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Platinum and Palladium Complexes Bearing New (1R,2R)-(−)-1,2-Diaminocyclohexane (DACH)-Based Nitrogen Ligands: Evaluation of the Complexes Against L1210 Leukemia

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Abstract. The reaction of (1R,2R)-(−)-1,2-diaminocyclohexane (**1**) [*DACH*] with the aldehyde (1R)-(−)-myrtenal (**2**) in MeOH afforded the bidentate diimine ligand, (1R,2R)-(−)-N^1,N^2-bis{(1R)-(−)myrtenylidene}-1,2-diaminocyclohexane (**3**) in a high yield. Reduction of **3** using $LiAlH_4$ led to the formation of the desired ligand (**4**) (1R,2R)-(−)-N^1,N^2-bis{(1R)-(−)myrtenyl}-1,2-diaminocyclohexane. Treatment of compound **4** with K_2PtCl_4 or K_2PdCl_4 yielded the corresponding platinum(II) and palladium(II) complexes, **Pt-5** and **Pd-6**, respectively. The reaction of compound **3** with K_2PtCl_4 gave the diimine complex **Pt-7**. The cytotoxic activity of the complexes **Pt-5**, **Pd-6** and **Pt-7** was tested and compared to the approved drugs, cisplatin (***Cis*-Pt**) and oxaliplatin (**Ox-Pt**). The complexes (**Pt-5**, **Pd-6** and **Pt-7**) inhibit L1210 cell line proliferation with an IC_{50} of 0.6, 4.2, and 0.7 μL, respectively as evidenced by measuring thymidine incorporation.

Keywords: Platinum; Palladium; Bidentate nitrogen ligands; Anticancer effect

1 Introduction

Since the discovery of the activity of the anticancer compound cis-[$(NH_3)_2PtCl_2$], clinically called cisplatin [1], many new platinum complexes have been synthesized and evaluated for their antitumor activity [2]. However, a few of these complexes have entered clinical trials [3]. Platinum complexes which are based on 1,2-diaminocyclohexane (DACH) ligand, such as oxaliplatin [4] [*cis*(oxalato)(*trans-l*-1,2-diaminocyclohexane)platinum(II)], entered clinical trial based on their circumvention of acquired cisplatin resistance. Preclinical antitumour activity tests indicate that the antitumour spectrum of oxaliplatin was similar to that of cisplatin, with some notable differences [5]. Oxaliplatin was more effective toward L1210 leukemia than cisplatin, and it even produces cures after intraperitoneal administration. The corresponding dichloro platinum derivative of 1,2-diaminocyclohexane (DACH) is effective in vitro against L1210 leukemia and the line made resistant to cisplatin [6]. Investigations on this type of chiral complexes showed that the *trans* isomer *trans-l* (*trans*-(−)-1R,2R) is more efficacious than both the corresponding *trans-d*- (*trans*-(+)-1S,2S) and the *cis*-isomer (1R,2S) [7]. The conformation of the platinum complexes bearing DACH ligand may have relation to their activity. The cyclohexyl ring of *cis*-isomer is nearly perpendicular to the chelate ring, while in both enantiomers of *trans*-DACH complex the cyclohexyl ring lies in a common plane. Therefore the *trans* isomers could enter the large groove of the DNA double-helix more easily than the *cis*-isomer [8].

Recently, we found that the oxaliplatin which was isolated by reacting the enantiomerically pure isomer *trans-l* (*trans*-(−)-1R,2R) with the platinum salt K_2PtCl_4 in H_2O [9], does not consist only of the desired isomer, but of a mixture of both the trans-*l* and trans-*d* isomers. No retention of optical isomerism was observed despite that the enantiomerically pure DACH ligand was utilized. It is believed that the transformation of R,R-DACH takes place throughout the synthesis of the corresponding dichloride complex [(DACH)$PtCl_2$]. Therefore, it will be desirable to find a new methodology to synthesize the enantiomerically pure (1R,2R)-oxaliplatin-like complexes. In addition, there is still a need for other platinum antitumour agents in order to broaden the spectrum of activity, to improve clinical effectiveness, and to overcome the side-effects of Pt based chemotherapy.

