538.83): C 17.83, H 3.37, N 15.60; found: C 18.08, H 3.20, N 15.58%. FW: 538.83 g mol^{-1}.

Trichlorido[glyoxaldehyde bis(4-phenylthiosemicarbazonato)]bismuth(III) ([$Bi(H_2Gy3Ph)Cl_3$]; **6**). Yellow solid. Yield 90%. M.p. 255.0–256.2°. IR (KBr): 3297*m*, 3158*m* (N–H), 1602*s* (C=N), 761*m* (C=S). IR (CsI/nujol): 285*w* (Bi–N), 273*w* (Bi–S), 188*w* (Bi–Cl). ^{1}H-NMR (200 MHz, (D_6)DMSO): 7.89 (*s*, 2 CH=N); 10.19 (*s*, 2 H–N(4)); 12.15 (*s*, 2 H–N(2)). ^{13}C-NMR (200 MHz, (D_6)DMSO): 175.9 (C=S)); 140.6 (C=N). $\Lambda_M = 20.86\ \Omega^{-1}\ cm^2\ mol^{-1}$ in DMF. Anal. calc. for $C_{16}H_{16}BiCl_3N_6S_2$ (671.81): C 28.61, H 2.40, N 12.51; found: C 28.39, H 2.28, N 12.29%. FW: 671.81 g mol^{-1}.

3.3. *X-Ray Crystallography.* The crystal structure of **2a** was determined by single-crystal X-ray diffractometry[1]). Measurements were carried out on an *Oxford-Diffraction GEMINI-Ultra* diffractometer using graphite-enhance source MoK_α radiation (λ 0.71073 Å) at 293(2) K and 1 atm. The data collection, cell refinement, and data reduction were performed using the CRYSALISPRO software [34]. Semi-empirical from equivalents absorption correction method was applied [34].

The structure was solved by direct methods using SHELXS-97 [35]. Full-matrix least-squares refinement procedure on F^2 with anisotropic thermal parameters was carried on using SHELXL-97 [35]. Positional and anisotropic atomic displacement parameters were refined for all non-H-atoms. The H-atoms were placed geometrically and the positional parameters were refined using a riding model. *Table 4* contains the crystal data and refinement results for the determined structure of **2a**. Molecular graphics and packing figures were plotted using XP in SHELXTL-PC [36] and Mercury [27], resp.

3.4. *Antibacterial Activity.* Studies of antibacterial activity were performed in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) to determine broth microdilution minimum inhibitory concentration (*MIC*) values [37][38]. *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 19433), and *Pseudomonas aerugionosa* (ATCC 9027) stored on *Mueller–Hinton* agar (MHA) were inoculated into *Mueller–Hinton* Broth (MHB) and incubated aerobically at 35° for 24 h. Then, the bacterial cells were suspended, according to the *McFarland* protocol in saline soln. [38], to produce a suspension of *ca.* 10^5 CFU ml^{-1} (colony-forming units per ml). The cultures were further diluted tenfold to attain inoculum size of 1.2×10^4 CFU ml^{-1}. Serial dilutions of the compounds, previously dissolved in DMSO, were prepared in test tubes to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 μg ml^{-1}, then the inoculum (100 μl) was added to each tube. The *MIC*, defined as the lowest concentration of the test compound, which inhibits the visible growth, was determined visually after incubation for 20 h at 37°. Tests using tetracycline hydrochloride and ciprofloxacin as reference and DMSO as negative control were carried out in parallel. All tests were performed in triplicates with full agreement between the results.

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[1]) CCDC No. 859622 contains the supplementary crystallographic data for [Bi(2Ac4Ph)Cl_2DMSO] (**2a**). These data can be obtained free of charge from the *CCDC via* www.ccdc.cam.ac.uk/data_request/cif.

