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The interaction of antitumor active vanadocene dichloride with sulfur-containing amino acids

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Abstract

Vanadocene dichloride (**1**) reacts with sulfur-containing amino acids, cysteine and methionine, giving new complexes with five- or six-membered chelate ring, but the structure of isolated compounds is affected by the pH value of the reaction mixture. Methionine reacts with aqueous **1** in the pH range of 3–8 affording chelate structure $[Cp_2V(N,O\text{-met})]Cl$ (**4**). Similar reaction with cysteine gives two different products depending on pH. In the acidic solution, the complex $[Cp_2V(O,S\text{-cys})]Cl$ (**2**) is present, whereas in neutral media the compound $[Cp_2V(N,S\text{-cys})]$ (**3**) could be identified. On inspection of spectroscopic measurements, particularly EPR and vibrational spectroscopy, it is evident that sulfur atom of amino acid is bonded directly to the vanadium atom of $[Cp_2V]^{2+}$ moiety. For the purpose of comparison the complexes $[Cp_2V(O,S\text{-mpa})]$ (**5**) and $[Cp_2V(N,S\text{-csam})]^+$ (**6a**) with related chelating ligands, 3-mercaptopropionic acid (mpa) and cysteamine (csam), respectively, were prepared and spectroscopically characterized. The structure of the complex $[Cp_2V(N,S\text{-csam})]BPh_4$ (**6b**) was also determined by X-ray diffraction analysis.

Keywords: Antitumor drugs; Vanadocene dichloride; Cysteine; Methionine; EPR; X-ray

1. Introduction

Vanadocene dichloride Cp_2VCl_2 (VDC, **1**) belongs to the class of metallocene dihalides Cp_2MX_2 (M = Ti, Mo, Nb, V and X = halide), which show high antiproliferative properties against various animal or human tumors [1–4]. Extensive studies have evidenced that the cytostatic activity of metallocene compounds is related with their interaction toward DNA molecule or proteins responsible for DNA replication (e.g. proteinkinase C or topoisomerase II) [5–11]. Investigation of the interactions between the metallocenes and DNA was performed using so-called "model compounds". When various metallocenes are compared, there are some distinct differences in their hydrolysis chemistry [12], interactions with DNA fragment and amino acids. Although Cp_2TiCl_2 and Cp_2MoCl_2 form stable complexes with alkylated nucleobases and nucleotides [5,7,13–16], no such complexes were found for VDC [11].

The interaction of bent metallocenes with proteins was widely studied on the series of model amino acid complexes and it was found that the chemistry of each metallocene compound strongly depends on the nature of central metal atom. Highly oxophilic fragment $[Cp_2Ti]^{2+}$ prefers monodentate bonding of two amino acid (aa) *via* the oxygen atoms of carboxylic group [17–20]. The reaction of Cp_2TiCl_2 with sulfur-containing amino acids such as methionine or cysteine gives the same type of complexes $[Cp_2Ti(O\text{-aa})_2]Cl_2$ (aa = met, cys) with O-bounded amino acid ligands [28,29].

On the other hand, Cp_2MoCl_2 and Cp_2VCl_2 generally give *N*,*O*-chelated amino acid complexes [21–27]. Similarly, methionine complex $[Cp_2Mo(N,O\text{-met})]Cl$ has typical chelate bond previously described for molybdenocene derivatives with sulfur-free amino acids [22]. However, from the

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reaction of Cp_2MoCl_2 with cysteine two different compounds were isolated. Chelate structure of the complex $[Cp_2Mo(N,S\text{-cys})]Cl$ has been proved by X-ray diffraction analysis [23] whereas the structure of the second complex, $[Cp_2Mo(S\text{-cys})_2]$, was proposed only on the basis of NMR spectra [30].

As a part of broad investigation vanadocene chemistry, we recently described vanadocene complexes with simple amino acids [25,26]. Present work deals with the study of interaction of **1** with sulfur-containing biogenic amino acids, cysteine and methionine, respectively. We have also prepared related complexes with 3-mercaptopropionic acid (mpa) and cysteamine (csam). All isolated compounds were characterized by spectroscopic techniques (IR and Raman spectroscopy), electrospray ionization mass spectrometry (ESI-MS), electron paramagnetic resonance spectroscopy (EPR) and X-ray diffraction with the view to elucidate bonding pattern in these complexes. Obtained results can contribute to the understanding of the antiproliferative activity of VDC at the molecular level. In addition, prepared amino acid derivatives are air-stable and highly water-soluble analogues of **1**. This fact could be advantageously used in clinical studies.

2. Results and discussion

2.1. The reaction of vanadocene dichloride with L-cysteine in aqueous solutions at different pH

The behavior of L-cysteine in aqueous solution of vanadocene dichloride at various pH was investigated by isotropic electron paramagnetic resonance spectroscopy; see Scheme 1 and Fig. 1.

