Available online at www.sciencedirect.com
SCIENCE DIRECT®
Inorganica Chimica Acta 358 (2005) 4237–4246
Inorganica Chimica Acta
www.elsevier.com/locate/ica

Gold(I) and silver(I) complexes of 2,3-bis(diphenylphosphino)maleic acid: Structural studies and antitumour activity

Susan J. Berners-Price [a,b,*], Richard J. Bowen [b,c,d,*], Manuel A. Fernandes [c], Marcus Layh [c,*], William J. Lesueur [b], Sherika Mahepal [e], Matolwandile M. Mtotywa [d], Rodney E. Sue [b], Constance E.J. van Rensburg [e]

[a] School of Biomedical and Chemical Sciences, University of Western Australia, Perth, Western Australia 6009, Australia
[b] School of Science, Griffith University, Nathan, Brisbane, Qld 4111, Australia
[c] Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Private Bag 3, Wits, 2050 Johannesburg, South Africa
[d] Project AuTEK, Mintek, Private Bag X 3015, Randburg 2125, South Africa
[e] Department of Pharmacology, Faculty of Health Sciences, University of Pretoria, PO Box 2034, Pretoria 0001, South Africa

Received 31 January 2005; accepted 26 March 2005
Available online 24 May 2005

This contribution is dedicated to Prof. Hubert Schmidbaur.

Abstract

The 2:1 and 1:2 adducts of Au(I) and 1:2 adducts of Ag(I) with the diphosphine 2,3-bis(diphenylphosphino)maleic acid (dpmaa) have been prepared in high yields. Crystal structures have been determined for the neutral digold complex $(AuCl)_2$(dpmaa) · 2thf (**1**) and the bis-chelated complex $[Au(dpmaa)_2]Cl \cdot H_2O \cdot CH_3OH$ (**2**). For **1**, conformational rigidity imposed by the ethylenic bridge facilitates the formation of short intramolecular Au–Au contacts with no evidence of similar intermolecular contacts. Complex **2** crystallizes with $[Au(dpmaa)_2]^+$ cations hydrogen bonded through the carboxyl groups to a water molecule and chloride anion to form a H-bonded chain along the a axis. ^{31}P NMR titration of **1** with dpmaa in acetone shows conversion to **2** at Au:P–P ratios less than 1:1 indicating similar high thermodynamic and kinetic stabilities to other bis-chelated $[Au(P–P)_2]^+$ complexes containing 5- or 6-membered chelate rings. The ionic Au(I) complex **2** and the analogous Ag(I) complex $[Ag(dpmma)_2]NO_3$ (**3**) are highly water soluble. The in vitro cytotoxic activity of **2** was assessed against eight different cell lines and no significant activity was found. The solubility properties and solution behaviour of the complexes are compared to the analogous 1,2-bis(diphenylphosphino)ethane (dppe) complexes and the potential significance of these results to the antitumour properties of chelated 1:2 Au(I)diphosphine complexes are discussed.

Keywords: Gold complexes; Silver complexes; Phosphine ligands; Antitumour agents

1. Introduction

Generally, bis-chelated 1:2 M(I) diphosphine complexes of the type $[M\{R_2P(CH_2)_nPR_2\}]^+$, where M = Au(I), Ag(I) or Cu(I) and R = aryl, n = 2 or 3 and analogous complexes with *cis*-$R_2PCH{=}CHPR_2$, exhibit antitumour activity [1]. In particular, the Au(I) complex $[Au(dppe)_2]Cl$ (where dppe is 1,2-bis(diphenylphosphino)ethane) showed potent activity against a range of tumour models in mice, including a cisplatin-resistant subline of P388 leukaemia [2]. While there is evidence that mitochondria are implicated in the mechanism of cytotoxicity for this class of lipophilic cations [3], the clinical development of $[Au(dppe)_2]Cl$ was precluded

* Corresponding authors. Tel.: +61 8 6488 3258; fax: +61 8 6488 1005.
E-mail address: sbp@chem.uwa.edu.au (S.J. Berners-Price).

0020-1693/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ica.2005.03.056

as a result of toxic side effects associated with altered mitochondrial function [4]. The lipophilic cationic properties of $[Au(dppe)_2]^+$ promote its non-selective uptake into mitochondria in all cells, with heart and liver tissue being particularly sensitive owing to the high numbers of mitochondria. For other large lipophilic cations, such as bis quarternary ammonium heterocycles [5] and trialkylphosphonium salts [6] a relationship between antitumour selectivity and lipophilic–hydrophilic balance was demonstrated. Our strategy has been to develop more hydrophilic analogues of $[Au(dppe)_2]^+$ that may be less toxic than the parent compound and retain the antitumour activity. We have shown previously for Au(I) and Ag(I) complexes of type $[M\{R_2P(CH_2)_nPR_2\}_2]^+$ that replacing the phenyl groups with pyridyl substituents [7,8], resulted in a series of complexes with different hydrophilic–lipophilic balance, which exhibited differences in cellular uptake and hence differences in antitumour selectivity, potency and hepatotoxicity [9–11].

In this work, we have adopted a different approach, which also retains the aromatic substituents, which appear to be important for antitumour activity [1] but introduces hydrophilic groups into the ethane bridge of dppe to increase water solubility. Here, we describe the preparations, stabilities and structural studies in both solid state and in solution of the Au(I) and Ag(I) complexes of the known ligand, 2,3-bis(diphenylphosphino)maleic acid (dpmaa) [12]. The results obtained from cytotoxicity assays of the Au(I) complex against tumour cell lines in vitro are also discussed.

2. Experimental

All manipulations were carried out under argon, using standard Schlenk techniques. Solvents were distilled from drying agents and degassed. The NMR spectra were recorded on the following Bruker instruments: DRX 400 (^{1}H, 400.32; ^{31}P, 161.98 MHz), Avance 300 (^{1}H, 300.13; ^{13}C, 75.5; ^{31}P 121.5 MHz) or AC 200 (^{1}H, 200.13 MHz). ^{1}H and ^{13}C{^{1}H}NMR spectra were referenced using the residual solvent signal as the internal standard, whereas ^{31}P–{^{1}H}NMR spectra were referenced to external H_3PO_4 (85%). NMR data for the complexes are shown in Table 1. Elemental analyses were determined by the Institute for Soil, Climate and Water, Pretoria, South Africa or by the Australian Microanalytical Service, Victoria, Australia.

Data sets for compounds **1** and **2** were collected on a Bruker SMART 1K CCD area detector, using monochromatic Mo Kα radiation. Data reduction was carried out using the program SAINT [13a] and absorption corrections were made using the program SADABS [13b]. The structures were solved by direct methods using SHELXTL [14]. Refinement was based on F^2 with hydrogen atoms in riding mode, using SHELXTL [14]. Publication materials were generated using SHELXTL [14], PLATON [15] and SCHAKAL-97 [16]. Further details are found in Table 4.