Table 4. *Crystal Data and Refinement Results for [Bi(2Ac4Ph)(DMSO)Cl$_2$]* (**2a**)

Empirical formula		$C_{16}H_{19}BiCl_2N_4OS_2$
Formula weight		627.35
Crystal system		Triclinic
Space group		$P\bar{1}$
Unit cell dimensions	a [Å]	7.9293(2)
	b [Å]	10.3773(3)
	c [Å]	14.6909(4)
	α [°]	109.604(2)
	β [°]	90.301(2)
	γ [°]	108.125(2)
Volume [Å^3]		1074.11(5)
Z/Density calc. [Mg/m^3]		2/1.940
Absorption coefficient [mm^{-1}]		8.663
$F(000)$		600
Crystal size [mm]		$0.59 \times 0.13 \times 0.02$
θ Range for data coll. [°]		2.88 to 26.37
Index range		$-9 \le h \le 9$; $-12 \le k \le 12$; $-18 \le l \le 18$
Reflec. collect./unique (R_{int})		23945/4367 (0.0678)
Completeness [%]		99.9 (to 26.37°)
Max. and min. transmission		0.8478 and 0.0799
Data/restraints/parameters		4367/0/235
Goodness-of-fit on F^2		1.071
Final R indices [$I > 2\sigma(I)$]		$R1 = 0.0374$, $wR2 = 0.0967$
R indices (all data)		$R1 = 0.0446$, $wR2 = 0.0995$
Larg. peak/hole, [e Å^{-3}]		3.365/−1.322

REFERENCES

[1] L. B. Rice, *Biochem. Pharmacol.* **2006**, *71*, 991.
[2] H. Kresse, M. J. Belsey, H. Rovini, *Nat. Rev. Drug Discovery* **2007**, *6*, 19.
[3] K. J. Simmons, I. Chopra, C. W. G. Fishwick, *Nat. Rev., Microbiol.* **2010**, *8*, 501.
[4] A. R. Shaikh, R. Giridhar, M. R. Yadav, *Int. J. Pharm.* **2007**, *332*, 24.
[5] N. Yang, H. Sun, *Coord. Chem. Rev.* **2007**, *251*, 2354.
[6] W. G. Veloira, P. Domenico, J. J. LiPuma, J. M. Davis, E. Gurzenda, J. A. Kazzaz, *J. Antimicrob. Chemother.* **2003**, *52*, 915.
[7] P. Domenico, R. J. Salo, S. G. Novick, P. E. Schoch, K. Van Horn, B. A. Cunha, *Antimicrob. Agents Chemother.* **1997**, *41*, 1697.
[8] J. P. Folsom, B. Baker, P. S. Stewart, *J. Appl. Microbiol.* **2011**, *111*, 989.
[9] H. Beraldo, D. Gambino, *Mini. Rev. Med. Chem.* **2004**, *4*, 31, and refs. cit. therein.
[10] G. L. Parrilha, J. G. da Silva, L. F. Gouveia, A. K. Gasparoto, R. P. Dias, W. R. Rocha, D. A. Santos, N. L. Speziali, H. Beraldo, *Eur. J. Med. Chem.* **2011**, *46*, 1473.
[11] I. C. Mendes, F. B. Costa, G. M. de Lima, J. D. Ardisson, I. Garcia-Santos, A. Castiñeiras, H. Beraldo, *Polyhedron* **2009**, *28*, 1179.
[12] I. C. Mendes, J. P. Moreira, A. S. Mangrich, S. P. Balena, B. L. Rodrigues, H. Beraldo, *Polyhedron* **2007**, *26*, 3263.
[13] T. O. Bastos, B. M. Soares, P. Silva Cisalpino, I. Castro Mendes, R. G. dos Santos, H. Beraldo, *Microbiol. Res.* **2010**, *165*, 573.
[14] K. Nomiya, K. Sekino, M. Ishikawa, A. Honda, M. Yokoyama, N. C. Kasuga, H. Yokoyama, S. Nakano, K. Onodera, *J. Inorg. Biochem.* **2004**, *98*, 601.
[15] R. Diemer, U. Dittes, B. Nuber, V. Seifried, W. Opferkuch, B. K. Keppler, *Metal-Based Drugs* **1995**, *2*, 271.