Freshly prepared aqueous solution of **1** gives simple eight-line EPR spectrum of vanadocene diaquacomplex $[Cp_2V(H_2O)_2]^{2+}$ ($A_{iso} = 74.5 \times 10^{-4}\,cm^{-1}$, $g_{iso} = 1.979$) [31]. After the addition of one equivalent of L-cysteine to this solution the new paramagnetic species (**2**) with EPR parameters $A_{iso} = 65.8 \times 10^{-4}\,cm^{-1}$ and $g_{iso} = 1.986$ was occurred; pH value of this solution was found to be 2.5. Successive addition of carbonate-free NaOH solution led to the diminishing of diaquacomplex signal and increasing of the signal of **2**. Further increasing of pH to a value of 5 leads to the appearance of new paramagnetic compound **3** ($A_{iso} = 59.9 \times 10^{-4}\,cm^{-1}$, $g_{iso} = 1.994$). In the pH range

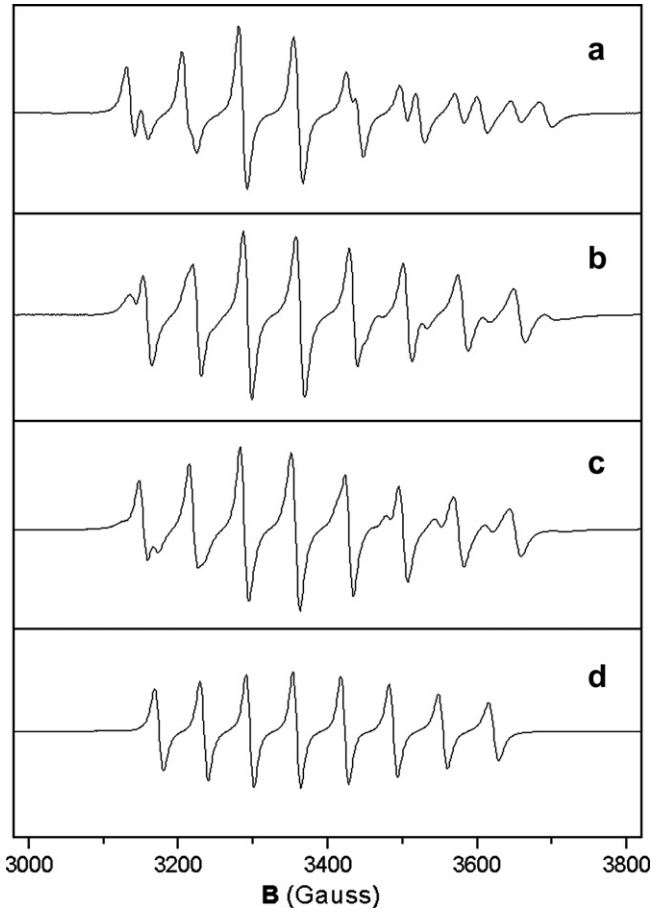
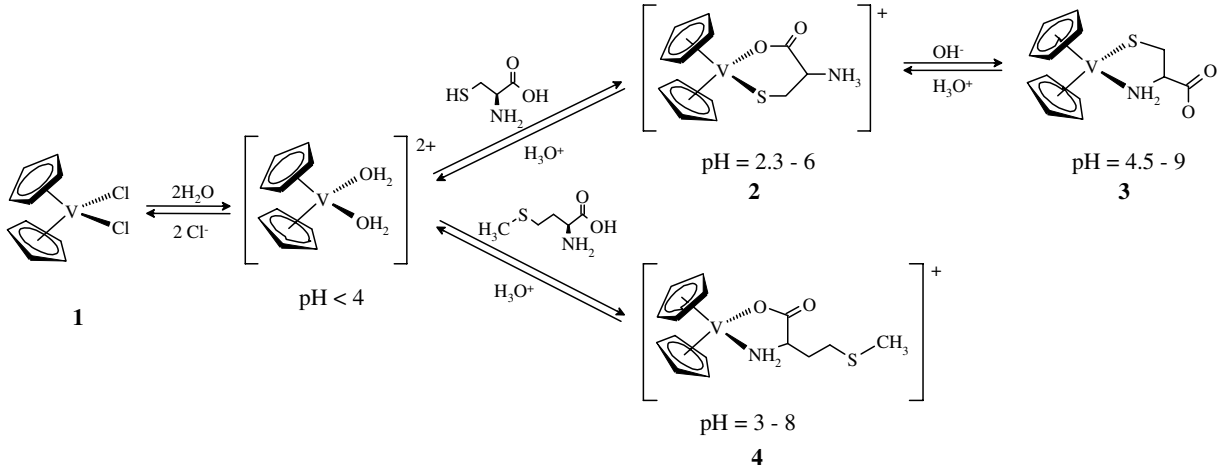


Fig. 1. EPR spectra of aqueous solutions of **1** with L-cysteine at different pH ($\nu_0 = 9.46$ GHz). (a) pH 2.6, (b) pH 3.6, (c) pH 5, and (d) pH 7.4.



