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Syntheses and structural studies of platinum(II) complexes of *O*-methylselenomethionine and related ligands

Michael Carland [a], Brendan F. Abrahams [a], Teresa Rede [b], Jodie Stephenson [b], Vincent Murray [b], William A. Denny [c], W. David McFadyen [a,*]

[a] *School of Chemistry, The University of Melbourne, Melbourne, Vic. 3010, Australia*
[b] *School of Biochemistry and Molecular Genetics, University of New South Wales, Sydney, NSW 2052, Australia*
[c] *Auckland Cancer Society Research Centre, Faculty of Medicine and Health Science, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

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Abstract

The complexes dichloro[2-(phenylselanyl)ethanamine]platinum(II), dichloro[2-(benzylselanyl)ethanamine]platinum(II) and dichloro(*O*-methylselenomethionine)platinum(II) have been prepared and the structure of dichloro(*O*-methylselenomethionine)platinum(II) has been determined by single crystal X-ray diffraction. The Pt(II) is in a square planar environment and is coordinated by two *cis* chloride ligands and a chelating *O*-methylselenomethionine ligand. The cytotoxicities of the compounds have been assessed in the human cell lines HeLa and K562 and they are at least threefold less toxic than cisplatin in both cell lines.

Keywords: Selenium–nitrogen chelates; *O*-Methylselenomethionine; Platinum(II) complexes, Synthesis; Crystal structure

1. Introduction

For some time now there has been considerable interest in the use of sulfur containing ligands as platinum rescue agents and it has been shown, for instance, that L-MetH may be used to reduce cisplatin toxicity [1–3]. Given the chemical similarities between sulfur and selenium it is not surprising that selenium containing compounds have also been exploited in an effort to reduce the toxicity of platinum drugs and selenomethionine **1** has been shown to reduce the renal toxicity of cisplatin in rats and mice [4]. It is with these considerations in mind that we have begun to investigate the chemistry of platinum(II) with bidentate ligands containing both amine and selenium donor groups.

The preparation of the palladium(II) and platinum(II) dihalide complexes of selenomethionine **1** has been reported by Faraglia and Fregona [5] who showed that complexes of the form $M(sem)X_2$ ($M = Pd(II)$, $X = Cl^-$ or Br^-; $M = Pt(II)$, $X = Cl^-$) (for example **2)** were readily formed when MX_2 reacted with selenomethionine. Structurally related complexes derived from methionine, such as **3,** have also been described and the cytotoxic properties and in vivo activity of this compound have been examined [6]. In a recent report we have described our structural studies on hydrogen-bonded networks formed by dimerization of platinum(II) dichloro complexes formed from the ligands 2-(phenylselanyl)ethanamine (**4**) and 2(benzylselanyl)ethanamine (**5**) [7]. These complexes, like those of composition $M(sem)X_2$, also contain a bidentate —Se-$(CH_2)_n$—NH_2 ligand system. In the present work, we describe the preparation of these ligands and the corresponding platinum (II) dichloro complexes **10** and **11**. We also describe the synthesis of *O*-methylselenomethionine **6,** the corresponding platinum(II) complex **12** [dichloro-(*O*-methylselenomethionine)platinum(II)] as well as the crystal structure obtained for this complex.

* Corresponding author. Tel.: +61 3 8344 4222; fax: +61 3 9347 5180.
E-mail addresses: wdmf@chemistry.unimelb.edu.au, wdmf@unimelb.edu.au (W.D. McFadyen).

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2. Experimental

2.1. General

NMR spectra were obtained on a Varian Unityplus 400 spectrometer at 400 MHz for ^{1}H spectra, 100 MHz for ^{13}C spectra, 85 MHz for ^{195}Pt spectra and 76.2 MHz for ^{77}Se spectra. ^{195}Pt spectra were externally referenced to potassium tetrachloroplatinate at −1630 ppm. ^{77}Se spectra were externally referenced to diphenyldiselenide at 464 ppm. ^{195}Pt spectra were recorded in *N*-methylpyrrolidinone (NMP). ESI-MS spectra were recorded on a quadrupole ion trap Finnigan-MAT LCQ mass spectrometer equipped with electrospray ionisation (ESI). Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Elemental analyses were performed by the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand and by CMAS, Belmont, Vic.

