

# Ruthenium-Nitrosyl Complexes with Glycine, L-Alanine, L-Valine, L-Proline, D-Proline, L-Serine, L-Threonine, and L-Tyrosine: Synthesis, X-ray Diffraction Structures, Spectroscopic and Electrochemical Properties, and Antiproliferative Activity

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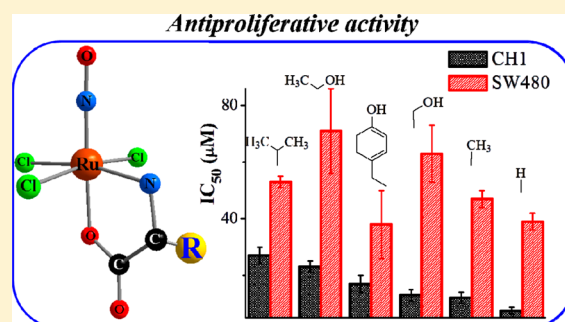
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## Supporting Information

**ABSTRACT:** The reactions of  $[\text{Ru}(\text{NO})\text{Cl}_5]^{2-}$  with glycine (Gly), L-alanine (L-Ala), L-valine (L-Val), L-proline (L-Pro), D-proline (D-Pro), L-serine (L-Ser), L-threonine (L-Thr), and L-tyrosine (L-Tyr) in *n*-butanol or *n*-propanol afforded eight new complexes (1–8) of the general formula  $[\text{RuCl}_3(\text{AA}-\text{H})(\text{NO})]^-$ , where AA = Gly, L-Ala, L-Val, L-Pro, D-Pro, L-Ser, L-Thr, and L-Tyr, respectively. The compounds were characterized by elemental analysis, electrospray ionization mass spectrometry (ESI-MS),  $^1\text{H}$  NMR, UV–visible and ATR IR spectroscopy, cyclic voltammetry, and X-ray crystallography. X-ray crystallography studies have revealed that in all cases the same isomer type (from three theoretically possible) was isolated, namely *mer*(Cl),*trans*(NO,O)- $[\text{RuCl}_3(\text{AA}-\text{H})(\text{NO})]$ , as was also recently reported for osmium analogues with Gly, L-Pro, and D-Pro (see *Z. Anorg. Allg. Chem.* **2013**, 639, 1590–1597). Compounds **1**, **4**, **5**, and **8** were investigated by ESI-MS with regard to their stability in aqueous solution and reactivity toward sodium ascorbate. In addition, cell culture experiments in three human cancer cell lines, namely, A549 (nonsmall cell lung carcinoma), CH1 (ovarian carcinoma), and SW480 (colon carcinoma), were performed, and the results are discussed in conjunction with the lipophilicity of compounds.



## INTRODUCTION

Nitric oxide plays important roles in biochemical processes<sup>1</sup> and, in particular, in progression of human tumors.<sup>2</sup> The antimetastatic activity of NAMI-A, an investigational drug in phase II clinical trials,<sup>3</sup> was suggested to be related to its interaction with NO *in vivo*.<sup>4</sup> Given the importance of NO as a noninnocent ligand in coordination chemistry,<sup>5</sup> the occurrence of structural trans effects (STEs), the role of the metal-nitrosyl unit as a reaction mediator or regulator of geometry around the metal ion,<sup>6</sup> as well as linkage isomerization of the N- and O-bound nitrosyl ligand,<sup>7</sup> surprisingly little is known about the reactivity of ruthenium(II)- and osmium(II)-nitrosyl complexes with respect to amino acids. Although a few ruthenium-nitrosyl complexes with amino acids and related ligands have been reported in the literature, for example,  $\text{K}[\text{Ru}(\text{Gly})(\text{OH})_3\text{NO}]$ ,<sup>8</sup>  $\text{K}[\text{Ru}(\text{L-Ala})(\text{OH})_3\text{NO}]$ ,<sup>9</sup>  $[\text{RuCl}_2(\text{L-His})(\text{NO})]$ ,<sup>10</sup>  $[\text{RuCl}_2(\text{L-Met})(\text{NO})]$ ,<sup>11</sup> and  $(\text{C}_2\text{H}_5)_4\text{N}$

$[\text{RuCl}_3(\text{pyca})(\text{NO})]$ ,<sup>12</sup> where pycaH = 2-pyridinecarboxylic acid, their antiproliferative activity remains unknown. All this prompted us to continue our recently initiated study<sup>13</sup> on the interaction of  $[\text{MCl}_5(\text{NO})]^{2-}$  with different amino acids as a benchmark for further investigation of the reactivity of ruthenium and osmium nitrosyl complexes with azole heterocycles toward amino acids (AA).

Moreover, we reported recently on the synthesis of two series of transition metal complexes, namely (cation) $[\text{cis-MCl}_4(\text{Hazole})(\text{NO})]$  and (cation) $[\text{trans-MCl}_4(\text{Hazole})(\text{NO})]$ , where M = Ru, Os and Hazole = 1*H*-indazole, 1*H*-pyrazole, 1*H*-imidazole, or 1*H*-benzimidazole. Ruthenium and osmium analogues showed a striking difference in antiproliferative activity in three human cancer cell lines, A549 (nonsmall cell

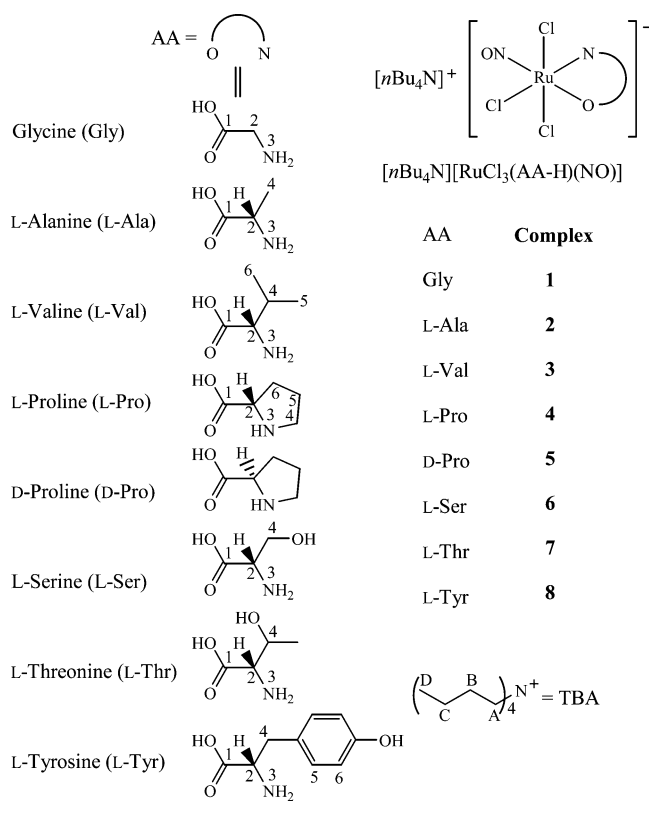
Received: December 23, 2013

Published: February 20, 2014

lung carcinoma), CH1 (ovarian carcinoma), and SW480 (colon carcinoma).<sup>14,15</sup> These results were in strong contrast to previous comparative studies on homologous ruthenium and osmium complexes (with metal ion in different oxidation states) showing either similar activities<sup>16,17</sup> or much smaller differences<sup>18–20</sup> than those observed for compounds reported in reference 15. We are now trying to find out whether their behavior toward amino acids can provide an explanation for their different antiproliferative activity. Amino acids are the basic units of proteins and the most important low-molecular-weight biological ligands. They are major ingredients of the media used in cell culture experiments.<sup>21</sup> In addition, reactions with amino acids are likely to be involved in speciation of metal complexes during biotransformation in the body. Knowledge about these reactions will therefore help in elucidating the species delivered into the cell and in better understanding the mechanisms of drug metabolism or detoxification.<sup>22</sup> For example, a  $[(\text{Pt}(\text{L-Met})_2)]$  species was isolated from the urine of cancer patients treated with cisplatin. This is one of the few known metabolites of the drug.<sup>23</sup>

Herein we report on the synthesis of eight new ruthenium(II)-nitrosyl complexes with Gly, L-Ala, L-Val, L-Pro, D-Pro, L-Ser, L-Thr, and L-Tyr (Chart 1), their X-ray diffraction

Chart 1



structures, spectroscopic and electrochemical properties, lipophilicity, behavior in aqueous solution, and antiproliferative activity in human cancer cell lines in vitro. The latter was compared to that of osmium-nitrosyl complexes with Gly (**1\***), L-Pro (**4\***), and D-Pro (**5\***).

## EXPERIMENTAL SECTION

**Materials.** The starting compounds  $\text{Na}_2[\text{RuCl}_5\text{NO}] \cdot 6\text{H}_2\text{O}$  and  $(n\text{Bu}_4\text{N})_2[\text{RuCl}_5\text{NO}]$  were synthesized as previously reported in the

literature.<sup>24</sup>  $\text{RuCl}_3 \cdot \text{H}_2\text{O}$  was purchased from Johnson Matthey, sodium nitrite (97%), tetrabutylammonium chloride (97%), L-Thr, L-Ala, and Gly (99%) were from Sigma-Aldrich. L-Ser was from Serva, L-Pro (99%), and D-Pro (99%) were from Alfa Aesar, and L-Tyr (99%), formic acid, and sodium ascorbate were from Fluka. All chemicals were used without further purification. Methanol (HPLC grade, Fisher) and ultrapure water (18.2 M $\Omega$ , Advantage A10, 185 Ultrapure Water System, Millipore, France) were used for the ESI-MS study.

**Synthesis of Complexes.**  $(n\text{Bu}_4\text{N})[\text{RuCl}_3(\text{Gly-H})(\text{NO})]$  (**1**). A mixture of  $\text{Na}_2[\text{RuCl}_5\text{NO}] \cdot 6\text{H}_2\text{O}$  (400 mg, 0.86 mmol),  $n\text{Bu}_4\text{NCl}$  (362 mg, 1.31 mmol), and Gly (121 mg, 1.61 mmol) was refluxed in *n*-butanol (10 mL) for 1.5 h. The solution was allowed to cool to room temperature. The separated salt was filtered off. The solution was transferred into a beaker. Dark red crystals formed after several days were filtered off and washed with water/ethanol 1:3 (4 mL), diethyl ether (4 mL), and dried in vacuo. Yield: 75 mg, 15.5%. Anal. Calcd for  $\text{C}_{18}\text{H}_{40}\text{Cl}_3\text{N}_3\text{O}_3\text{Ru}$  ( $M = 553.96$  g/mol): C, 39.03; H, 7.28; N, 7.59. Found: C, 38.77; H, 6.96; N, 7.43%. ESI-MS in MeOH (negative):  $m/z$  312.7  $[\text{RuCl}_3\text{NO}(\text{Gly-H})]^-$  ( $m_{\text{theor}} = 312.8$ ), 274.7  $[\text{RuCl}_2\text{NO}(\text{Gly-2H})]^-$  ( $m_{\text{theor}} = 274.8$ ), 238.7  $[\text{RuClNO}(\text{Gly-3H})]^-$  ( $m_{\text{theor}} = 238.9$ ). IR,  $\text{cm}^{-1}$ : 886, 1160, 1301, 1490, 1669 (vs)  $\nu_{\text{as}}(\text{COO}^-)$ , 1862 (vs)  $\nu(\text{NO})$ , 2955 (m)  $\nu(\text{CH})$ , 3124 (m)  $\nu_{\text{s}}(\text{NH}_2)$ , and 3193 (m)  $\nu_{\text{as}}(\text{NH}_2)$ . UV-vis (buffer),  $\lambda_{\text{max}}$  nm ( $\epsilon$ ,  $\text{M}^{-1} \text{cm}^{-1}$ ): 279 (1790), 453 (104).  $^1\text{H}$  NMR (500.32 MHz, DMSO- $d_6$ ):  $\delta$  0.95 (t, 12H<sub>D</sub>,  $J = 7.5$  Hz), 1.32 (sxt, 8H<sub>C</sub>,  $J = 7.3$  Hz), 1.58 (qui, 8H<sub>B</sub>,  $J = 7.8$  Hz), 3.17 (m, 8H<sub>A</sub>,  $J = 8.2$  Hz), 3.36 (t,  $J = 6.5$  Hz, 2H, H<sub>2</sub>), 5.89 (s, 2H, H<sub>3</sub>) ppm. For assignment of proton resonances see atom numbering in Chart 1.