Scheme 1. Behavior of L-cysteine and L-methionine in aqueous solution of vanadocene dichloride at different pH.

from 6 to 8.5 only one EPR active species in the reaction mixture is complex **3**.

By the integration of the EPR spectra measured during the reaction was evidenced that the starting diaquacomplex was completely transformed into cysteine products. No other vanadium species (e.g. EPR inactive oxidized or reduced reaction products) were observed and the signal intensity was constant up to pH value 8.5. At higher pH the EPR silent vanadium compounds are formed.

The study of reaction mixtures containing an excess of L-cysteine (molar ratios 2:1, 5:1 or 10:1) did not evidence formation of another complexes and observed EPR spectra were identical to those recorded for stoichiometric mixtures of VDC and L-cysteine.

2.2. Synthesis and characterization of 2

Complex $[Cp_2V(O,S\text{-cys})]Cl$ (**2**) was synthesized in methanol by the reaction of vanadocene dichloride (**1**) with an equimolar amount of L-cysteine after neutralization by one equivalent of sodium hydroxide. When the reaction was carried out in water, inseparable mixture of **2** and **3** was obtained.

EPR parameters of isolated compound **2** dissolved in methanol are similar to those observed during above-mentioned study, see Table 1.

Both the base peak $[Cp_2V(cys)]^+$ in positive-ion ESI mass spectrum and results of elemental analysis of **2** showed that only one molecule of L-cysteine is presented.

The infrared (IR) and Raman spectra (Ra) of **2** confirmed the presence of vanadocene fragment as well as amino acid ligand. The presence of η^5-bonded cyclopentadienyl ring is evident from medium to strong bands of ν_a(C–H) (IR: ~3140 cm^{-1}), ν_s(C–H) (IR: ~3096 cm^{-1}, Ra: ~3105 cm^{-1}), δ(C–H) (IR: ~1025 cm^{-1}) and γ(C–H) (IR: ~840 cm^{-1}) modes, the integrity of bent vanadocene moiety $[Cp_2V]^{2+}$ verifies observed very strong Raman lines ν_s(C–C) (Ra: ~1132 cm^{-1}, ring breathing) and κ(Cp) (Ra: ~285 cm^{-1}, Cp rings tilting). These vibrations are typical for bent bis(η^5-cyclopentadienyl)metal complexes [32].

Very intensive IR band of ν_a(COO) vibration at ~1640 cm^{-1} proved the presence of amino acid carboxylic group in **2**. The absence of strong vibration ν(SH) at ~2545 cm^{-1} in both IR and Raman spectra indicates that thiolic group is deprotonised and participates in bond to the vanadocene moiety [33].

For the purpose of identification of complex **2**, we prepared and spectroscopically characterized vanadocene complex with 3-mercaptopropionic acid, $[Cp_2V(O,S\text{-mpa})]$ (**5**). 3-mercaptopropionic acid contains exactly two donor groups (carboxylic and thiolic function) that are can be used for chelate bond realized *via* oxygen and sulfur atoms. Since EPR parameters of **5** with **2** are very similar, we anticipate identical coordination environment around the vanadium(IV) atom in these complexes, see Table 1.

Table 1
The isotropic EPR parameters A_{iso} (10^{-4} cm^{-1}) and g_{iso} for studied complexes

	Solvent	A_{iso}	g_{iso}
2	CH_3OH	65.9	1.984
	H_2O	65.8	1.986
3	CH_3OH	59.6	1.992
	H_2O	59.9	1.994
4	CH_3OH	62.5	1.985
	H_2O	62.5	1.984
5	CH_3OH	66.0	1.988
6a	CH_3OH	60.3	1.992
6b	CH_3OH	60.1	1.990

2.3. Synthesis and identification of 3

Neutralization of equimolar mixture of **1** and L-cysteine by 2 equiv. of NaOH led to the formation of $[Cp_2V(N,S\text{-cys})]$ (**3**). EPR parameters of compound **3** isolated from methanolic solution are similar to those observed in aqueous solution of **1** and L-cysteine at neutral pH.

Observation of the base peak $[Cp_2V(cys)]^+$ in positive-ion ESI mass spectrum and results of elemental analysis showed that **3** contains only one molecule of L-cysteine.