Several reports on asymmetric catalysis have shown that terminal chiral groups in the ligand system [10, 11] are more efficient for the transfer of the chiral information to the metal center than chirality in the backbone bridge, which is normally the center for tuning the electronic properties of the metal. However, the available literature shows no inves-

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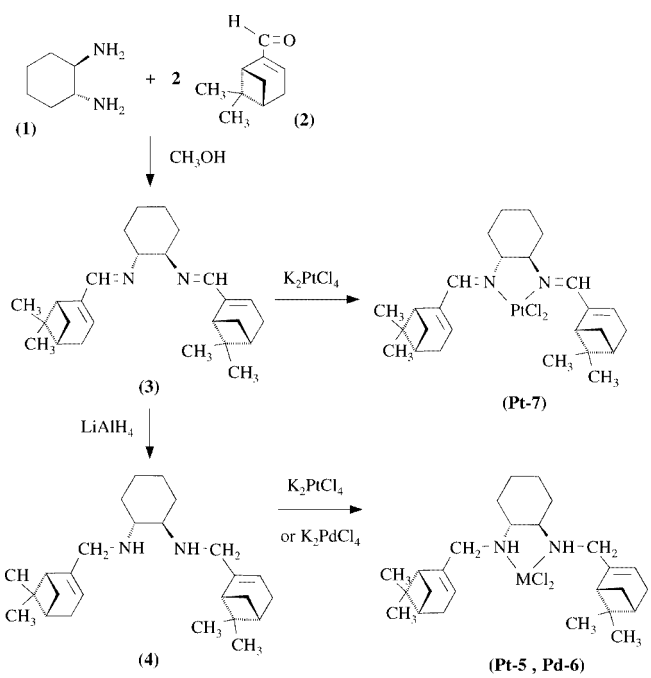
tigation on the influence of chiral-bulky terminal substituents on the 1,2-diaminocyclohexane on both the retention of isomerism and the cytotoxic activity towards tumor cell lines in the third generation of antitumor complexes.

As a continuation of our intrinsic interest in the synthesis of chiral square-planar platinum(II) and palladium(II) complexes [12−14] and the investigation of their biological activity [9, 15, 16], we describe here the preparation of some platinum(II) and palladium(II) complexes with new *trans-l*-dach based diamine and diimine donor ligands containing the enantiomerically pure myrtenyl groups as terminal substituents. To confirm the identity of the compounds prepared in the present study, a variety of techniques including elemental analysis, MS (EI), IR, ^{1}H- and ^{13}C-NMR spectroscopy have been used. Furthermore, antitumor evaluation for the isolated complexes against L1210 cell lines and their pro-apoptotic properties have been investigated.

Results and Discussion

2.1 Ligand synthesis

The condensation reaction of the compounds (1R,2R)-(−)-1,2-diaminocyclohexane (DACH, **1**) and (1R)-(−)-myrtenal (**2**) in MeOH at 50 °C afforded the bidentate diimine ligand, (1R,2R)-(−)-N^1,N^2-bis{(1R)-(−)myrtenylidene}-1,2-diaminocyclohexane (**3**) in a high yield (Scheme 1). Reduction of compound **3** using $LiAlH_4$ led to the formation of the desired ligand **4**, where only the imine groups have been reduced (Scheme 1) as indicated by IR and ^{1}H NMR analysis.



Scheme 1 Synthesis of the diimine and diamine bidentate nitrogen ligands (**3** and **4**) and their corresponding platinum(II) (**Pt-5** and **Pt-7**) and palladium(II) (**Pd-6**) complexes.

The condensation reaction between **1** and **2** was followed by IR spectroscopy. The total vanishing of the carbonyl band and the appearance of a new band at 1632 cm^{-1}, which is assigned to the imine bond (ν C=N), indicates the formation of the diimine ligands (**3**).

The IR- spectra of **4** is characterized by a broad medium band at about 3280 cm^{-1} assigned to the NH stretching vibration. The presence of a medium band at 1614 cm^{-1}, which is assigned to the olefinic C=C bond in the myrtenyl moiety, indicate that only the imine groups were reduced. The ^{1}H-NMR spectrum for **4** n $CDCl_3$ showed one set of signals indicating the presence of one enantiomer. The spectrum is lacking the signals due to HC=N (7.54 ppm) protons that are characteristic for compound **3**, while the signals due to C=CH of the mertenyl groups appear at 5.29 ppm.

2.2 Complex synthesis

The platinum(II) complex **Pt-5** and the corresponding palladium(II) complex **Pd-6** were prepared in a good yield by treating the ligand (**4**) with the metal salts K_2PtCl_4 and K_2PdCl_4, respectively (Scheme 1). For comparison, the platinum(II) complex **Pt-7** of the diimine ligand **3** was also prepared following the same procedure.