$(n\text{Bu}_4\text{N})[\text{RuCl}_3(\text{L-Ala-H})(\text{NO})]$  (**2**). A mixture of  $\text{Na}_2[\text{RuCl}_5\text{NO}] \cdot 6\text{H}_2\text{O}$  (400 mg, 0.86 mmol),  $n\text{Bu}_4\text{NCl}$  (450 mg, 1.62 mmol), and L-Ala (115 mg, 1.29 mmol) was refluxed in *n*-butanol (10 mL) for 1.5 h. The solvent was removed under reduced pressure, and the remaining oil was dried in vacuo. Water (7 mL) was added. The solution was decanted into a beaker and allowed to stand at room temperature. Five days later orange crystals were filtered off, and a second fraction was collected 2 d later. The product was washed with water/ethanol 1:1 (4 mL), diethyl ether (4 mL), and dried in vacuo. Yield: 102 mg, 21.0%. Anal. Calcd for  $\text{C}_{19}\text{H}_{42}\text{Cl}_3\text{N}_3\text{O}_3\text{Ru}$  ( $M = 567.98$  g/mol): C, 40.18; H, 7.45; N, 7.40. Found: C, 40.15; H, 7.72; N, 7.05%. ESI-MS in MeOH (negative):  $m/z$  326.7  $[\text{RuCl}_3\text{NO}(\text{L-Ala-H})]^-$  ( $m_{\text{theor}} = 326.9$ ), 288.7  $[\text{RuCl}_2\text{NO}(\text{L-Ala-2H})]^-$  ( $m_{\text{theor}} = 288.9$ ), 252.7  $[\text{RuClNO}(\text{L-Ala-3H})]^-$  ( $m_{\text{theor}} = 252.9$ ). IR,  $\text{cm}^{-1}$ : 873, 1181, 1266, 1224, 1470, 1577, 1666 (vs)  $\nu_{\text{as}}(\text{COO}^-)$ , 1858 (vs)  $\nu(\text{NO})$ , 2874, 2960  $\nu(\text{CH})$ , 3120 (m)  $\nu_{\text{s}}(\text{NH}_2)$ , and 3190 (m)  $\nu_{\text{as}}(\text{NH}_2)$ . UV-vis (buffer),  $\lambda_{\text{max}}$  nm ( $\epsilon$ ,  $\text{M}^{-1} \text{cm}^{-1}$ ): 279 (1857), 453 (104).  $^1\text{H}$  NMR (500.32 MHz, DMSO- $d_6$ ):  $\delta$  0.95 (t, 12H<sub>D</sub>,  $J = 7.4$  Hz), 1.32 (m, 12H, 8H<sub>C</sub>, 3H<sub>4</sub>), 1.58 (qui, 8H<sub>B</sub>,  $J = 7.8$  Hz), 3.17 (t, 8H<sub>A</sub>,  $J = 8.2$  Hz), 3.59 (qua, 1H, H<sub>2</sub>,  $J = 7.3$  Hz), 5.28 (m, 1H, H<sub>3</sub>) and 6.39 (m, 1H, H<sub>3</sub>'') ppm.

$(n\text{Bu}_4\text{N})[\text{RuCl}_3(\text{L-Val-H})(\text{NO})]$  (**3**). A mixture of  $\text{Na}_2[\text{RuCl}_5\text{NO}] \cdot 6\text{H}_2\text{O}$  (400 mg, 0.86 mmol),  $n\text{Bu}_4\text{NCl}$  (450 mg, 1.62 mmol), and L-Val (151 mg, 1.29 mmol) was refluxed in *n*-butanol (10 mL) for 2 h. The solvent was removed under reduced pressure, and the remaining oil was dried in vacuo. Water (7 mL) was added. The solution was decanted into a beaker and allowed to stand at room temperature. Seven days later orange crystals formed were filtered off, washed with water/ethanol 1:1 (4 mL), diethyl ether (4 mL), and dried in vacuo. Yield: 179 mg, 35.0%. Anal. Calcd for  $\text{C}_{21}\text{H}_{46}\text{Cl}_3\text{N}_3\text{O}_3\text{Ru} \cdot 0.5\text{H}_2\text{O}$  ( $M = 605.05$  g/mol): C, 41.69; H, 7.83; N, 6.94. Found: C, 41.69; H, 8.14; N, 6.73%. ESI-MS in MeOH (negative):  $m/z$  355  $[\text{RuCl}_3\text{NO}(\text{L-Val-H})]^-$  ( $m_{\text{theor}} = 354.9$ ), 317  $[\text{RuCl}_2\text{NO}(\text{L-Val-2H})]^-$  ( $m_{\text{theor}} = 316.9$ ), 281  $[\text{RuClNO}(\text{L-Val-3H})]^-$  ( $m_{\text{theor}} = 280.9$ ). IR,  $\text{cm}^{-1}$ : 806, 894, 1012, 1180, 1299, 1372, 1467, 1663 (vs)  $\nu_{\text{as}}(\text{COO}^-)$ , 1852 (vs)  $\nu(\text{NO})$ , 2878, 2962 (m)  $\nu(\text{CH})$ , and 3187 (m)  $\nu(\text{NH}_2)$ . UV-vis (buffer),  $\lambda_{\text{max}}$  nm ( $\epsilon$ ,  $\text{M}^{-1} \text{cm}^{-1}$ ): 279 (1883), 453 (104).  $^1\text{H}$  NMR (500.32 MHz, DMSO- $d_6$ ):  $\delta$  0.86 (d, 3H, H<sub>6</sub>,  $J = 7.9$  Hz), 0.95 (t, 12H<sub>D</sub>,  $J = 7.4$  Hz), 0.99 (d, 3H, H<sub>5</sub>,  $J = 7.9$ ), 1.32 (sxt, 8H<sub>C</sub>,  $J = 7.4$  Hz), 1.58 (qui, 8H<sub>B</sub>,  $J = 7.8$  Hz), 2.19 (m, 1H, H<sub>4</sub>), 3.17 (t, 8H<sub>A</sub>,  $J = 8.2$  Hz), 3.44 (m, 1H, H<sub>2</sub>), 4.67 (m, 1H, H<sub>3</sub>'), 6.44 (m, 1H, H<sub>3</sub>'') ppm.

$(n\text{Bu}_4\text{N})[\text{RuCl}_3(\text{L-Pro-H})(\text{NO})]$  (**4**). A mixture of  $(n\text{Bu}_4\text{N})_2[\text{RuCl}_5\text{NO}]$  (350 mg, 0.44 mmol) and L-Pro (76 mg, 0.66

mmol) was refluxed in *n*-butanol (6 mL) for 3.5 h. The solvent was removed under reduced pressure. The remaining oil was dissolved in water (5 mL). The solution was transferred into a beaker and allowed to stand at room temperature. Orange crystals formed were filtered off, and a second fraction was collected after 24 h. The product was washed with water/ethanol 1:1 (4 mL), diethyl ether (4 mL), and dried in vacuo. Yield: 94 mg, 36%. Anal. Calcd for  $C_{21}H_{43}Cl_3N_3O_3Ru$  ( $M = 593.01$  g/mol): C, 42.53; H, 7.31; N, 7.09. Found: C, 42.48; H, 7.37; N, 6.78%. ESI-MS in MeOH (negative):  $m/z$  352.7  $[RuCl_3NO(L-Pro-H)]^-$  ( $m_{theor} = 352.9$ ), 314.8  $[RuCl_2NO(L-Pro-2H)]^-$  ( $m_{theor} = 314.9$ ), 278.7  $[RuClNO(L-Pro-3H)]^-$  ( $m_{theor} = 278.9$ ). IR,  $cm^{-1}$ : 740, 883, 1353, 1464, 1644, 1647 (vs)  $\nu_{as}(COO^-)$ , 1845 (vs)  $\nu(NO)$ , 2874 and 2960 (m)  $\nu(CH)$ , 3101 (m)  $\nu_s(NH_2)$ , and 3169 (m)  $\nu_{as}(NH_2)$ . UV-vis (buffer),  $\lambda_{max}$  nm ( $\epsilon$ ,  $M^{-1} cm^{-1}$ ): 279 (1981), 253 (104).  $^1H$  NMR (500.32 MHz, DMSO- $d_6$ ):  $\delta$  0.95 (t, 12H<sub>D</sub>,  $J = 7.4$  Hz), 1.32 (sxt, 8H<sub>C</sub>,  $J = 7.4$  Hz), 1.58 (qui, 8H<sub>B</sub>,  $J = 7.8$  Hz), 1.69 (m, 1H, H<sub>5</sub>), 1.85 (m, 2H, H<sub>6</sub>, H<sub>5'</sub>), 2.05 (m, 1H, H<sub>6''</sub>), 2.87 (m, 1H, H<sub>4</sub>), 3.17 (t, 8H<sub>A</sub>,  $J = 8.2$  Hz), 3.42 (m, 1H, H<sub>4'</sub>), 3.88 (qua, 1H, H<sub>2</sub>,  $J = 7.1$  Hz), 7.08 (m, 1H, H<sub>3</sub>) ppm.

