Directed coordination study of $[Pd(en)(H_2O)_2]^{2+}$ with hetero-tripeptides containing C-terminus methyl esters employing NMR spectroscopy

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Keywords: GSH, Pd(II), NMR, potentiometry, tripeptide, AspAlaGly, β-AspAlaGly, TrpAlaGly

Abbreviations:

Ala – alanine
Asp – aspartic acid
Cys – cysteine
En – ethylene diamine
Fmoc – fluorenylmethoxycarbonyl
Glu – glutamic acid
Gly – glycine
GSH – glutathione
GSMe – S-methylated glutathione
Trp – tryptophan
Z – Benzyl carbamate

Electronic Supplementary Material Figure SI 1 shows a summary of pH titration data. Figures SI 2 to 5 of pH dependent NMR spectra of **1**-**4**. Figures SI 6A-9C of spectroscopic characterization data for **1**-**4**, and SI Figures 10-19 of MS data for complexes.

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Abstract

Alkylation of the C-terminus acids in small peptides allows direction to amine and amide coordination while changing the peptide composition to form tetradentate κ^4[n,5,5], where n = 5, 6, 7, or 8 membered ring coordination geometries, can be achieved. The alkylated tripeptide ligands, TrpAlaGly(OMe), β-Asp(OtBu)AlaGly(OMe), Asp(OtBu)AlaGly(OMe), and the fully methylated GSH, γ-Glu(OMe)Cys(SMe)Gly(OMe), were synthesized and their coordination properties to $[Pd(en)(H_2O)_2]^{2+}$ were studied. pH-dependent coordination was analyzed by NMR spectroscopy and the coordination to the alkylated tripeptides at selected pH values inferred from their NMR spectra. If selective coordination of amine/amide donors results in metal complexation, allowing for flexible and adjustable ligand frameworks, then this strategy could potentially be extended to other metal ions and peptide system.

Introduction

The complexation of biological peptides with various transition metal ions has been studied for many decades, specifically in the context of metallodrugs and metal toxicity [1-4]. In general, when complexed, peptides form stable 5-membered ring chelates through the amino, amide backbone or the carboxylate moiety [5]. Some amino acids have side chain groups that are capable of additional coordination, allowing for a variety of geometries [6-15].

The formation of metal amide bonds requires the presence of a primary ligating group, and for simple oligopeptides, once amine coordination takes place the amide coordination is facilitated [14-16,5]. Pd(II) ions have a high affinity for nitrogen and soft sulfur donor atoms found in various organic ligands [17-22]. One characteristic feature found in the complexation of palladium(II) and small peptides is the ability of the metal ion to induce deprotonation of the amides [23,24]. In the absence of coordinating anions, like chloride, the chelation of amides to the palladium ion is complete at pH values below 2, forming planar complexes with remarkable thermodynamic stability [23]. For tripeptides,

typical chelation takes place via amine, amide, and carboxylate donor groups to form κ^4(NH_2, N, N, O) tetradentate complexes [10].

Glutathione (GSH) is an omnipresent biologically active tripeptide and serves many functions in the body [25]. One distinct function of the GSH tripeptide is metal ion transport, this functionality is due to the fact that GSH has eight different possible binding sites for metals and its conformational flexibility [26-29]. Soft metal ions like palladium are known to coordinate through the thiolate, a soft base, whereas transition metal ions can also coordinate through the amide functions [25,30,31]. In aqueous solutions this coordination is pH dependent since it requires deprotonation of the amide donors.

An unusual characteristic of glutathione is that it is an *iso*-peptide, where the glutamic acid residue forms a γ-peptide bond through its side chain, which increases the distance from the N-terminus amine to the adjacent amide and thiol moieties. This allows both the N-terminus amine and the thiol to act as an anchoring site for the metal ion [14,32]. Glutathione is well studied in its native state [33-37,25] as well as the glutathione thioether derivative [38-40]. When GSH is reacted with K_4PdCl_2 in aqueous solution, $[Pd(GSH)Cl]\cdot 3H_2O$ is formed which then dimerizes to form a chloride bridged species of the $M_2X_2L_2$ type, where Pd(II) is coordinated through the thiol sulfur and amide group on the glycyl residue at a pH of 7-10, forming a five membered ring [26]. When reacted with Ni(II), glutathione yielded a $Ni(HL)\cdot 2H_2O$ complex, which was reported to have octahedral geometry with the water molecules coordinated, one axial and one equatorial, and glutathione forming κ^4[5,7,8] tetradentate coordination [41].

The *iso*-peptide structure of GSH, and its function as a metal ion transport brings up an interesting research question; Whether it is possible, through pH manipulations and protection of the carboxylate and thiol moieties, to direct chelation of modified GSH. If

selective coordination to amine/amide donors results in metal coordination of a κ^4[7,5,5] tetradentate coordination; and if this strategy could potentially be extended to other metal ions and peptide systems. The thiol/thioether side chain of GSH imparts challenges for future bioinspired applications and therefore this work was extended by exchanging the cysteinyl residue for alanyl residue, and the glutamyl residue was exchanged for aspartic acid which has carboxylic acid side chain that could be coupled to form either the *iso*- or C-terminus coupled peptide. In this way, the formation of either κ^4[6,5,5] or κ^4[5,5,5] membered ring tetradentate coordination geometries can be achieved, allowing for a flexible, adjustable ligand framework for potential bioinspired catalyst design [42]. A similar approach has been employed featuring histidine on the C-terminus of di- and tri- peptides with κ^4[5,5,6] type coordination [8,43].

To this effect, the novel alkylated tripeptides TrpAlaGly(OMe), **1**, β-Asp(O^{t}Bu)AlaGly(OMe), **2**, Asp(O^{t}Bu)AlaGly(OMe), **3**, and the fully methylated GSH, γ-Glu(OMe)Cys(SMe)Gly(OMe), **4** were synthesized (**Figure 1**). Using the aa-AlaGly(OMe) framework allowed distinctive NMR resonances for the alanyl and glycyl residues to be identified and interpreted, while avoiding hydrophobicity introduced by larger side chains. Alkylation of the C-terminus both increases non-aqueous solubility and directs coordination to the N-donors. Additionally, a weaker ester donor group potentially serves as a non-rigid donor offering an open coordination site at the metal center for application such as bioinspired catalysis [42]. Tryptophan was selected due to its five possible coordination sites; in addition to the primary amino and carbonyl functional groups of the acid, indole complexation has been reported through the secondary amine, [44] the C2 carbon, [45,46] and the C3 carbon [47]. The amino acids β-Asp(O^{t}Bu) and Asp(O^{t}Bu) both have five different coordination sites possible, the amine, two amide groups, and the *two* esters. The *iso*-peptide, **2**, and the C-terminus coupled **3** allow for the possibility of a direct comparison

of chemical reactivities based on the five versus six membered chelates in future studies. TrpAlaGly(OMe), **1**, was chosen as a sulfur free alternative for coordination to form chelates larger than six. Currently, it was of interest to see the coordination behavior of these ligands and confirm the different chelate ring sizes could be verified where the ring size is increased at the N-terminus rather than at the C-terminus [8,43,41].

The coordination properties of tripeptides **1-4** to $[Pd(en)(H_2O)_2]^{2+}$ were studied in water. $[Pd(en)(H_2O)_2]^{2+}$ was selected to prevent dimerization of the metal ion [48], and study the initial formation of the Pd complex. pH-dependent coordination was analyzed by NMR spectroscopy and the coordination to the alkylated tripeptides at selected pH values inferred from their NMR spectra and reported literature. The molecular ions of the complexes formed were identified in mass spectra.

Materials and methods

Instrumentation

The NMR spectra were measured at ambient temperature. ^{1}H, COSY and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker Avance 400 MHZ spectrometer at 400 and 101 MHz, respectively. Solvents used were D_2O, DCl, and NaOD. Infrared spectra were recorded on a Nicolet Avatar 360 FT-IR (E.S.P.) spectrophotometer using KBr pellets. Mass spectra were recorded on a micrOTOF-Q spectrometer, equipped with E-spray atmospheric pressure ionization chamber (ESI). All pH measurements were performed at 298 K with Mettler Toledo pH and conductivity meter using certified buffer solutions of pH 4.01, 7.00, and 10.01.

Solvents and reagents used were purchased from Sigma-Aldrich and used without further purification unless otherwise stated. Alanylglycine, Fmoc-Asp(O^{t}Bu)-OH, Fmoc-

Asp(OH)-O′Bu, Z-Trp, and EDC-Cl use in peptide coupling were purchased from Bachem. Solvents used were distilled under nitrogen and dried using standard methods [49]. Thiol methylation of GSH was performed with modification of a published procedure [50]. Alanylglycine esterification was completed by reacting it with trimethylchlorosilane [51] and **4** was isolated as the hydrochloride salt. $[Pd(en)Cl_2]$ [52] and $[Pd(en)(H_2O)_2](NO_3)_2$ [53,54] were prepared according to published procedures.

pH Titrations

The pH potentiometric titrations were carried out in 15 ml samples using four different metal to ligand ratios (0:1, 1:1, 2:1, 4:1). During titration argon was bubbled through the samples to prevent oxygen and carbon dioxide and to prevent aggregation of the samples. The concentration of the ligand was fixed at ~$3.2x10^{-3}$ M and the metal concentration adjusted to fit desired ratios. The ionic strength of the samples was adjusted with KCl to 0.2 M in a 60-fold excess to suppress complex formation due to competitive binding of the Cl^-. The titrations were carried out at constant temperature (298 K). The use of acid to lower the pH before titration was omitted because the *t*-butyl ester is subject to acid catalyzed hydrolysis. The analytes were titrated with standardized potassium hydroxide and 30 to 40 data points were obtained for each titration curve. The apparent equilibrium constants were evaluated from the titration data (Table 1) as defined by Equations 1 and 2, using the evaluation function on the Excel sheet CurTiPot, developed by Prof. Gutz,[55] where M, L and H represent $[Pd(en)(H_2O)_2]^{2+}$ ion, the ligand and protons, respectively:

$$pM + qL = rH \leftrightharpoons MpLqHr$$

$$\beta_{pqr} = \frac{M_p L_q H_r}{[M]^q [L]^q [H]^r}$$

NMR experimental procedure

The experiments were performed in D_2O. A stock solution of the ligand (0.020M) and a stock solution of the Pd(II) (0.040M) complex were mixed in a 1:1 ratio, for a final concentration of 5.0 x 10^{-3}. The ionic concentration was increased to 0.100M with KCl and the pH adjusted with NaOD. Stock solutions were prepared fresh daily. The reaction mixture was monitored over a 48 h period using NMR spectroscopy. The results were analyzed using ^1H and COSY NMR techniques.

General procedure for compound characterization by MS

The $[Pd(en)(H_2O)_2]^{2+}$ complex was dissolved in water, the ligand added in 1:1 ratio, and the pH increased to 10 to 11 with 0.1 M NaOH. The solution was allowed to stand for 1 hour, then the water distilled off under reduced pressure, and the residue washed with ether to remove free ethylenediamine. The complex was extracted from the residue with MeOH. The methanol solution was diluted for ESI-MS spectra. Complexes with ligands **1**-**3** were obtained in the negative scan mode, and complexes with **4** in the positive scan mode. The complex molecular peaks were simulated to compare with found peaks. The spectra are shown in SI Information Figures 10-19.

