Addition of (Pyrazol-1-yl)acetyl and (Pyridin-2-yl)acetyl Groups to the Terminal Amino Group of a Phe−Gly Dipeptide Affords ATCUN-Like Copper(II) Binding Sites

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(Pyrazol-1-yl)acetic acid (HL^1, **1**) and (pyridin-2-yl)acetic acid have been successfully condensed with the terminal amino group of a Phe−Gly dipeptide to generate the derivatives $PzCH_2$–Phe−Gly (H_3L^2, **7**) and $PyCH_2$–Phe−Gly (H_3L^3, **8**) respectively, which contain ATCUN-like metal ion binding sites. The crystal structures of **1** and the copper(II) complexes $Cu(L^1)_2(H_2O)_2$ (**2**) and $[nBu_4N][Cu(L^2)]$ (**9**) are described. Complex **2** has a tetragonally-elongated, Jahn–Teller distorted octahedral geometry and complex **9** is square-planar. The structural similarity of the copper(II) complexes of **7** and **8** is demonstrated by their almost identical electronic absorption and EPR spectra.

Introduction

The amino terminal copper(II) and nickel(II)-binding protein motif (H_2N)–Xaa–Xaa–His (ATCUN), where Xaa is an α-amino acid other than Pro, occurs naturally in albumens (human, bovine and rat), histatins, human sperm protamine P2a, and neuromedins (C and K).[1] In the albumens its prominent role is proposed to be the binding and transport of metal ions, usually copper(II) and nickel(II). These ions have been the most extensively studied for ATCUN and ATCUN-like systems.[2–6] The presence of the ATCUN motif in the other proteins raises the possibility that the binding of metal ions may have some influence, presumably adverse, upon the normal function of these systems. It is also worthy of note that although the copper(II) binding sites identified in the prion protein of Creutzfeld–Jakob disease (CJD) are not terminal, they are nevertheless structurally very similar to the ATCUN motif, and it has been proposed that this may indicate the function of the normal cellular form of this protein is copper(II) binding, storage and/or transport.[7]

The investigation of the role of the ATCUN and related motifs in biological systems is well established, but of particular chemical importance are: (i) The observations that both the copper(II) and nickel(II) bound forms of the ATCUN motif are able to mediate oxidative DNA damage, including cleavage and cross-linking, and (ii) even simple ATCUN-like complexes display selective binding to, and cleavage of, DNA and RNA.[1,2] Indeed this has inspired the synthesis and study of ATCUN-containing proteins as selective DNA binding/cleavage systems.[2,5,6] In addition, polydentate ligands predominantly based upon the ATCUN motif have been demonstrated to act as highly selective, fluorescent chemosensors for copper(II).[8,9]

We report herein the synthesis of novel copper(II) binding peptides (Scheme 1) that do not contain a His residue within the amino-terminal protein sequence. In the peptide systems (**7** and **8**) the heterocyclic *N*-donor ligand for the metal ion is provided by the straightforward condensation of (pyrazol-1-yl)acetic acid (**1**) or (pyridin-2-yl)acetic acid with the amino terminal of the peptide. In principle, this approach allows the generation of an ATCUN-like site at the amino terminal of any dipeptide. In the specific examples described here the chosen starting dipeptide was Phe−Gly and in the resulting tetradentate systems the terminal carboxylate group acts as the fourth ligand to the bound, square-planar copper(II) ion.

Results and Discussion

(Pyrazol-1-yl)acetic acid HL^1 (**1**) was prepared in good yield by the alkylation of pyrazole with bromoacetic acid, and X-ray quality crystals were obtained by recrystallisation from water. The structure of **1** (Figure 1) reveals that the pyrazole group is not sufficiently basic for the compound to crystallise in a zwitterionic form; the freely refined acidic proton H(1) is clearly located on the carboxylic acid