**(*nBu<sub>4</sub>N*)[RuCl<sub>3</sub>(*D-Pro-H*)(NO)] (5).** A mixture of Na<sub>2</sub>[RuCl<sub>5</sub>NO]·6H<sub>2</sub>O (400 mg, 0.86 mmol), *n*Bu<sub>4</sub>NCl (450 mg, 1.62 mmol), and *D-Pro* (148 mg, 1.29 mmol) was refluxed in *n*-propanol (10 mL) for 2 h. The solvent was removed under reduced pressure. Water (7 mL) was added to the residue. The solution was decanted into a beaker and allowed to stand at room temperature. Orange crystals formed were filtered off after 72 h, washed with water/ethanol 1:1 (4 mL), diethyl ether (4 mL), and dried in vacuo. Yield: 175 mg, 34.0%. Anal. Calcd for  $C_{21}H_{43}Cl_3N_3O_3Ru \cdot 0.75H_2O$  ( $M = 606.52$  g/mol): C, 41.54; H, 7.33; N, 6.92. Found: C, 41.70; H, 7.68; N, 7.07%. ESI-MS in MeOH (negative):  $m/z$  352.7  $[RuCl_3NO(D-Pro-H)]^-$  ( $m_{theor} = 352.9$ ), 314.8  $[RuCl_2NO(D-Pro-2H)]^-$  ( $m_{theor} = 314.9$ ), 278.7  $[RuClNO(D-Pro-3H)]^-$  ( $m_{theor} = 278.9$ ). IR,  $cm^{-1}$ : 740, 883, 1353, 1464, 1644, 1647 (vs)  $\nu_{as}(COO^-)$ , 1845 (vs)  $\nu(NO)$ , 2874, 2960 (m)  $\nu(CH)$ , 3198 (m)  $\nu(NH_2)$ . UV-vis (buffer),  $\lambda_{max}$  nm ( $\epsilon$ ,  $M^{-1} cm^{-1}$ ): 279 (1846), 253 (90).  $^1H$  NMR (500.32 MHz, DMSO- $d_6$ ):  $\delta$  0.95 (t, 12H<sub>D</sub>,  $J = 7.4$  Hz), 1.32 (sxt, 8H<sub>C</sub>,  $J = 7.4$  Hz), 1.58 (qui, 8H<sub>B</sub>,  $J = 7.8$  Hz), 1.69 (m, 1H, H<sub>5</sub>), 1.85 (m, 2H, H<sub>6</sub>, H<sub>5'</sub>), 2.05 (m, 1H, H<sub>6''</sub>), 2.87 (m, 1H, H<sub>4</sub>), 3.17 (t, 8H<sub>A</sub>,  $J = 8.2$  Hz), 3.42 (m, 1H, H<sub>4'</sub>), 3.88 (qua, 1H, H<sub>2</sub>,  $J = 7.1$  Hz), 7.08 (m, 1H, H<sub>3</sub>) ppm.

**(*nBu<sub>4</sub>N*)[RuCl<sub>3</sub>(*L-Ser-H*)(NO)] (6).** A mixture of Na<sub>2</sub>[RuCl<sub>5</sub>NO]·6H<sub>2</sub>O (400 mg, 0.86 mmol), *n*Bu<sub>4</sub>NCl (450 mg, 1.62 mmol), and *L-Ser* (137 mg, 1.29 mmol) was refluxed in *n*-butanol (10 mL) for 1.5 h. The solvent was removed under reduced pressure, and the remaining oil was dried in vacuo. The remaining oil was dissolved in water (10 mL). The solution was decanted into a beaker and allowed to stand at room temperature. Four days later orange crystals were filtered off, washed with water/ethanol 1:1 (4 mL), diethyl ether (4 mL), and dried in vacuo. Yield: 111 mg, 22.0%. Anal. Calcd for  $C_{19}H_{42}Cl_3N_3O_4Ru$  ( $M = 583.98$  g/mol): C, 39.08; H, 7.25; N, 7.20%. Found: C, 39.30; H, 6.90; N, 6.93. ESI-MS in MeOH (negative):  $m/z$  342.7  $[RuCl_3NO(L-Ser-H)]^-$  ( $m_{theor} = 342.8$ ), 304.7  $[RuCl_2NO(L-Ser-2H)]^-$  ( $m_{theor} = 304.9$ ). IR,  $cm^{-1}$ : 878, 1070, 1369, 1477, 1644 (vs)  $\nu_{as}(COO^-)$ , 1855 (vs)  $\nu(NO)$ , 2875, 2956 (m)  $\nu_a(CH)$ , 3120 (m)  $\nu_s(NH_2)$ , 3190 (m)  $\nu_{as}(NH_2)$ , and 3448 (m)  $\nu_s(OH)$ . UV-vis (buffer),  $\lambda_{max}$  nm ( $\epsilon$ ,  $M^{-1} cm^{-1}$ ): 279 (1721), 453 (87).  $^1H$  NMR (500.32 MHz, DMSO- $d_6$ ):  $\delta$  0.95 (t, 12H<sub>D</sub>,  $J = 7.4$  Hz), 1.32 (sxt, 8H<sub>C</sub>,  $J = 7.4$  Hz), 1.58 (qui, 8H<sub>B</sub>,  $J = 7.8$  Hz), 3.17 (t, 8H<sub>A</sub>,  $J = 8.2$  Hz), 3.59 (m, 1H, H<sub>4</sub>), 3.75 (m, 1H, H<sub>4'</sub>), 4.98 (m, 1H, H<sub>3</sub>), 5.05 (t, 1H, H<sub>2</sub>,  $J = 5.35$  Hz), 6.45 (m, 1H, H<sub>3''</sub>) ppm.

**(*nBu<sub>4</sub>N*)[RuCl<sub>3</sub>(*L-Thr-H*)(NO)] (7).** A mixture of Na<sub>2</sub>[RuCl<sub>5</sub>NO]·6H<sub>2</sub>O (400 mg, 0.86 mmol), *n*Bu<sub>4</sub>NCl (450 mg, 1.62 mmol), and *L-Thr* (154 mg, 1.29 mmol) was refluxed in *n*-butanol (10 mL) for 1.5 h. The solvent was removed under reduced pressure, and the remaining oil was dried in vacuo. The remaining oil was dissolved in water (10 mL). The solution was decanted into a beaker and allowed to stand at room temperature. Six days later orange crystals were filtered off, washed with water/ethanol 1:1 (4 mL), diethyl ether (4 mL), and dried in vacuo. Yield: 88 mg, 17.0%. Anal. Calcd for  $C_{20}H_{44}Cl_3N_3O_4Ru$  ( $M = 598.01$  g/mol): C, 40.17; H, 7.42; N, 7.03. Found: C, 40.02; H, 7.81; N, 6.78%. ESI-MS in MeOH (negative):  $m/z$  356.7  $[RuCl_3NO-$

$(L-Thr-H)]^-$  ( $m_{theor} = 356.9$ ), 318.7  $[RuCl_2NO(L-Thr-2H)]^-$  ( $m_{theor} = 318.9$ ). IR,  $cm^{-1}$ : 592, 742, 890, 1066, 1173, 1257, 1372, 1459, 1642 (vs)  $\nu_{as}(COO^-)$ , 1849 (vs)  $\nu(NO)$ , 2875, 2966 (m)  $\nu(CH)$ , 3233 (m)  $\nu(NH_2)$ , and 3440 (m)  $\nu(OH)$ . UV-vis (buffer),  $\lambda_{max}$  nm ( $\epsilon$ ,  $M^{-1} cm^{-1}$ ): 279 (1761), 453 (89).  $^1H$  NMR (500.32 MHz, DMSO- $d_6$ ):  $\delta$  0.95 (t, 12H<sub>D</sub>,  $J = 7.4$  Hz), 1.17 (d, 3H, H<sub>5</sub>,  $J = 6.75$ ), 1.32 (sxt, 8H<sub>C</sub>,  $J = 7.4$  Hz), 1.58 (qui, 8H<sub>B</sub>,  $J = 7.8$  Hz), 3.17 (t, 8H<sub>A</sub>,  $J = 8.2$  Hz), 4.15 (m, 1H, H<sub>4</sub>), 4.92 (m, 1H, H<sub>3</sub>), 5.16 (d, 1H, H<sub>2</sub>,  $J = 5.33$ ), 6.46 (m, 1H, H<sub>3'</sub>) ppm.

**(*nBu<sub>4</sub>N*)[RuCl<sub>3</sub>(*L-Tyr-H*)(NO)] (8).** A mixture of Na<sub>2</sub>[RuCl<sub>5</sub>NO]·6H<sub>2</sub>O (500 mg, 1.08 mmol), *n*Bu<sub>4</sub>NCl (598 mg, 2.16 mmol), and *L-Tyr* (294 mg, 1.62 mmol) was refluxed in *n*-butanol (10 mL) for 2 h. The solution was allowed to cool to room temperature, filtered, and transferred into an Erlenmeyer flask. After 12 d dark-red crystals were filtered off, washed with water (5 mL), ethanol (5 mL), diethyl ether (5 mL), and dried in vacuo. Yield: 274 mg, 38%. Anal. Calcd for  $C_{24}H_{44}Cl_3N_3O_4Ru$  ( $M = 660.08$  g/mol): C, 45.49; H, 7.02; N, 6.37. Found: C, 45.33; H, 6.85; N, 6.12%. ESI-MS in MeOH (negative):  $m/z$  418.7  $[RuCl_3NO(L-Tyr-2H)]^-$  ( $m_{theor} = 418.9$ ), 380.8  $[RuCl_2NO(L-Tyr-2H)]^-$  ( $m_{theor} = 380.9$ ), 344.8  $[RuClNO(L-Tyr-3H)]^-$  ( $m_{theor} = 344.9$ ). IR,  $cm^{-1}$ : 740, 827, 1183, 1270, 1366, 1466, 1641 (vs)  $\nu_{as}(COO^-)$ , 1885 (vs)  $\nu(NO)$ , 2962 m  $\nu(CH)$ , 3101 (m)  $\nu_s(NH_2)$ , and 3169 (m)  $\nu_{as}(NH_2)$ . UV-vis (buffer),  $\lambda_{max}$  nm ( $\epsilon$ ,  $M^{-1} cm^{-1}$ ): 279 (2109), 453 (99).  $^1H$  NMR (500.32 MHz, DMSO- $d_6$ ):  $\delta$  0.95 (t, 12H<sub>D</sub>,  $J = 7.4$  Hz), 1.32 (sxt, 8H<sub>C</sub>,  $J = 7.4$  Hz), 1.58 (qui, 8H<sub>B</sub>,  $J = 7.8$  Hz), 2.96 (m, 2H, H<sub>4</sub>, H<sub>4'</sub>), 3.17 (t, 8H<sub>A</sub>,  $J = 8.2$  Hz), 3.75 (m, 1H, H<sub>2</sub>), 4.71 (m, 1H, H<sub>3</sub>), 6.41 (m, 1H, H<sub>3''</sub>), 6.69 (d, 2H, H<sub>5</sub>,  $J = 7.4$  Hz), 7.09 (d, 2H, H<sub>5</sub>,  $J = 8.4$  Hz), 9.22 (s, 1H, H<sub>1</sub>) ppm.