Table 1
NMR data for dpmaa and the Au(I) complexes **1** and **2** (in $CDCl_3$)

	dpmaa	$(AuCl)_2$(dpmaa) (**1**)	$[Au(dpmaa)_2]Cl$ (**2**)
δ^{13}C (J_{PC}, Hz)[a]			
C_i	132.1	125.9 (32)	128.6 (m)
C_o	134.1 (11)	133.9 (7)	131.5 (m)
C_m	128.7 (4)	128.5 (6)	128.1 (m)
C_p	129.9	131.8	130.0
C=C	150.0 (m)	143.5 (23)	151.2 (m)
COOH	169.1 (4)	164.7 (10)	165.0 (m)
δ^1H			
ArH	7.25 (m)	7.52 (m)	7.20 (m)
COOH	9.11	–[b]	–[b]
δ^{31}P[c]			
	−10.9	21.9	28.1

[a] J_{PC} couplings (where resolved) are observed as virtual triplets for **1** and dpmaa and second-order multiplets for **2**.
[b] Not observed.
[c] In d_6-acetone.

2.1. Preparation of compounds

The ligands 2,3-bis(diphenylphosphino)maleic anhydride (dpma) [17] and 2,3-bis(diphenylphosphino)maleic acid (dpmaa) [12] were prepared by literature methods. $Na[AuCl_4] \cdot 2H_2O$ was purchased from Johnson Matthey, while $ClAuSMe_2$ was prepared by the oxidation of gold metal with dimethylsulfoxide, as described elsewhere [18].

2.1.1. $(AuCl)_2$(dpmaa) (**1**)

(a) Thiodiglycol (0.19 g, 1.62 mmol) in thf (2 ml) was quickly added to an orange solution of $NaAuCl_4 \cdot H_2O$ (0.307 g, 0.808 mmol) in deionised water (5 ml) at 0 °C. After stirring for 5 min, the resulting colourless solution was added to a light yellow solution of dpmaa · $1.5Et_2O$ (0.241 g, 0.404 mmol) in thf (10 ml). The solution became dark orange, and after 10 min, the solvents were removed in vacuo to give a pale yellow gum. The material was redissolved in a thf:Et_2O mixture (1:1) and after refrigeration at 4 °C overnight, yellow crystals were produced and collected by filtration. Elemental analysis *Anal.* Calc. for $C_{28}H_{22}O_4P_2Au_2Cl_2 \cdot 1.5C_4H_8O$: C, 38.7; H, 3.1. Found: C, 38.5; H, 2.9%. The presence of thf solvate was verified by ^{1}H NMR spectroscopy. IR (nujol): $\bar{\nu} = 3378$ (ν_{OH}), 2728 (ν_{COOH}), 2594 (ν_{COOH}), 1719 (ν_{CO}), 1555 (ν_{ArH}) cm^{-1}.

(b) $ClAuSMe_2$ (0.238 g, 0.808 mmol) was added to dpmaa · $1.5Et_2O$ (0.241 g, 0.404 mmol) in thf (20 ml) at room temperature. The reaction mixture was stirred for 30 min, the solvents removed in vacuo, and the resultant residue re-dissolved in a 1:1 thf:Et_2O mixture (30 ml

total). Colourless crystals of **1** · 2thf suitable for crystallographic analysis were formed upon standing overnight, and were collected by filtration (0.330 g, 75% yield). m.p. 131–142 °C. The presence of two molecules of thf was further confirmed by ^{1}H NMR spectroscopy.

*2.1.2. [Au(dpmaa)$_2$]Cl (**2**)*

(a) Thiodiglycol (0.048 g, 0.404 mmol) in thf (2 ml) was added quickly to an orange solution of $NaAuCl_4 \cdot H_2O$ (0.077 g, 0.202 mmol) in deionised water (3 ml) at 0 °C. After 10 min, the resulting colourless solution was added to a light yellow solution of dpmaa · 1.5Et$_2$O (0.241 g, 0.404 mmol) in thf (10 ml). The solution became deep orange, and after 10 min the solvents were removed in vacuo to give an orange gum. Treatment with ether (25 ml) provided an orange suspension which was filtered, washed with ether and air dried to give **2** · 2H$_2$O (0.214 g, 88% yield). Elemental analysis *Anal.* Calc. for $C_{56}H_{44}O_8P_4AuCl \cdot 2H_2O$: C, 54.4; H, 3.9; P, 10.0. Found: C, 54.4; H, 3.8; P, 8.9%. m.p. 195–196 °C (sublimed). IR (nujol): $\bar{\nu} = 3378$ (ν_{OH}), 2728 (ν_{COOH}), 2594 (ν_{COOH}), 1719 (ν_{CO}), 1555 (ν_{ArH}) cm^{-1}.

(b) A solution of dpmaa · 1.5Et$_2$O (0.4 g, 0.67 mmol) in Et$_2$O (20 ml) was cooled to 0 °C and $ClAuSMe_2$ (0.097 g, 0.33 mmol) was added in one quantity. The reaction was stirred for 30 min at room temperature and the solvent and volatiles were removed in vacuo yielding **2** · Et$_2$O (0.412 g, 98 % yield). The presence of a monoether solvate was confirmed by ^{1}H NMR.

(c) dpma (0.4 g, 0.858 mmol) was dissolved in thf (10 ml) and the solution cooled to 0 °C. $ClAuSMe_2$ (0.124 g, 0.42 mmol) was added in one quantity, resulting in a dark coloured reaction mixture. After stirring for 30 min at room temperature H_2O (ca. 2 ml) was added, resulting in an immediate colour change to give a light orange solution. All solvents and volatiles were removed in vacuo, affording a dark orange solid, which was recrystallized from either ethylacetate or acetonitrile at −20 °C. The resultant orange coloured microcrystalline material was collected by filtration and dried in vacuo (0.46 g, 89% yield). Crystals of $[Au(dpmaa)_2]Cl \cdot H_2O \cdot CH_3OH$ suitable for X-ray crystallography were obtained by slow evaporation of a saturated methanol solution of the product. Elemental analysis, *Anal.* Calc. for $C_{56}H_{44}O_8P_4AuCl \cdot H_2O \cdot CH_3OH$: C, 54.7; H, 4.2. Found: C, 54.2; H, 4.1%.

(d) $[(AuCl)_2(dpmaa)] \cdot 2thf$ (0.01 g, 0.00915 mmol) was dissolved in d_6-acetone (0.75 ml) and to this was added 3 mol equiv of dpmaa · 1.5Et$_2$O (0.0164 g, 0.0275 mmol). ^{31}P NMR analysis confirmed the complete conversion to the bis-chelated cation $[Au(dpmaa)_2]^+$.