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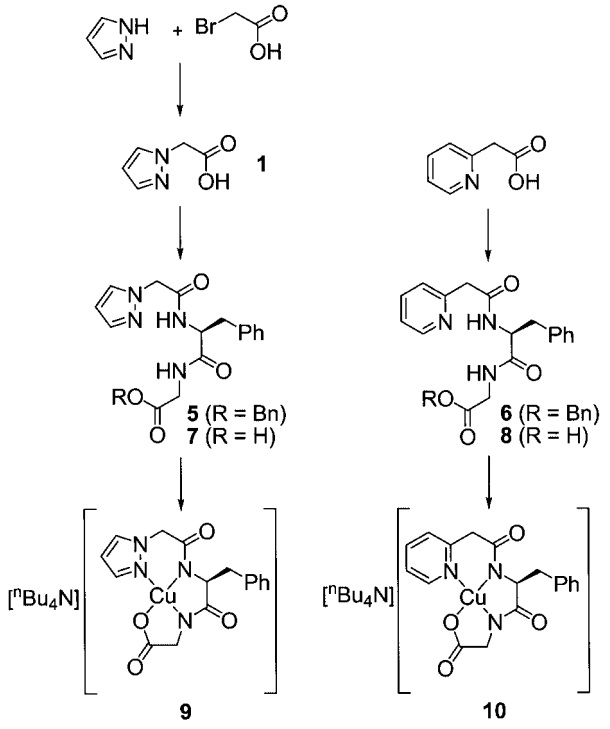

Scheme 1

group with a strong intermolecular hydrogen bond to N(1) of a neighbouring molecule. Thus **1** crystallises as parallel, 1-D, hydrogen-bonded chains that are helical and homochiral (non-centrosymmetric space group $P2_12_12_1$). The least-squares planes of the pyrazole and carboxylic acid groups are almost mutually perpendicular with a dihedral angle of 80.76(6)° and N(2) lies only 0.0276(14) Å out of the plane of the carboxylic group. In all these regards the structure is similar to that reported for the substituted analogue 3,5-dimethyl-(pyrazol-1-yl)acetic acid.[10]

The complexation of **1** with copper(II) yields the Jahn–Teller distorted, centrosymmetric, octahedral structure $Cu(L^1)_2(H_2O)_2$ (**2**), the molecular structure of which is shown in Figure 2. The ligand bite angle is 90.58(7)° and the neutral pyrazole Cu(1)–N(1) bond length of 1.954(2) Å is significantly shorter than the Cu(1)–O(1) bond length of 1.980(2) Å involving the anionic carboxylate ligand. This is consistent with the delocalisation of the carboxylate negative charge over both oxygen atoms as evinced by the similar C(5)–O(1) and C(5)–O(2) bond lengths of 1.274(3) and 1.246(2) Å respectively [Δd = 0.028(4) Å], and this delocalisation is stabilised by the presence of two hydrogen-bonds to the non-coordinated carboxylate oxygen atom O(2). Upon complexation the dihedral angle between the least-squares planes of the pyrazole and carboxylic acid groups has changed from 80.76(6)° in the free ligand (**1**) to 32.35(5)° in **2**. Due to the non-planarity of the ligand the dihedral angle between the pyrazole group and the copper square-plane is 22.55(4)°. The electronic absorption spectrum of **2** in aqueous solution is typical of axially elongated octahedral copper(II) with a single broad absorption band at 14700 cm^{-1} (ε = 32 $M^{-1}cm^{-1}$). There are no reported complexes of either (pyrazol-1-yl)acetic acid (**1**) or its simple derivatives in the literature, however several transition metal complexes (VO^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+} and Cu^{2+}) of the similar ligand imidazol-4-yl-acetic acid have been described.[11–16] The crystal structures of many of these have been reported,[17] but surprisingly not for Cu^{2+}. Furthermore, there is only one unequivocally characterised transition metal complex (Pt^{2+}) of (pyridin-2-yl)acetic acid or its simple derivatives.[18] To date, attempts to prepare and characterise other first row transition metal complexes of **1** have been unsuccessful, thus at present it is difficult to compare di-