[16] D. C. Reis, M. C. X. Pinto, E. M. Souza-Fagundes, S. M. S. V. Wardell, J. L. Wardell, H. Beraldo, *Eur. J. Med. Chem*. **2010**, *45*, 3904.
[17] A. P. Rebolledo, M. Vieites, D. Gambino, O. E. Piro, E. E. Castellano, C. L. Zani, E. M. Souza-Fagundes, L. R. Teixeira, A. A. Batista, H. Beraldo, *J. Inorg. Biochem*. **2005**, *99*, 698.
[18] A. E. Graminha, C. Rodrigues, A. A. Batista, L. R. Teixeira, E. S. Fagundes, H. Beraldo, *Spectrochim. Acta, Part A* **2008**, *69*, 1073.
[19] A. P. Rebolledo, G. M. de Lima, L. N. Gambi, N. L. Speziali, D. F. Maia, C. B. Pinheiro, J. D. Ardisson, M. E. Cortés, H. Beraldo, *Appl. Organomet. Chem*. **2003**, *17*, 945.
[20] J. A. Lessa, D. C. Reis, I. C. Mendes, N. L. Speziali, L. F. Rocha, V. R. A. Pereira, C. M. L. Melo, H. Beraldo, *Polyhedron* **2011**, *30*, 372.
[21] K. Nakamoto, 'Infrared Spectra of Inorganic and Coordination Compounds', Wiley-Interscience, New York, 1970.
[22] G. Q. Zhong, S. R. Luan, P. Wang, Y. C. Guo, Y. R. Chen, Y. Q. Jia, *J. Therm. Anal. Calorim.* **2006**, *86*, 775.
[23] J. G. Shao, Y. X. Yang, B. W. Li, L. P. Zhang, Y. R. Chen, X. L. Liu, *J. Therm. Anal. Calorim*. **2009**, *96*, 277.
[24] H. P. S. Chauhan, N. M. Shaik, U. P. Singh, *Appl. Organomet. Chem*. **2006**, *20*, 142.
[25] X. Li, A. A. Gewirth, *J. Am. Chem. Soc.* **2003**, *125*, 7086; R. P. Oertel, R. A. Plane, *Inorg. Chem.* **1968**, *7*, 1192.
[26] E. Bermejo, A. Castiñeiras, R. Domíguez, R. Carballo, C. Maichle-Moessmer, J. Straehle, D. X. West, *Z. Anorg. Allg. Chem.* **1999**, *625*, 961.
[27] C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek, P. A. Wood, *J. Appl. Crystallogr.* **2008**, *41*, 466.
[28] C. Silvestru, H. J. Breunig, H. Althaus, *Chem. Rev.* **1999**, *99*, 3277.
[29] J. R. Eveland, K. H. Whitmire, *Inorg. Chim. Acta* **1996**, *249*, 41.
[30] K. O. S. Ferraz, G. M. M. Cardoso, C. M. Bertollo, E. M. Souza-Fagundes, N. Speziali, C. L. Zani, I. C. Mendes, M. A. Gomes, H. Beraldo, *Polyhedron* **2011**, *30*, 315.
[31] J. A. Lessa, J. C. Guerra, L. F. de Miranda, C. F. D. Romeiro, J. G. Da Silva, I. C. Mendes, N. L. Speziali, E. M. Souza-Fagundes, H. Beraldo, *J. Inorg. Biochem.* **2011**, *105*, 1729.
[32] A. Pérez-Rebolledo, G. M. de Lima, N. L. Speziali, O. E. Piro, E. E. Castellano, J. D. Ardisson, H. Beraldo, *J. Organomet. Chem.* **2006**, *691*, 3919.
[33] H. Beraldo, L. P. Boyd, D. X. West, *Transition Met. Chem.* **1998**, *23*, 67.
[34] CRYSALISPRO, Oxford Diffraction Ltd., Version 1.171.34.34 (release 05-01-2010 CrysAlis171.-NET).
[35] G. M. Sheldrick, *Acta Crystallogr., Sect. A* **2008**, *64*, 112.
[36] SHELXTL, Reference Manual (Version 6.14), Bruker Analytical X-Ray Systems, Inc., Madison, WI, USA, 1998.
[37] NCCLS, 'Method for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically' – Approved Standard-sixth Edition, M7-A6, National Committee for Clinical Laboratory Standards, Villanova, PA, 2005.
[38] Clinical and Laboratory Standards Institute, 'Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement', CLSI document M100-S15 (ISBN 1-56238-556-9). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2005.

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