IR and Raman spectra proved the presence $[Cp_2V]^{2+}$ fragment and L-cysteine in the molecule of **3**. Similarly as in the case of **2**, the absence of strong SH vibration at ~2540 cm^{-1} in vibrational spectra indicates that the sulfur atom of thiolic group participates in chelate bond to the vanadium(IV) centre [33].

For the purpose of identification of complex **3**, related compound with cysteamine, $[Cp_2V(N,S\text{-csam})]Cl$, (**6a**) was prepared and characterized. The molecule of cysteamine contains only two donor functions (amino and thiolic group) and preferential formation of *N*,*S*-chelate bond could be expected. This presumption was unambiguously validated by X-ray diffraction analysis on the single crystal of tetraphenylborate salt, $[Cp_2V(N,S\text{-csam})]BPh_4$ (**6b**), see below. An agreement of EPR parameters for **6a**, **6b** and **3** evidenced that coordination environment at the V^{IV+} center is identical and we can assume that all these complexes contain five-membered *N*,*S*-chelate ring.

2.4. Interaction of vanadocene dichloride with L-methionine in aqueous solutions at different pH

The behavior of L-methionine in aqueous solution of **1** at different pH was investigated by EPR spectroscopy (Scheme 1, Fig. 2).

Addition of L-methionine to the aqueous solution of **1** does not change its EPR spectrum and signal corresponding to $[Cp_2V(H_2O)_2]^{2+}$ was solely observed. Adjusting of pH to a value of 3 led to the appearance of new paramagnetic vanadocene complex **4** ($A_{iso} = 62.5 \times 10^{-4}$ cm^{-1}, $g_{iso} =$ 1.984). When pH value of 4.5 was reached, the signal of diaquacomplex disappeared and complex **4** was only one the EPR active specie in the solution. The complex **4** is stable in the solution at the pH up to 8.

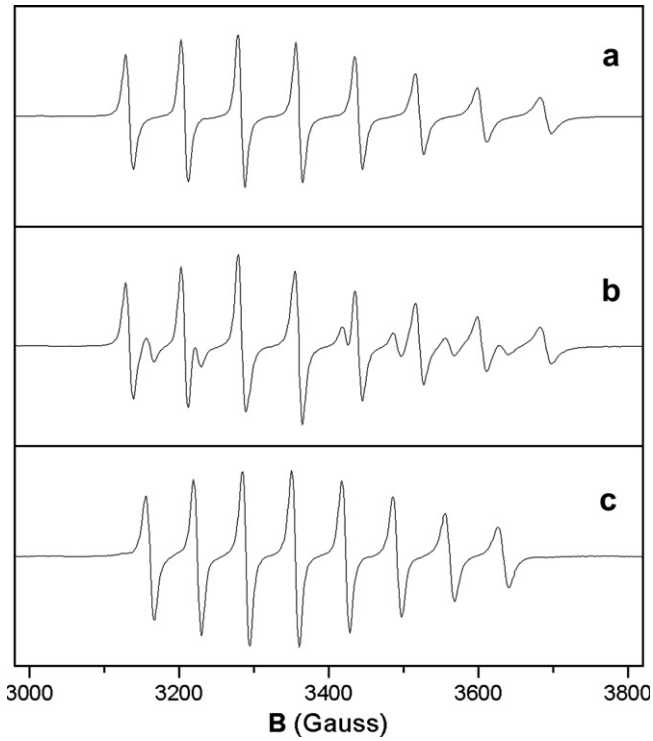


Fig. 2. EPR spectra of aqueous solutions of **1** with L-methionine at different pH ($v_0 = 9.46$ GHz). (a) pH 2.6, (b) pH 4, and (c) pH 6.5.

2.5. Synthesis and characterization of *4*

Complex [$Cp_2V(N,O$-met)]Cl (**4**) was prepared analogously to compound **2**. Its structure was proposed on the basis of spectroscopic measurements (IR, Raman and EPR), mass spectrometry and elemental analysis.

Raman and IR spectroscopies validated the presence of both $[Cp_2V]^{2+}$ fragment and amino acid, additionally, ESI-MS spectrum and elemental analysis of **4** confirmed the presence of one L-methionine molecule.

The EPR spectrum recorded in methanol is identical to those observed in aqueous solution during above-mentioned acidobasic study (Table 1). The agreement of EPR parameters of **4** with those known vanadocene amino acid complexes [$Cp_2V(O,N$-aa)]Cl (aa = gly, ala, val, leu, ile, phe, his, trp; $A_{iso} = 62.6 \pm 0.2 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1{,}986 \pm 0.006$) evidenced the presence of N,O-chelate bond in **4** [25,26].