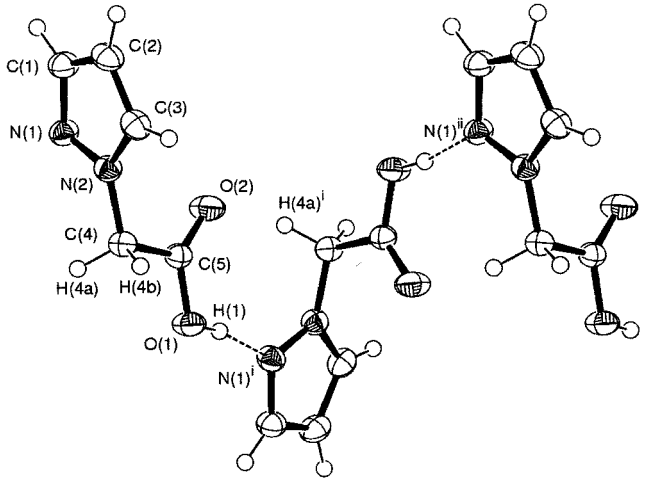

Figure 1. ORTEP[25] view of the molecular structure and hydrogen-bonding of **1** with thermal ellipsoids shown at 50 %. Selected bond lengths [Å] and angles [°]: O(1)–C(5), 1.309(2); O(2)–C(5), 1.207(2); O(1)–H(1), 0.95(3); H(1)···N(1)^i, 1.69(3); O(1)···N(1)^i, 2.628(2); O(1)–H(1)···N(1)^i, 170(3). Symmetry transformations: [i] $-x$, y + ½, $-z$ + 1½; [ii] x, y + 1, z.

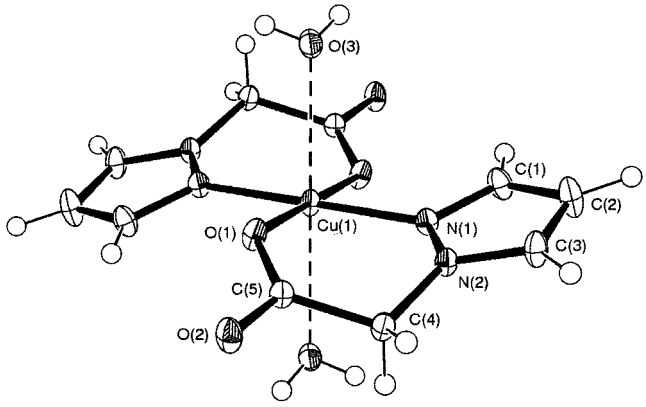

Figure 2. ORTEP[25] view of the molecular structure of **2** with thermal ellipsoids shown at 50 %. Selected bond lengths [Å] and angles [°]: Cu(1)–O(1), 1.980(2); Cu(1)–N(1), 1.954(2); Cu(1)–O(3), 2.503(2); O(1)–Cu(1)–N(1), 90.52(4); O(1)–Cu(1)–O(3), 92.57(4); N(1)–Cu(1)–O(3), 93.60(4); O(3)–H(3d), 0.80(2); H(3d)···O(2)^i, 2.11(2); O(3)···O(2)^i, 2.886(1); O(3)–H(3d)···O(2)^i, 167(2); O(3)–H(3e), 0.77(2); H(3e)···O(2)^{ii}, 2.03(2); O(3)···O(2)^{ii}, 2.801(1); O(3)–H(3e)···O(2)^{ii}, 176(2). Symmetry transformations: [i] x + ½, $-y$ + 1½, z + ½; [ii] $-x$ + 1½, y – ½, $-z$ – ½.

rectly the coordination behaviour of these related ligand systems.