Synthesis

***Coupling procedure*:** Coupling reactions were executed by adding N-protected amino acids to HOBt in a 1:1 molar equivalent and stirring in chloroform at 0°C. Once the solution cooled, EDC-HCl (1.1 mol eq) was added and stirred for 30 min. AlaGly(OMe)-HCl (1 mol eq) was added, followed by drop-wise addition of triethylamine (1.05 mol eq). The solution was stirred under slight N_2 flow at ambient temperature for 24 hrs. Product was washed with water, dried with $MgSO_4$ and the $CHCl_3$ removed in vacuo. The product was then washed with ether and dried in a vacuum desiccator to obtain clean product.

Z-TrpAlaGly(OCH_3) [56] **1a**: Coupling reaction was executed using coupling procedure. Reaction was ran with 50 ml of $CHCl_3$. Z-Trp (1.153 g, 3.407 mmol), HOBt (0.460 g, 3.407 mmol), EDC-HCl (0.719 g, 3.748 mmol), ala-gly(OMe)-HCl (0.670 g, 3.407 mmol), and triethylamine (0.50 mL, 3.578 mmol). The product was purified by washing with water (3 x 20 mL), drying with MgSO4, then removing the chloroform under reduced pressure. The yield was 1.607 g (98.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H, NH_{indole}), 7.61 (d, J = 7.9 Hz, 1H, $H_{indole\text{-}C5}$), 7.32 (p, J = 3.9, 2.9 Hz, 6H, Ar_{CBZ}), 7.29 (d, J = 8.1 Hz, 2H, $H_{indole\text{-}C8}$), 7.20 – 7.12 (t, J = 7.5 Hz, 1H, $H_{indole\text{-}C7}$), 7.07 (t, J = 7.5 Hz, 1H, $H_{indole\text{-}C6}$), 7.03 – 6.99 (m, 1H, $H_{indole\text{-}C2}$), 6.77 (d, J = 5.6 Hz, 1H, NH_{Gly}), 6.61 (s, 1H, NH_{Ala}), 5.78 – 5.71 (m, 1H, NH_{Asp}), 5.16 – 5.03 (m, 2H, $CH_{2\text{-}CBZ}$), 4.56 (d, J = 6.9 Hz, 1H, α-H_{Asp}), 4.46 (q, J = 7.2 Hz, 1H, α-H_{Ala}), 3.85 (dd, J = 18.1, 5.6 Hz, 1H, α-H_{Gly}), 3.77 – 3.64 (m, 1H, α-H_{Gly}), 3.68 (s, 3H, $ROCH_{3\ Gly}$), 3.24 (qd, J = 14.6, 6.4 Hz, 2H, β-H_{Trp}), 1.20 (d, J = 7.0 Hz, 3H, β-H_{Ala}). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.27($C{=}O_{Ala}$), 171.62($C{=}O_{Trp}$), 170.31($C{=}O_{Gly}$), 156.39($C{=}O_{CBZ}$), 136.34($C9_{indole}$), 136.23(Ar_{CBZ}), 128.69(Ar_{CBZ}), 128.63(Ar_{CBZ}), 128.38(Ar_{CBZ}), 128.26(Ar_{CBZ}), 127.39($C4_{indole}$), 123.65($C2_{indole}$), 122.38($C7_{indole}$), 119.87($C6_{indole}$), 118.86($C5_{indole}$), 111.43($C3_{indole}$), 110.09($C8_{indole}$), 100.11(Ar_{CBZ}), 77.48, 77.16, 76.84($CDCl_3$), 67.31(CH_{2CBZ}), 55.92(α-C_{Trp}), 52.43($OCH_{3\ Gly}$), 48.92(α-C_{Ala}), 41.18(α-C_{Gly}), 28.67(β-C_{Trp}), 18.01(β-C_{Ala}).

Fmoc-β-Asp($OC(CH_3)_3$)AlaGly(OCH_3) 2a: Coupling reaction was executed using coupling procedure. Fmoc-Asp- O^{t}Bu (2.783 g, 6.764 mmol), (HOBt) (0.914 g, 6.764), EDC-HCl (1.426 g, 7.440 mmol), ala-gly(OMe)-HCl (3) (1.330 g, 6.764) and TEA (0.99 mL, 7.102 mmol). The yield was 3.682 g (98.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H, Ar_{Fmoc}), 7.63 (d, *J* = 7.6 Hz, 2H, Ar_{Fmoc}), 7.41 (t, *J* = 7.5 Hz, 2H, Ar_{Fmoc}), 7.37 – 7.31 (m, 2H, Ar_{Fmoc}), 7.11 (d, *J* = 5.7 Hz, 1H, NH_{Gly}), 6.61 (d, *J* = 7.5 Hz, 1H, NH_{Ala}), 6.20 (d, *J* = 8.3 Hz, 1H, NH_{Asp}), 4.67 (h, *J* = 7.3 Hz, 1H, α-H_{Ala}), 4.54 (dt, *J* = 9.1, 4.7 Hz, 1H, α-H_{Asp}),

4.36 (q, *J* = 10.4, 9.1 Hz, 2H, CH_{2Fmoc}), 4.23 (t, *J* = 7.2 Hz, 1H, CH_{Fmoc}), 4.18 – 3.97 (m, 2H, α-H_{Gly}), 3.71 (s, 3H, $ROCH_{3\ Gly}$), 2.99 – 2.76 (m, 2H, β-H_{Asp}), 1.49 (s, 9H, $RO(CH_3)_{3Asp}$), 1.42 (d, *J* = 7.0 Hz, 3H, β-H_{Ala}). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.56(γ-$C{=}O_{Asp}$), 170.43($C{=}O_{Ala}$), 170.16($C{=}O_{Asp}$), 169.96($C{=}O_{Gly}$), 156.36($C{=}O_{Fmoc}$), 143.99(Ar_{Fmoc}), 141.37(Ar_{Fmoc}), 127.80(Ar_{Fmoc}), 127.19(Ar_{Fmoc}), 125.33(Ar_{Fmoc}), 120.05(Ar_{Fmoc}), 82.51($ROC(CH_3)_{3Asp}$), 67.31(CH_{2Fmoc}), 52.48(OCH_{3Gly}), 51.53(α-C_{Ala}), 48.83(α-C_{Asp}), 47.26(CH_{Fmoc}), 41.23(α-C_{Gly}), 38.31(β-C_{Asp}), 28.06($OC(CH_3)_{3Asp}$), 18.60(β-C_{Ala}). IR (KBr, cm^{-1}) 3300 (s, N-H, N-H_2), 1744 (s, C=O-OCH_3), 1730 (sh, C=O-$OC(CH_3)_3$), 1693 (s, Amide I), 1539 (s, Amide II), 1249 (sh, C=O-$OC(CH_3)_3$), 1220 (s, C=O-$OC(CH_3)_3$), 1158 (s, C=O-OCH_3). MS (ESI/Positive) M ($C_{29}H_{35}N_3O_8$) = 553.6120, M/Z found(calc) = 576.2318(576.2316) [$M+Na^+$]

Fmoc-Asp(OC(CH₃)₃)AlaGly(OCH₃) 3a: Coupling reaction was executed using coupling procedure. Reactant amounts are as follows. Fmoc-Asp(O′Bu)-OH (1.406 g, 3.418 mmol), HoBt (0.462 g, 3.418 mmol), EDC-HCl (0.462 g, 3.418 mmol), ala-gly(OMe)-HCl (0.672 g, 3.418 mmol), TEA (0.5 mL, 3.588 mmol). The yield was 1.807 g (95.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.69 (m, 2H), 7.66 – 7.51 (m, 2H), 7.40 (td, J = 7.6, 1.1 Hz, 2H), 7.31 (td, J = 7.5, 1.2 Hz, 2H), 6.89 (s, 2H), 5.88 (d, J = 8.3 Hz, 1H), 4.51 (p, J = 7.0 Hz, 1H), 4.43 (d, J = 7.0 Hz, 1H), 4.22 (t, J = 6.9 Hz, 1H), 4.12 – 3.89 (m, 2H), 3.72 (s, 3H), 2.94 – 2.66 (m, 2H), 1.52 – 1.32 (m, 11H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.09(γ-$C{=}O_{Asp}$), 171.24($C{=}O_{Ala}$), 170.75($C{=}O_{Asp}$), 170.20($C{=}O_{Gly}$), 143.83($C{=}O_{Fmoc}$), 143.76(Ar_{Fmoc}), 141.45(Ar_{Fmoc}), 127.94(Ar_{Fmoc}), 127.24(Ar_{Fmoc}), 125.15(Ar_{Fmoc}), 120.18(Ar_{Fmoc}), 82.30($ROC(CH_3)_{3Asp}$), 67.48(CH_{2Fmoc}), 52.47(OCH_{3Gly}), 51.50(α-C_{Ala}), 49.20(α-C_{Asp}), 47.24(CH_{Fmoc}), 41.28(α-C_{Gly}), 37.53(β-C_{Asp}), 28.17($OC(CH_3)_{3Asp}$), 17.73(β-C_{Ala}). IR (KBr, cm^{-1}) 3307 (s, N-H, N-H_2), 1754 (sh, C=O-OCH_3), 1732 (s, C=O-$OC(CH_3)_3$), 1654 (s, Amide I), 1533 (s, Amide II), 1246 (sh, C=O-$OC(CH_3)_3$), 1216 (s, C=O-$OC(CH_3)_3$), 1153 (s,

C=O-OCH_3). MS (ESI/Positive) M ($C_{29}H_{35}N_3O_8$) = 553.6120, M/Z found(calc) = 576.2316(576.2313) [$M+Na^+$]