2.6. Crystal structure of *[Cp$_2$V(N,S-csam)]BPh$_4$ (**6b**)*

A typical bent metallocene structure of the cationic part of **6b** is shown in Fig. 3, crystallographic data summarizes Table 2. In this structure the vanadium(IV) atom is pseudo-tetrahedrally coordinated by sulfur and nitrogen atoms of cysteamine and two η^5-bonded Cp rings. The structural features of the vanadocene core (V–Cg of 1.968 and 1.960 Å, Cg–V–Cg angle 132.5°) are in good agreement with other vanadocene compounds (Cg–V ~1.96–1.97 Å; Cg–V–Cg ~131–135°) [26,34–37]. The S–V–N bond angle

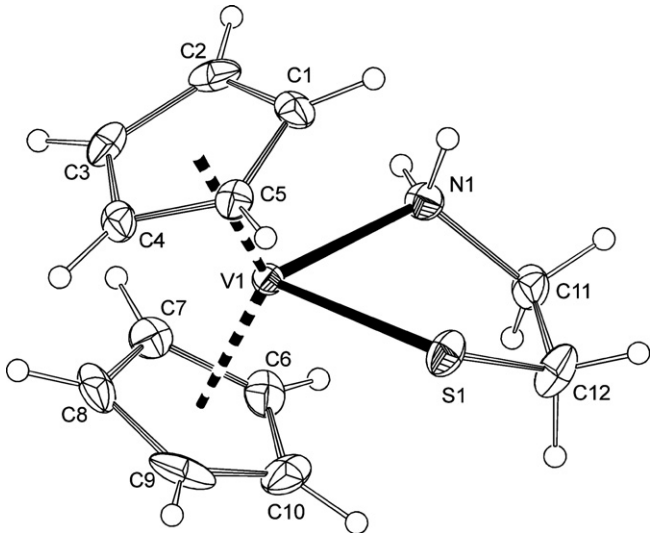


Fig. 3. ORTEP drawing of the molecular structure and atom numbering scheme of the cationic part of **6b** (ellipsoids: 30% probability). Selected bond distances (Å) and angles (deg): Cg(1)–V(1) 1.9685(11), Cg(2)–V(1) 1.9597(10), Cg(1)–V(1)–Cg(2) 132.52(5), V(1)–N(1) 2.181(2), V(1)–S(1) 2.3997(7), N(1)–V(1)–S(1) 76.25(5).

Table 2
Crystal data of **6b**, measurement and refinement details[a]

Compound	**6b**
Moiety formula	$C_{12}H_{16}NSV$, $C_{24}H_{20}B$
Crystal system	Orthorhombic
Space group	$Pna2_1$ (No. 33)
a (Å)	20.18700(10)
b (Å)	14.6480(2)
c (Å)	9.8000(3)
Z	4
V (Å^3)	2897.85(10)
D_{calc} (g cm^{-3})	1.321
Crystal size (mm)	$0.5 \times 0.25 \times 0.15$
Color	Green
Shape	Prism
μ (mm^{-1})	0.441
h Range	−26, 26
k Range	−18, 19
l Range	−12, 12
Reflections measured	49 013
– Independent (R^a_{int})	6425(0.050)
– Observed [$I > 2\sigma(I)$]	5737
Number of parameters	370
GOF[b]	1.071
R^c, wR^c	0.034, 0.081
$\Delta\rho$ (e Å^{-3})	0.249, −0.297

[a] $R_{int} = \sum|F_o^2 - F_{o,mean}^2|/\sum F_o^2$.
[b] GOF = $[\sum(w(F_o^2 - F_c^2)^2)/(N_{diffrs} - N_{params})]^{1/2}$ for all data.
[c] $R(F) = \sum||F_o| - |F_c||/\sum|F_o|$ for observed data, $wR(F^2) = [\sum(w(F_o^2 - F_c^2)^2)/(\sum w(F_o^2)^2)]^{1/2}$ for all data.

is constrained by the presence of five-membered chelate ring to a value of 76.25° when compared to vanadocene dichloride (Cl–V–Cl 87.2°). The V–N bond length 2.181 Å is close to the values found in chelate vanadocene or 1,1′-dimethylvanadocene amino acids complexes (~2.14–2.18 Å) [26,38] and is longer than in vanadocene

complexes with monodentate *sp-nitrogen* bonded ligands $Cp_2V(NCNCN)_2$ (2.05 Å) [39], $(MeCp)_2V(NCO)_2$ (2.03 Å) [40], $Cp_2V(N_3)_2$ (2.08 Å) [41]. The V–S distance 2.40 Å is slightly shorter than corresponding one reported for $Cp_2V(SPh)_2$ (2.445 Å) [42] and $Cp_2V(SMe)_2$ (2.442 Å) [43]. The structural parameters for **6b** and its molybdenocene analogues [$Cp_2Mo(N,S$-csam)]I (Mo–N = 2.21 Å; Mo–S = 2.44 Å; N–Mo–S = 78.4°) and [$Cp_2Mo(N,S$-cys)]Cl (Mo–N = 2.26 Å; Mo–S = 2.44 Å; N–Mo–S = 78.4°) are comparable [23,44].