The complexes prepared in the present study were characterized by their physical properties, infrared spectroscopy, mass spectroscopy and elemental analysis. All the data support the formation of the desired dichloro complexes.

IR-analyses of the complexes indicate the presence of the ligands. A slight shift of the peaks due to the C=C bond (**Pt-5**, **Pd-6**, and **Pt-7**) and the imine bond (νC=N) (**Pt-7**) was observed due to complexation. Also elemental analyses showed that the metal to ligand ratio in the dichloro complexes is 1:1. Therefore, a square planar arrangement around the metal center is formed. 1H-NMR measurements were not feasible due to the very low solubility of the platinum complexes in most organic solvents.

2.3 Biological investigation

The anti-proliferative effect of compounds **Pt-5**, **Pd-6** and **Pt-7** together with the commercial drugs cisplatin (**Cis-Pt**) and oxaliplatin (**Ox-Pt**) were investigated in L1210 Cell line using 3H-thymidine incorporation. As shown in Figure 1, the platinum compounds **Pt-5** and **Pt-7** suppress proliferation more efficiently than the commercial platinum-based drugs with an IC_{50} of 0.6 and 0.7 μL, respectively (Table 1). Compound **Pt-5** is 17-folds more potent than the commercial oxaplatin and cisplatin. No significant difference could be observed between the complex that contains the diamine nitrogen ligand and the one holding the corresponding diimine ligand. The palladium complex (**Pd-6**) also suppresses proliferation efficiently with an IC_{50} of 4.2 μL. This is about 2-folds more potent than the commercial oxaplatin and cisplatin.

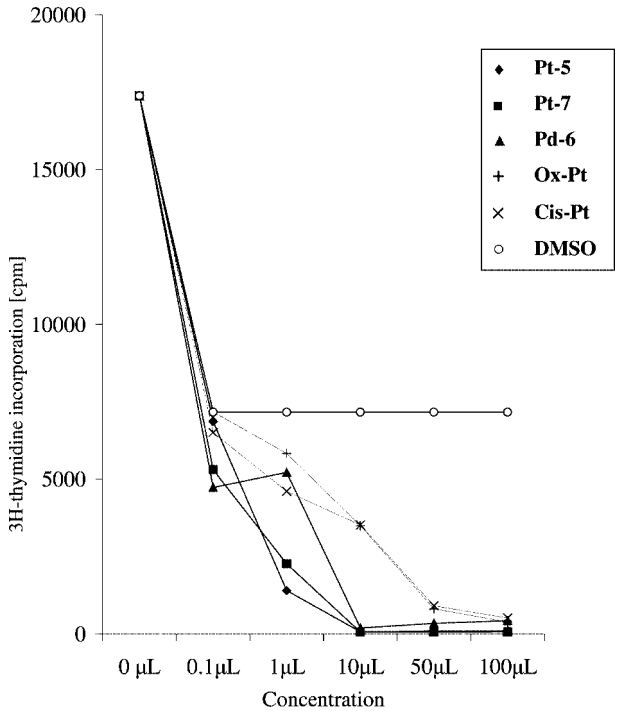


Figure 1 3H-thymidine incorporation utilizing the complexes: **Pt-5**, **Pd-6**, **Pt-7**, oxaliplatin (**Ox-Pt**), and Cisplatin (**Cis-Pt**) against L1210, 0.1−100 µM, 24h, 1% DMSO.

The pro-apoptotic properties of compounds **Pt-5**, **Pd-6**, and **Pt-7** were investigated and compared to those of cisplatin and oxaliplatin drugs. We initially screened various concentrations of each compound and then investigated the pro-apopototic properties of all compounds after 8 and 16 hours of one single concentration (0.3 µL). This concentration is lower than the IC_{50} obtained for compound **Pt-5**.

Table 1 IC_{50} values (µL) for the compounds **Pt-5**, **Pd-6**, and **Pt-7** compared to the standards oxaliplatin (**Ox-Pt**) and cisplatin (**Cis-Pt**).

Entry	Complex	IC_{50} (µL)
1	(Pt-5)	0.6
2	(Pd-6)	4.2
3	(Pt-7)	0.7
4	(Ox-Pt)	10.1
5	(Cis-Pt)	10.2

Apoptotic cell death in cellular systems was measured using the photometric enzyme-immunoassay Cell Death Detection, ELISA^{PLUS}. As presented in Figure 2, compound **Pt-5** was the most potent agent among the tested compounds. Taken together, the cytotoxic activity of compound **Pt-5** may be explained by inducing programmed cell death.