*2.1.3. [Ag(dpmaa)$_2$]NO$_3$ (**3**)*

(a) dpma (0.4 g, 0.858 mmol) was dissolved in thf (10 ml) and $AgNO_3$ (0.066 g, 0.39 mmol) in H_2O (1 ml) was added. The colour changed initially from orange to dark orange as the silver salt was added and then clarified, becoming light orange after 10 min of stirring. After stirring for 4 h the solvent was removed in vacuo, affording a light brown solid. An analytically pure yellow sample of **3** was obtained by washing the solid with water and drying it over P_2O_5 (0.39 g, 88% yield). m.p. 166–170 °C (dec.). Elemental analysis, *Anal.* Calc. for $C_{56}H_{44}NAgO_{11}P_4$: C, 59.1; H, 3.9; N, 1.2. Found: C, 59.0; H, 4.2; N 1.0%.

(b) To a light yellow solution of dpmaa (0.256 g, 0.404 mmol) in acetone (20 ml) was added $AgNO_3$ (0.034 g, 0.202 mmol) followed by H_2O (0.5 ml). The dissolution of $AgNO_3$ was accompanied by an intensification of the yellow colouration. After 12 h of stirring at room temperature, the solvents were removed in vacuo. The resultant yellow gum was treated with Et$_2$O, producing a yellow precipitate. The suspension was filtered, washed with Et$_2$O and dried by air, thereby affording the pentahydrate of the title compound (0.22 g, 96% yield). Elemental analysis, *Anal.* Calc. for $C_{56}H_{44}NAgO_{11}P_4 \cdot 5H_2O$: C, 54.7; H, 4.4; N, 1.1. Found: C, 54.7; H, 4.1; N, 0.9%.

2.2. Cell culture and treatment

The following cell lines were used to investigate the in vitro cytotoxic potency of $[Au(dpmaa)_2]Cl$: human ovarian carcinoma (A2780)(ECACC 93112519) and the cisplatin resistant subline (A2780cis)(ECACC 93112517); breast carcinoma (MCF-7)(HTB-22); colon cancer (COLO 320 DM)(CCL-220); cervical carcinoma cells (HeLa)(CCL-2); human primary fibroblasts (MRC-5) (CCL-171); breast non-tumourogenic cell line (MCF-12A)(CRL-10782) and mouse melanoma (B16) (ECACC 92101203). Unless stated the reagents have been obtained from Sigma Chemical Company (St. Louis, MO).

A2780, A2780cis, COLO 320 DM, were maintained in RPMI 1640, while HeLa and MRC-5 were maintained in EMEM and B16 was maintained in DMEM (National Institue for Communicable Diseases, Johannesburg, SA). The media for the above-mentioned cell lines were supplemented with 10% fetal calf serum (FCS) (Sterilab, Johannesburg, SA) and 1% penicillin–streptomycin (Sterilab, Johannesburg, SA). MCF-7 was maintained in DMEM that was supplemented with 10% FCS, 1% penicillin–streptomycin and 2% essential amino acids (National Institue for Communicable Diseases, Johannesburg, SA). MCF-12A was maintained in DMEM/HAMS F12 solution that was prepared by adding HAMS F12 solution (HAMS F12, $NaHCO_3$ and Milli Q water) to DMEM, which was supplemented with 10 ml of DMEM that contained 0.5% Hydrocortisone, 1% cholera toxin, 5% insulin and 2% epidermal growth factor. For growth

inhibition studies, $[Au(dpmaa)_2]Cl$ was dissolved in dimethylsulfoxide (Merck, Darmstadt, Germany) and then diluted with supplemented medium to reach the planned concentrations (0.25 μM–200 μM).

2.2.1. Cytotoxicity

Cells (100 μl) were seeded in 96-well round-bottomed plates and incubated for 24 h at 37 °C with 5% CO_2. After 24 h, 80 μl of supplemented medium and 20 μl of increasing concentrations of $[Au(dpmaa)_2]Cl$ (**2**) were added to each well and cells were further incubated for 96 h. Growth inhibition was assayed with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (20 μl of a 5 mg/ml MTT solution), which detects only viable cells [19]. The plates were read on a UV 900 Micro-ELISA reader using a test wavelength of 540 nm. Untreated control wells (100% viability) were included in all the experiments and all the results represent an average of 3–5 assays. The average inhibitory concentration (IC_{50}) was determined.

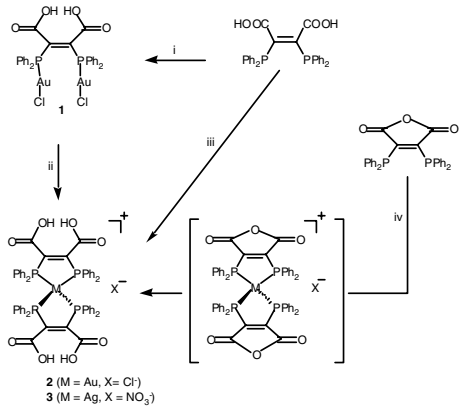
3. Results and discussion

3.1. Synthesis of compounds

In these studies, the Au(I) complexes **1** and **2** were prepared either by a similar method described previously for $(AuCl)_2(dppe)$ [20] involving the addition of stoichiometric amounts of dpmaa to either a solution of $Na[AuCl_4]$ that had been reduced to Au(I) in situ by reaction of Au(III) with thiodiglycol, or by direct reaction with the Au(I) salt, $ClAuSMe_2$ [(i) and (iii) in Scheme 1]. While preparations utilising the thiodiglycol reduction are suitable for synthesising less water soluble complexes such as $(AuCl)_2(dppe)$, the water soluble cationic complexes derived from dpmaa are difficult to separate from the water soluble thiodiglycol oxidation product, thus accounting for the lower yields. These complexes are better prepared by the reaction with previously prepared anhydrous gold(I) salts, for example, in the present studies $[Au(dpmaa)_2]Cl$ is best formed by additions of stoichiometric quantities of dpmaa to the di-gold bridged complex **1** [(ii) in Scheme 1] or the thioether complex $ClAuSMe_2$ [(iii) in Scheme 1]. Reactions with $ClAuSMe_2$ are particularly attractive because $ClAuSMe_2$ is an anhydrous, easily prepared air stable compound [18] which when reacted with a phosphine gives a clean product owing to the concomitant facile elimination of SMe_2.