3. Conclusions

The interaction of vanadocene dichloride with sulfur-containing α-amino acids (L-cysteine and L-methionine) was studied. The prepared amino acid complexes [Cp_2V-(O,S-cys)]Cl, [$Cp_2V(N,S$-cys)] and [$Cp_2V(N,O$-met)]Cl were isolated and characterized by spectroscopic techniques (EPR, IR, Raman, MS). Obtained results confirmed that both L-cysteine and L-methionine are able to coordinate the vanadocene(IV) moiety by stable chelate bond.

The reaction of **1** with cysteine gives two different products whose structure depends on pH of the reaction mixture. In acidic media, complex [$Cp_2V(O,S$-cys)]Cl was identified, but from mixture of neutral pH the compound [$Cp_2V(N,S$-cys)] could be isolated. The reaction with methionine afforded only [$Cp_2V(N,O$-met)]Cl in the pH range 3–8. Since the methionine contains the sulfur atom involved in sulfidic bond, possibility of its transformation into highly nucleophilic thiolate anion is disabled. This fact explains observation, that no N,S- or O,S-chelate complexes of L-methionine were observed during this study.

The bonding manner of polydentate ligands such as aforesaid amino acids, cysteamine and 3-mercaptopropionic acid in prepared complexes was determined particularly by EPR spectroscopy using the comparison of EPR parameters of amino acid complexes with model vanadocene compounds containing a known bond. On the basis of agreement of EPR parameters of amino acid complexes **2**, **3** and **4** with related compounds [$Cp_2V(O,S$-mpa)], [$Cp_2V(N,S$-csam)]Cl and [$Cp_2V(O,N$-aa)]Cl (aa = gly, ala, val, leu, ile, phe, his, trp), respectively, structures of each of them were proposed. The structure of related complex [$Cp_2V(N,S$-cysam)]BPh_4 was unambiguously determined by X-ray diffraction analysis.

4. Experimental

4.1. Methods and materials

All operations were routinely carried out using conventional Schlenk-line and vacuum techniques. The solvents were purified and deoxygenated by standard methods. Water was deionised, double distilled and saturated with argon. Carbonate-free sodium hydroxide was prepared by the reaction of pure sodium metal with the excess of water under argon atmosphere. The α-amino acids (L-cysteine, L-methionine), 3-mercaptopropionic acid and cysteamine were obtained commercially (Fluka) and used without further purification. Vanadocene dichloride (**1**) was prepared by published method [45] and purified by crystallization from dichloromethane. Elemental analysis, IR, Raman and EPR spectroscopy checked its purity.

4.2. Measurements

IR spectra were recorded in the 4000–350 cm^{-1} region on a Perkin–Elmer 684 using KBr pellets. Raman spectra of solid samples at 50–3500 cm^{-1} were recorded on a Bruker IFS 55 equipped with FRA 106 extension.

EPR spectra were run on ERS 221 (ZWG Berlin) apparatus in microwave X-band (~9.5 GHz) in flat quartz cuvettes (width 0.3 mm) at room temperature. The apparatus was calibrated with DPPH value ($g = 2.0036 \pm 2$). Obtained EPR spectra were simulated using EPR simulation software SimFonia v.1.2 (Bruker). A second-order perturbation theory for interaction of unpaired electronic spin with vanadium nuclear spin, anisotropic line widths and mixed Lorentzian/Gaussian lineshapes were used.

Positive-ion electrospray ionization (ESI) mass spectra were measured on an Esquire 3000 ion trap analyzer (Bruker Daltonics, Bremen, Germany) in the m/z range 50–1000. The samples were dissolved in methanol and analyzed by direct infusion at a flow rate 1 μl/min. The selected precursor ions were further analyzed by MS/MS analyses under the following conditions: the isolation width $m/z = 4$, the collision amplitude 0.9 V, the ion source temperature 300 °C, the flow rate and the pressure of nitrogen 4 l/min and 10 psi, respectively.

The pH adjustments of aqueous solutions were made with carbonate-free NaOH solution ($c = 0.5$ mol l^{-1}). The pH measurements were performed on Hanna pH 211 pH-meter using a glass electrode HI 1131.