The two tetradentate square planar ligands H_3L^2 (**7**) and H_3L^3 (**8**) were prepared in two steps, from **1** and (pyridin-2-yl)acetic acid, respectively, by standard peptide coupling with the dipeptide H–Phe−Gly−OBn, followed by hydrolysis of the benzyl ester. The complexation of **7** with copper(II) yields the deep pink, square-planar complex $[Cu(L^2)]^-$ as the tetra-n-butylammonium salt **9**. The crystal structure of the complex anion of **9** (Figure 3) reveals that the copper(II) centre is close to planar; the sum of the angles subtended at the metal is 360.0° and the maximum deviation from the least-squares coordinate plane for Cu(1) and its four donor atoms is only 0.017(2) Å for N(4). There is a very slight tetrahedral distortion of the Cu(1) coordination geometry with the pairs of opposite donor atoms lying on opposite sides of the least-squares plane and N(1)–Cu(1)–N(4) and N(3)–Cu(1)–O(3) angles of 178.79(6) and 168.71(5)° respectively. As expected, the shortest bond lengths in the copper coordination sphere involve the deprotonated amide nitrogen atoms. The strength of these bonds is further illustrated by the short C=O [average 1.244(2) Å] and long C–N [average 1.324(2) Å] bond lengths within the amide groups showing very little delocalisation of the formal negative charge onto the oxygen atom, and hence relatively little contribution to the electronic structure by the oxy-imine [RN=C(R′)–O⁻] resonance form. Due to the square-planar geometry of the copper centre in **9** and the consequent absence of any axial ligand interactions, the neutral pyrazole Cu(1)–N(1) bond length of 1.9321(13) Å is shorter than in **2**, despite being *trans* to the shortest Cu(1)–N(amide) bond. The carboxylate Cu(1)–O(3) bond length is the longest in the coordination sphere, yet this is also shorter than the similar bond in **2**. In addition the carboxylate C(8)–O bond lengths are more dissimilar [Δd = 0.067(3) Å] than in **2**, consistent with the absence of any stabilisation of negative charge on O(4) by hydrogen bonding. Due to the non-planar nature of the (pyrazol-1-yl)acetyl group the dihedral angle between the pyrazole group and the copper square-plane is 17.81(5)°, similar to that found in **2**. The phenyl substituent of the ligand is positioned above one face of the copper square plane in the solid state and the angle between their respective least-squares planes is 61.59(5)°. Of the three possible staggered conformations about the C(6)–C(10) bond, this is the one that minimises the unfavourable steric interactions between the phenyl ring and the carbonyl oxygen atoms O(1) and O(2). The Flack parameter determined from the structure refinement confirms the known (*S*)-chirality of the ligand at C(6), being derived from (*S*)-phenylalanine. The pink colour of **9** is retained in dichloromethane solution and arises from a single absorption band at 19500 cm^{-1} in the visible region of the electronic absorption spectrum. There is no significant change to the spectrum either in dichloromethane/methanol (1:1) or upon the addition of ten equivalents of a good N-donor ligand (*N*-methylimidazole), indicating that the square-planar geometry is retained under these conditions. In aqueous solution however, the complex is pale violet and the absorption band shifts to 18000 cm^{-1}. This shift of 1500 cm^{-1} to lower energy is similar to that observed for the 1,4-diazacycloheptane (LL) complexes $[Cu(LL)_2]^{2+}$ and $[Cu(LL)_2(H_2O)]^{2+}$,[19] and may therefore indicate the coordination of one water ligand to give a five coordinate, square pyramidal complex in aqueous solution.

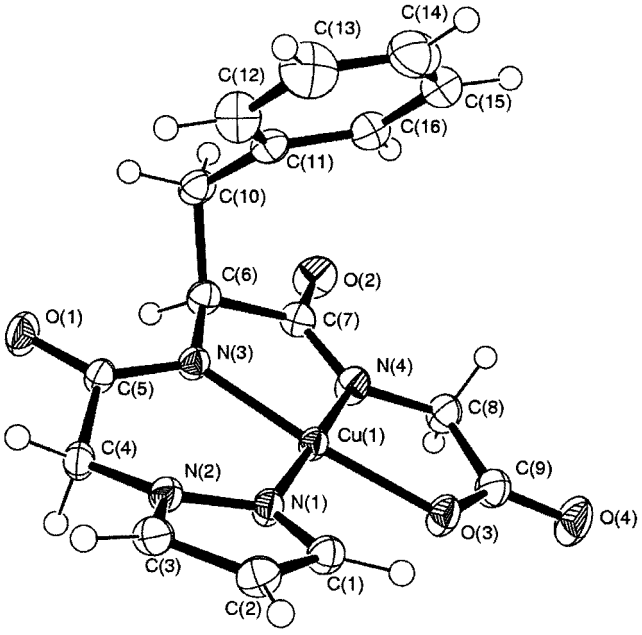

Figure 3. ORTEP[25] view of the structure of the complex cation of **9** with thermal ellipsoids shown at 50 %. Selected bond lengths [Å] and angles [°]: Cu(1)–O(3), 1.9606(12); Cu(1)–N(1), 1.9321(13); Cu(1)–N(3), 1.9299(13); Cu(1)–N(4), 1.8654(13); O(1)–C(5), 1.246(2); N(3)–C(5), 1.325(2); O(2)–C(7), 1.242(2); N(4)–C(7), 1.323(2); O(3)–C(9), 1.298(2); O(4)–C(9), 1.231(2); N(1)–Cu(1)–N(3), 94.42(5); N(3)–Cu(1)–N(4), 84.66(6); O(3)–Cu(1)–N(4), 84.09(6); O(3)–Cu(1)–N(1), 96.85(5).