Alternatively, $[Au(dpmaa)_2]Cl$ (**2**) is prepared by the reaction of two mol. equivalents of dpma with an aqueous mixture of Au(I) [(iv) in Scheme 1]. The reaction proceeds through a series of colour changes, with the initial orange anhydrous thf solution of dpma darkening upon the addition of $Au(I)_{aq}$ and then lightening to become a light clear yellow solution. For previously reported CuCl complexes of dpma, a dark species was obtained under anhydrous conditions corresponding to $[CuCl(dpma)_2]$ but water addition was accompanied by lightening of the product and ring opening of the anhydride bridge to form $[Cu(dpmaa)_2]^+$ [21]. Similarly, it is reasonable that complexation of dpma to Au(I) greatly enhances the susceptibility of the anhydride functionality toward nucleophilic attack by water and subsequent ring opening to form the carboxylate species $[Au(dpmaa)_2]^+$. The presence of water on its own is not sufficient to ring-open dpma, since reflux under basic conditions is required to effect the transformation to dpmaa [12]. The $AgNO_3$ analogue $[Ag(dpmaa)_2]NO_3$, (**3**) is prepared similarly in high yield by the reaction of $AgNO_3$ with either two mol. equivalents of dpmaa or dpma [(iii) and (iv) in Scheme 1] in an aqueous solvent.

While all the preparations undertaken in this work are best carried out under argon, the complexes formed are stable in air and in solution. The ionic species, **2** and **3** are highly water-soluble. On the other hand the neutral complex **1** has limited aqueous solubility, but is soluble in polar organic solvents, such as acetone,



Reactions and conditions

Compound	Reaction	Reagents	Solvent
1	(i)	2 mol $NaAuCl_4/S(CH_2CH_2OH)_2$	thf, H_2O
		or	
		2 mol $ClAuSMe_2$	thf
2	(ii)	3 mol dpmaa	acetone
	(iii)	0.5 mol $NaAuCl_4/S(CH_2CH_2OH)_2$	thf, H_2O
		or	
		0.5 mol $ClAuSMe_2$	Et_2O
	(iv)	0.5 mol $ClAuSMe_2$	thf, H_2O
3	(iii)	0.5 mol $AgNO_3$	acetone, H_2O
	(iv)	0.5 mol $AgNO_3$	thf, H_2O

Scheme 1. Preparations of Au(I) and Ag(I) complexes of dpmaa.

thf and methanol. Crystals suitable for X-ray crystallographic analysis of $[Au(dpmaa)_2]Cl \cdot H_2O \cdot CH_3OH$ (**2**) and $(AuCl)_2(dpmaa) \cdot 2thf$ (**1**) were obtained at room temperature from saturated methanol and 1:1 thf:Et_2O solutions, respectively.

3.2. Structures

The molecular structures of $[Au(dpmaa)_2]Cl \cdot H_2O \cdot CH_3OH$ (**2**) and $(AuCl)_2(dpmaa) \cdot 2thf$ (**1**) with the atom numbering scheme are shown in Figs. 1 and 2, while selected bond distances are compared in Table 2. Complex **2** crystallized with one cation, a chloride counterion, a water and a methanol molecule in the asymmetric unit, while **1** crystallized as a neutral dpmaa bridged dinuclear species with the two halves of the molecule related by a two fold rotation axis, containing bonded chlorides, and two tetrahydrofuran molecules in the unit cell. In the crystal structure of complex **2**, discrete $[Au(dpmaa)_2]^+$ cations are hydrogen bonded through the carboxyl groups to a water molecule and chloride anion to form a H-bonded chain along the *a*-axis (Fig. 1(b), Table 3).

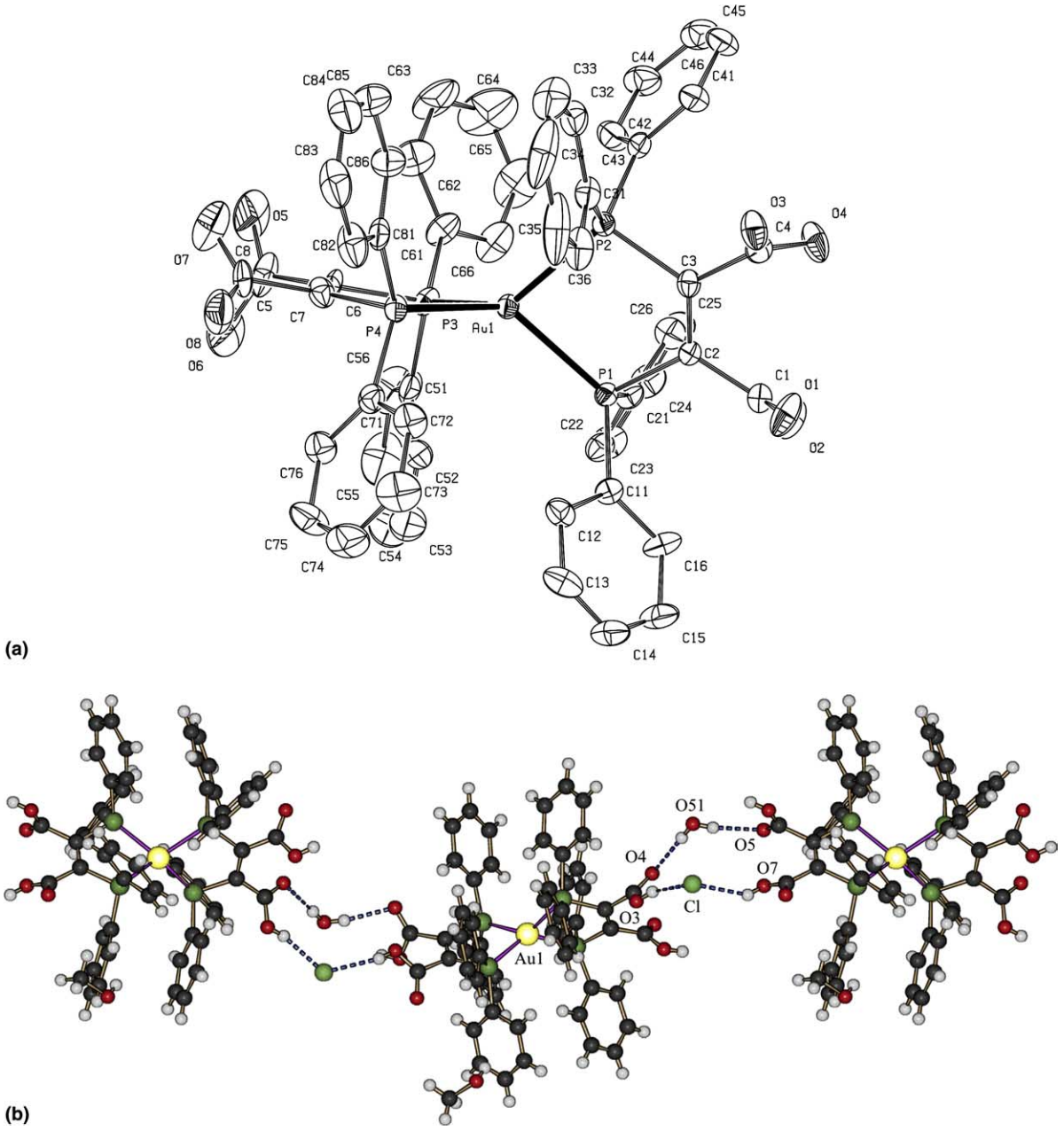


Fig. 1. (a) Molecular structure of the cation of complex **2** (thermal ellipsoids at 20% probability). H atoms have been omitted for clarity (b) H-bonding network linking $[Au(dpmaa)_2]^+$ entities in complex **2**.