2.2. Synthesis of O-methylselenomethionine (**6**)

This was prepared from L-(+)-selenomethionine in quantitative yield in an analogous manner to that described [9] for the preparation of *O*-methylmethionine and was characterised as the hydrochloride salt **9**. ^{1}H NMR (D_2O) δ 1.98 (s, 3H), 2.24 (m, 2H), 2.63 (t, 2H, $J = 4.2$ Hz), 3.81 (s, 3H), 4.27 (t, 1H, $J = 3.8$ Hz) ^{13}C NMR ($CDCl_3$) δ 3.91, 21.01, 34.12, 52.01, 53.93, 175.88; ^{77}Se NMR (D_2O) δ 78.50; MS *m*/*z* (relative intensity) 212.0 (M+H, 47), 195.0 (91) 151.9 (100); $C_6H_{14}ClNO_2Se$ requires: C, 29.20; H, 5.47; N, 5.54. Found: C, 28.79; H, 5.38; N, 5.46%. HRMS (M+H) calculated: 212.0190. Found: 212.0194.

2.3. Synthesis of N-tert-butoxycarbonyl-2-[(phenylmethyl)seleno]ethanamine (**8**)

Dibenzyldiselenide (1.00 g, 2.94 mmol) was suspended in stirred ethanol (100 mL) and enough sodium borohydride was added to discharge the intense yellow color, at which time the cloudy mixture became pale yellow. 2-Bromo-*N*-(boc)ethanamine (**7**) [8] (1.30 g, 5.70 mmol) dissolved in ethanol (10 mL) was added and the mixture stirred under a nitrogen atmosphere at ambient temperature for 5 h. The reaction was quenched with 10% aqueous $NaHCO_3$ solution (50 mL), then most of the solvents removed under reduced pressure. The residue was partitioned between water and diethyl ether and the collected solvent layers dried ($MgSO_4$). The solvent was then removed and the residue was then subjected to flash chromatography (1:2 ethyl acetate–hexane) followed by removal of solvent to afford the title compound as a pale yellow solid, 1.60 g (88%), m.p. 46–47 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.58 (t, 2H, $J = 3.6$ Hz), 3.33 (m, 2H), 3.78 (s, 2H), 4.84 (bs, 1H), 7.2–7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 24.07, 26.84, 28.35, 40.18, 64.40, 126.79, 128.53, 128.78, 138.98, 155.62; ^{77}Se NMR ($CDCl_3$) δ 225.57; $C_{14}H_{21}NO_2Se$ requires: C, 53.50; H, 6,73; N, 4.46. Found: C, 53.54; H, 6.68; N, 4.39%.

2.4. Synthesis of dichloro[2-(phenylselanyl)ethanamine]-platinum(II) (**10**)

2-(Phenylselanyl)ethanamine (**4**) (340 mg, 1.70 mmol) was dissolved in water (20 mL) containing 1 drop of conc. HCl and the pH was adjusted to 7.6 with dilute sodium hydroxide solution. Potassium tetrachloroplatinate(II) (776 mg, 1.87 mmol) dissolved in water (10 mL) was added with immediate formation of a buff-colored solid. The mixture was stirred for 16 h, then the solid was collected via centrifugation and washed with acetone. The product was recrystallised by diffusing acetone into a NMP solution of the complex. This yielded the title complex as yellow crystals, 515 mg (71%); ^{195}Pt NMR (NMP) δ −3088 (major), −2967 (minor); $C_8H_{11}Cl_2NPtSe$ requires C, 20.61; H, 2.38; Cl, 15.21; N, 3.00. Found: C, 20.48; H, 2.25; Cl, 15.32; N, 2.70%.