**Physical Measurements.**  $^1H$  NMR spectra were recorded on a Bruker Avance III instrument (Ultraschield Magnet) at 500.13 MHz at room temperature. DMSO- $d_6$  was used as a solvent. Standard pulse programs were applied.  $^1H$  chemical shifts were measured relative to the residual solvent peaks. The hydrolytic stability of complex 8 in 20 mM phosphate buffer at pH 7.4 (0.1 M (KCl) ionic strength) and in pure water, both containing 10% D<sub>2</sub>O, was followed by recording  $^1H$  NMR spectra over 24 h. Complex concentration was 1.0 mM. Watergate water suppression program and 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) internal standard were used. ATR-IR spectra were measured on a Bruker Vertex spectrometer.  $D_{7.4}$  values were determined by the traditional shake-flask method in *n*-octanol/buffered aqueous solution at pH 7.4 (*N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES) buffer) and 298.0 ± 0.2 K, as described previously.<sup>25</sup> In the case of the complexes of *L-Ala* (2) and *L-Val* (3) the  $D_{7.4}$  values were determined in the presence of 0.1 M KCl as well. Two parallel experiments were performed for each sample. The complexes were dissolved at 0.3 mM in the *n*-octanol presaturated aqueous solution of the buffer (0.02 M). The aqueous solutions and *n*-octanol with 1:1 phase ratio were gently mixed with 360° vertical rotation for 3 h to avoid emulsion formation, and the mixtures were centrifuged at 5000 rpm for 3 min by a temperature-controlled centrifuge at 298 K. After separation, UV spectra of the complexes in the aqueous phase were compared to those of the original aqueous solutions, and  $D_{7.4}$  values were calculated as the mean of [absorbance (original solution)/absorbance (aqueous phase after separation) - 1] obtained in the region of  $\lambda \approx (250-290$  nm). Circular dichroism (CD) and UV-vis spectra under physiological conditions (0.02 M phosphate buffer, pH 7.40 with 0.1 M KCl) were recorded on a Jasco J-815 spectrometer in an optical cell of 2 cm path length (*l*) in the wavelength range from 220 to 600 nm. The analytical concentration for the CD measurement of the complexes was 400 μM in aqueous solution. CD data are given as the differences in molar absorptivities between left and right circularly polarized light, based on the concentration of the ligand ( $\Delta\epsilon = \Delta A/l/c_{complex}$ ). The concentrations for the UV-vis measurements amounted to 403 (1), 401 (4), 401 (5), 400 (8), 399 (3), 401 (2), 403 (7), and 401 (6) μM.

ESI-MS measurements for the characterization of the complexes were carried out with a Bruker Esquire 3000 instrument; the samples were dissolved in methanol. Cyclic voltammetry measurements were performed at room temperature using an AMEL 7050 all-in-one potentiostat. The concentrations amounted to 1.5–2.5 mM, the

Table 1. Crystal Data and Details of Data Collection for Complexes 1–4

complex	1	2	3	4
empirical formula	C <sub>18</sub> H <sub>40</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>3</sub> Ru	C <sub>19</sub> H <sub>42.34</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>3.17</sub> Ru	C <sub>21</sub> H <sub>46</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>3</sub> Ru	C <sub>21</sub> H <sub>44</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>3</sub> Ru
Fw	553.95	570.98	596.03	594.01
space group	<i>Pna</i> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
<i>a</i> , [Å]	10.1942(5)	15.3062(8)	8.6937(8)	10.2263(4)
<i>b</i> , [Å]	16.8268(9)	17.0885(8)	13.8069(12)	15.6517(6)
<i>c</i> , [Å]	15.6678(8)	31.3660(16)	22.711(2)	17.9281(7)
$\beta$ , [deg]		91.371(3)		
<i>V</i> [Å <sup>3</sup> ]	2687.6(2)	8201.7(7)	2726.1(4)	2869.55(19)
<i>Z</i>	4	12	4	4
$\lambda$ [Å]	0.71073	0.71073	0.71073	0.71073
$\rho_{\text{calcd}}$ [g cm <sup>-3</sup> ]	1.369	1.387	1.452	1.375
crystal size, [mm <sup>3</sup> ]	0.08 × 0.07 × 0.05	0.20 × 0.15 × 0.05	0.15 × 0.05 × 0.05	0.20 × 0.18 × 0.10
<i>T</i> [K]	293(2)	100(2)	100(2)	120(2)
$\mu$ , [mm <sup>-1</sup> ]	0.902	0.890	0.895	0.850
<i>R</i> <sub>1</sub> <sup>a</sup>	0.0321	0.0566	0.0634	0.0147
<i>wR</i> <sub>2</sub> <sup>b</sup>	0.0844	0.1346	0.1656	0.0418
Flack parameter	−0.02(5)	0.02(3)	0.01(8)	0.015(16)
GOF <sup>c</sup>	1.001	1.132	1.092	1.025

<sup>a</sup> $R_1 = \sum |F_o| - |F_c| / \sum |F_o|$ . <sup>b</sup> $wR_2 = \{ \sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2] \}^{1/2}$ . <sup>c</sup>GOF =  $\{ \sum [w(F_o^2 - F_c^2)^2] / (n - p) \}^{1/2}$ , where *n* is the number of reflections and *p* is the total number of parameters refined.

Table 2. Crystal Data and Details of Data Collection for Complexes 5–8

complex	5	6	7	8
empirical formula	C <sub>21</sub> H <sub>44</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>3</sub> Ru	C <sub>19</sub> H <sub>42.15</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>4.08</sub> Ru	C <sub>20</sub> H <sub>44</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>4</sub> Ru	C <sub>25</sub> H <sub>46</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>4</sub> Ru
Fw	594.01	585.33	598.00	660.07
space group	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	<i>P</i> 1	<i>P</i> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
<i>a</i> , [Å]	10.1919(19)	9.7963(4)	12.6677(12)	9.9542(3)
<i>b</i> , [Å]	15.628(3)	10.7133(4)	10.7195(10)	17.1180(6)
<i>c</i> , [Å]	17.930(4)	13.6446(6)	20.253(2)	17.8215(6)
$\alpha$ , [deg]		75.440(2)		
$\beta$ , [deg]		85.146(2)	102.943(5)	
$\gamma$ , [deg]		79.953(2)		
<i>V</i> [Å <sup>3</sup> ]	2855.9(10)	1363.52(10)	2680.3(4)	3036.71(17)
<i>Z</i>	4	2	4	4
$\lambda$ [Å]	0.71073	0.71073	0.71073	0.71073
$\rho_{\text{calcd}}$ [g cm <sup>-3</sup> ]	1.382	1.426	1.482	1.444
crystal size, [mm <sup>3</sup> ]	0.30 × 0.05 × 0.03	0.15 × 0.10 × 0.08	0.30 × 0.10 × 0.06	0.20 × 0.10 × 0.07
<i>T</i> [K]	120(2)	100(2)	100(2)	120(2)
$\mu$ , [mm <sup>-1</sup> ]	0.854	0.896	0.913	0.814
<i>R</i> <sub>1</sub> <sup>a</sup>	0.0539	0.0211	0.0430	0.0318
<i>wR</i> <sub>2</sub> <sup>b</sup>	0.1326	0.0508	0.1110	0.0816
Flack parameter	0.05(6)	0.01(1)	−0.06(2)	0.01(3)
GOF <sup>c</sup>	1.010	1.003	1.065	1.003

<sup>a</sup> $R_1 = \sum |F_o| - |F_c| / \sum |F_o|$ . <sup>b</sup> $wR_2 = \{ \sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2] \}^{1/2}$ . <sup>c</sup>GOF =  $\{ \sum [w(F_o^2 - F_c^2)^2] / (n - p) \}^{1/2}$ , where *n* is the number of reflections and *p* is the total number of parameters refined.

samples were dissolved in acetonitrile, and 0.1 to 0.2 M *n*Bu<sub>4</sub>N[BF<sub>4</sub>] was added as supporting electrolyte. Further a 3 mm glassy carbon (GC) working electrode, a Pt auxiliary electrode, and a saturated calomel electrode (SCE) reference electrode were used. The same electrode types were used for coulometry. In this case, the compartment of the auxiliary electrode was separated from the study compartment. Ferrocene was used as an internal standard.

**Crystallographic Structure Determination.** X-ray diffraction measurements were performed on a Bruker X8 APEXII CCD diffractometer. Single crystals were positioned at 40 mm from the detector, and 1348, 1526, 1100, 2183, 961, 2191, 1606, and 1391 frames were measured, each for 30, 30, 80, 20, 10, 60, 30, and 30 s over 1 (or 0.5° for 4) scan width for 1–8, respectively. The data were processed using SAINT software.<sup>26</sup> Crystal data, data collection parameters, and structure refinement details are given in Tables 1 and

2. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were inserted in calculated positions and refined with a riding model. Two carbon atoms C5 and C6 in the tetrabutylammonium cation in 1 were found to be disordered over three positions with site occupation factors (s.o.f.) of 0.4:0.4:0.2, while C20, C21, and C22 were found in one of six crystallographically independent TBA cations in 2 over two positions with s.o.f. 0.5:0.5. In complex 4 the C2 atom of the prolinic ring and atoms C6 and C8 of L-Ser in one crystallographically independent complex anion in 6 were found to be disordered over two positions with populations of 0.8:0.2. The carbon atoms C12, C13, C16, C17, C21, and C24, C25 in the TBA cation in 8 were found to be disordered over 2 positions with s.o.f. of 0.6:0.4. The disorder was resolved by using restraints SADI and EADP implemented in

SHELXL. The following computer programs and hardware were used: structure solution, *SHELXS-97* and refinement, *SHELXL-97*;<sup>27</sup> molecular diagrams, ORTEP;<sup>28</sup> computer, Intel CoreDuo.

**Mass Spectrometry.** The stability of four compounds, namely **1**, **4**, **5**, and **8**, in aqueous solution and in the presence of 4 equiv of sodium ascorbate was investigated using an AmaZon SL ESI ion trap mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany). For this purpose, the compounds were diluted from 400  $\mu\text{M}$  stock solution (1% DMSO) to 50  $\mu\text{M}$  in water and in the presence of 200  $\mu\text{M}$  sodium ascorbate. The solutions were incubated at 310 K in the dark, and samples were measured after 0.5, 1, 2, 6, and 24 h after a second dilution step to 5  $\mu\text{M}$  of the metal compound. The samples were introduced by direct infusion into the mass spectrometer at 280  $\mu\text{L}/\text{h}$ , and mass spectra were recorded over 0.5 min and averaged. Typical experimental conditions were as follows: high voltage (HV) capillary  $\pm 4.5$  kV, dry temp 180  $^{\circ}\text{C}$ , nebulizer 8 psi, dry gas 6 L/min, radio frequency (RF) level 77%, trap drive 57.6, average accumulation time 25 ms (negative ion mode) and 120  $\mu\text{s}$  (positive ion mode). Mass spectra were acquired and processed using ESI Compass 1.3 and DataAnalysis 4.0 (Bruker Daltonics GmbH, Bremen, Germany). The theoretically most abundant signal of the isotopic pattern is annotated.

**Antiproliferative Activity.** CH1 cells (human ovarian carcinoma) were a generous gift from Lloyd R. Kelland, CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK. SW480 (human adenocarcinoma of the colon) and A549 (human nonsmall cell lung cancer) cells were kindly provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). All cell culture media and reagents were purchased from Sigma-Aldrich Austria and plastic ware from Starlab Germany. Cells were grown in 75  $\text{cm}^2$  culture flasks as adherent monolayer cultures in minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal calf serum, 1 mM sodium pyruvate, 4 mM L-glutamine, and 1% nonessential amino acids (from 100 $\times$  ready-to-use stock). Cultures were maintained at 310 K in humidified atmosphere containing 95% air and 5%  $\text{CO}_2$ .