TrpAlaGly(OCH_3) 1: The protecting group Z was removed by adding Z-TrpAlaGly(OMe) (1.515 g, 3.255 mmol) and 150 mg 10% catalyst loaded Pd/C together and evacuating the flask, backfilling with argon. Dry methanol (~20 mL) was syringed in. A balloon filled with H_2, attached to a needle, was inserted into the top of the septum and the N_2 was flushed out of the flask and replaced with H_2.[57] (Care must be taken because Pd/C is pyrophoric and can cause organic solvents to ignite when air is present) The reaction mixture was stirred overnight with the H_2 balloon attached. When finished, the H_2 is flushed out by argon and the product is filtered through Celite on a fritted filter and washed with an additional 20 mL of methanol. Methanol was removed under reduced pressure with slight heat. Yield was 0.912 g (85%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H, NH_{indole}), 7.69 (d, J = 7.8 Hz, 1H, NH_{Ala}), 7.65 (dd, J = 7.9, 1.0 Hz, 1H, $H_{indole\text{-}C5}$), 7.36 (dt, J = 8.1, 1.0 Hz, 1H, $H_{indole\text{-}C8}$), 7.20 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H, $H_{indole\text{-}C7}$), 7.11 (ddd, J = 8.0, 7.0, 1.1 Hz, 1H, $H_{indole\text{-}C6}$), 7.07 (d, J = 2.4 Hz, 1H, $H_{indole\text{-}C2}$), 6.99 (t, J = 5.4 Hz, 1H, NH_{Gly}), 4.51 (p, J = 7.2 Hz, 1H, α-H_{Ala}), 4.07 – 3.91 (m, 2H, α-H_{Gly}), 3.78 – 3.73 (m, 1H, α-H_{Asp}), 3.73 (s, 1H, $ROCH_{3\ Gly}$), 3.35 (ddd, J = 14.5, 4.4, 0.9 Hz, 1H, β-H_{Trp}), 2.97 (dd, J = 14.5, 8.6 Hz, 1H, β-H_{Trp}), 1.72 – 1.65 (m, 2H, NH_{2Trp}), 1.31 (d, J = 7.0 Hz, 3H, β-H_{Ala}). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.44(C=O_{Trp}), 172.66(C=O_{Ala}), 170.35(C=O_{Gly}), 136.53($C9_{indole}$), 127.56($C4_{indole}$), 123.29($C2_{indole}$), 122.45($C7_{indole}$), 119.79($C6_{indole}$), 119.03($C5_{indole}$), 111.51($C3_{indole}$), 111.42($C8_{indole}$), 55.45(α-C_{Trp}), 52.48($ROCH_{3\ Gly}$), 48.51(α-C_{Ala}), 41.28(α-C_{Gly}), 30.67(β-C_{Trp}), 17.36(β-C_{Ala}). IR (KBr, cm^{-1}) 3389 (sh, N-H, N-H_2), 3296 (b, N-H, N-H_2), 3056 (m, aromatic C-H stretch), 1749 (s, C=O-OCH_3), 1655 (s, Amide I), 1517 (s, Amide II), 1213 (s, C=O-OCH_3), 745 (s, aromatic C-H bend). UVVis($CHCl_3$), ε_{281} = 6114 L/mol·cm. Specific rotation $[\alpha]_D$ = -18.76° (2.5mg/100mL, DMSO). MS (ESI/Positive) MW ($C_{17}H_{22}N_4O_4$) = 346.164, M/Z found(calc)

= 347.1714(347.1709) [M+H$^+$]. CHN: ($C_{17}H_{22}N_4O_4$) found(calc) %, C: 58.78(58.95), H: 6.38(6.40), N: 15.91(16.17).

β-Asp(OC(CH$_3$)$_3$)AlaGly(OCH$_3$) 2: The protecting group Fmoc was removed by stirring Fmoc-β-Asp(O′Bu)AlaGly(OMe) (3.778 g, 6.764 mmol) (2a) in 10 mL of DMF at 110 ºC for 40 min [58]. Methanol was added to the DMF and was washed with 3x10 mL hexane. The DMF was removed in vacuo and the product purified with flash chromatography. Yield for this reaction was 2.120 g (94%) and after purification 1.296 g. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J = 6.3 Hz,1H, NH_{Ala}),8.03(d,J=7.2Hz,1H, NH_{Gly}),4.63(q,J=7.2Hz,1H, α-H_{Ala}), 4.38 (d, J = 6.9 Hz, 1H, α-H_{Asp}), 4.05 (qd, J = 17.6, 5.5 Hz, 2H, α-H_{Gly}), 3.75 (d, J = 5.7 Hz, 3H, $ROCH_{3\ Gly}$), 3.26 (ddd, J = 72.9, 17.2, 4.4 Hz, 2H, β-H_{Asp}), 1.51 (s, 9H, RO(CH$_3$)$_3$), 1.47 (d, J = 7.1 Hz, 3H, β-H_{Ala}). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.86(γ-C=O_{Asp}), 171.90(C=O_{Ala}), 170.60(C=O_{Asp}), 170.49(C=O_{Gly}), 82.83($ROC(CH_3)_{3Asp}$), 52.46(OCH_{3Gly}), 51.68(α-C_{Asp}), 49.12(α-C_{Ala}), 41.20(α-C_{Gly}), 38.51(β-C_{Asp}), 28.05($OC(CH_3)_{3Asp}$), 17.76(β-C_{Ala}). IR (KBr, cm^{-1}) 3385 (b, N-H, N-H_2), 3307 (b, N-H, N-H_2), 1743 (sh, C=O-OCH_3), 1728 (s, C=O-$OC(CH_3)_3$), 1653 (s, Amide I), 1541 (s, Amide II), 1254 (s, C=O-$OC(CH_3)_3$), 1210 (s, C=O-$OC(CH_3)_3$), 1155 (s, C=O-OCH_3). UVVis($CHCl_3$), ε_{258} = 1638.3 L/mol·cm. Specific rotation $[\alpha]_D$ = -353.1° (2.5g/100mL, DMSO). MS (ESI/Positive) M ($C_{14}H_{25}N_3O_6$) = 331.368, M/Z found(calc) = 354.1633(354.1636) [M+Na$^+$]. CHN: ($C_{14}H_{25}N_3O_6$) found(calc) %, C: 50.28(50.75), H: 7.11(7.60), N: 13.86(12.68).

Asp(OC(CH$_3$)$_3$)AlaGly(OCH$_3$) 3: The protecting group Fmoc was removed using the same as procedure used above. Fmoc-Asp(O^{t}Bu)AlaGly(OMe) (2.453 g, 4.443 mmol) (**3a**). The yield was 82%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 – 8.29 (d, J = 6.7 Hz, 1H, NH_{Ala}), 7.87 (t, J = 5.9 Hz, 1H, NH_{Gly}), 7.26 ($CDCl_3$), 4.58 (p, J = 7.0 Hz, 1H, α-H_{Ala}), 4.50 (d, J = 6.2 Hz, 1H, α-H_{Asp}), 3.98 (qd, J = 17.8, 5.6 Hz, 2H, α-H_{Gly}), 3.71 (s, 3H, $ROCH_{3\ Gly}$), 3.11 (t, J = 6.6 Hz, 2H, β-H_{Asp}), 1.42 (d, J = 4.1 Hz, 13H, RO(CH$_3$)$_3$ Asp, β-H_{Ala}). ^{13}C NMR (101 MHz,

$CDCl_3$) δ 172.75(γ-C=O_{Asp}), 170.77(C=O_{Ala}), 170.24(C=O_{Asp}), 168.44(C=O_{Gly}), 82.93(ROC(CH_3)$_{3\ Asp}$), 52.30($OCH_{3\ Gly}$), 50.23(α-C_{Asp}), 49.95(α-C_{Ala}), 41.04(α-C_{Gly}), 36.23(β-C_{Asp}), 28.00(OC(CH_3)$_{3\ Asp}$), 17.59(β-C_{Ala}). IR (KBr, cm^{-1}) 3360 (b, N-H, N-H_2), 3235 (b, N-H, N-H_2), 1755 (sh, C=O-OCH_3), 1728 (s, C=O-OC(CH_3)$_3$), 1670 (s, Amide I), 1545 (s, Amide II), 1249 (s, C=O-OC(CH_3)$_3$), 1214 (s, C=O-OC(CH_3)$_3$), 1158 (s, C=O-OCH_3). UVVis($CHCl_3$), ε_{286} = 157.97 L/mol·cm. Specific rotation [α] = -1.4°(2.5g/100mL, DMSO). MS (ESI/Positive) M ($C_{14}H_{25}N_3O_6$) = 331.368, M/Z found(calc) = 332.1816(332.1816) [M+H^+]. CHN ($C_{14}H_{25}N_3O_6$)·1/2 H2O found(calc) %, C: 49.60(49.40), H: 7.58(7.70), N: 12.31(12.35).

γ-Glu(OMe)Cys(SMe)Gly(OMe) 4: The methyl esterification of GSH(SMe) was completed by adding GSH(SMe) (0.520 mg, 1.620 mmol) to distilled MeOH (20ml) along with trimethylchlorosilane (0.825 mL, 6.510 mmol) [51]. This mixture was stirred under N2 for 24 h. An additional 0.825 mL of Me_3SiCl was added and the reation stirred for another 24h. The solvent was removed in vacuo, and the product stirred in diethylether. The ether was decanted off resulting in isolation of a white hydroscopic powder as the hydrochloride salt of **4**. The yield was 0.514 g (82%). ^{1}H NMR (400 MHz, D_2O) δ 4.52 (dd, J = 8.7, 5.3 Hz, 1H, α-H_{Cys}), 4.13 (t, J = 6.7 Hz, 1H, α-H_{Glu}), 3.98 (d, J = 1.5 Hz, 2H, α-H_{Gly}), 3.79 (s, 3H, $ROCH_{3\ Glu}$), 3.69 (s, 3H, $ROCH_{3\ Gly}$), 2.99 – 2.91 (m, 1H, β-H_{Cys}), 2.80 (dd, J = 14.1, 8.7 Hz, 1H, β-H_{Cys}), 2.62 – 2.45 (m, 2H, β-H_{Glu}), 2.27 – 2.12 (m, 2H, γ-H_{Glu}), 2.08 (s, 3H, $RSCH_{3\ Cys}$). ^{13}C NMR (101 MHz, D_2O) δ 174.15(δ-C=O_{Glu}), 173.06(C=O_{Gly}), 171.76(C=O_{Cys}), 170.14(C=O_{Glu}), 53.65(OCH_{3Glu}), 52.79(OCH_{3Gly}), 52.62(α-C_{Gly}), 52.16(α-C_{Cys}), 41.20(α-C_{Glu}), 34.87(β-C_{Cys}), 30.65(SCH_3), 25.26(β-C_{Glu}), 14.63(γ-C=O_{Glu}). IR (KBr, cm^{-1}): 3396(ms), 3257(ms) (N-H, R_2-NH), 3059(ms, N-H, R-NH_3^+), 1748 (s, C=O-OCH_3), 1645(s, Amide I). UVVis(H_2O), ε_{258} = 30.8 L/mol·cm. Specific rotation $[\alpha]_D$ = -209.75°

(2.5g/100mL, DMSO). MS (ESI/Positive): M ($C_{13}H_{24}N_3O_6S$) = 349.40, M/Z found(calc.) = 350.1380(350.1370) [$M+H^+$].

Results and discussion

The synthesis and characterization of three new tripeptide ligands (Figure 1, **1**-**3**) is reported here. Although the parent tripeptides have been identified as fragments in protein digestion, [59-61] the ligands **1**-**3** were not synthesized previously. The thioether of glutathione is reported [62] and has been studied, [38-40] while the product of complete methylation is reported here for the first time (Figure 1, **4**).

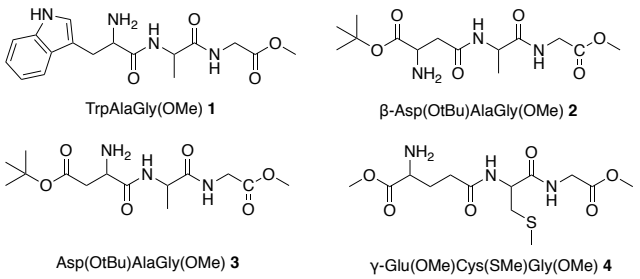


Figure 1. The tripeptides synthesized and used in Pd binding studies

Synthesis

The synthesis of the tripeptides was achieved using solution phase peptide synthesis. Solution phase synthesis has many benefits for short peptide synthesis, including larger scale synthesis and less consumption of materials [63-65]. Either Z- or Fmoc-protecting groups were used in coupling reactions (Scheme 1), based on availability and cost of the protected amino acid.