In summary, the platinum(II) (**Pt-5** and **Pt-7**) and palladium(II) (**Pd-6**) complexes of new chiral diamine donor ligands which are based on the enantiomerically pure (1R,2R)-diaminocyclohexane (DACH) as a backbone and the bulky myrtenyl groups as auxiliary ligands were prepared. Screening the compounds for their antitumor activity against L1210 leukemia showed that both compounds **Pt-5** and **Pt-7** suppress proliferation more efficiently than the commercial drugs cisplatin and oxaliplatin. The isolated complexes are a subject for further derivatization toward the more soluble oxalato and carboxylato species.

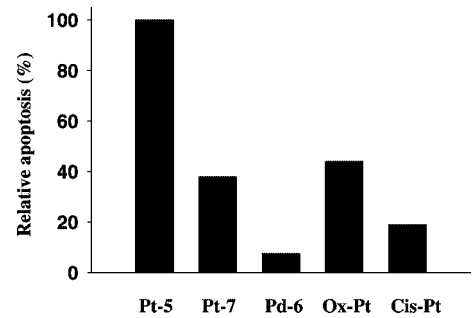


Figure 2 Relative apoptosis (%) of the complexes **Pt-5**, **Pd-6**, and **Pt-7**.

3 Experimental Section

3.1 General

The chemicals, (1R,2R)-(−)-1,2-diaminocyclohexane, (1R)-(−)-myrtenal, potassium tetrachloropalladate(II) and potassium tetrachloroplatinate were purchased from Aldrich.

NMR spectra were recorded on a Varian Gemini 200 spectrometer using $CDCl_3$ with TMS as an international standard. The IR spectra were recorded on a Nicolet 205 FT-IR Spectrometer. Mass spectra (EI) were acquired with a JEOL JMS-SX102 mass spectrometer. Elemental analysis was performed using a (EA 1110 CHNS-O CE instrument).

3.2 Synthesis of ligands and complexes

3.2.1 (1R,2R)-(−)-N^1,N^2-bis{(1R)-(−)myrtenylidene}-1,2-diaminocyclohexane (3)

A solution of (1R,2R)-(−)-1,2-diaminocyclohexane (1.0 g, 8.75 mmol) in dry methanol (25 ml) is added to a solution of (1R)-(-)-myrtenal (2.8 ml, 18.39 mmol) in methanol (25 ml). After stirring for 70 hours at room temperature and refluxing for 3 hours, the solvent was evaporated and the product was extracted with n-pentane (4 × 200 ml). Evaporation of the solvent followed by washing with hexane (30 ml) and drying under vacuum gave a yellow solid. Yield: 2.73 g (83 %). Anal. Calcd. for $C_{26}H_{38}N_2$: C, 82.49; H, 10.12; N, 7.39 %. Found: C, 82.75; H, 9.94; N, 7.53 %.

^{1}H-NMR (200 MHz, $CDCl_3$): δ: 7.54 (s, 2H, N=CH), 5.77 (m, 2H, C=CH), 3.0 (m, 2H, N-CH-cyclohex.), 2.8 and 2.4 (t, J = 5 Hz, 2H, CH_2-myrt.), 2.31 (m, 4H, CH-bridge-myrt.), 2.05 (m, 2H, cyclohex.), 1.67 (m, 6H, cyclohex.), 1.35 (t, 2H, C-CH-myrt.), 1.24 (s, 6H, CH_3-myrt.), 1.0 (m, 2H, CH-myrt.), 0.64 (s, 6H, CH_3-myrt.). **^{13}C NMR** ($CDCl_3$): δ : 162.6, 148.6, 134.9, 73.7, 55.2, 44.6, 40.6, 38.1, 33.8, 32.8, 32.1, 26.6, 25.2, 21.6. **IR** ν/cm^{-1}: = 2853, 2972 (s), 1632 (s, sh), 1614(m), 1447 (m), 1366 (m), 1263 (m), 1092 (m), 1031 (m), 795 (s).