Cytotoxic effects of the test compounds were determined by means of a colorimetric microculture assay [MTT assay; MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] as described previously.<sup>13</sup> Cells were harvested from culture flasks by trypsinization and seeded by using a pipetting system (Biotek Precision XS Microplate Sample Processor) in densities of  $1 \times 10^3$  (CH1),  $2 \times 10^3$  (SW480), and  $3 \times 10^3$  (A549) in 100  $\mu\text{L}$ /well aliquots in 96-well microculture plates. For 24 h, cells were allowed to settle and resume proliferation. Test compounds were then dissolved in DMSO, diluted in complete culture medium, and added to the plates where the final DMSO content did not exceed 0.5%. After 96 h of drug exposure, the medium was replaced with 100  $\mu\text{L}$ /well of a 1:7 MTT/RPMI 1640 mixture (MTT solution, 5 mg/mL of MTT reagent in phosphate-buffered saline; RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine), and plates were incubated for further 4 h at 310 K. Subsequently, the solution was removed from all wells, and the formazan crystals formed by viable cells were dissolved in 150  $\mu\text{L}$  of DMSO per well. Optical densities at 550 nm were measured with a microplate reader (Biotek ELx808) by using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed relative to untreated controls, and 50% inhibitory concentrations ( $\text{IC}_{50}$ ) were calculated from concentration-effect curves by interpolation. Evaluation is based on means from three independent experiments.

## RESULTS AND DISCUSSION

We were interested in the study of the reactions of ruthenium-nitrosyl complexes with all amino acids except two already reported in the literature with L-His and L-Met,<sup>10,11</sup> isolation of the resulted products, and testing antiproliferative activity of all prepared products, including  $[\text{RuCl}_2(\text{L-His-H})(\text{NO})]$  and  $[\text{RuCl}_2(\text{L-Met-H})(\text{NO})]$ , reported previously. Amino acids are potential biological ligands for ruthenium anticancer drugs.

The interactions with amino acids deserve to be investigated, as they can help in elucidating the underlying mechanism of their antitumor activity. These reactions and the biological effects of the resulting species are still little understood.

It is known, however, that cisplatin and carboplatin have a high affinity for sulfur-containing biological molecules, such as methionine, glutathione, and sulfur-containing proteins. These interactions have been associated with toxic side effects, detoxification, and resistance mechanisms, as well as with delivery of active species to the cell for ultimate binding to DNA.<sup>23a,29–31</sup>

In the case of ruthenium, it has been found that  $[(\eta^6\text{-bip})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$  (bip = biphenyl, en = ethylenediamine) reacts slowly with L-Cys, L-Met, and L-His in water at 310 K to partial (22–50%) completion.<sup>32,33</sup> Comparison of the equilibrium constants measured suggested that the affinity of the  $[(\eta^6\text{-bip})\text{Ru}(\text{en})]^{2+}$  moiety for these amino acids decreases in the order of L-Cys > L-Met > L-His.<sup>34</sup> The observed reactions were largely suppressed in the presence of 0.1 M NaCl, indicating that these amino acids may not be able to inactivate the complex in blood plasma or in cells.<sup>30</sup> The low reactivity of these amino acids toward  $[(\eta^6\text{-bip})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$  may be the reason for low toxic side effects of this and related ruthenium-arene complexes.<sup>35</sup> These interactions cannot impede the transport and delivery of the drugs to the cancer cells and allow at least for some amino acids to act as drug reservoirs for DNA ruthenation.<sup>33</sup> In stark contrast, ruthenium hexacationic prism reacts with His, which binds to the  $(\eta^6\text{-p-cymene})\text{Ru}$  moiety with release of the 2,4,6-tri(pyridin-4-yl)-1,3,5-triazine and 1,4-benzoquinonato ligands, while it remains intact in the presence of Met.<sup>36</sup> The resulting  $(\eta^6\text{-p-cymene})\text{Ru-His}$  complex was found to catalyze oxidation of cysteine to cystine more efficiently than the original complex, and this process may play a role in the antiproliferative activity of the complex since amino acids represent a significant component of the cytosol. Sequence-specific catalytic peptide synthesis with the half-sandwich ruthenium complex is another example well-documented in the literature.<sup>37</sup> NAMI-A treated with histidine or glutamine at their MEM concentrations was shown to result in a reduced uptake by KB carcinoma cells presumably because of formation of adducts with these amino acids or competition between MEM components and NAMI-A upon transport through the cell membrane.<sup>38</sup> Thus, reactions with amino acids may also have an impact on intracellular chemistry of ruthenium-based drugs.

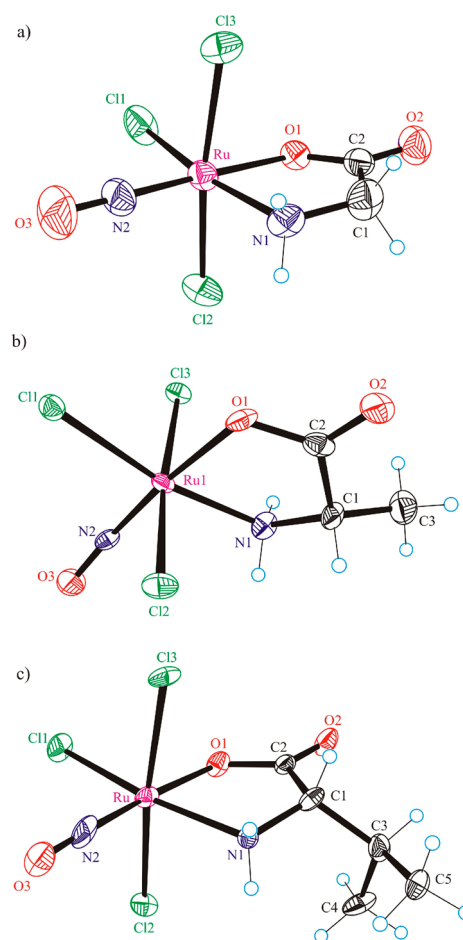
In this work we report on the preparation of eight ruthenium-nitrosyl complexes with Gly, L-Ala, L-Val, L-Pro, D-Pro, L-Ser, L-Thr, and L-Tyr. As starting material,  $\text{Na}_2[\text{RuCl}_5(\text{NO})]\cdot 6\text{H}_2\text{O}$  was used, which was prepared by reaction of  $\text{RuCl}_3\cdot n\text{H}_2\text{O}$  with  $\text{NaNO}_2$  in the presence of 6 M HCl as reported previously, or  $(n\text{Bu}_4\text{N})_2[\text{RuCl}_5(\text{NO})]$ .<sup>24</sup> Complexes **1–8** were synthesized by boiling  $\text{Na}_2[\text{RuCl}_5(\text{NO})]\cdot 6\text{H}_2\text{O}$  with 1.5 equiv of tetrabutylammonium chloride and 1.1 equiv of the corresponding amino acid or by reaction of  $(n\text{Bu}_4\text{N})_2[\text{RuCl}_5(\text{NO})]$  with AA in *n*-butanol or *n*-propanol with 15–38% yields. Compounds **1** and **8** crystallized directly from the reaction mixture after reaction completion. All other complexes were obtained by evaporating the solvent under reduced pressure and recrystallization of the residue from water at room temperature over 96 h on average. ESI mass spectra measured in positive ion mode showed a peak at  $m/z$  242 due to  $n\text{Bu}_4\text{N}^+$ , while those measured in negative ion mode showed strong peaks at  $m/z$  312, 324, 353, 351, 351, 342, 355,

and 419 for 1–8, respectively, attributed to  $[\text{RuCl}_3(\text{AA}-\text{H})(\text{NO})]^-$ . Other signals of moderate intensity usually found in the mass spectra were assigned to  $[\text{RuCl}_2(\text{AA}-\text{H})(\text{NO})-\text{H}]^-$  and  $[\text{RuCl}(\text{AA}-\text{H})(\text{NO})-2\text{H}]^-$ . Coordination of an amino acid to ruthenium in  $[\text{RuCl}_5(\text{NO})]^{2-}$  leads to a shift of the stretching vibration  $\nu(\text{NO})$  from  $1902\text{ cm}^{-1}$  to  $1837\text{--}1852\text{ cm}^{-1}$  for 1–8. All complexes are diamagnetic. The number of proton resonances in the  $^1\text{H}$  NMR spectra of 1–8 in  $\text{DMSO}-d_6$  is in accordance with the proposed structures for these compounds (see Chart 1 and Experimental Section).

**X-ray Crystallography.** As reported for osmium-nitrosyl complexes with Gly, L-Pro, and D-Pro,<sup>13</sup> three isomeric structures are theoretically possible for  $[\text{RuCl}_3(\text{AA}-\text{H})(\text{NO})]^-$  (AA = Gly, L-Ala, L-Val, L-Pro, D-Pro, L-Ser, L-Thr, and L-Tyr acting as bidentate ligands): one *fac* isomer with three chlorido ligands coordinated to ruthenium in facial configuration and two others with three chlorido ligands bound to the central atom in meridional fashion. In the first hypothetical meridional isomer NO is located trans to the N atom of the amino acid, while in the second NO is bound trans to the carboxylic oxygen atom of the AA ligand. According to X-ray crystallography all studied compounds (1–8) have a similar ionic crystal structure, which is built up from coordination anions  $[\text{RuCl}_3(\text{AA}-\text{H})(\text{NO})]^-$  and tetrabutylammonium cations. No cocrystallized solvent has been found in the crystals of the compounds studied, except the structures 2 and 6, which contain some statistically distributed water molecules. The results of X-ray diffraction studies of complexes 1–8, together with atom numbering schemes, are shown in Figures 1–3. The crystallographic data and refinement details are given in Tables 1 and 2, while selected geometrical parameters are in Table 3. The asymmetric part in 6 and 7 contains two cation/anion pairs, while in 2 six chemically identical but crystallographically independent cation/anion pairs are found. Figures 1b and 3a,b show the structures of only one asymmetric component in the unit cell.

Each ruthenium atom in 1–8 adopts a slightly distorted octahedral coordination geometry, being coordinated by the (AA-H)<sup>-</sup> nitrogen atom and carboxylate oxygen donor, one nitrosyl and one chlorido ligand in the equatorial plane, and two Cl<sup>-</sup> ligands in axial positions. Three chlorido ligands are bound meridionally with the average distances of Ru–Cl at 2.37 Å. The NO ligand is coordinated almost linearly to ruthenium trans to the carboxylic oxygen atom of the (AA-H)<sup>-</sup> ligand, with a Ru–O bond length of about 1.71 Å (see Table 3). The equatorial coordination plane is practically planar. The maximum deviation from the mean-square plane in all structures does not exceed  $\pm 0.03$  Å. In structures 1 and 8 the five-membered chelate ring formed upon the coordination of (AA-H)<sup>-</sup> is almost planar, while in all other cases it adopts a half-chair conformation. Thus, in 2, 3, 6, and 7 the angle between the Ru1O1C2C1 and Ru1N1C1 planes is equal to 24.8°, 18.9°, 29.8°, and 24.6°, while between Ru1O1C4C5 and Ru1N1O1 the angle in 4 and 5 is 17.1° and 17.5°, respectively. As found in earlier reported osmium complexes,<sup>13</sup> the two chiral centers located on C1 and N1 atoms have the same configuration  $S_C S_N$  and  $R_C R_N$  in 4 and 5, respectively. The configuration of asymmetric atoms C1 and C3 of L-Thr is also preserved in complex 7. Selected bond lengths and angles summarized in Table 3 suggest that there are no marked geometrical parameter variations among complexes 1–8.