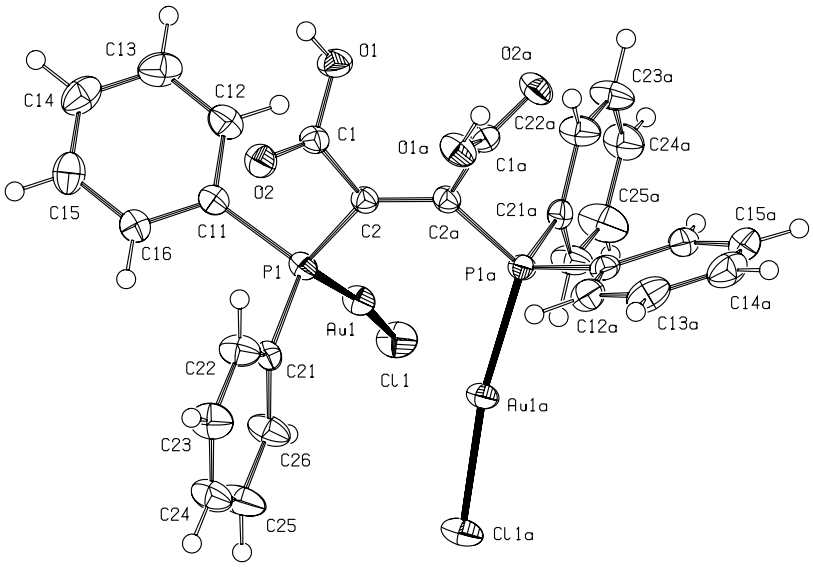


Fig. 2. Molecular structure of complex **1** (thermal ellipsoids at 50% probability).

Similarly, for another hydrophilic cation, [Au(d4pype)$_2$]Cl · HCl · 6H$_2$O [where d4pype is 1,2-bis(di-4-pyridylphosphino)ethane], strong interactions between the 4-pyridyl substituents and the solvated water molecules lead to a well defined hydrogen-bond network between the water molecules, chloride anions and pyridyl nitrogens [8]. Unlike **2**, the structure of **1** is void of hydrogen bonding arrays between (AuCl)$_2$(dpmaa) entities, with discrete hydrogen bonding occurring only between thf molecules and the dpmaa hydroxyl groups. Similar stabilization is provided by a single methanol–hydroxyl group interaction for the bis-chelated species. For digold species, such as (AuCl)$_2$(P–P) or annular [{(AuCl)(P–P)}$_2$] compounds (where P–P is bisphosphine), intramolecular and/or intermolecular Au–Au interactions are conformation determining forces depending on the restrictions imposed by the geometry of the ligand, and become pertinent at interatomic distances of ca. 3.5 Å [22,23]. For example, short intramolecular Au–Au distances are formed with $Ph_2PCH_2PPh_2$ [24] and *cis*-$Ph_2PCH{=}CHPPh_2$ [25] owing to the presence of only one carbon atom in the backbone of the former ligand and the conformational rigidity imposed by the ethylenic bridge in the latter complex [23]. In contrast, for the propane and ethane bridged derivatives, the P–Au–X moieties point away from one another, precluding the possibility of intramolecular Au–Au interactions. In these cases, short intermolecular Au–Au contacts represent the dominating influence in dictating both the crystal packing and the observed molecular conformations, considering the absence of intramolecular Au–Au effects and the lack of significant rigidifying influence of these ligands [23,26]. For (AuCl)$_2$(dpmaa) · 2thf **1**, like (AuCl)$_2$(*cis*-$Ph_2PCH{=}CHPPh_2$), conformational rigidity imposed by the ethylenic bridge facilitates the formation of intramolecular Au–Au contacts [3.1913(4) Å] with no evidence of similar intermolecular

Table 2
Selected bond distances (Å) and angles (°) of complex **1** and **2**

[Au(dpmaa)$_2$]Cl (**2**)		(AuCl)$_2$(dpmaa) (**1**)	
Au(1)–P(1)	2.387(3)	Au(1) ··· Au(1′)[a]	3.1913(4)
Au(1)–P(2)	2.381(2)	Au(1)–P(1)	2.2301(8)
Au(1)–P(3)	2.402(3)	Au(1)–Cl(1)	2.2889(9)
Au(1)–P(4)	2.384(3)		
P(1)–Au(1)–P(3)	109.4(1)	P(1)–Au(1)–Cl(1)	171.73(3)
P(2)–Au(1)–P(1)	86.3(1)		
P(2)–Au(1)–P(4)	115.5(1)		
P(2)–Au(1)–P(3)	131.9(2)		
P(4)–Au(1)–P(1)	134.3(1)		
P(4)–Au(1)–P(3)	86.1(1)		

[a] Symmetry code: ′ $-x+2$, y, $-z+1/2$.

Table 3
H-bonding in complex **2** (bond lengths in Å and angles in °)

D–H	*d*(D–H)	*d*(H ··· A)	∠*d*(D ··· A)	*d*(D ··· A)	A
O(1)–H(1)	0.82	2.21	173	3.03(3)	O(50)
O(7)–H(7)	0.82	2.32	140	3.00(2)	Cl
O(3)–H(3)	0.82	2.19	156	2.95(1)	Cl
O(51)–H(51a)	0.82	2.14	179	2.96(1)	O(4)
O(51)–H(51b)	0.82	2.10	168	2.91(2)	O(5)