The choice to use both the methyl ester and *t*-butyl ester on **2** and **3** presented itself with a variety of challenges in the synthesis. The *t*-butyl ester was selected as a commercially available protection on the Fmoc protected aspartic acid, and the methyl ester protection was

chosen over the *t*-butyl or ethyl because of facile completion of the esterification reaction for the methyl ester with quantitative yields. The reaction scheme is shown in Scheme 1. Dipeptide AlaGly was alkylated to AlaGly(OMe), and coupled with Z-(**1**) or Fmoc-protected α- or β-Asp(O^{t}Bu)(OH) (**2**,**3**) starting material in a coupling reaction that was optimized for facile workup.

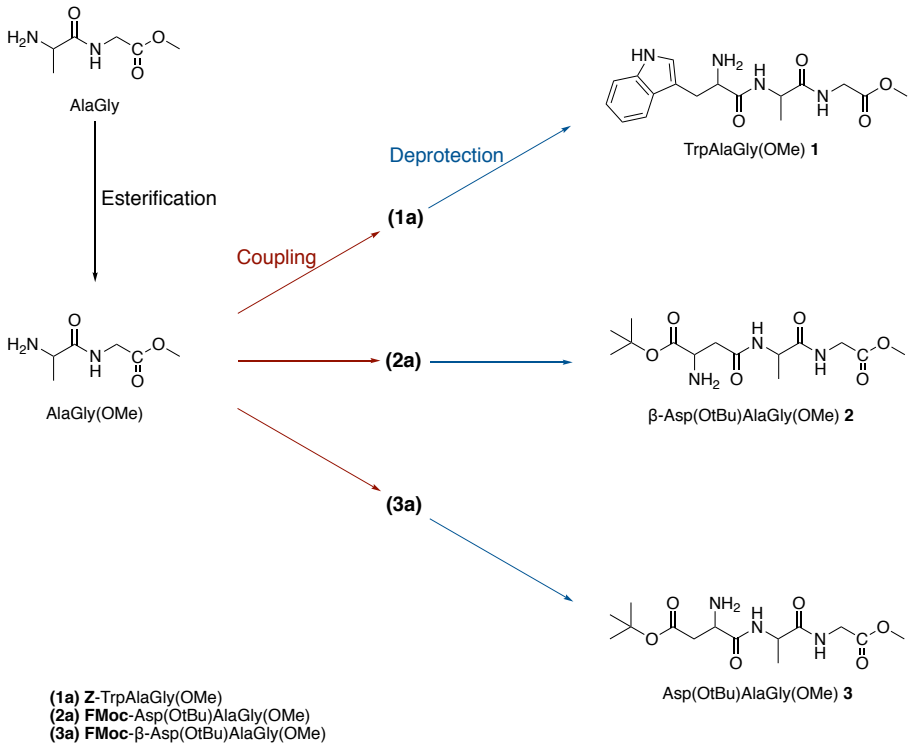
Esterification reactions to produce *t*-butyl or ethyl esters required long reaction times, and resulted in incomplete esterification, and loss of yields. Challenges presented themselves in the selection of a deprotection method for the Fmoc or Z and work-up procedures to obtain pure compounds. Methyl ester hydrolysis is catalyzed by basic conditions above a pH of 10, whereas *t*-butyl esters are catalytically hydrolyzed in acidic conditions below a pH of 2 and is very sensitive to heating [66]. Complications arose during work-up due to the common solubilities of the product and side products, making separation difficult, this was solved through selection of solvent and base for the reaction.

Standard Fmoc cleavage procedures in basic medium could not be employed. Attempted deprotection of Fmoc-Asp(O^{t}Bu)AlaGly(OMe) and Fmoc-β-Asp(O^{t}Bu)AlaGly(OMe) using standard basic conditions resulted in the formation of aspartimide. DMF solvent mediated Fmoc cleavage worked well although residual DMF content likely contributed to very hygroscopic behavior of the crude compounds. Dry, well behaved, analytically pure compounds were isolated after washing them thoroughly with dry solvent after flash chromatography.

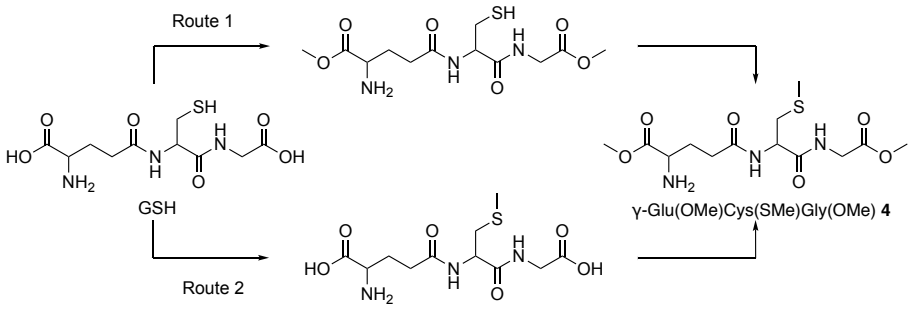
Attempts to precipitate out the HCl salt of **2** and **3** produced the partially hydrolyzed *t*-butyl ester species. Dry reaction conditions under inert air were used to precipitate the products using a HCl/Et_2O solution. Synthesis and purification of **1**, (Scheme 1) was straightforward using standard methods when employing the Z amine protecting group. After isolation and purification **1**-**3** were stored under nitrogen in the freezer.

Two possible synthetic routes were explored to achieve complete alkylation of GSH to form **4** (Scheme 2). S-alkylated GSH, (GSMe) is commercially available, while fully methylated glutathione is not. For the desired experimental quantity, GSH was used as a starting material and both carboxylate esters and the thiol were alkylated to form the methyl esters of glutathione. In Route 1, esterification is followed by thiol methylation. Complete alkylation using Route 1 proved unsuitable, since the basic aqueous conditions employed caused hydrolysis of the methyl esters. In the second approach, (Route 2) the thioether is formed first, followed by the esterification reaction. These successive reactions showed complete alkylation forming the hydrochloride salt of **4**.

All tripeptides were fully characterized using ^{1}H, ^{13}C, COSY, and HSQC, NMR spectroscopy, as well as ESI-MS, IR, UV-vis, and elemental analysis. MS, ^{1}H and ^{13}C are provided in the supplementary information (Figures SI 6A-9C).



Scheme 1. Synthesis scheme for ligands **1-3**



Scheme 2. Synthesis scheme for ligand **4**

Binding motifs and coordination geometries were investigated for compounds **1**-**4** in water with the metal ion $[Pd(en)(H_2O)_2]^{2+}$ employing ^{1}H-NMR spectroscopy and potentiometric titrations.

Potentiometric studies

Determination of the pK_a *of ligands using pH titrations*

Ligands **1** - **4** contain methyl esters on the C-terminus of the tripeptides. With the *t*-butyl esters protecting the side chain of the aspartic acid residue on **2** and **3**, and the cysteinyl of **4** transformed into a thioether, only the amine deprotonation needs to be determined. The experimentally determined pK_a values are listed in Table 1. The pH titrations curves are shown in SI information Figure SI 1. Conventionally, acid would be added to the mixture to ensure the entire pH range is covered. However, this was not possible here due to the acid hydrolytic sensitivity of the *t*-butylester. As a consequence, the titrations begin in the mid-pH range. Amino acid titrations normally need to cover the full pH value range in order to determine the pK_a of the carboxylate, which is observed below a pH of 3 [67]. However, in compounds **1**-**4**, this moiety has been converted to an ester, which should not affect the collection of data.

Ligand **1** has an amine and two amide groups as well as an indole amine group. By itself, ligand **1** shows amine deprotonation at a pH of 9.25. The reported pK_a of free tryptophan is 9.34 [67]. Again for **2**, the amine group is the only measurable pK_a, this was found to be 8.78. For **3** the experimentally determined pK_a was 8.56, where the pK_a for aspartic acid is 9.66 [67]. Compound **2** amine, being further away from the peptide bond experiences less effects than **3**. For compound **4** two deprotonations seem to occur, one at 8.26 and another at 9.95. The pK_a value at 8.26 may correspond to deprotonation of the partially hydrolyzed thioether, where the pK_a for the thiol on GSH is 8.75 [67]. The second pK_a found is the amine deprotonation, where for GSH this value is 9.65. These differences in the pK_a values of the amino acid and the corresponding peptides are due to peptide bond formation.

Titrations of the ligands with $[Pd(en)(H_2O)_2]^{2+}$

Titrations of **1**-**4** with $[Pd(en)(H_2O)_2]^{2+}$ were performed in three different ligand to metal ratios. The titration curves are shown in Figure SI 1 along with the ligand titrations. As $[Pd(en)(H_2O)_2]^{2+}$ is added to the ligand solution, there is a drop in pH due to the Lewis acid nature of Pd(II). When more ligand is added, the shift to lower pH values is diluted for 2:1 ratio of ligand to metal and insignificant at a 4:1 ratio. This observation suggests that the plots for a 2:1 ratio is a combination of the free ligand and the 1:1 ratio titration. This pattern is also observed in the 4:1 titration data. It is clear from the plots that the en successfully inhibits the formation of bis type complexes, even with the addition of excess ligand. Therefore, it is sufficient to study the species present in the 1:1 ratio in order to identify all of the metal coordinated species in the mixtures. As before the initial pH was not adjusted with the addition of acid, preventing observation of amine deprotonation, and allowing only the observation of amide deprotonation. The amide deprotonation takes place at significantly lower pH upon coordination, in the range of 7.33 to 7.65, as the Pd(II) coordination drives the

deprotonation at a lower pH. No discernible deprotonation could be identified for solution **4** with $[Pd(en)(H_2O)_2]^{2+}$

Table 1. Protonation values of the Pd(II) complexes of tripeptide ligands

	1	**2**	**3**	**4**
Ligand				
pK_{a1} (amine)	9.25	8.79	8.56	8.21
Ligand + M				
pK_{a1} (amide)	7.65	7.33	7.42	~
pK_{a2} (amide)	7.65	7.33	7.42	~

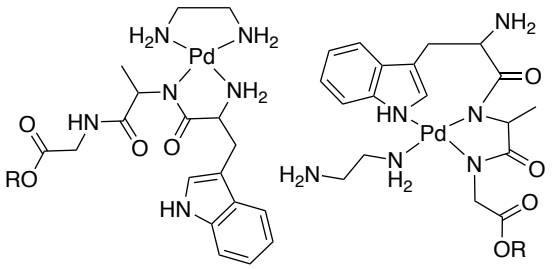
pH dependent NMR studies

pH-dependent NMR studies were performed in D_2O. A stock solution of the ligands (**1**-**4**) (0.020M) and a stock solution of the Pd(II) (0.040M) complex were mixed in a 1:1 ratio, for final concentrations of 5.0 x 10^{-3} M. The ionic concentration was increased to 0.100 M with KCl and the pH adjusted with NaOD. The reaction mixture was monitored over a 48-h period using NMR spectroscopy.

*pH dependent coordination of $[Pd(en)(H_2O)_2](NO_3)_2$ with **1**.*

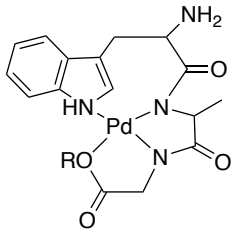
The initial pD of the mixture of $[Pd(en)(H_2O)_2]^{2+}$ and **1** resulted in a pD value of 4.76. This resulted in the formation of two metal complex isomers, in approximately a 1:1 ratio (Figure 2). One of the isomers shows a large downfield shift for the α-C protons on the tryptophan residue, indicating primary amine coordination, followed by amide chelation, as indicated by the shift of the β-C*H* on the alanine residue. Furthermore, the en group is still present, as seen by the presence of its bound singlet at 2.71 ppm, leading to proposed structure **Pd-1A**. The second isomer present at this pH has a mono-coordinated ethylenediamine.