2.5. Synthesis of dichloro[2-(benzylselanyl)ethanamine]-platinum(II) (**11**)

2-[(Phenylmethyl)selanyl]-*N*-(boc)ethanamine (**8**) (361 mg, 1.15 mmol) was dissolved in methanol (10 mL) and added dropwise to a solution of HCl in methanol/methylacetate (40 mL) (prepared through dropwise addition of acetyl chloride to methanol). The mixture was stirred at room temperature for 3 h, at which time TLC indicated the absence of starting material. The solvents were removed under reduced pressure to afford a solid, which was used immediately without further purification. The solid was dissolved in water (50 mL) and the pH adjusted to 7.6 with dilute NaOH solution at which time the solution became cloudy. Potassium tetrachloroplatinate(II) (524 mg, 1.26 mmol) dissolved in water (10 mL) was added to this stirred solution with prompt formation of a pink-grey precipitate. Stirring was continued in the dark for 16 h, then the precipitate was collected and washed with water and then acetone. The product was recrystallised by diffusing acetone into a DMF solution of the complex. This yielded the title complex as yellow prisms (monohydrate), 388 mg (68%); ^{195}Pt NMR (NMP) δ −3057 (major), −2745 (minor); $C_9H_{15}Cl_2NOPtSe$ requires: C, 21.70; H, 3.03; N, 2.81; Cl, 14.23. Found: C, 21.41; H, 2.77; N, 2.94; Cl, 14.93%.

2.6. Synthesis of dichloro(O-methylselenomethionine)-platinum(II) (**12**)

O-Methylselenomethionine hydrochloride (**9**) (190 mg, 0.770 mmol) was dissolved in water (10 mL) and the pH adjusted to 8 with dilute NaOH solution, at which time the stirred solution became cloudy. Potassium tetrachloroplatinate(II) (351 mg, 0.846 mmol) dissolved in water (10 mL) was added and a solid began to precipitate after

15 min. Stirring was continued overnight, then the solid was collected and washed with water and acetone, then recrystallised from NMP–acetone to afford the title compound as colorless crystals, 209 mg (57%); ^{195}Pt NMR (NMP) δ −2978, −2950; $C_6H_{13}Cl_2NO_2PtSe$ requires C, 15.14; H, 2.75; N 2.94. Found: C, 15.07; H, 2.92; N, 2.93%.

2.7. X-ray crystallography

Single crystal X-ray data of a pale yellow crystal of **12** ($0.04 \times 0.06 \times 0.40$ mm) were collected on a Bruker CCD diffractometer using Mo Kα radiation, $\lambda = 0.71073$ Å (graphite monochromator) at 293(2) K. The crystal structure was solved using direct methods and refined using a full-matrix least-squares refinement procedure based upon F^2 using all data [10].

*Crystal data and structure determination details for **12**:* Formula $C_6H_{13}NO_2Cl_2SePt$; $M = 476.1$; monoclinic, $P2_1$, $a = 8.8109(15)$, $b = 7.2134(13)$, $c = 9.1222(16)$ Å; $\beta = 96.549(3)°$; $V = 575.99(17)$ Å^3, $Z = 2$; Calc. density = 2.745 g cm^{-3}; $\mu = 15.77$ mm^{-1}; $F(000)$ 436; Mo Kα-radiation: $2\theta_{max} = 55°$; 3635 reflections measured; 2344 independent reflections (all included in refinement); parameters = 118; Flack parameter = −0.006(14); goodness-of-fit = 0.98; $wR_2 = 0.053$; $R_1 = 0.026$; maximum and minimum residual electron density = 1.01 (1.00 Å from Pt(1)) and −0.72 e Å^{-3}.