Table 4
Crystallographic data for compounds **1** and **2**

Compound	**1** · 2thf	**2** · H_2O · CH_3OH
Formula	$C_{36}H_{38}Au_2Cl_2O_6P_2$	$C_{57}H_{50}AuClO_{10}P_4$
Formula weight	1093.44	1251.27
Temperature (K)	173(2)	293(2)
Wavelength	0.71073	0.071073
Crystal system	monoclinic	orthorhombic
Space group	*C*2/*c*	$Pna2_1$
Unit cell dimensions		
a (Å)	17.979(2)	29.600(3)
b (Å)	15.284(2)	11.669(1)
c (Å)	15.335(2)	17.230(2)
β (°)	117.181(2)	–
U (Å^3)	3748.4(7)	5951.3(10)
Z	4	4
D (Mg/m^3)	1.938	1.397
μ (mm^{-1})	8.090	2.677
F(000)	2096	2512
θ Range (°)	1.84–28.29	1.38–25.00
Index ranges	$-9 \leqslant h \leqslant 23, -20 \leqslant k \leqslant 20, -20 \leqslant l \leqslant 16$	$-35 \leqslant h \leqslant 35, -13 \leqslant k \leqslant 13, -20 \leqslant l \leqslant 16$
Reflections collected	12927	46535
Unique reflections [R_{int}]	4634 [0.040]	8488 [0.083]
Reflections with $I > 2\sigma(I)$	3943	5734
Data/restraints/parameter	4634/0/217	8488/1/587
Final *R* indices (for $I > 2\sigma(I)$)	$R_1 = 0.022$	$R_1 = 0.048$
	$wR_2 = 0.054$	$wR_2 = 0.116$
R indices (all data)	$R_1 = 0.031$	$R_1 = 0.089$
	$wR_2 = 0.054$	$wR_2 = 0.132$
Largest differences in peak/hole (e Å^{-3})	0.705, −1.143	1.155, −0.523

contacts. The configuration at the gold atoms is close to linear in the dpmaa complex (see Table 2), and the individual atom P–Au–Cl triples are crossed like swords with their neighbouring units, as observed previously, for example, for $(TP)(AuCl)_2$ [TP = bis{2-(diphenylphosphino)phenylene}phenylphosphane] [27]. The Au–P and Au–Cl bond lengths of 2.2301(8) Å and 2.2889(9) Å compare well with Au–P and Au–Cl distances in other $(AuCl)_2$(P–P) complexes [25–28].

By comparison, the four coordinated Au(I) complex, $[Au(dpmaa)_2]Cl \cdot H_2O \cdot CH_3OH$ contains longer Au–P bonds [Au–$P_{ave.}$ = 2.389(3) Å], which reflects the expected lengthening upon increasing the coordination number to four. The gold atom lies in a distorted-tetrahedral array of phosphorus atoms, with all P–Au–P angles exhibiting deviations from the ideal angles of 109.5° (see Table 2). The smaller bite angles of 86.3(1)° and 86.1(1)° for P(1)–Au(1)–P(2) and P(3)–Au(1)–P(4), respectively, are values largely imposed by the geometry of the five-membered ring formed by chelating dpmaa ligands. All Au–P bond lengths and P–Au–P angles in this complex correspond closely to those reported for other monomeric bis-chelated tetrahedral $[Au(P–P)_2]^+$ complexes, ranging from 2.36(1) to 2.417(9) Å and 85.4(1)° to 137.5(3)°, respectively [8,29,30]. Previously, the flexibility of the C–C–P angles in dpmaa has been noted [12]. These angles are 131.1(3)° and 130.2(3)° in the free ligand, while upon coordination to Au(I), these angles average 127.25(9)° and 122(1)° [121.3(9)° and 121.9(12)° for each ligand] in $(AuCl)_2(dpmaa) \cdot 2thf$ (**1**) and $[Au(dpmaa)_2]Cl \cdot H_2O \cdot CH_3OH$ (**2**), respectively.

3.3. Solution behaviour

In previous studies, we have shown that the formation of stable bis-chelated $[Au(P–P)_2]^+$ complexes in the solution state can be conveniently monitored by ^{31}P NMR titration of the bridged di-gold complex $(AuCl)_2$(P–P) with free ligand (P–P) [8,20,29]. With the aim of investigating the formation of the four coordinate $[Au(dpmaa)_2]^+$ complex a similar study was conducted as part of the present work. The ^{31}P NMR spectrum of $(AuCl)_2(dpmaa)$ (**1**) in acetone consists of a single resonance at δ 21.9. With addition of 0.5 mol. equivalents of dpmaa this peak broadens marginally and shifts to slightly higher frequency and a new sharp peak appears at δ 28.1. With further addition of dpmaa the high field peak broadens further and decreases in intensity, while the peak at δ 28.1 increases in intensity and remains sharp with each addition until the Au:dpmaa ratio is greater than 1:2, when a peak for free dpmaa (δ −10.9) appears. Both peaks remained sharp showing a slow rate of ligand

exchange on the NMR time scale. The resonance at δ 28.1 is assigned to the four-coordinate bis-chelated complex ion $[Au(dpmaa)_2]^+$ on the basis of the chemical shift which is the same as that of the isolated compound **2**. This behaviour is analogous to that observed previously for the bridged di-gold complex $(AuCl)_2(dppe)$ on titration with dppe [20,29], in which the tetrahedral cation $[Au(dppe)_2]^+$ is formed on addition of dppe:

$$(AuCl)_2(dppe) + 3dppe \rightarrow 2[Au(dppe)_2]Cl$$

The bis-chelated four-coordinated species was present in solution on addition of less than stoichiometric amounts of dppe and exchange of free and bound ligand occurred at a rate of $\ll 800\ s^{-1}$ at room temperature, demonstrating much enhanced thermodynamic and kinetic stability compared to that of $[Au(PR_3)_4]^+$ containing monodentate phosphines. Similar behaviour was found for other $[Au(P–P)_2]^+$ with five or six-membered chelate rings [8,20]. In the case of dpmaa, as for the ligand *cis*-$Ph_2PCH{=}CHPPh_2$ (dppey) [20], chelation is facilitated since the geometry of the ligand is constrained to favour ring closure, while for dppe the most populated rotamers would be expected to be those of the *trans*-P configuration about the C–C single bond. A noteworthy observation is that the coordination chemical shift (ccs) for **1** (32.8 ppm) is less than that of **2** (39.0 ppm) showing a similar trend to that of $(AuCl)_2(dppey)$ (ccs 36.1 ppm) and $[Au(dppey)_2]^+$ (ccs 45.7 ppm) [20], whereas for other bidentate phosphines the ccs of the bridged di-gold complex $(AuCl)_2(P–P)$ is greater than that of the bis-chelated species $[Au(P–P)_2]^+$ [20]. The increased shielding of the ^{31}P nuclei in the ethylene bridged complexes may be a consequence of the intermolecular Au–Au interactions and it is notable that the ethylenic carbons in **1** are significantly shielded in comparison to those in **2** and the free ligand dpmaa (Table 1). The observation of deshielded aromatic protons in **1** and slightly shielded aromatic protons in **2**, relative to free dpmaa, is consistent with noted observations for analogous dppe complexes which were attributed to ring current effects involving adjacent phenyl rings in the tetrahedral bis-chelated complexes [20].