In a mono-coordinated ethylenediamine, the methylene protons are no longer equivalent, and they split to form three resonances [68]. For the second isomer, the multiplet at 2.50 ppm integrates to 2 protons corresponding to the distant CH_2 group of en. The multiplets at 2.14 ppm and 2.30 ppm integrate to one H each and represent the two non-equivalent protons closer to the Pd ion.

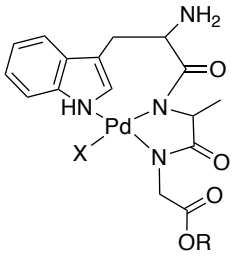


Pd-1A

Pd-1B



Pd-1C



Pd-1D

pD	Species present		% Ester Hydrolysis	
			R (OMe)	R' (O^{t}Bu)
4.76	A ~50%	B ~50%	~10	~
6.52	B ~100%	~	~19	~
7.92	C ~100%	~	~75	~
11.54	C ~75%	D ~25%	100	~

Figure 2. Possible coordination geometries of **1** (5 mmol L^{-1}) with $[Pd(en)(H_2O)_2]^{2+}$ (5 mmol L^{-1}) (c_{KCl} = 100 mmol L^{-1}) over the pD range of 4.75-11.54, with hydrolysis values present after 1 hour.

Evidence for indole amine coordination is seen in the doubling of all resonances for the entire indole moiety, with downfield shifts ~0.10 ppm. The indole coordination appears to be accompanied by both amides coordinating as seen by the shift in the α, and β-C protons for the tryptophan, alanine, and glycine residues. The corresponding coordination geometry is shown for **Pd-1B**, where an 8 membered ring has formed between the indole amine and the

alanine amide, which then continues to the glycine amide, replacing one of the ethylenediamine donors to form a tridentate species. As the pD is increased to 6.52, this second isomer, **Pd-1B**, is the only species present in the NMR spectrum.

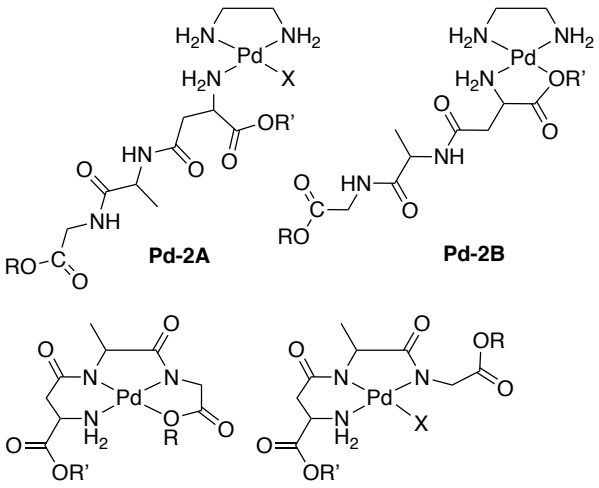
Increasing the pD to 7.92 results in complete dissociation of en as evidenced by appearance of free en signals at 2.77, leading to likely carboxylate ligation and suggests **Pd-1C** as the best model for this pH. The disappearance of the methyl ester signal (3.78 ppm) and the parallel appearance of MeOD at 3.37 ppm confirms ~75% methyl ester hydrolysis. Palladium catalyzed hydrolysis of methyl esters for alanylglycine is known to occur at a pH of 4-5 [69,70], however base catalyzed hydrolysis of methyl esters tends to start at pH values greater than 10 [66], indicating observed ester hydrolysis is Pd(II) catalyzed.

At pD of 11.54 the methyl ester is completely hydrolyzed and a new minor species appears (**Pd-1D**), as indicated by a shift in the methylene group of glycine. This new species is tentatively a hydrolyzed Pd(II), i.e. containing [71] a coordinated hydroxo group rather than the carboxylate group. At basic pH values, the fourth coordination site in Pd(II) peptides complexes has been reported as occupied by hydroxide ions [23][72,73].

*pH dependent coordination of $[Pd(en)(H_2O)_2](NO_3)_2$ with **2***

Initial pD of the $[Pd(en)(H_2O)_2]^{2+}$ and **2** solution was 5.01. Observed coordination geometry of **2** and $[Pd(en)(H_2O)_2]^{2+}$ is shown in Figure 3. Complexation was seen immediately by the downfield shift of the α-C Asp protons as well as the β-C Asp protons and the broadening and doubling of the en methylene protons of the $[Pd(en)]^{2+}$, due to the *trans* effect. This suggests a monodentate binding geometry as as seen in **Pd-2A**. However, some methyl ester hydrolysis (10%) and *t*-butyl hydrolysis (< 8%) is also occurring. No evidence of bidentate coordination of the peptide was observed at this pH.

At pD of 6.93 an upfield shift of the Asp α-C*H* and hydrolysis of the Asp *t*-butyl ester was observed, as indicated by the appearance of a *t*-butanol signal. Two species appear to be present, both with the coordination geometry **Pd-2B**; the majority (>80%) showing probable $\kappa^2(NH_2, O^tBu)$ binding and the other (<20%) showing $\kappa^2(NH_2, O\text{-})$ binding due to *t*-butyl ester hydrolysis. Hydrolysis of the methyl ester reaches ~20% as indicated by the growing MeOH signal. Negligible change was associated with the chemical shifts of Ala and Gly moieties. No evidence of amide chelation was present.



pD	Species present		% Ester Hydrolysis	
			R (OMe)	R' (O^{t}Bu)
5.01	A ~100%	~	~10	~8
6.93	B ~100%	~	~20	~20
9.21	C ~100%	~	~65	~35
11.06	C ~70%	D ~30%	100	~70

Figure 3. Possible coordination geometries of 2 (5 mmol L^{-1}) with $[Pd(en)(H_2O)_2]^{2+}$ (5 mmol L^{-1}) (c_{KCl} = 100 mmol L^{-1}) over the pD range of 5.01-11.06, with hydrolysis values present after 1 hour.

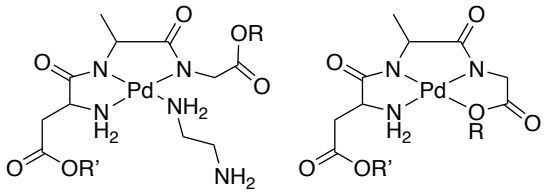
As many as 4 isomers were present at a pD of 9.61 due to varying degrees of hydrolysis of the methyl and *t*-butyl ester. The coordination geometry of all of the isomers is represented by **Pd-2C**. This assignment is deduced by dissociation of en and a concomitant shift of the Ala and Gly protons signaling amide binding, suggesting a $\kappa^4(NH_2, N, N, O)$

coordination geometry. Methyl and *t*-butyl ester hydrolysis was estimated to be ~65%/35% respectively, where the *t*-butyl ester hydrolysis was likely Pd-catalyzed, induced by the proximity of the ester to the Pd-NH_2 group.

At highly basic conditions of pD at 11.20 complete hydrolysis of the methyl ester was observed, and *t*-butyl ester hydrolysis was ~70%, while the major species maintained the coordination geometry shown as **Pd-2C**. Another minor species was observed via the shift of the Gly methylene protons suggesting dissociation of the Gly carboxylate from the metal. This species exhibits probable formation of a Pd-hydroxo species, as suggested by coordination geometry **Pd-2D**.

*pH dependent coordination of $[Pd(en)(H_2O)_2](NO_3)_2$ with **3***

A mixture of $[Pd(en)(H_2O)_2]^{2+}$ with **3** at a pD of 3.77 did not result in complexation judged by the fact that the resonances from the $[Pd(en)(H_2O)_2]^{2+}$ complex remained intact. The amine protons shifted by 0.04 ppm, due to the change in pH, but all other resonances remain unchanged. The overall integration for ethylene diamine was low, due to precipitation of $[Pd(en)(H_2O)_2]^{2+}$ from solution. This precipitation is consistent with reports stating that amine coordination to the $[Pd(en)]^{2+}$ fragment does not take place until a pH of 4 due to competitive binding to Cl^- [23].



Pd-3A **Pd-3B**

pD	Species present		% Ester Hydrolysis	
			R (OMe)	R' (O^{t}Bu)
3.77	**3** ~100%	~	~0	~0
4.71	A ~55%	**3** ~45%	~12	~5
7.34	A ~100%	~	~35	~5
11.20	B ~100%	~	100	~30

Figure 4. Possible coordination geometries of **3** (5 mmol L^{-1}) with $[Pd(en)(H_2O)_2]^{2+}$ (5 mmol L^{-1}) (c_{KCl} = 100 mmol L^{-1}) over the pD range of 3.75-11.25 with hydrolysis values present after 1 hour.

At a pD of 4.71 the formation of a new species, **Pd-3A** (Figure 4) was observed. The tripeptide formed a tridentate species with Pd(II) that has a mono-coordinated ethylenediamine occupying the fourth coordination site. Evidence for this structure was seen in a significant shift of α- and β-C protons of the three amino acid residues, along with the doubling and broadening of the ethylene diamine resonance at 2.77 ppm. Free tripeptide **3** was still present amounting to about ~50% based on integrations. Convergence of the NMR resonances at pD of 7.34 evidenced coordination geometry **Pd-3A** becomes the major species. At this pD the methyl ester starts hydrolyzing, and after 8h about 35% of the ester has been hydrolyzed.

After raising the pD to 11.20, the formation of tetradentate species **Pd-3B** is evidenced by the upfield shift of the α-C protons for glycine by 0.3 ppm and the complete dissociation of coordinated ethylene diamine, which was observed as free en at 2.78 ppm. At this pD, the methyl ester was completely hydrolyzed, and the *t*-butyl ester was ~30% hydrolyzed after 8h.

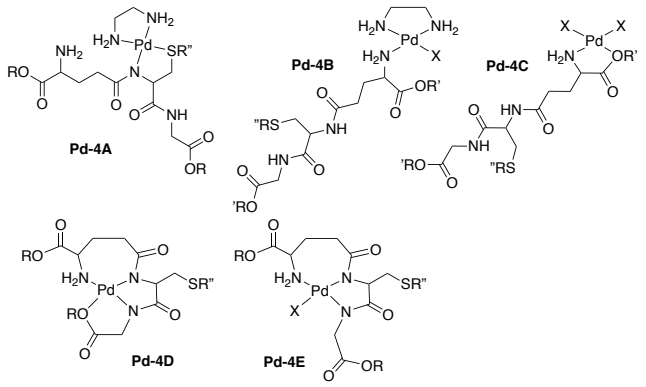
*pH dependent coordination of $[Pd(en)(H_2O)_2](NO_3)_2$ with **4***

The reaction of $[Pd(en)(H_2O)_2]^{2+}$ with **4** at a pD of 2.30, observed via NMR, formed a species that appears to have κ^2(S,N) coordination (**Pd-4A,** Figure 5). Initially, glutathione was bound to the metal through the thioether, as seen in the downfield S-methyl shift from 2.18 ppm to 2.46 ppm, as time progresses the thioether hydrolyzes resulting in the formation of MeOD. This hydrolysis started at ~5%, and after 48 hours had increased to ~72%. The en remains coordinated but shifts due to *trans* effects from 2.71 ppm to 2.85 ppm, this shift is accompanied by broadening of the signal. The evidence for cysteinyl amide coordination was

seen in the downfield shift of the α-C*H* by 0.38 ppm, as well as the downfield shift of the β-C protons.