There are different groups that can play the role of potential proton donors or proton acceptors in the crystal structures of

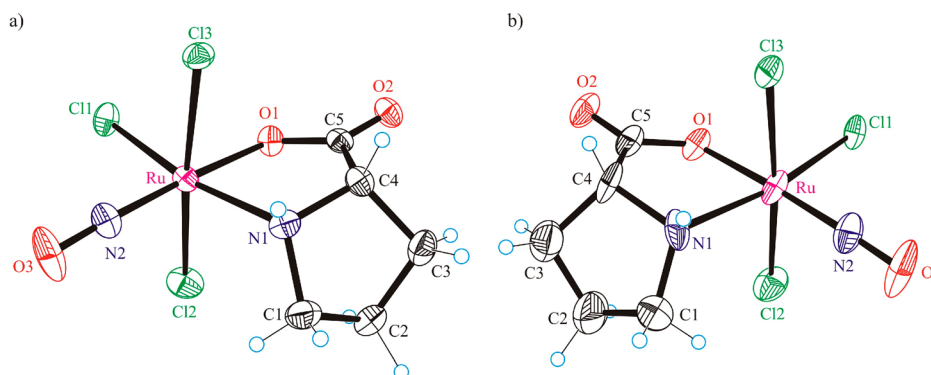


**Figure 1.** ORTEP drawings of the complex anion (a)  $[\text{RuCl}_3(\text{Gly}-\text{H})(\text{NO})]^-$  in 1, (b)  $[\text{RuCl}_3(\text{L-Ala}-\text{H})(\text{NO})]^-$  in 2, and (c)  $[\text{RuCl}_3(\text{L-Val}-\text{H})(\text{NO})]^-$  in 3 with atom labeling. The thermal ellipsoids are drawn at 50% probability level.

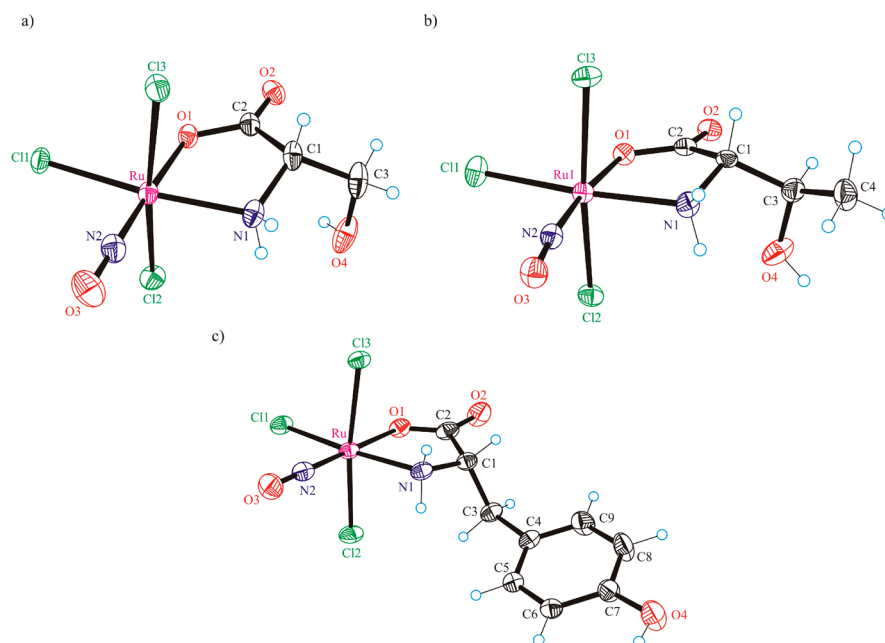
complexes 1–8. The relevant hydrogen bonding parameters are collected in Supporting Information, Table S1. The common crystal structure motif for 1–8 is determined by the parallel packing of one-dimensional polymeric chains, assembled via hydrogen bonding of the complex anions. The perspective views of these supramolecular architectures are shown in Supporting Information, Figures S1–S4. These chains are of four types, depending on the nature of hydrogen-bonding interactions. Supporting Information, Figure S1 shows infinite chains formed in crystals of 1–3 via  $\text{N}-\text{H}\cdots\text{Cl}$  contacts. Polymeric chains in complexes 4–6 are built up via H-bonds of two types  $\text{N}-\text{H}\cdots\text{O}$  and  $\text{N}-\text{H}\cdots\text{Cl}$ , as shown in Supporting Information, Figure S2, while those in complexes 7 and 8 shown in Supporting Information, Figures S3 and S4 are formed via interactions of the types  $\text{N}-\text{H}\cdots\text{O}$ ,  $\text{O}-\text{H}\cdots\text{O}$ , and  $\text{N}-\text{H}\cdots\text{Cl}$ ,  $\text{O}-\text{H}\cdots\text{O}$ , respectively. Note that all possibilities for hydrogen bonding formation are exhausted in 1–8.

The diamagnetic behavior of 1–8, the  $\nu_{\text{NO}}$  wavenumbers, and the linearity of the Ru–NO group provide strong evidence for the formulation  $\{\text{Ru}(\text{NO})\}^6$  containing  $\text{Ru}^{\text{II}}$  ( $S = 0$ ) bound to  $\text{NO}^+$  ( $S = 0$ ) or  $\text{Ru}^{\text{III}}$  ( $S = 1/2$ ) coupled antiferromagnetically to  $\text{NO}^0$  ( $S = 1/2$ ).

**Electrochemistry.** The redox properties of complexes 2–8 have been investigated by cyclic voltammetry at a GC electrode in a 0.1–0.2 M  $[\text{nBu}_4\text{N}][\text{BF}_4]/\text{CH}_3\text{CN}$  solution at 25 °C. For 1, 3–7 similar electrochemical behavior was observed, as shown



**Figure 2.** ORTEP drawings of the complex anion (a)  $[\text{RuCl}_3(\text{L-Pro-H})(\text{NO})]^-$  in **4** and (b)  $[\text{RuCl}_3(\text{D-Pro-H})(\text{NO})]^-$  in **5** with atom labeling. The thermal ellipsoids are shown at 50% probability level.



**Figure 3.** ORTEP drawings of the complex anion (a)  $[\text{RuCl}_3(\text{L-Ser-H})(\text{NO})]^-$  in **6**, (b)  $[\text{RuCl}_3(\text{L-Thr-H})(\text{NO})]^-$  in **7**, (c) and  $[\text{RuCl}_3(\text{L-Tyr-H})(\text{NO})]^-$  in **8** with atom labeling. The thermal ellipsoids are shown at 50% probability level.

in Supporting Information, Figures S5–S8. The compounds show one to three irreversible oxidation waves with peak potential values higher than 1.6 V versus SCE (Table 4). At these potentials, the ruthenium(II) ion is usually oxidized.<sup>39</sup> The processes are irreversible due to chemical reactions that follow the electron transfer(s). The oxidation of the metal-bound amino acid largely depends on the experimental conditions.<sup>40</sup> Dissociation of the amino acid from ruthenium results in the formation of electrode deposit. This was encountered upon several coulometry experiments performed. The number of electrons involved in all the oxidation waves (determined by coulometry or with the use of the  $\text{Fc}^+/\text{Fc}$  couple as reference) gave generally an apparent electron number  $n_{\text{app}} = 3$ . A similar value was found for  $[\text{RuCl}_3(\text{AA-H})(\text{NO})]^-$  (AA = amino acid), with one irreversible electron transfer followed by one reversible process.<sup>13</sup> For osmium complexes, the peak separation was dependent on the nature of the coordinated amino acid. In addition, the reaction  $[\text{Os}(\text{NO})]^{6+} \rightarrow [\text{Os}(\text{NO})]^{5+}$ , generally reversible or quasi-reversible, could also be identified. Here, all the processes are irreversible, and the accurate determination of the peak

potential values depends on the degree of the overlapping of the oxidation waves. In particular, a more distinct separation of the oxidation waves (at ca. 1.90 and 2.30 V vs SCE) is observed for **3** (Supporting Information, Figure S5) than for **6** (1.80 and 1.87 V vs SCE). Taking all this into account we suggest that the anodic waves can be attributed to both the oxidation of the ruthenium ion and the oxidation of the amino acid. Upon reduction we observe a one-electron irreversible wave at ca.  $-0.8$  V versus SCE (except for **3** and **8**). Note that more positive values were seen for related osmium complexes.<sup>13</sup> This reduction process presumably takes place on the metal center and is followed by chemical transformations. For **3** the general pattern of reduction peaks seems to be dependent on the state of the electrode area (see Supporting Information, Figure S5).

**UV-vis and CD Spectra.** The complexes possess fairly similar UV-vis spectra with a well-defined  $\lambda_{\text{max}}$  at 452 nm (Figure 4). CD spectra of the complexes of the L-amino acids show similarities as well, namely, negative peaks with  $\lambda_{\text{max}}$  at  $\sim 440$  and 313 nm, while the complex of D-Pro shows positive peaks at the same wavelengths.

Table 3. Selected Bond Distances (Å) and Bond Angles in Compounds 1–8

	1	2 <sup>c</sup>	3	4	5	6 <sup>b</sup>	7 <sup>b</sup>	8
Ru–O1	2.001(3)	2.009(5), 1.993(9)	1.997(5)	1.998(1)	1.988(4)	2.008(2), 2.011(3)	2.011(3), 2.009(1)	2.019(3)
Ru–N1	2.051(4)	2.078(6), 2.073(4)	2.097(6)	2.107(2)	2.132(6)	2.068(1), 2.074(7)	2.078(3), 2.086(8)	2.077(3)
Ru–N2	1.702(4)	1.707(6), 1.714(3)	1.731(7)	1.725(2)	1.726(6)	1.7256(17), 1.7249(7)	1.732(4), 1.729(3)	1.730(3)
N2–O3	1.149(6)	1.178(8), 1.164(7)	1.158(8)	1.147(2)	1.141(7)	1.149(2), 1.1485(5)	1.145(5), 1.147(2)	1.137(3)
Ru–Cl1	2.361(2)	2.376(2), 2.367(3)	2.363(2)	2.3756(4)	2.383(2)	2.3724(4), 2.374(1)	2.365(1), 2.369(4)	2.3771(7)
Ru–Cl2	2.361(2)	2.370(2), 2.381(6)	2.376(2)	2.3803(4)	2.368(2)	2.3539(4), 2.36(1)	2.367(1), 2.3664(9)	2.3713(8)
Ru–Cl3	2.380(1)	2.364(2), 2.360(5)	2.374(2)	2.3636(5)	2.383(2)	2.3789(4), 2.37(3)	2.376(1), 2.371(4)	2.3732(6)
atom1–atom2–atom3								
O1–Ru–N1	80.1(1)	80.0(2), 81.0(4)	80.5(2)	80.67(5)	81.4(2)	79.35(6), 79.2(1)	80.0(1), 79.7(3)	81.28(9)
Ru–N2–O3	179.1(5)	171.9(6), 176(1)	177.7(6)	176.52(16)	176.4(6)	178.30(2), 177.5(9)	174.1(3), 174.8(7)	179.4(3)
Cl2–Ru–Cl3	172.77(5)	173.71(7), 173.1(5)	173.96(8)	174.90(2)	175.37(7)	172.42(2), 172.9(5)	173.93(4), 173.1(6)	172.74(3)

<sup>a</sup>Parameters for one of the six crystallographically independent complex anions and average values are quoted. <sup>b</sup>Parameters for one of the two crystallographically independent complex anions and average values are quoted. <sup>c</sup>In comparison with the ferrocene these values are close. <sup>d</sup>No clear oxidation wave was observed; sh = shoulder.