The ^{31}P NMR spectrum of the bis-chelated Ag(I) complex **3** in acetone consists of two overlapping doublets (δ 3.0, intensity ratio 51:49) with ^1J ($^{107/109}Ag–^{31}P$) values (233, 266 Hz) typical of those expected for bis-chelated complexes with tetrahedral AgP_4 coordination [7,31]. The observation of resolved $^{107/109}Ag–^{31}P$ spin–spin couplings at room temperature is consistent with the behaviour observed for other 1:2 adducts of $AgNO_3$ with bidentate arylphosphines [7,31] and shows remarkably enhanced kinetic and thermodynamic stability when compared to similar Ag(I) complexes of monodentate phosphines.

3.4. Antitumour activity

$[Au(dpmaa)_2]Cl$ (2) exhibited no cytotoxicity ($IC_{50} > 200\ \mu M$) in six of the eight cell lines tested. The only two cell types that were sensitive to this poorly active compound are MCF-7 and HeLa cells with IC_{50} values of 91 and 164 μM, respectively. These results are in stark contrast to that of $[Au(dppe)_2]Cl$ which is cytotoxic to cultured tumour cells in vitro at concentrations as low as 0.08 μM [11], with an IC_{50} value of 2 μM reported for B16 cells following a 2 h exposure [2]. An important consideration for biological evaluation of metal compounds is whether they are stable in solution and especially under the testing conditions, as a lack of activity may simply be a reflection of the instability of the complex. Gold(I) compounds, in particular, often react readily with thiols and cytotoxicity can be reduced by reaction with –SH groups in serum and cell culture media. For bis-chelated Au(I) complexes the high thiol reactivity is considerably reduced and $[Au(dppe)_2]Cl$ has been shown to be stable in the presence of thiols and to remain intact in human plasma [2,32]. The ^{31}P NMR titration data show that the dpmaa complex **2** has similar high thermodynamic and kinetic stabilities compared to $[Au(dppe)_2]^+$ and ^{31}P NMR studies confirmed that the complex remains intact after prolonged incubation in RPMI-1640 cell culture media containing 5% fetal calf serum. The lack of cytotoxicity may be a reflection of a low degree of cellular uptake of this hydrophilic complex. For pyridyl phosphine analogs of $[Au(dppe)_2]^+$, a direct correlation was observed between cellular uptake and growth inhibition of CH-1 ovarian cancer cells and the lipophilicity of the Au(I) phosphine complex [11]. The Au(I) 4-pyridyl complex $[Au(d4pype)_2]Cl$ is highly water soluble and exhibited selective activity (500-fold range) against a panel of human ovarian cells showing activity against CH1 and CH1cisR (IC_{50} 3.09, 6.82 μM) but no activity ($IC_{50} > 1000\ \mu M$) in 41M, 41McisR or SKOV3 [10]. From the crystal structure of $[Au(dpmaa)_2]Cl$, it is apparent that the high water solubility is a consequence of the exposed carboxyl groups and their propensity to hydrogen bond as evident by well defined hydrogen bonding networks in the crystal lattice. Similar H-bonding networks account for the hydrophilicity of the Au(I) 4-pyridyl complex. It is possible that complex **2** may also exhibit selective cytotoxicity to only certain cell lines such as the CH1 pair. However, an important distinction is that for the 4-pyridyl analog of $[Au(dppe)_2]^+$ hydrophilicity is achieved whilst retaining the positive charge on the complex and the likely protonation of at least one of the 4-pyridyl nitrogens at physiological pH [8] will increase the overall charge. On the other hand for **2** the carboxyl groups are likely to be deprotonated under physiological conditions so that the complex has an overall negative charge. Thus the lack of antitumour

activity of **2** supports the hypothesis that $[Au(dppe)_2]^+$ and related antitumour compounds act by an antimitochondrial mechanism and accumulate in tumour cell mitochondria as a result of the lipophilic–cationic properties [33]. All known active analogs of $[Au(dppe)_2]^+$ are lipophilic cations (albeit with different hydrophilic–lipophilic character and charge), which are stable in the presence of thiols, with aromatic substituents reducing the likelihood of oxidative side reactions in comparison to alkyl-substiututed phosphines [1,34]. The studies reported here suggest that attempts to reduce the toxicity of $[Au(dppe)_2]^+$ by the introduction of negatively charged hydrophilic groups into the ethane bridge of dppe will lead to a loss of antitumour activity.

Acknowledgements

This work was supported by the Australian Research Council, the Australian National Health & Medical Research Council, Mintek (project AuTEK), CANSA, THRIP and the University of the Witwatersrand. We thank Mr Messai Mamo for assistance with some of the experiments.

Appendix A. Supplementary data

Full crystallographic data (CCDC No. 261257 for compound **1**, 261256 for compound **2**) have been deposited at the Cambridge Crystallographic Database Centre and are available on request from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc,cam.ac.uk or http://www.ccdc.cam.ac.uk). Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.ica.2005.03.056.