3.2.2 (1R,2R)-(−)-N^1,N^2-bis{(1R)-(−)myrtenyl}-1,2-diaminocyclohexane (4)

To a suspension of lithium aluminum hydride (1.33 g, 35.17 mmol) in dry THF (200 ml) was added carefully the diimine (**3**) (2.69 g, 7.03 mmol). Continuous stirring for 50 hours at room temperature

and then for 25 hours refluxing was performed. The mixture, which turned into dark green, was filtered over Na_2SO_4 and then evaporated to dryness. The residue was extracted with diethyl ether (800 ml) and hydrolyzed with distilled water (150 ml). The organic layer was separated, dried over Na_2SO_4, filtered and then evaporated under vacuum to give a yellow oil. Yield: 1.4 g (52 %). Anal. Calcd. for $C_{26}H_{42}N_2$: C, 81.62; H, 11.06; N, 7.32 %. Found: C, 81.58; H, 11.35; N, 7.34 %.

^{1}H-NMR (200 MHz, $CDCl_3$): δ: 5.29 (m, 2H, C=CH-myrt.), 3.20 and 2.85 (2 d, J = 6 Hz, 4H, N-CH_2), 2.3 (m, 4H, CH_2-bridge-myrt.), 2.1 (m, 4H, CH_2-myrt.),2.0 (m, 6H, cyclohex.), 1.6 (m, 4H, cyclohex.), 1.21 (s, 6H, CH_3-myrt.), 1.13 (m, 2H, myrt.), 0.85 (m, 2H, myrt.), 0.77 (s, 6H, CH_3-myrt.). **IR** ν/cm^{-1}: 3280 (m), 2931, 2832 (s), 1614 (w), 1450 (m, sh), 1365 (m), 1109 (w), 803 (w).

3.2.3 Dichloro[(1R,2R)-(−)-N^1,N^2-bis{(1R)-(−)myrtenyl}-1,2-diaminocyclohexane]-platinum(II)·3H_2O (Pt-5)

To a solution of potassium tetrachloroplatinate (0.96 g, 2.31 mmol) in water (30 ml) a solution of **4** (1.0 g, 2.58 mmol) in ethanol (30 ml) was added. Stirring was continued for three days at room temperature. The resulted yellowish product was filtered, washed with water (75 ml), ethanol (50 ml), petroleum ether (75 ml), and dried under vacuum. Yield: 0.83 g (51 %). Anal. Calcd. for $C_{26}H_{42}N_2PtCl_2 \cdot 3\ H_2O$: C, 44.44; H, 6.89; N, 3.99. Found: C, 43.77; H, 6.85; N, 4.86 %.

IR ν/cm^{-1}: = 3121 (m), 2933, 2866 (s), 1624 (w), 1450 (m, sh), 1383 (w), 1365 (w). **MS** (EI): (M / z, intensity %): M^+−Cl (612, 10), M^+−2Cl (576, 20), M^+−$PtCl_2$ (382, 20)

3.2.4 Dichloro[(1R,2R)-(−)-N^1,N^2-bis{(1R)-(−)myrtenyl}-1,2-diaminocyclohexane]-palladium(II)·1.5H_2O (Pd-6)

A solution of compound (**4**) (1.18 g, 3.05 mmol) in ethanol (30 ml) was added to a solution of potassium tetrachloropalladate (0.87 g, 2.66 mmol) in distilled water (30 ml) with continuous stirring. Upon addition an orange solid started to form. Stirring was continued for 5 hours at room temperature then the product was filtered, washed with water (50 ml), ethanol (50 ml), petroleum ether (75 ml), and dried under vacuum. Yield: 0.9 g (42 %). Anal. Calc. for $C_{26}H_{42}N_2PdCl_2 \cdot 1.5H_2O$: C, 53.20; H, 7.73; N, 4.77. Found: C, 53.17; H, 8.22; N, 5.29 %.

^{1}H-NMR (200 MHz, $CDCl_3$): δ: 5.64 (m, 2H, C=CH), 3.4 (m, 2H), 2.0-2.4 (m, 10H), 1.5-2.0 (m, 6H), 1.2-1.5 (m, 6H), 1.17 (s, 6H, CH_3-myrtanyl), 0.8-1.0 (m, 4H), 0.77 (s, 6H, CH_3-myrtanyl). **IR** ν/cm^{-1} = 3127 (m), 2926, 2866 (s), 1625 (m), 1449 (m, sh), 1381 (w), 1364 (w). **MS** (EI): (M / z, intensity %): M^+−2Cl (495, 8), M^+−$PdCl_2$ (382, 70).