As the pD was raised to 3.06, **Pd-4A** only achieves ~50% formation, and mono-coordinated complex **Pd-4B** accounts for the other 50% speciation. Support for the formation of this species is the upfield shift in the α-C Glu residue to 3.8 ppm and the downfield shift of the Glu β-, and γ-methylene protons. This coordination was also supported by the en resonance at 2.72 ppm. Here the fourth coordination could be stabilized by a chloride ion, a water molecule or possibly by the formation of a dimer. The thioether is hydrolyzed by approx. 55% after 1 hour. Although challenging to integrate accurately, the esters appear intact at pH values lower than 3.60.

At a pD of 7.70 the **Pd-4A** species was no longer present. The en group shifts to 2.78 ppm, signifying dissociation from the metal center. The slight upfield shift of the methyl ester protons of the Glu residue from 3.89 to 3.83 ppm signify chelation to the Glu carboxylate, forming a 5 membered chelate with the coordination geometry **Pd-4C**. Methyl ester hydrolysis was quantified at 21% after 1 hour forming methanol. The thioether remains intact.



pD	Species present			% Ester/Thioether Hydrolysis	
				R (OMe)	R" (SMe)
2.30	A ~ 100%	~	~	~ 0	~ 30
3.06	A ~ 50%	B ~ 50%	~	~ 0	~ 55

7.70	C ~ 90	B ~ 10	~	~ 21	~ 0
8.70	C ~ 80	D ~ 20	~	~ 80	~ 0
11.30	C ~ 30	D ~ 30	E ~ 30	100	~ 0

Figure 5. Possible coordination geometries of **4** (5 mmol L^{-1}) with $[Pd(en)(H_2O)_2]^{2+}$ (5 mmol L^{-1}) (c_{KCl} = 100 mmol L^{-1}) over the pD range of 2.30-11.30 with hydrolysis values present after 1 hour.

Pd-4C was still the major species at a pD value of 8.70, but a new minor species with tetradentate $\kappa^4(NH_2,N,N,O)$ was formed, as represented by **Pd-4D**. This observation was supported by a quadruplet observed at 4.19 ppm corresponding to Cys α-C, and by a Glu α-C protons triplet at 4.55 ppm. Further coordination was evidenced an upfield shift of the Glu β- (0.15 ppm), and γ- (0.25 ppm) methylene protons.

Once the basic pD of 11.30 is reached, the formation of a third species was observed; the probable Pd-hydroxo species (**Pd-4E**). At these basic conditions the methyl esters were fully hydrolyzed, while the thioether remains intact.

Mass Spectra of Pd(II) complexes with 1-4.

The complexes were not amenable to isolation from aqueous solutions because of the multiple species present in solution and separation of products proved a serious challenge. As identification of the species present, mass spectra were obtained. The results summarized in Table 2 and SI Figures 10-19 show the found and simulated spectra for the complexes. pH of 10.5 was chosen assuming that molecular peaks of the expected complexes could be identified in the mass spectrum. At high pH it is expected to see the carboxylate of the C-terminus amino acid as the fourth donor to Pd(II) or alternatively, either Cl^- or OH^- from the solution mixture.

The methyl ester hydrolysis is rapid and only ligand **1** showed peaks with the methyl ester intact. The t-butyl ester was observed in spectra of **2** and **3**. Ligand **4** confirmed an intact thioether.

Table 2. Summary of expected and found molecular ion peaks in the mass spectra of **1**-**4** with $[Pd(en)(H_2O)_2]^{2+}$ at pH of ~10.5.

Ligand	Species expected by NMR			Species found by MS		
1	**Pd-1C** *major*	**Pd-1D** *minor*	~	**Pd-1C** *major*	**Pd-1D** *minor*	**Pd-1B** [1] *minor*
2	**Pd-2C** *major*	**Pd-2D** *minor*	~	**Pd-2C** *major*	**Pd-2D** *minor*	~
3	**Pd-3B** *major*	~	~	**Pd-3B** *major*	**Pd-3A** [2] *minor*	~
4	**Pd-4C** *One third*	**Pd-4D** *One third*	**Pd-4E** *One third*	**Pd-4C/E** *major*	**Pd-4D** *minor*	~

1) Hydrolyzed methyl ester

2) With and without ester hydrolysis

The complexes predicted by NMR did show their molecular ion peaks in the mass spectrum. Ligands **1** and **3** showed presence of ethylene diamine in minor peaks that could be caused by incomplete removal of free ethylene diamine during sample preparation.

Discussion

Coordination of $[Pd(en)(H_2O)_2]^{2+}$ with **1** through the indole nitrogen led to an unusual 8 membered chelate. Predictions could be made for 5-, 7- or 8-membered chelates with **1**, although 8-membered chelates are rare [74]. Formation of an initial 5-membered ring via the amine and the Ala amide was observed at the lowest pH (Figure 2, **Pd-1A**). Five membered chelate coordination of Trp with the $[Pd(en)]^{2+}$ fragment has been reported, from the indole C3 to the carboxyl O [47], as well as Pd(II) and Pt(II) bis-tryptophan complexes, in which coordination proceeds through the amine N, and carboxyl O [75,76].

The indole C2 proton is the most acidic proton and is most likely to leave when interacting with a Lewis acid [45]. C2-indole coordination together with Ala amide would lead to a less-strained 7-membered ring. Palladacycles with indoles have been reported, where the Pd(II) center is sigma bonded to the C2 carbon and the indole nitrogen is

functionalized to form a 6-membered chelate with the palladium [46]. One more possibility is an 8-membered ring with the indole nitrogen and the nearest amide. Examples of η^1 coordination of Ru(II) with indole nitrogen show that it is not deprotonated [44] in such coordination, and is in that way comparable to amine coordination. The pK_a of the indole proton in $[(cymene)Ru(\eta^1\text{-indoline})(CH_3CN)_2]^{2+}$ was determined to be 5.2 or much lower than for free indole [77]. An NMR spectrum of **1** and Pd(II) mixed with two equivalences of base in DMSO-d_6 confirmed the loss of the amide resonances, but the N1 proton was still present. The C2 proton was located in the NMR spectrum of **1** with $[Pd(en)(H_2O)_2]^{2+}$ leading to the conclusion that **1** formed an 8 membered chelate with Pd^{2+} at all pH values, albeit a minor species at pH of 4.7. The κ^4[8,5,5] coordination is dominant in the mixture at high pH as well. An interesting result here is that the indole nitrogen is a strong donor for the Pd(II) center competing efficiently with the normally dominating amine donor group in peptide coordination.

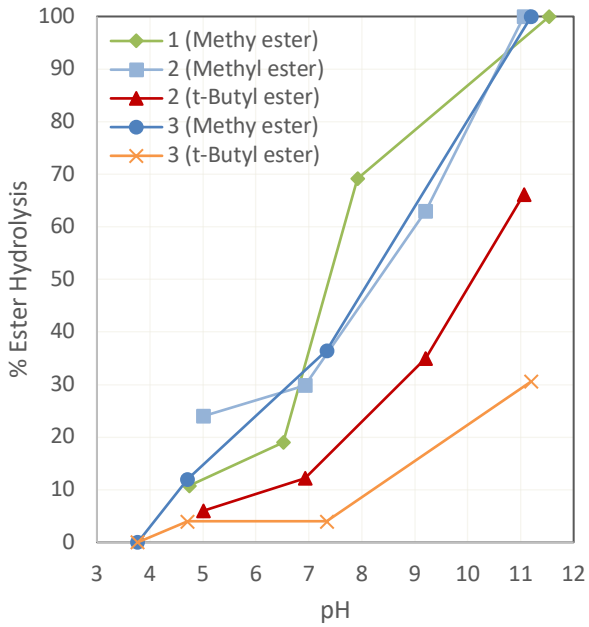


Figure 6. Percent ester hydrolysis as a function of pH.

Ligands **2** and **3** were expected to form respectively six and five membered chelates with the amine and the nearest amide. This was achieved but in an unpredictable manner. At

moderate pH values, **2** preferred a 5-membered chelate with the amine and the side-chain ester carbonyl oxygen (Figure 3, **Pd-2B**) promoting ester hydrolysis. This coordination type (N,O) is well known for simple amino acids [78]. Ligand **3** shows significantly less Pd^{2+} promoted *t*-butyl ester hydrolysis and formed a conventional 5-membered chelate. This chelation begins with the amine and continues through both amides, immediately rearranging the en ligand from bidentate to a mono-coordinated mode. The en was completely dissociated by a pD of 11.20, at which point the free carboxylate coordinates to form κ^4[5,5,5] chelate. Ligand **3** shows multiple isomers that represent various forms of hydrolyzed ester combinations. Ligand **2** coordination is more strongly pH driven, where the **Pd-2B** coordination prevails until a pH of 7.33 (Table 1), at which point the amides are deprotonated and the en dissociates completely, leading to tetradentate κ^4[6,5,5] complexation of **2** with Pd^{2+}. No mono-coordinated en was observed with **2**. The importance of the five membered chelate over the six membered chelate is only overcome at high pH where the amides are deprotonated regardless.

Considering both carboxylates and the thiol were alkylated, ligand **4** showed coordination preferences that were highly pH dependent. At low pH the amine/thioether coordination prevails (Figure 5, **Pd-4A**) forming five-membered κ^2(S,N) chelate, promoting Pd-catalyzed thioether hydrolysis. This hydrolysis is analogous with the *t*-butyl ester coordination and subsequent hydrolysis seen for **2**. The thiol group on GSH normally has strong influence on GSH coordination chemistry [35,38,39,53]; however, by forming the thioether, this reactivity was curbed at neutral and high pH values by preventing formation of the 5-membered κ^2(S,N) ring normally favored for GSH complexation. Both being *iso*-peptides, ligands **2** and **4** show similar coordination preferences at neutral and high pH values, where coordination starts at the amine, but the chelation to the carboxylate (Figure 5**,** **Pd-4C**) is preferable to either 6- (**2**) or 7- (**4**) membered chelate formation with the nearest

amide. Only at high pH values for **4** does chelation proceed through the amides and the formation of the κ^4[7,5,5] chelate was observed.

Methyl ester hydrolysis (Figure 6) significantly impacts coordination preferences of **1**-**4** at high pH where the free carboxylate competes to displace ethylenediamine and complete tetradentate coordination around the Pd^{2+} ion. At neutral pH, methyl ester hydrolysis was more prominent for **1** compared to the other ligands (Figure 6). The methyl ester hydrolysis for **2** and **3** was comparable, while the *t*-butyl ester for **2** experienced significantly more Pd(II) catalyzed hydrolysis [17,18] driven by the coordination preference of **2** to form a 5-membered ring at a pH lower than the pK_a of the ligand (Table 1, Figure 5).