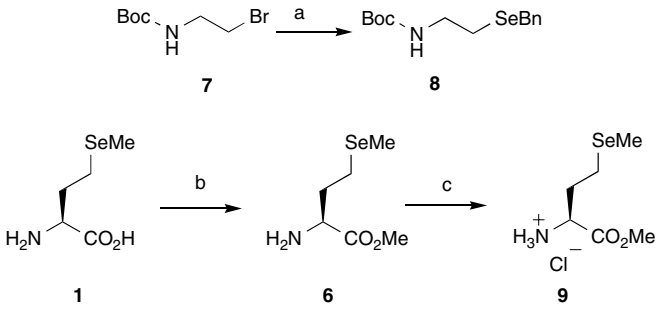
2.8. Cytotoxicity assays

RPMI-1640 medium supplemented with 20 mM HEPES, 10% (v/v) foetal calf serum and $NaHCO_3$ (0.85 g/L) were used to culture the K562 and HeLa cell lines in at 37 °C with 5% CO_2 tension in a humidified incubator. The cells were passaged every 2–3 days.

Exponentially growing K562 cells and HeLa cells were seeded at 3×10^4 cells per well and 0.5×10^4 cells per well, respectively, into 96 well microtitre plates in 100 μL aliquots. The HeLa cells were incubated at 37 °C overnight to provide sufficient time to attach. The test compound was initially dissolved in DMF; 2-fold serially diluted in RPMI-1640 media; and a volume of 100 μl was added in triplicate to each cell line. The cells were then incubated for 3 days at 37 °C with 5% CO_2 tension in a humidified incubator. The cell viability was then assessed via an alamar blue assay (Trek Diagnostic Systems) using fluorescence with excitation at 544 nm and emission at 590 nm. Control wells were treated as above except that 100 μl of RPMI-1640 media replaced the test compound. The IC_{50} was defined as the concentration that resulted in 50% inhibition of growth. An average IC_{50} was determined based on at least two independent experiments [11].

3. Results and discussion

The ligand 2-(phenylselanyl)ethanamine (**4**) was prepared according to a published procedure [12] and the synthetic routes to the other ligands are given in Scheme 1. The ligand 2-(benzylselanyl)ethanamine (**5**) was prepared from reaction of 2-bromo-(*N*-boc)ethanamine (**7**) [8] with sodium benzylselenolate (prepared through reduction of dibenzyldiselenide with sodium borohydride), followed by acidic deprotection. As both the free amine and the hydrochloride salt of **5** proved hygroscopic and difficult to handle the ligand was characterized as its Boc protected precursor **8**. The ligand **5** was generated in situ by acid deprotection of **8** prior to its use in the synthesis of the platinum complexes. *O*-Methylselenomethionine (**6**) was prepared via acid-catalysed esterification of selenomethionine **1** with methanol and amberlyst-15 [9]. This compound also proved difficult to handle and as a result full characterisation was performed on its hydrochloride salt **9**.



Scheme 1. *Reagents and conditions:* (a) $Bn_2Se_2/NaBH_4$, EtOH, 88%; (b) MeOH, amberlyst-15, 68%; (c) HCl/MeOH, 2 min, 100%.

Reaction of the ligands **4**–**6** with potassium tetrachloroplatinate(II) in water at ambient temperature afforded the dichloroplatinum(II) complexes **10**–**12**. As neutral species, none of the complexes was soluble in water and as such were readily isolated and yielded satisfactory elemental analyses. The complexes were of low solubility in common solvents such as DMF (in which molecules like cisplatin are soluble). It was found that NMP was the most effective 'inert' solvent, although heating and/or sonication was needed in order to achieve full dissolution. Once dissolved, the solutions could be diluted with DMF without the formation of any precipitate. The complexes **10**–**12** could be recrystallised through diffusion of acetone into the NMP–DMF solutions of the complexes and in this way crystals suitable for X-ray diffraction were obtained (see Fig. 1).