3.2.5 Dichloro[1R,2R)-(−)-N^1,N^2-bis{(1R)-(−)myrtenylidene}-1,2-diaminocyclohexane]-platinum(II) (Pt-7)

A solution of compound (**3**) (0.50 g, 1.33 mmol) in ethanol (30 ml) was added to a solution of potassium tetrachloroplatinate (0.45 g, 1.10 mmol) in distilled water (30 ml). Upon addition, a pale yellow solid started to form. Stirring was continued for 5 hours at room temperature then the product was filtered, washed with water (50 ml), ethanol (50 ml), petroleum ether (75 ml), and dried under vacuum. Yield: 0.46 g (66 %). Anal. Calcd. for $C_{26}H_{38}N_2PtCl_2$: C, 48.45; H, 5.94; N, 4.35. Found: C, 48.26; H, 5.72; N, 4.58 %.

IR ν/cm^{-1} = 1594 (s, sh) (C=N). **MS** (EI): (M / z, intensity %): M^+−2Cl (573, 8), M^+−$PtCl_2$ (378, 70).

3.3 Biology

The compounds **Pt-5**, **Pd-6**, and **Pt-7** together with commercial drugs cisplatin and oxaliplatin were tested for their antitumor efficiency on L1210 cancer cell lines. 3H-thymidine was used to measure the prolifiration. IC_{50} values for the compounds were read from the graph obtained (Fig.1). In addition, photometric enzyme-immunoassay Cell Death Detection ELISAPLUS was applied in measurement of apoptotic cell death in cellular systems. 20 µL stock solutions in DMSO were prepared from the compounds. The desired concentrations were obtained by dilution of the stock solution with DMSO. One µL of each dillution was added to 100 µL of medium containing 1×10^4 cells. 3-8 replicants and 96 well culture plates were used in the experiments. The plates were incubated at 37 °C in a humidified 5 % CO_2 atmosphere for 20, 44 or 68 hours. Then, 10 µL of 0.05 % 3H-thymidine solution in medium was added to each well and the plates were incubated for 4 hrs. After harvesting, the cells were calculated with Top Count device. Obtained data was processed with Microsoft Excel. The wells lacking drug served as a control for cell growth.

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References

[1] B. Rosenberg, L. Van Camp, J. E. Trosko, V. H. Mansour, *Nature* **1969**, *222*, 385.
[2] E. Wong, C. M. Giandomenico, *Chem. Rev.* **1999**, *99*, 2451.
[3] A. S. Abu-Surrah, M. Kettunen, *Curr. Med. Chem.* **2006**, *13*, 1337.
[4] Y. Kidani, M. Noji, T. Tashiro, *Jpn. J. Cancer Res.* **1980**, *71*, 637.
[5] T. Tashiro, Y. Kawada, Y. Sakurai, Y. Kidani, *Biomed. Pharmacother.* **1989**, *43*, 251.
[6] J. H. Burchenal, K. Kalaher, K. Dew, L. Lokys, *Cancer Treat. Reports* **1979**, *63*, 1493.
[7] Y. Kidani, K. Inagaki, M. Iigo, A. Hoshi, K. Kuretani, *J. Med. Chem.* **1978**, *21*, 1315.
[8] M. A. Bruck, R. Bau, M. Noji, K. Inagaki, Y. Kidani, *Inorg. Chim. Acta* **1984**, *92*, 279.
[9] A. S. Abu-Surrah, T. A. K. Al-Allaf, M. Klinga, M. Ahlgren, *Polyhedron* **2003**, *22*, 1529.
[10] A. S. Abu-Surrah, B. Rieger, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2475.
[11] For application of chiral amine ligands in catalysis see: H. Brunner, H. Nishiyama, K. Itoh, in "Catalytic Asymmetric Synthesis", I. Ojima (Ed.), Wiley-VCH, **1993**, p. 303−322.
[12] A. S. Abu-Surrah, K. Lappalainen, T. Repo, M. Klinga, M. Leskelä, H. A. Hodali, *Polyhedron* **2000**, *13*, 1601.
[13] A. S. Abu-Surrah, M. Kettunen, K. Lappalainen, U. Piironen, M. Klinga, M. Leskelä, *Polyhedron* **2002**, *21*, 27.
[14] A. S. Abu-Surrah, M. Klinga, M. Leskelä, *Z. Kristallog. NCS* **2002**, *217*, 257.
[15] T. A. K. Al-Allaf, L. J. Rashan, A. S. Abu-Surrah, R. Fawzi, M. Steiman, *Transition Met. Chem.* **1998**, *23*, 403.
[16] A. S. Abu-Surrah, T. A. K. Al-Allaf, L. J. Rashan, M. Klinga, M. Leskelä, *Eur. J. Med. Chem.* **2002**, *37*, 919.