The (pyridin-2-yl)acetyl analogue, **10**, could be prepared as a pink complex by the same method as used for **9**. Unfortunately, a pure or crystalline sample of **10** could not be obtained due to its slow decomposition during attempted recrystallisation, resulting in a pale green solution. Electronic absorption spectra of **10** in dichloromethane/methanol (3:1) and water were recorded for freshly prepared samples and were found to be almost identical to the corresponding spectra of **9**, consistent with the formation of a structurally very similar complex. Additionally, the negative ion electrospray mass spectrum of complex **10** in dichloromethane solution revealed the desired molecular ion. For further confirmation of the structure of **10**, and for comparison with **9**, the EPR spectra of both were measured at X-band. Complexes **9** and **10** gave near identical spectra in the same solvents systems (Figure 4).

At room temperature the Cu hyperfine (to both isotopes), as well as ligand superhyperfine splittings to ^{14}N and ^{1}H can be resolved, in both aqueous and in CH_2Cl_2 solution. In frozen solution at ca. 110 K (adding ca. 10 % glycerol to H_2O, or ca. 10 % toluene to CH_2Cl_2, to aid glassing) we observed well resolved spectra which are axial within the resolution of the X-band experiment. The ^{14}N

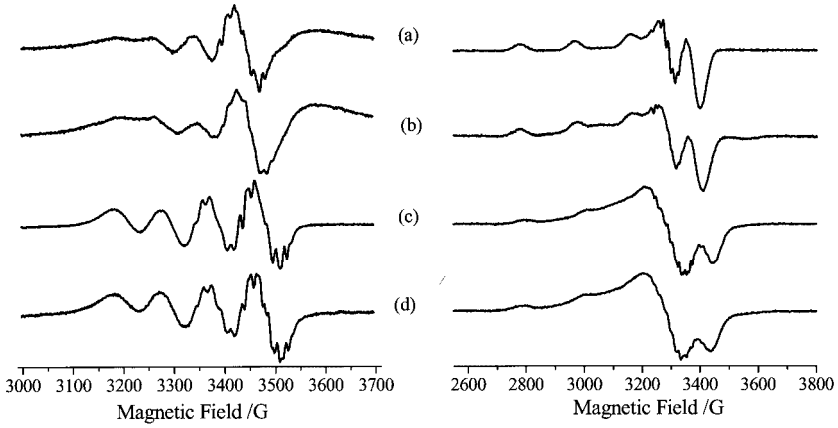

Figure 4. Fluid solution EPR spectra at 290 K and 9.773 GHz (left) and frozen solution EPR spectra at 120 K and 9.457 GHz (right). (a) **9** in H_2O/glycerol; (b) **10** in H_2O/glycerol; (c) **9** in CH_2Cl_2/toluene; (d) **10** in CH_2Cl_2/toluene.

Table 1. Simulation of EPR parameters for complex **9** (A in Gauss). Complex **10** gave nearly identical spectra in the same solvent systems.

Solvent	g_{iso}	A_{iso}^{Cu}	A_{iso}^{3N}	$A_{iso}^{2/3H}$	g_z	g_{xy}	A_z^{Cu}	A_{xy}^{Cu}	A_{xy}^{3N}
H_2O	2.104	79	14	8	2.206	2.040	190	20	16
CH_2Cl_2	2.088	90	16	8	2.165	2.050	216	35	16

Table 2. Comparison of visible and EPR parameters for complexes **9** and related Cu^{II} complexes (data taken from reference 1. *pH = 6.5; HSA, human serum albumin; DSA, dog serum albumin; A values in Gauss).