The structure determination of **12** indicates the expected square planar Pt(II) centre (Fig. 2). The Pt centre is coordinated by 2 *cis* chloride ions and the chelating *O*-methylselenomethionine ligand, which binds to the metal centre through the selenium and nitrogen atoms. The Pt centre lies within 0.043(4) Å of the mean plane defined by the four donor atoms. The bite angle of the chelating ligand is 95.6(1)°. The chelate ring is somewhat puckered, with atoms C(2), C(3) and C(4) lying 0.78(1), 0.26(1) and 0.75(1) Å respectively from the mean plane defined by the four coordinated atoms. The platinum to donor bond distances, (Pt(1)—Se(1) 2.3697(8), Pt(1)—Cl(1) 2.294(2), Pt(1)—Cl(2) 2.322(2) and Pt(1)—N(1) 2.043(5) Å) all fall

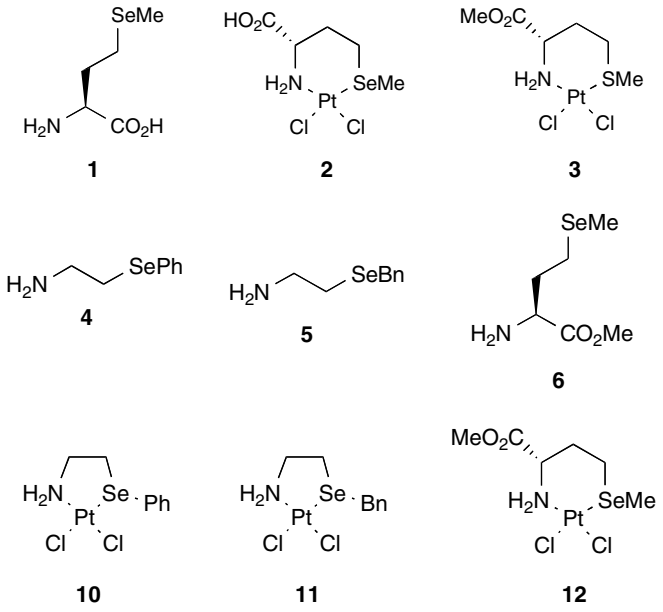


Fig. 1. Selenium containing ligands **4**–**6**, derived complexes **10**–**12**, and related compounds **1**–**3**.

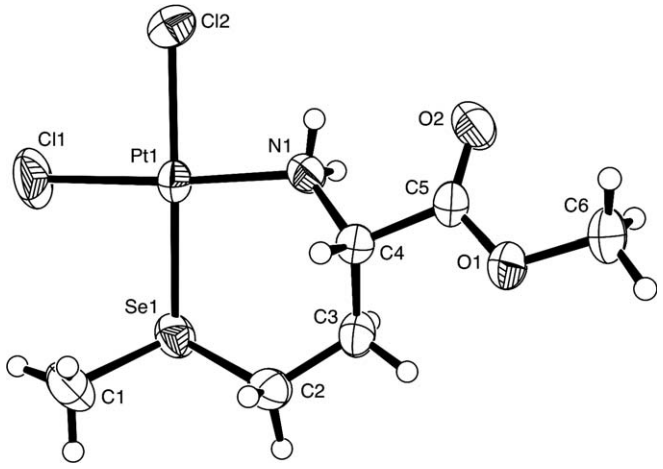


Fig. 2. ORTEP diagram of **12**. Ellipsoids are at the 50% probability level.

within expected values. The complex is essentially flat, with no non-hydrogen atom further than 1.5 Å from the mean plane defined by the four donor atoms. Interestingly, all non-hydrogen atoms of the complex lie on the same side of the plane defined by the metal atom donor set. The complexes stack along a 2_1-axis (the *b*-axis) with the Pt centres displaced by 0.70 Å from the axis. Platinum centres belonging to adjacent complexes within the stack are 3.87 Å apart. Perhaps the most significant interaction between complexes within the stack is between Se(1) and Cl(2) centres which are separated by 3.54 Å.