4.3. Synthesis of compounds

4.3.1. [$Cp_2V(O,S$-cys)]Cl (2)

To a solution of **1** (0.5 g, 1.98 mmol) in 20 ml of deoxygenated methanol L-cysteine (0.24 g, 1.98 mmol) was added and neutralized by addition of NaOH solution (3.96 ml, $c = 0.5$ mol l^{-1}, 1.98 mmol). Solvent was evaporated *in vacuo* and the solid residue was crystallized from the acetone–methanol mixture. The brown-green solid was then washed with 10 ml acetone and dried *in vacuo*. Yield: 0.62 g (1.84 mmol, 93%). Anal. Calc. for $C_{13}H_{16}ClNO_2SV$ (MW 336.7): C, 49.4; H, 4.8; N, 4.2; Cl, 10.5. Found: C, 49.3; H, 4.9; N, 4.0; Cl, 10.5%. EPR (CH_3OH solution): $A_{iso} = 71.1$ G, $g_{iso} = 1.984$. IR (KBr): 3104m, 3068w, 3032w, 2962w, 2930w, 2346w, 1734w, 1647m, 1635s, 1506m, 1436m, 1394m, 1340m, 1286w, 1260m, 1187w, 1130m, 1103m, 1024m, 971m, 839s, 665w, 615m, 565w, 553w, 512m, 488w, 419s, 380m. Raman: 3256(2), 3231(1),

3187(2), 3115(3), 3095(3), 2964(2), 2919(1), 1130(10), 496(2), 353(3), 287(7), 273(8). Positive-ion MS: *m/z* 301 $[M-Cl]^+$ (100%), 284 $[M-Cl-NH_3]^+$. Positive-ion MS/MS of 284: *m/z* 268; 240 $[M-Cl-NH_3-CO_2]^+$; 198; 181 $[Cp_2V]^+$ (100%), 127.

4.3.2. [Cp_2V(N,S-cys)] (**3**)

Complex **3** was prepared *via* the procedure described for **2** using 0.5 g of **1**, 0.24 g of L-cysteine. After neutralization by 7.98 ml (3.96 mmol) of NaOH solution ($c = 0.5\ mol\ l^{-1}$) and evaporation of solvent was obtained dark-brown solid. Yield: 0.58 g (1.72 mmol, 87%). Anal. Calc. for $C_{13}H_{16}NO_2SV \cdot NaCl$ (MW 359.7): C, 43.4; H, 4.5; N, 3.9; Cl, 9.9. Found: C, 43.6; H, 4.4; N, 4.0; Cl, 9.9%. EPR (CH_3OH solution): $A_{iso} = 64.1$ G, $g_{iso} = 1.992$. IR (KBr): 3126w, 3112w, 2965m, 2929m, 2856w, 2380w, 2346w, 1772m, 1645m, 1635s, 1539m, 1506m, 1436m, 1394m, 1340w, 1260m, 1102m, 1024m, 960m, 876w, 806s, 755w, 701w, 667w, 544w, 448w. Raman: 3269(6), 3252(7), 3164(2), 3141(2), 3112(5), 3096(5), 2978(3), 2930(5), 2916(1), 1452(4), 1298(4), 1182(3), 1129(10), 632(4), 293(8), 275(6), 165(1), 121(4). Positive-ion MS: *m/z* 323 $[M-Cl+Na]^+$ (100%), 301 $[M-Cl]^+$, 284 $[M-Cl-NH_3]^+$; 258 $[M-Cl-Cp+Na]^+$. Positive-ion MS/MS of 284: *m/z* 240 $[M-Cl-NH_3-CO_2]^+$; 198; 181 $[Cp_2V]^+$ (100%).

4.3.3. [Cp_2V(O,N-met)]Cl (**4**)

The reaction was carried out as described for complex **2** using 0.5 g of **1** and 0.30 g (1.98 mmol) L-methionine. The dark-green solid was obtained. Yield: 0.65 g (1.78 mmol, 90%). Anal. Calc. for $C_{15}H_{20}ClNO_2SV$ (MW 364.8): C, 49.4; H, 5.5; N, 3.8; Cl, 9.7. Found: C, 49.3; H, 5.4; N, 3.6; Cl, 9.8%. EPR (CH_3OH solution): $A_{iso} = 67.5$ G, $g_{iso} = 1.985$. IR (KBr): 3103m, 3090w, 3079w, 2965w, 2919m, 2856w, 1733w, 1645s, 1635s, 1435s, 1386s, 1362w, 1336m, 1303w, 1263m, 1227w, 1187w, 1149w, 1129m, 1094m, 1017m, 969m, 855s, 802m, 665w, 557s, 455w, 396w. Raman: 3113(4), 2983(1), 2917(5), 1435(3), 1369(2), 1350(<1), 1132(10), 1067(2), 717(<1), 700(<1), 598(<1), 436(2), 421(2), 280(9), 237(1), 117(1). Positive-ion MS: *m/z* 329 $[M-Cl]^+$ (100%). Positive-ion MS/MS of 329: *m/z* 263 $[M-Cl-CpH]^+$; 219 $[M-Cl-CpH-CO_2]^+$; 181 $[Cp_2V]^+$ (100%), 104.