Table 4. Cyclic Voltammetric Data<sup>a</sup> for Complexes 1–8

compound		oxidation peaks		reduction peaks <sup>b</sup>
1		1.90	2.40	−1.20
2	<i>d</i>			−0.79
3	1.8 <sup>sh</sup>	1.90	2.28	−1.31
4	1.63 <sup>c</sup>			−0.79
5	1.68 <sup>c</sup>			−0.82
6	1.8 <sup>sh</sup>	1.87		−0.80
7	1.8 <sup>sh</sup>	1.91		−0.83
8	<i>d</i>			−2.25

<sup>a</sup>Potential values in volts  $\pm$  0.02 vs SCE, in a 0.1–0.2 M [*n*Bu<sub>4</sub>N][BF<sub>4</sub>]/CH<sub>3</sub>CN solution, at a GC working electrode, determined by using the [Fe( $\eta^5$ -C<sub>5</sub>H<sub>5</sub>)<sub>2</sub>]<sup>0/+</sup> redox couple ( $E_{1/2}^{ox}$  = 0.525 V vs SCE)<sup>41,42</sup> as internal standard at a scan rate of 100 mV s<sup>−1</sup>; the values can be converted to the NHE reference by adding +0.245 V. <sup>b</sup>Determined in the experiment with several cycles of potential. <sup>c</sup>In comparison with the ferrocene these values are close. <sup>d</sup>No clear oxidation wave was observed; sh = shoulder.

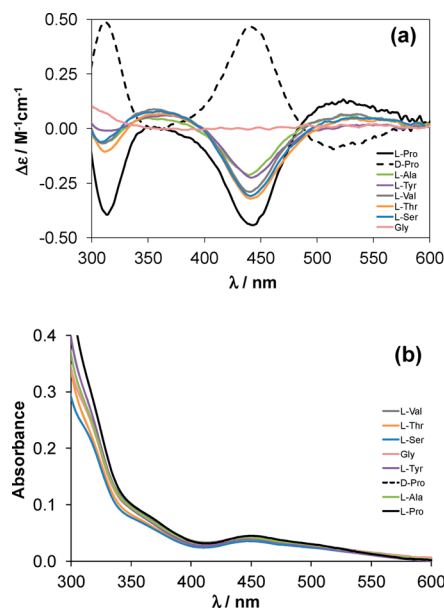
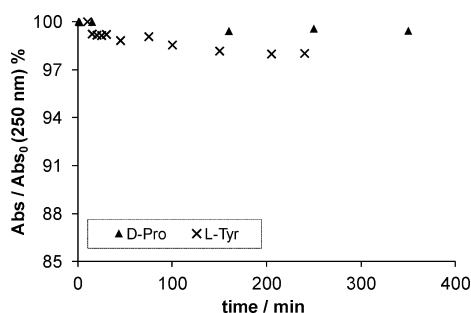


Figure 4. (a) CD and (b) UV–vis spectra of the studied (*n*Bu<sub>4</sub>N)[RuCl<sub>3</sub>(AA–H)(NO)] complexes at pH 7.40 [*c*<sub>complex</sub> = 400 μM; *l* = 2 cm; 0.02 M phosphate buffer; 0.1 M KCl; *T* = 298 K].

**Hydrolytic Stability, Lipophilicity, and Co-incubation with Sodium Ascorbate.** The hydrolytic stability of complex 8 was monitored in aqueous medium (0.1 M KCl), buffered at pH 7.4, by <sup>1</sup>H NMR spectroscopy over 24 h. Chemical shifts and shapes of all peaks remained unchanged within this time frame (see Supporting Information, Figure S9). In addition a solution of complex 8 in a 10% D<sub>2</sub>O/H<sub>2</sub>O mixture in nonbuffered medium (pH = 5.86) showed the same NMR spectra as in solutions buffered at pH 7.4. Hydrolytic stability of complexes 1–8 was further investigated by UV–vis spectroscopy (vide infra).

All the prepared complexes were found to be moderately water-soluble and stable in solution within the time frame of the measurements (5.5 h), since the normalized UV–vis spectra recorded after the partitioning were identical with the original ones. It is noteworthy that hydrolysis of complexes 5 and 8 is negligible over 24 h in the presence of 0.1 M KCl or in its absence, as illustrated in Figure 5.





**Figure 5.** Time dependence of absorbance values of  $(\text{Bu}_4\text{N})\text{-}[\text{RuCl}_3(\text{AA-H})(\text{NO})]$  complexes, where AA = D-Pro and L-Tyr, recorded at 250 nm at pH 7.40 [ $c_{\text{complex}} = 0.25 \text{ mM}$ ; 0.02 M HEPES;  $T = 298.0 \text{ K}$ ].

The  $\log D_{7.4}$  values of the complexes were determined by the traditional shake-flask method in *n*-octanol/buffered aqueous solution at pH 7.4 by analysis of the UV-vis spectra of the aqueous phases before and after separation (Supporting Information, Figure S10 and Table 5). Results revealed the fairly hydrophilic character of all the complexes studied. The  $\log D_{7.4}$  values for the complexes increase in the following order: Gly (**1**) < L-Ser (**6**), L-Thr (**7**), L-Ala (**2**) < D/L-Pro (**5/4**) < L-Tyr (**8**), L-Val (**3**), corresponding well to the expectations based on the hydrophilicity of the side chains of the coordinated amino acids. On the other hand the presence of chloride ion does not alter significantly the lipophilicity of the complexes (Table 5).

The aqueous stability of compounds **1**, **4**, **5**, and **8** was also confirmed by ESI mass spectrometry. Mass spectra recorded in the negative ion mode revealed  $[\text{RuCl}_3(\text{AA-H})(\text{NO})]^-$  as the major species (Supporting Information, Figures S11–S13) in all four incubations over 24 h, while TBA was the only mass signal in the positive ion mode. Similar mass spectra were observed for the co-incubation with 4 equiv of sodium ascorbate, a potent biological reducing agent. These results largely parallel the findings with analogous osmium-nitrosyl complexes with amino acids, which were also stable in water.<sup>13</sup> In the present study, however, additional mass signals were assigned to  $[\text{RuCl}_{2-n}\{\text{AA}-(2+n)\text{H}\}\text{NO}]^-$ , where  $n = 0$  or 1, and probably stem from the spraying process. The simultaneous cleavage of two HCl molecules from the parent mass

signal during ionization indicates that the ruthenium compounds might be activated by hydrolysis. This would also be in line with the increased antiproliferative activity of the  $[\text{RuCl}_3(\text{AA-H})(\text{NO})]^-$  series compared to the osmium counterparts. Obviously, compound **1** does only have four hydrogen atoms stemming from the coordinated Gly-H. We performed ESI-MS experiments of **1** in  $\text{D}_2\text{O}$  and  $\text{H}_2\text{O}$ , respectively, to investigate which hydrogens are abstracted to provide the negative charge of the observed gas-phase compounds (Figure 6). Dissolution of **1** in  $\text{D}_2\text{O}$  leads to the change of the labile  $-\text{NH}_2$  to  $-\text{ND}_2$ , and the resulting compound  $[\text{RuCl}_3(\text{N},\text{N}-d_2\text{-Gly-H})(\text{NO})]^-$  ( $m/z$  314.74 compared to  $m/z$  312.72 of **1**) was analyzed. As can be seen in Figure 6, DCl is cleaved in a first step from the parent ion following the deprotonation of the coordinated amine. The cleavage of HCl in the second step suggests imine formation. Incubation with sodium ascorbate did not induce ligand release over 24 h. Note that related ruthenium-nitrosyl complexes withazole heterocycles reacted quantitatively with sodium ascorbate within several hours.<sup>15</sup>