The ^{195}Pt NMR spectrum of compound **12** shows two signals of comparable size within 30 ppm of each other at δ −2978 and −2950 ppm. The relatively small separation between the signals suggests that the Pt centre is in a very similar environment in each of the species represented by the signals. On the basis of the crystal structure we would expect only a single resonance. We note however, that the environment of the Se atom is pyramidal with the methyl group lying above the plane of the donor atom set (as do all the other non-hydrogen atoms of the complex). We propose that in solution the methyl group on the selenium atom occupies one of two energetically similar positions; one above the plane and one below the plane resulting in the existence of two diastereoisomers [5]. In compounds **10** and **11** the terminal R group (R = phenyl in **10**; R = benzyl in **11**) on the pyramidal Se centre may lie above or below the plane of the donor atom set. The two sides of this plane are intrinsically different owing to the expected puckering of the 5-membered chelate ring and thus two distinct diastereoisomers are possible in each case (Fig. 3). As with compound **12**, the ^{195}Pt NMR spectra of **10** and **11** also provide evidence of diastereomeric forms. Compounds **10** and **11** each possess an intense signal around −3000 ppm in addition to a smaller secondary peak in each case at −2967 ppm for **10** and −2745 ppm for **11**, consistent with a second, less favorable diastereomeric geometry. It is also possible that the presence of two peaks in the ^{195}Pt spectra of these compounds is due to NMP entering the coordination sphere of platinum. This seems unlikely as NMP is expected to be a poor ligand for platinum.

The cytotoxicity of the selenium-containing compounds **11** and **12** was investigated using the two human cells lines, HeLa and K562. The IC_{50} values (Table 1) indicate that the selenium-containing complexes **11** and **12** are cytotoxic although at least threefold less so than cisplatin in both these cell lines. The sulfur analogue of **12**, that is, complex [Pt(*O*-methylmethionine)Cl_2] **3** has been reported to show moderate in vivo antitumour activity [6].

We also prepared the known complex dichloroselenomethionineplatinum(II) (**2**) [5], which we found to be effec-

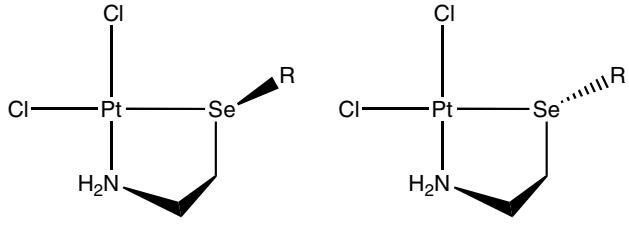


Fig. 3. The two diastereoisomeric forms of compounds **10** and **11**.

Table 1
Cytotoxicity data in the human K562 and HeLa cell lines

Cisplatin (μM)	Complex **10**	Complex **11** (μM)	Complex **12** (μM)
IC_{50} in K562 cells			
9	nd[a]	34	48
IC_{50} in HeLa cells			
16	nd[a]	>65[b]	>65[b]

[a] Not determined (due to the low solubility of this complex).
[b] The solvent DMF is cytotoxic to HeLa and K652 cells at an equivalent IC_{50} concentration present in 65 μM solutions of the complexes **11** and **12**.

tively insoluble in DMF, NMP and other non-coordinating solvents as well as in water, and this behaviour precluded an examination of its cytotoxic properties.

4. Supplementary material

Crystallographic data has been deposited with the Cambridge Crystallographic Data Centre. CCDC No. 279957. Copies of the data can be obtained, free of charge, on application to The Director, CCDC, Union Road 12, Cambridge CB2 1EZ, UK (fax: +44 1223/336 033 or e-mail: deposit@ccdc.cam.ac.uk).

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