Conclusions

Three new alkylated tripeptides **1**-**3** were synthesized and characterized fully using solution phase synthesis in high yields. A synthetic route to fully alkylate GSH, **4**, is reported as well. The four tripeptides were explored as ligands for Pd(II) in water at different pH using ethylenediamine complex $[Pd(en)(H_2O)_2]^{2+}$ to explore stepwise coordination and draw out differences in ligand properties.

The ligands **1**-**4** formed coordination geometries as κ^4[n,5,5] (n = 8,7,6,5) chelates. **1** and **3** formed κ^4[8,5,5] and κ^4[5,5,5] respectively, at low pH values and this chelation dominated at all pH values explored. The observed coordination appears driven by different viewpoints; Where **1** forms a strong bond as a neutral donor to Pd(II) through the sidechain indole nitrogen and the nearest amide, while **3** forms a traditional 5-membered chelate with N-terminus amine and the nearest amide. Compounds **2** and **4** chose the N-terminus amine with an ester to form five membered chelates preferably to the respective 6- and 7-membered chelates of the N-terminus amine and the nearest amide. These five membered chelates dominated the coordination until the pH was sufficiently high to deprotonate the amides

suggesting that 5-membered chelate stability is more important than the amine/amide coordination even for a soft ion such as Pd(II).

The tripeptides chosen exhibited their maximum expected chelating ring sizes at the N terminus and confirmed it is possible to form complexes with κ^4[n,5,5] (n = 8,7,6,5) chelates that may be employed to adjust ligand frameworks for bioinspired catalyst design in future work. The molecular ions of the complexes were found and matched with their simulated isotope patterns in the ESI MS of the complexes.

The esters showed significant hydrolysis that was both pH dependent and Pd(II) catalyzed. The Pd(II) catalyzed hydrolysis was significant for **2** and **4**, where the coordinated carboxylate-esters and thioether hydrolyzed at lower pH than the free functional groups. Despite the ester hydrolysis the study successfully drew out interesting differences in the coordination of these ligands and directed coordination geometries using pH manipulations was successfully carried out. The 8-membered chelate with **1** was unexpected as well as the differences in chelation amenability of **2** and **3**. Ligand **4** was expected to show κ^4[7,5,5] coordination when fully alkylated and this was successfully achieved, but only at highly alkaline conditions. The variety in coordination behavior of **4** observed agrees with the notion that the GSH amine and thiol moieties may be its most important donor groups.

Acknowledgements. Financial support by The Icelandic Centre of Research (Rannis) grant nr 152323 is gratefully acknowledged. SGS and GRR thank COST Action CM1105 for STSM Grant and Prof. Etelka Farkas for hosting the STMS at the early stages of this project. Dr Sigridur Jonsdottir is thanked for assistance with collection of mass spectrometry data.

Compliance with ethical standards.

Conflict of interest: The authors declare no conflict of interest

References

1. Soldevila-Barreda JJ, Sadler PJ (2015) Approaches to the design of catalytic metallodrugs. Current Opinion in Chemical Biology 25:172-183. doi:10.1016/j.cbpa.2015.01.024
2. Metzler-Nolte N, Guo Z (2016) Themed Issue on "Metallodrugs: Activation, Targeting, and Delivery". Dalton Transactions 45 (33):12965-12965. doi:10.1039/C6DT90135B
3. Bradford SS, Cowan JA (2014) From Traditional Drug Design to Catalytic Metallodrugs: A Brief History of the Use of Metals in Medicine. Metallodrugs 1:10-23
4. Mjos KD, Orvig C (2014) Metallodrugs in Medicinal Inorganic Chemistry. Chemical Reviews 114:4540-4563
5. Sigel H, Martin RB (1982) Coordinating properties of the amide bond. Stability and structure of metal ion complexes of peptides and related ligands. Chemical Reviews 82 (4):385-426
6. Sóvágó I, Kállay C, Várnagy K (2012) Peptides as complexing agents: Factors influencing the structure and thermodynamic stability of peptide complexes. Coordination Chemistry Reviews 256 (19):2225-2233. doi:https://doi.org/10.1016/j.ccr.2012.02.026
7. Griffith DM, Bíró L, Platts JA, Müller-Bunz H, Farkas E, Buglyó P (2012) Synthesis and solution behaviour of stable mono-, di-and trinuclear Pd(II) complexes of 2,5-pyridinedihydroxamic acid: X-ray crystal structure of a novel Pd(II) hydroxamato complex. Inorganica Chimica Acta 380 (1):291-300. doi:10.1016/j.ica.2011.09.050
8. Perinelli M, Guerrini R, Albanese V, Marchetti N, Bellotti D, Gentili S, Tegoni M, Remelli M (2020) Cu(II) coordination to His-containing linear peptides and related branched ones: Equalities and diversities. Journal of Inorganic Biochemistry 205:110980. doi:https://doi.org/10.1016/j.jinorgbio.2019.110980
9. Peana M, Gumienna-Kontecka E, Piras F, Ostrowska M, Piasta K, Krzywoszynska K, Medici S, Zoroddu MA (2020) Exploring the Specificity of Rationally Designed Peptides Reconstituted from the Cell-Free Extract of Deinococcus radiodurans toward Mn(II) and Cu(II). Inorganic Chemistry. doi:10.1021/acs.inorgchem.9b03737
10. Vicatos GM, Jackson GE, Hammouda AN, Bonomo RP, Valora G (2019) Potentiometric and spectroscopic studies of the complex formation between copper(II) and Gly-Leu-Phe or Sar-Leu-Phe tripeptides. Polyhedron 170:553-563. doi:https://doi.org/10.1016/j.poly.2019.06.011
11. Gavrish SP, Lampeka YD, Babak MV, Arion VB (2018) Palladium Complexes of N,N′-Bis(2-aminoethyl)oxamide (H2L): Structural (PdIIL, PdII2L2, and PdIVLCl2), Electrochemical, Dynamic 1H NMR, and Cytotoxicity Studies. Inorganic Chemistry 57 (3):1288-1297. doi:10.1021/acs.inorgchem.7b02732
12. Gonzalez P, Vileno B, Bossak K, El Khoury Y, Hellwig P, Bal W, Hureau C, Faller P (2017) Cu(II) Binding to the Peptide Ala-His-His, a Chimera of the Canonical Cu(II)-Binding Motifs Xxx-His and Xxx-Zzz-His. Inorganic Chemistry 56 (24):14870-14879. doi:10.1021/acs.inorgchem.7b01996
13. Park GY, Lee JY, Himes RA, Thomas GS, Blackburn NJ, Karlin KD (2014) Copper–Peptide Complex Structure and Reactivity When Found in Conserved His-Xaa-His Sequences. Journal of the American Chemical Society 136 (36):12532-12535. doi:10.1021/ja505098v
14. Sóvágó I, Ősz K (2006) Metal ion selectivity of oligopeptides. Dalton Transactions (32):3841-3854. doi:10.1039/B607515K

15. Kozlowski H, Bal W, Dyba M, Kowalik-Jankowska T (1999) Specific structure-stability relations in metallopeptides. Coordination Chemistry Reviews 184 (1):319-346. doi:10.1016/S0010-8545(98)00261-6
16. Murphy JM, Powell BA, Brumaghim JL (2020) Stability constants of bio-relevant, redox-active metals with amino acids: The challenges of weakly binding ligands. Coordination Chemistry Reviews 412:213253-213221. doi:10.1016/j.ccr.2020.213253
17. El-Sherif AA (2012) Coordination Chemistry of Palladium(II) Ternary Complexes with Relevant Biomolecules. In: Innocenti A (ed) Stoichiometry and Research - The Importance of Quantity in Biomedicine. InTech, intechopen.com, Rijeka, Croatia, pp 79-120
18. Jószai V, Nagy Z, Ősz K, Sanna D, Di Natale G, La Mendola D, Pappalardo G, Rizzarelli E, Sóvágó I (2006) Transition metal complexes of terminally protected peptides containing histidyl residues. Journal of Inorganic Biochemistry 100 (8):1399-1409. doi:10.1016/j.jinorgbio.2006.04.003
19. Livingstone SE, Chemistry JNI, 1968 (1968) Metal chelates of biologically important compounds. I. Complexes of DL-ethionine and S-methyl-L-cysteine.
20. Chandrasekharan M (1973) Cysteine complexes of palladium (II) and platinum (II). Elsevier 7:88-90. doi:10.1016/S0020-1693(00)94785-6
21. Pettit LD, Bezer M (1985) Complex formation between palladium(II) and amino acids, peptides and related ligands. Coordination Chemistry Reviews 61:97-114. doi:https://doi.org/10.1016/0010-8545(85)80003-5
22. McAuliffe CA (1967) The infrared spectra of palladium(II) and platinum(II) complexes of (±)-methionine. Journal of the Chemical Society A: Inorganic, Physical, Theoretical (0):641-642. doi:10.1039/J19670000641
23. Ágoston CG, Jankowska TK, Sóvágó I (1999) Potentiometric and NMR studies on palladium (II) complexes of oligoglycines and related ligands with non-co-ordinating side chains. J Chem Soc, Dalton Trans, (18):3295-3302
24. Wilson EW, Martin RB (1970) Circular dichroism of palladium(II) complexes of amino acids and peptides. Inorganic Chemistry 9 (3):528-532. doi:10.1021/ic50085a019
25. Krężel A, Bal W (1999) Coordination chemistry of glutathione. Acta Biochimica Polonica 46 (3):567-580
26. Chow ST, McAuliffe CA, Sayle BJ (1975) Metal complexes of amino acids and derivatives—IX: Reactions of the tripeptide, glutathione, with divalent cobalt, nickel, copper and palladium salts. Journal of Inorganic and Nuclear … 37 (2):451-454. doi:10.1016/0022-1902(75)80354-x
27. Bresson C, Spezia R, Solari PL, Jankowski CK, Den Auwer C (2015) XAS examination of glutathione–cobalt complexes in solution. Journal of Inorganic Biochemistry 142:126-131. doi:https://doi.org/10.1016/j.jinorgbio.2014.10.006
28. Jalilehvand VMaF (2012) Lead(II) Complex Formation with Glutathione. Inorganic Chemistry 51 (11):1-14. doi:10.1021/ic300496t
29. Shoeib T, Sharp BL (2013) Monomeric cisplatin complexes with glutathione: Coordination modes and binding affinities. Inorganica Chimica Acta 405:258-264. doi:https://doi.org/10.1016/j.ica.2013.06.006
30. Jószai V, Sóvágó I (2011) Palladium(II) complexes of oligopeptides containing aspartyl and glutamyl residues. Polyhedron 30 (12):2114-2120. doi:10.1016/j.poly.2011.05.032