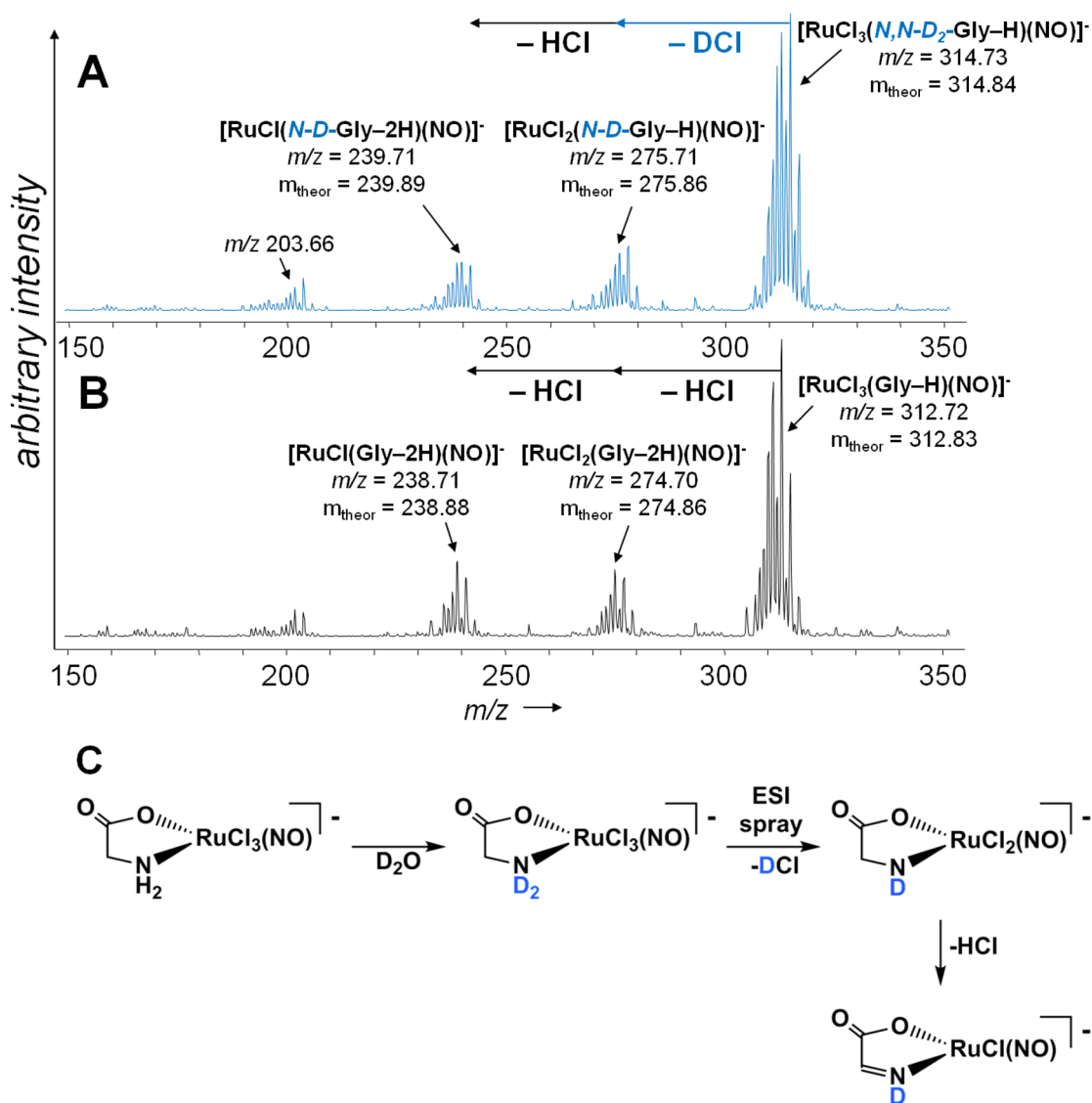
**Inhibition of Cancer Cell Growth.** The *in vitro* anticancer activity of complexes **1–8** was assessed in the human cancer cell lines CH1 (ovarian carcinoma), SW480 (colon carcinoma), and A549 (nonsmall cell lung carcinoma) by means of the colorimetric MTT assay, yielding the  $\text{IC}_{50}$  values listed in Table 5. All compounds show a higher effect in the generally more chemosensitive CH1 cells ( $\text{IC}_{50}$ : 7.5–27  $\mu\text{M}$ ) than they do in SW480 cells ( $\text{IC}_{50}$ : 20–71  $\mu\text{M}$ ) and in the generally more chemoresistant A549 cells ( $\text{IC}_{50} > 100 \mu\text{M}$ ). With regard to variation of the amino acid ligand, differences between  $\text{IC}_{50}$  values within each of the cell lines CH1 and SW480 are all smaller than 4-fold. In CH1 cells, the  $\text{IC}_{50}$  is 3.6 times higher for the glycinate complex **1**, which is the most hydrophilic compound, than it is for the L-valinate complex **3**, which is the most hydrophobic compound. The L-prolinate (**4**) and D-prolinate (**5**) analogues show differences in antiproliferative activity, with the latter being more active than the former by factors of 1.5 and 2.7 in CH1 and SW480 cells, respectively. In two of the three cancer cell lines, complex **5** shows the strongest growth inhibitory effect in all three cancer cell lines.

The antiproliferative activity of these ruthenium complexes is particularly remarkable in comparison with osmium analogues published previously.<sup>13</sup> The ruthenium complex ( $n\text{Bu}_4\text{N}$ )-

**Table 5.** In Vitro Anticancer Activity of the Compounds **1–8** and Three Osmium Analogues **1\***, **4\***, and **5\***<sup>a</sup>

complex	$\text{IC}_{50}$ values $\pm$ SD ( $\mu\text{M}$ )			partition coefficients	
	A549	CH1	SW480	$\log D_{7.4}$	$\log D_{7.4}^b$
<b>1</b>	196 $\pm$ 27	7.5 $\pm$ 1.2	39 $\pm$ 3	−2.04 $\pm$ 0.08	
<b>2</b>	>320	12 $\pm$ 2	47 $\pm$ 3	−1.63 $\pm$ 0.08	−1.47 $\pm$ 0.11
<b>3</b>	>320	27 $\pm$ 3	53 $\pm$ 2	−1.13 $\pm$ 0.02	−1.31 $\pm$ 0.07
<b>4</b>	>320	20 $\pm$ 3	54 $\pm$ 10	−1.55 $\pm$ 0.08	
<b>5</b>	108 $\pm$ 5	13 $\pm$ 1	20 $\pm$ 3	−1.43 $\pm$ 0.08	
<b>6</b>	>320	13 $\pm$ 2	63 $\pm$ 10	−1.77 $\pm$ 0.12	
<b>7</b>	>320	23 $\pm$ 2	71 $\pm$ 15	−1.75 $\pm$ 0.02	
<b>8</b>	>320	17 $\pm$ 3	38 $\pm$ 12	−1.16 $\pm$ 0.02	
<b>1*</b> <sup>c</sup>	629 $\pm$ 13	89 $\pm$ 11	140 $\pm$ 36		
<b>4*</b> <sup>c</sup>	>320	114 $\pm$ 37	237 $\pm$ 47		
<b>5*</b> <sup>c</sup>	>640	148 $\pm$ 38	274 $\pm$ 40		

<sup>a</sup>In human ovarian (CH1), colon (SW480), and non-small cell lung (A549) carcinoma cell lines, with  $\log D_{7.4}$  values for the complexes; 50% inhibitory concentrations (means  $\pm$  standard deviations), obtained by the MTT assay (exposure time 96 h),  $\log D_{7.4}$  values were estimated in 0.02 M HEPES;  $T = 298.0 \text{ K}$ . <sup>b</sup>In the presence of 0.1 M KCl. <sup>c</sup>Data taken from reference 13.



**Figure 6.** ESI mass spectra of **1** in (A)  $D_2O$  and (B)  $H_2O$  are shown. (C) Dissolution of **1** in  $D_2O$  leads to the exchange of the labile hydrogen atoms of the amino group by deuterium introducing two neutrons in the compound, thereby increasing the molecular mass. The mass-to-charge ratio of the fragments indicates the position of H/D abstraction.

$[RuCl_3(Gly-H)(NO)]$  (**1**) turned out to be more active than the corresponding osmium analogue  $(n-Bu_4N)[Os(NO)Cl_3(Gly)]$ , with a maximum factor of 12 in CH1 cells (3.6 and 3.2 in SW480 and A549 cells, respectively). In the mentioned publication the  $(n-Bu_4N)[Os(NO)Cl_3(L-Pro)]$  and  $(n-Bu_4N)[Os(NO)Cl_3(D-Pro)]$  complexes showed no pronounced activity and no differences between isomers. In contrast, the complexes  $(n-Bu_4N)[RuCl_3(L-Pro-H)(NO)]$  and  $(n-Bu_4N)[RuCl_3(D-Pro-H)(NO)]$  presented here show pronounced effects and a slight dependence on L-/D-isomerism. The D-isomer is 11-fold and 14-fold more active in CH1 and SW480 cells, respectively, whereas the L-isomer is 5.7-fold and 4.4-fold more active than the respective osmium analogue. A synopsis of all comparisons reveals that the impact of changing the metal center on cytotoxic potency is much bigger than that of varying the amino acid ligand.

## CONCLUSIONS

Reactions of potential anticancer drugs with amino acids have been studied by different groups,<sup>32,43,44</sup> but mainly in solution

by  $^1H$  NMR spectroscopy without isolation of the resulting products. We have succeeded in preparing a series of ruthenium-nitrosyl complexes with amino acids of the general formula  $(n-Bu_4N)[RuCl_3(AA-H)(NO)]$ , where AA = Gly, L-Ala, L-Val, L-Pro, D-Pro, L-Ser, L-Thr, and L-Tyr, in addition to two complexes documented in the literature with L-His and L-Met, namely,  $[RuCl_2(L-His)(NO)]^{10}$  and  $[RuCl_2(L-Met)(NO)]^{11}$ . X-ray crystallographic studies have shown that in crystal structures of **1–8**, as in the previously reported osmium counterparts  $(n-Bu_4N)[OsCl_3(AA-H)(NO)]^{13}$  where AA = Gly, L-Pro, and D-Pro, the carboxylate oxygen is a more preferred ligand trans to the coordinated nitrosyl ligand than it is to the chloride ligand, secondary or primary amine. Likewise, the only isomer isolated from reactions of  $[RuCl_5(NO)]^{2-}$  with eight amino acids is *mer*(Cl),*trans*(NO,O)- $(n-Bu_4N)[OsCl_3(AA-H)(NO)]$ . The results of the present study demonstrate that amino acids used in this work are potential biological ligands for ruthenium-nitrosyl-based drug candidates in the blood serum and in the cytosol. A comparison with previously reported osmium analogues reveals a favorable

influence of ruthenium on antiproliferative activity in human cancer cell lines in vitro, probably via hydrolysis pathways, although the cytotoxicity of ruthenium complexes with amino acids is either moderate or low, depending on the cell line. Whether this is a result of their low uptake into the cells (taking into account their reduced lipophilicity) or effective efflux as a part of detoxification mechanisms should be clarified in further research. Variation of the amino acid ligand has a smaller impact on this activity within the range of amino acids employed. Nevertheless, the synthesis of ruthenium- and osmium-nitrosyl complexes with other amino acids and, in particular, Met, His, and Cys deserves attention, as this will provide the opportunity to investigate their biological effects, which may differ from those studied in the present Work. Collectively this may help in elucidating the mechanism of action of ruthenium and osmium-nitrosyl complexes with azole heterocycles. Activation of amino acidate ligands upon coordination to the metal may lead to specific intracellular chemistry, and the resulting species may play a major role in either detoxification or therapeutic activity. According to other authors<sup>45</sup> oxidation of the sulfur atom of the tripeptide glutathione afforded sulfenato complexes, and binding to DNA mediated by these complexes may play a role in the mechanism of action of RM175. Similar behavior of coordinated cysteine has not been documented, but may also be envisaged.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

One-dimensional chains in (a) 1, (b) 2, and (c) 3 assembled via N–H···Cl hydrogen bonding (Figure S1), one-dimensional chains assembled via N–H···O hydrogen bonding in (a) 4 and (b) 5 (Figure S2), one-dimensional chains assembled via N–H···O and O–H···Cl hydrogen bonding in (a) 6, (b), and (c) 7 (two independent supramolecular chains) (Figure S3), one-dimensional supramolecular chains assembled via N–H···Cl and O–H···O hydrogen bonding in 8 (Figure S4), cyclic voltammograms with several cycles of potential for 3, 5, 6, and 7 at 100 mV/s on glass carbon electrode (Figures S5–S8), <sup>1</sup>H NMR spectra for 8 (Figure S9), log *D* values for 1–8 (Figure S10), mass spectra for 1, 8, 4, and 5 (Figures S11 and S12), experimental and theoretical mass signals of the identified species (Figure S13), hydrogen bonding parameters in 1–8 (Table S1), crystallographic data in CIF format. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Alexander Roller for collection of X-ray data and Ricarda Bugl for support in acquisition of the mass spectra. We are also indebted to the Austrian Science Fund (FWF) and the Agence Nationale de Recherche (France) for financial support of the bilateral Project I374–N19 and ANR-09-BLAN-0420-01 (VILYGRu), respectively. The PHC Amadeus and OEAD (Project No. FR01/2012), as well as the Hungarian Research

Foundation OTKA 103905 are also acknowledged for their support.

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