References

[1] S.J. Berners-Price, P.J. Sadler, Struct. Bond. (Berlin) 70 (1988) 27.
[2] S.J. Berners-Price, C.K. Mirabelli, R.K. Johnson, M.R. Mattern, F.L. McCabe, L.F. Faucette, C.-M. Sung, S.-M. Mong, P.J. Sadler, S.T. Crooke, Cancer Res. 46 (1986) 5486.
[3] (a) Y. Dong, S.J. Berners-Price, D.R. Thorburn, T. Antalis, J. Dickinson, T. Hurst, L. Qui, S.K. Khoo, P.G. Parsons, Biochem. Pharmacol. 53 (1997) 1673;
(b) M.J. McKeage, L. Maharaj, S.J. Berners-Price, Coord. Chem. Rev. 232 (2002) 127.
[4] (a) G.D. Hoke, G.F. Rush, G.E. Bossard, J.V. McArdle, B.D. Jensen, C.K. Mirabelli, J. Biol. Chem. 263 (1988) 11203;
(b) P.F. Smith, G.D. Hoke, D.W. Alberts, P.J. Bugelski, S. Lupo, C.K. Mirabelli, G.F. Rush, J. Pharmacol. Exp. Therap. 249 (1989) 944.
[5] W.A. Denny, G.J. Atwell, B.C. Baguely, B.F. Cain, J. Med. Chem. 22 (1979) 134.
[6] D.C. Rideout, T. Calogeropoulou, J.S. Jaworski, R.J. Dagino, M.R. McCarthy, Anti-Cancer Drug Design 4 (1989) 265.
[7] S.J. Berners-Price, R.J. Bowen, P.J. Harvey, P.C. Healy, G.A. Koutsantonis, J. Chem. Soc., Dalton Trans. (1998) 1743.
[8] S.J. Berners-Price, R.J. Bowen, T.W. Hambley, P.C. Healy, J. Chem. Soc., Dalton Trans. (1999) 1337.
[9] S.J. Berners-Price, R.J. Bowen, M.J. McKeage, P. Galettis, L. Ding, C. Baguley, W. Brouwer, J. Inorg. Biochem. 67 (1997) 154.
[10] S.J. Berners-Price, R.J. Bowen, P. Galettis, P.C. Healy, M.J. McKeage, Coord. Chem. Rev. 185–186 (1999) 823.
[11] M.J. McKeage, S.J. Berners-Price, P. Galettis, R.J. Bowen, W. Brouwer, L. Ding, L. Zhuang, B.C. Baguley, Cancer. Chemother. Pharmacol. 46 (2000) 343.
[12] A. Avey, D.M. Schut, J.R. Weakly, D.R. Tyler, Inorg. Chem. 32 (1993) 233.
[13] (a) Bruker, SAINT+. Version 6.02 (includes XPREP and SADABS), Bruker AXS Inc., Madison, WI, USA, 1999;
(b) G.M. Sheldrick, SADABS, University of Göttingen, Göttingen, 1996.
[14] Bruker, SHELXTL, Version 5.1 (includes XS, XL, XP, XSHELL), Bruker AXS Inc., Madison, WI, USA, 1999.
[15] A.L. Spek, J. Appl. Cryst. 36 (2003) 7.
[16] (a) E. Keller, J. Appl. Crystallogr. 22 (1989) 19;
(b) E. Keller, University of Freiburg, Germany, 1997.
[17] D. Fenske, H.J. Becher, Chem. Ber. 107 (1974) 117.
[18] (a) T.E. Müller, J.C. Green, D.M.P. Mingos, C.M. McPartlin, C. Wittingham, D.J. Williams, T.M. Woodroffe, J. Organomet. Chem. 551 (1998) 313;
(b) K.C. Dash, H. Schmidbaur, Chem. Ber. 106 (1973) 1221.
[19] T. Mosmann, J. Immunol. Methods 65 (1983) 55.
[20] S.J. Berners-Price, P.J. Sadler, Inorg. Chem. 25 (1986) 3822.
[21] (a) J.S. Lewis, J. Zweit, L.J. Dearling, B.C. Rooney, P.J. Blower, Chem. Commun. (1996) 1093;
(b) J.S. Lewis, S.L. Heath, A.K. Powell, J. Zweit, P.J. Blower, J. Chem. Soc., Dalton Trans. (1997) 855.
[22] (a) P.G. Jones, Gold Bull. 14 (1981) 102;
(b) P.G. Jones, Gold Bull. 14 (1981) 159;
(c) P.G. Jones, Gold Bull. 16 (1983) 114;
(d) P.G. Jones, Gold Bull. 19 (1986) 46;
(e) P. Pyykkö, Chem. Rev. 88 (1988) 563;
(f) H. Schmidbaur, Gold Bull. 23 (1990) 11.
[23] H. Schmidbaur, G. Reber, A. Schier, F.E. Wagner, G. Müller, Inorg. Chim. Acta 147 (1988) 143.
[24] H. Schmidbaur, A. Wohlleben, F. Wagner, O. Orama, G. Huttner, Chem. Ber. 110 (1977) 1748.
[25] P.G. Jones, Acta. Cryst. B36 (1980) 2775.
[26] D.S. Eggleston, D.F. Chodosh, G.R. Girard, D.T. Hill, Inorg. Chim. Acta 108 (1985) 221.
[27] J. Zank, A. Schier, H. Schmidbaur, J. Chem. Soc., Dalton Trans. (1998) 323.
[28] (a) V. Gee, A.G. Orpen, H. Phetmung, P.G. Pringle, R.I. Pugh, Chem. Commun. (1999) 901;
(b) P.A. Bates, J.M. Waters, Inorg. Chim. Acta 98 (1985) 125;
(c) D.S. Eggleston, J.V. McArdle, G.E. Zuber, J. Chem. Soc., Dalton Trans. (1987) 677;
(d) A.L. Balch, E.Y. Fung, Inorg. Chem. 29 (1990) 4764;
(e) H. Schmidbaur, K. Dziwok, A. Grohmann, G. Müller, Chem. Ber. 122 (1989) 893;
(f) K. Dziwok, J. Lachmann, D.L. Wilkinson, G. Müller, H. Schmidbaur, Chem. Ber. 123 (1990) 423;
(g) G. He, K.F. Mok, P.-H. Leung, Organometallics 18 (1999) 4027;
(h) E. Cerrada, C. Diaz, M.C. Diaz, M.B. Hursthouse, M. Laguna, M.E. Light, J. Chem. Soc., Dalton Trans. (2002) 1104.
[29] S.J. Berners-Price, M.A. Mazid, P.J. Sadler, J. Chem. Soc., Dalton Trans. (1984) 969.
[30] (a) P.A. Bates, J.M. Waters, Inorg. Chim. Acta 81 (1984) 151;
(b) C.S.W. Harker, E.R.T. Tiekink, M.W. Whitehouse, Inorg. Chim. Acta 181 (1991) 23;

(c) S.J. Berners-Price, L.A. Colquhoun, P.C. Healy, K.A. Byriel, J.V. Hanna, J. Chem. Soc., Dalton Trans. (1992) 3357;
(d) D.E. Berning, K.V. Katti, C.L. Barnes, W.A. Volkert, A.R. Ketring, Inorg. Chem. 36 (1997) 2765;
(e) F.P. Gabbai, S.-C. Chung, A. Schier, S. Kruger, N. Rosch, H. Schmidbaur, Inorg. Chem. 36 (1997) 5699.
[31] (a) S.J. Berners-Price, C. Brevard, A. Pagelot, P.J. Sadler, Inorg. Chem. 24 (1985) 4278;
(b) D. Affandi, S.J. Berners-Price, Effendy, P.J. Harvey, P.C. Healy, B.E. Ruch, A.H. White, J. Chem. Soc., Dalton Trans. (1997) 1411.
[32] S.J. Berners-Price, P.S. Jarrett, P.J. Sadler, Inorg. Chem. 26 (1987) 3074.
[33] P.J. Barnard, M.V. Baker, S.J. Berners-Price, D.A. Day, J. Inorg. Biochem. 98 (2004) 1642.
[34] S.J. Berners-Price, G.R. Girard, D.T. Hill, B.M. Sutton, P.S. Jarrett, L.F. Faucette, R.K. Johnson, C.K. Mirabelli, P.J. Sadler, J. Med. Chem. 33 (1990) 1386.