4.3.4. [Cp_2V(O,S-mpa)] (**5**)

Complex **5** was prepared according the procedure described for compound **2** from **1** (0.5 g), 3-mercaptopropionic acid (0.17 ml, 1.98 mmol) and NaOH (3.96 mmol). Yield: 0.47 g (1.64 mmol, 83%). Anal. Calc. for $C_{13}H_{14}O_2SV$ (MW 285.2): C, 54.7; H, 5.0. Found: C, 54.7; H, 5.1%. EPR (CH_3OH solution): $A_{iso} = 71.2$ G, $g_{iso} = 1.988$. IR (KBr): 3110s, 3102w, 2934w, 2925w, 2903w, 1733w, 1714w, 1700w, 1589vs, 1575w, 1569w, 1554m, 1506w, 1495w, 1435s, 1386s, 1305m, 1272m, 1199m, 1188m, 1151w, 1130m, 1067m, 1023m, 980w, 959m, 827vs, 673w, 666m, 594w, 542w, 442w. Raman: not measured due to fluorescence.

4.3.5. [Cp_2V(N,S-csam)]Cl (**6a**)

The reaction was carried out as described for complex **4** using 0.22 g (1.98 mmol) of cysteamine. Yield: 0.52 g (1.76 mmol, 89%). Anal. Calc. for $C_{12}H_{16}ClNSV$ (MW 292.7): C, 49.2; H, 5.5; N, 4.8; Cl, 12.1. Found: C, 49.1; H, 5.5; N, 4.9; Cl, 12.4%. EPR (CH_3OH solution): $A_{iso} = 64.8$ G, $g_{iso} = 1.992$. IR (KBr): 3093w, 3040w, 2965m, 2928w, 1734w, 1622vs, 1506m, 1487m, 1456m, 1384m, 1322m, 1262s, 1128w, 1095m, 1020vw, 979vs, 877vw, 803m, 712s, 667w, 623w, 453w, 396w. Raman: not measured due to fluorescence.

4.3.6. [Cp_2V(N,S-csam)]BPh_4 (**6b**)

Complex **6a** (0.2 g, 0.68 mmol) was dissolved in 2 ml of methanol and saturated methanolic solution (1 ml) of $NaBPh_4$ was added. After stirring of the mixture for several minutes the crystals suitable for X-ray diffraction analysis were grown. Yield: 0.16 g (0.29 mmol, 42%). Anal. Calc. for $C_{36}H_{20}BNSV$ (MW 566.3): C, 76.3; H, 3.6; N, 2.5%. Found: C, 76.1; H, 3.5; N, 2.5%. EPR (CH_3OH solution): $A_{iso} = 64.7$ G, $g_{iso} = 1.990$. IR (KBr): 3093w, 3041w, 2965w, 1734w, 1635m, 1580m, 1558w, 1539w, 1506w, 1480s, 1444m, 1428s, 1384w, 1366w, 1263m, 1185m, 1154m, 1121w, 1099m, 1071m, 1030s, 1020m, 971w, 884w, 842s, 832m, 806m, 747vs, 735vs, 714vs, 667w, 626m, 614 m, 602s, 487m, 464w. Raman: 3321(<1), 3250(<1), 3202(<1), 3168(<1), 3121(1), 3081(1), 3037(5), 2964(<1), 2946(1), 2929(1), 2829(1), 2703(<1), 2435(<1), 2313(<1), 1581(3), 1183(1), 1132(4), 1071(<1), 1030(1), 1000(10), 671(<1), 603(2), 531(<1), 494(<1), 354(1), 307(1), 280(3), 255(1), 189(1), 123(3).

4.4. X-ray crystallography

The X-ray data for **6b** was obtained at 150 K using Oxford Cryostream low-temperature device on a Nonius KappaCCD diffractometer with Mo Kα radiation ($\lambda = 0.71073$ Å), a graphite monochromator, and the ϕ and ω scan mode. Data reductions were performed with DENZO-SMN [46]. The absorption was neglected. The structures were solved by direct methods (Sir92) [47] and refined by full matrix least-square based on F^2 (SHELXL97) [48]. The symmetry of the crystal is non-centrosymetric, however the chirality parameter was close to 0.5 and therefore crystal was refined as racemic twin. Crystal data are summarized in Table 2.

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Appendix A. Supplementary material

CCDC 625114 contains the supplementary crystallographic data for **6b**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2007.05.026.

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