31. Shimazaki Y, Yamauchi O (2012) Group-10 metal complexes of biological molecules and related ligands: structural and functional properties. Chemistry & biodiversity 9 (9):1635-1658. doi:10.1002/cbdv.201100446
32. Lihi N, Lukács M, Szűcs D, Várnagy K, Sóvágó I (2017) Nickel(II), zinc(II) and cadmium(II) complexes of peptides containing separate aspartyl and cysteinyl residues. Polyhedron 133:364-373. doi:10.1016/j.poly.2017.05.044
33. Picquart M, Grajcar L, Baron MH, Abedinzadeh Z (1999) Vibrational spectroscopic study of glutathione complexation in aqueous solutions. Biospectroscopy 5 (6):328-337. doi:10.1002/(SICI)1520-6343(1999)5:6<328::AID-BSPY2>3.0.CO;2-J
34. Mukhtiar M, Jan SU, Khan MF, Ullah N, Hussain A, Rehman SU, Qureshi MM (2018) Palladium glutathione, N-acetylcysteine, D-penicillamine conjugation chemistry. Pakistan Journal of Pharmaceutical Sciences 31 (1):213-219
35. Mirzahosseini A, Somlyay M, Noszál B (2015) The comprehensive acid–base characterization of glutathione. Chemical Physics Letters 622:50-56. doi:https://doi.org/10.1016/j.cplett.2015.01.020
36. Singh G, Dogra SD, Kaur S, Tripathi SK, Prakash S, Rai B, Saini GSS (2015) Structure and vibrations of glutathione studied by vibrational spectroscopy and density functional theory. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 149:505-515. doi:https://doi.org/10.1016/j.saa.2015.04.062
37. Rubino FM (2015) Toxicity of Glutathione-Binding Metals: A Review of Targets and Mechanisms. Toxics 3 (1):20-62. doi:10.3390/toxics3010020
38. Lemma K, Elmroth S, Elding L (2002) Substitution reactions of [Pt(dien)Cl]+, [Pt(dien)(GSMe)]2+, cis- [PtCl2(NH3)2] and cis-[Pt(NH3)2(GSMe)2]2+ (GSMe = S-methylglutathione) with some sulfur-bonding chemoprotective agents. Journal of the Chemical Society, Dalton Transactions:1281-1286
39. Teuben J-M, Rodriguez i Zubiri M, Reedijk J (2000) Glutathione readily replaces the thioether on platinum in the reaction with [Pt(dien)(GSMe)]2+ (GSMe = S-methylated glutathione); a model study for cisplatin–protein interactions. (3):369-372. doi:10.1039/a908135f
40. Zabel R, Weber G (2016) Comparative study of the oxidation behavior of sulfur-containing amino acids and glutathione by electrochemistry-mass spectrometry in the presence and absence of cisplatin. Analytical and Bioanalytical Chemistry 408 (4):1237-1247. doi:10.1007/s00216-015-9233-x
41. Foulds G (1998) Nickel 1987–1989. Coordination Chemistry Reviews 169 (1):3-127. doi:https://doi.org/10.1016/S0010-8545(98)00003-4
42. Ward TR (2019) ACS Central Science Virtual Issue on Bioinspired Catalysis. ACS Central Science 5 (11):1732-1735. doi:10.1021/acscentsci.9b01045
43. Gonzalez P, Bossak K, Stefaniak E, Hureau C, Raibaut L, Bal W, Faller P (2018) N-Terminal Cu-Binding Motifs (Xxx-Zzz-His, Xxx-His) and Their Derivatives: Chemistry, Biology and Medicinal Applications. Chemistry – A European Journal 24 (32):8029-8041. doi:10.1002/chem.201705398
44. Chen S, Vasquez L, Noll BC, Rakowski DuBois M (1997) Synthesis and Characterization of Mononuclear Indoline Complexes. Studies of σ and π Bonding Modes. Organometallics 16 (8):1757-1764. doi:10.1021/om960744e
45. Sundberg R (2010) Electrophilic Substitution Reactions of Indoles, vol 26. Top. Heterocycl. Chem. Springer-Verlag. doi:10.1007/7081_2010_52

46. Singh MP, Saleem F, Pal RS, Singh AK (2017) Palladacycles having normal and spiro chelate rings designed from bi- and tridentate ligands with an indole core: structure, synthesis and applications as catalysts. New Journal of Chemistry 41 (19):11342-11352. doi:10.1039/c7nj02116j
47. Kaminskaia NV, Ullmann GM, Fulton DB, Kostic NM (2000) Spectroscopic, kinetic, and mechanistic study of a new mode of coordination of indole derivatives to platinum(II) and palladium(II) ions in complexes. Inorganic Chemistry 39 (22):5004-5013. doi:10.1021/ic000254l
48. Zhang L, Lin Y-J, Li Z-H, Jin G-X (2015) Rational Design of Polynuclear Organometallic Assemblies from a Simple Heteromultifunctional Ligand. Journal of the American Chemical Society 137 (42):13670-13678. doi:10.1021/jacs.5b08826
49. Armarego WLF (2017) Purification of Laboratory Chemicals. Purification of Laboratory Chemicals (Eighth Edition). Butterworth-Heinemann. doi:https://doi.org/10.1016/B978-0-12-805457-4.50008-2
50. Azizi N, Khajeh Amiri A, Bolourtchian M, Saidi MR (2009) A green and highly efficient alkylation of thiols in water. Journal of the Iranian Chemical Society 6 (4):749-753. doi:10.1007/BF03246165
51. Li J, Sha Y (2008) A Convenient Synthesis of Amino Acid Methyl Esters. Molecules 13 (5). doi:10.3390/molecules13051111
52. Mccormick BJ, Jaynes EN, Kaplan RI, Clark HC, Ruddick JD (1972) Dichloro(ethylenediamine)palladium(II) and (2,2′-Bipyridine)dichloropalladium (II). , vol 13. Inorganic Syntheses. John Wiley & Sons, Ltd, Hoboken, NJ, USA
53. F. M. Siebert A, S. Sheldrick W (1997) pH-Dependent competition between N,S and N,N′ chelation in the reaction of [Pt(en)(H2O)2]2+ (en = H2NCH2CH2NH2) with methionine-containing di- and tri-peptides. Journal of the Chemical Society, Dalton Transactions (3):385-394. doi:10.1039/A604689D
54. Lim MC, Bruce Martin R (1976) The nature of cis amine Pd(II) and antitumor cis amine Pt(II) complexes in aqueous solutions. Journal of Inorganic and Nuclear Chemistry 38 (10):1911-1914. doi:10.1016/0022-1902(76)80121-2
55. Gutz IGR CurTiPot – pH and Acid–Base Titration Curves. 4.2 edn., on-line
56. Sakina K, Kawazura K, Morihara K (1988) Enzymatic synthesis of delta sleep-inducing peptide. International Journal of Peptide and Protein Research 31 (2):245-252. doi:10.1111/j.1399-3011.1988.tb00030.x
57. Sultane PR, Mete TB, Bhat RG (2015) A convenient protocol for the deprotection of N-benzyloxycarbonyl (Cbz) and benzyl ester groups. Tetrahedron Letters 56 (16):2067-2070. doi:https://doi.org/10.1016/j.tetlet.2015.02.131
58. Höck S, Marti R, Riedl R, Simeunovic M (2010) Thermal Cleavage of the Fmoc Protection Group. CHIMIA International Journal for Chemistry 64 (3):200-202. doi:10.2533/chimia.2010.200
59. Bajusz S, Medzihradszky K, Kisfaludy L, Low M, Paulay Z, Lang MT, Szporny L (1968) Total synthesis of human corticotropin. Hungary Patent HU155254,
60. Masignani V, Scarlato V, Scarselli M, Galeotti C, Mora M (2001) Antigenic determinants of antigenic proteins of Neisseria meningitidis and their diagnostic, prophylactic and therapeutic use.
61. Chekalin SV, Golovlev VV, Kozlov AA, Matveets YA, Yartsev AP, Letokhov VS (1988) Femtosecond laser photoionization mass spectrometry of tryptophan-containing proteins. The Journal of Physical Chemistry 92 (24):6855-6858. doi:10.1021/j100335a001

62. Kermack WO, Matheson NA (1957) The synthesis of some analogues of glutathione. Biochemical Journal 65 (1):45-48. doi:10.1042/bj0650045
63. El-Faham A, Albericio F (2011) Peptide Coupling Reagents, More than a Letter Soup. Chemical Reviews 111 (11):6557-6602. doi:10.1021/cr100048w
64. Montalbetti CAGN, Falque V (2005) Amide bond formation and peptide coupling. Tetrahedron 61 (46):10827-10852. doi:https://doi.org/10.1016/j.tet.2005.08.031
65. Joullié MM, Lassen KM (2010) Evolution of amide bond formation. ARKIVOC viii:189-250
66. Wuts PGM, Greene TW (2006) Green's Protective Groups in Organic Synthesis. Green's Protective Groups in Organic Synthesis, 4th edn. John Wiley & Sons, Inc,
67. Lide DR (2004) CRC Handbook Chemistry and Physics. 85 edn. CRC Press,
68. Alexander MD, Spillert CA (1970) Monodentate ethylenediamine complex of cobalt(III). Inorganic Chemistry 9 (10):2344-2346. doi:10.1021/ic50092a028
69. Hay RW, Pujari MP (1986) The palladium (II) promoted hydrolysis of methyl, ethyl and isopropyl glycylglycylglycinate. Inorganica Chimica Acta 123 (1):47-51. doi:10.1016/s0020-1693(00)81314-6
70. Hay RW, acta MPIc (1986) The palladium (II) promoted hydrolysis of the methyl esters of glycyl-L-leucine, glycyl-L-alanine and L-alanylglycine. Elsevier 123 (3):175-179. doi:10.1016/S0020-1693(00)86341-0
71. Ozsváth A, Farkas E, Diószegi R, Buglyó P (2019) Versatility and trends in the interaction between Pd(II) and peptide hydroxamic acids. New J Chem 43:8239-8250
72. Martin RB, Pitner TP (1971) Inversion and proton exchange at asymmetric nitrogen centers in palladium (II) complexes. 93 (18):4400-4405. doi:10.1021/ja00747a010
73. Pitner TP, Wilson EW, Martin RB (1972) Properties of Palladium8II) Complexes of Peptides and Histidine in Basic Solutions. Inorganic Chemistry 11 (4):738-742
74. Haas K, Ponikwar W, Nöth H, Beck W (1998) Facile Synthesis of Cyclic Tetrapeptides from Nonactivated Peptide Esters on Metal Centers. Angewandte Chemie International Edition 37 (8):1086-1089. doi:10.1002/(SICI)1521-3773(19980504)37:8<1086::AID-ANIE1086>3.0.CO;2-V
75. Carvalho MA, Souza BC, Paiva REF, Bergamini FRG, Gomes AF, Gozzo FC, Lustri WR, Formiga ALB, Rigatto G, Corbi PP (2012) Synthesis, spectroscopic characterization, DFT studies, and initial antibacterial assays in vitro of a new palladium(II) complex with tryptophan. Journal of Coordination Chemistry 65 (10):1700-1711. doi:10.1080/00958972.2012.679660
76. Carvalho MA, Shishido SM, Souza BC, de Paiva REF, Gomes AF, Gozzo FC, Formiga ALB, Corbi PP (2014) A new platinum complex with tryptophan: Synthesis, structural characterization, DFT studies and biological assays in vitro over human tumorigenic cells. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 122:209-215. doi:https://doi.org/10.1016/j.saa.2013.11.044
77. Vasquez LD, Noll BC, Rakowski DuBois M (1998) Mononuclear Indoline Complexes. 2. Synthesis, Structure, and Reactivity of [(Cymene)Ru(η1-N-indoline)(CH3CN)2](OTf)2. Organometallics 17 (5):976-981. doi:10.1021/om970968c
78. Farkas E, Sóvágó I (2017) Metal complexes of amino acids and peptides. In: Amino Acids, Peptides and Proteins: Volume 41, vol 41. The Royal Society of Chemistry, pp 100-151. doi:10.1039/9781782626619-00100