Complex*	$\lambda_{max.}$ (nm)	$\varepsilon_{max.}$ ($M^{-1}cm^{-1}$)	g_z	g_{xy}	A_z^{Cu}
9 (H_2O)	555	110	2.206	2.040	190
9 (CH_2Cl_2)	513	135	2.165	2.050	216
HSA−Cu^{II}	525	101	2.166	2.051	214
DSA−Cu^{II}	600 (br)	−	2.256	2.059	163
Gly−Gly−His−Cu^{II}	525	103	2.170	2.051	211
Asp−Ala−His−Cu^{II}	525	103	2.167	2.050	211

coupling in the "perpendicular" region of the spectra is also resolved. The frozen solution EPR spectra were modeled assuming coupling of the unpaired electron with Cu and to three equivalent in-plane coordinated ^{14}N nuclei (equivalent within the resolution of the EPR experiment). These simulations were sensitive to both the ^{14}N and Cu hyperfine coupling in the "perpendicular" region (the latter having been estimated initially from the values of A_z^{Cu} and A_{iso}^{Cu}). In order to simulate the isotropic spectra it was also necessary to include coupling to ^1H (this is not resolved in the anisotropic spectra). Simulation parameters are in Table 1. The large values of A_z^{Cu} are consistent with the N_3O in-plane coordination, and the A^N values are typical for (in-plane) N-bound Cu complexes. The important trends are that g_z increases in switching from CH_2Cl_2 to H_2O solution and A_z^{Cu} (and A_{xy}^{Cu}) decreases in going from CH_2Cl_2 to H_2O solution. These trends are consistent with going from a square-planar complex in dichloromethane to square pyramidal species in water and thus support the conclusions drawn from the electronic spectra. The EPR spectroscopic data from **9** in dichloromethane compared with data from human serum albumin, Gly−Gly−His−Cu^{II} and Asp−Ala−His−Cu^{II} are very similar, and are consistent with square-planar coordination in these systems (Table 2). The switch to a square pyramidal structure for complex **9** in water is clearly revealed with the expected changes in g_z, A_z^{Cu} and λ_{max}. Dog serum albumin does not show the ATCUN motif as it does not contain histidine as the third residue from the *N* terminus. The EPR and visible absorption data for this albumin is clearly divergent from the other complexes.

In conclusion, the condensation of (pyrazol-1-yl)acetic acid and (pyridin-2-yl)acetic acid with the terminal amino group of a dipeptide has been shown to generate copper(II) ion binding sites. The extension of this methodology to larger oligopeptides, the investigation of the chemical reactivity of the metal centre, the cytotoxicity of these complexes and their interaction/reaction with DNA are in progress.

Experimental Section

The protected dipeptide [(*S*)-2-*tert*-butoxycarbonylamino-3-phenylpropanoylamino]acetic acid benzyl ester (Boc−Phe−Gly−OBn, **3**) was prepared using standard procedures.[20] Elemental analyses were obtained with a Fisons EA1108 CHNS analyser. IR spectra were recorded as KBr disks with a Perkin–Elmer Paragon-100 FT-IR spectrophotometer. Electronic absorption spectra were recorded

in the range 1100–300 nm with a Perkin–Elmer Lambda-40 spectrophotometer. NMR spectra were recorded with a Jeol JNM-LA400 spectrometer at room temperature (20 °C) and are reported relative to tetramethylsilane. Mass spectra were recorded with a Finnigan MAT-1020 or Thermo Finnigan LCQ ion trap spectrometer. EPR spectra were measured with a Bruker EMX X-band spectrometer.

(Pyrazol-1-yl)acetic Acid (HL^1, 1): Sodium hydroxide (16.2 g, 0.404 mol) was dissolved in water (185 mL) and pyrazole (12.5 g, 0.184 mol) was added. Next bromoacetic acid (28.1 g, 0.202 mol) was added in portions with stirring and the resulting mixture then heated at reflux for 2 hours. After cooling, the mixture was carefully acidified (2 M HCl) to about pH 3 whereupon the product crystallized from the solution. After drying under vacuum, **1** was isolated as a pale yellow crystalline solid. Yield: 15.26 g (67 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.0–4.5 (br. s, 1 H, OH), 4.95 (s, 2 H, CH_2), 6.25 [dd, ^3J(H,H) = 2.3 and 1.8 Hz, 1 H, PzH], 7.42 (d, $^3J_{H,H}$ = 1.4 Hz, 1 H, PzH), 7.70 (d, $^3J_{H,H}$ = 2.3 Hz, 1 H, PzH) ppm. IR (KBr): $\tilde{\nu}$ = 3423, 2509, 1733, 1071, 780 cm^{-1}. MS (EI): *m*/*z* (%) = 126 (20) [M$^+$].

Synthesis of the Copper(II) Complex $Cu(L^1)_2(H_2O)_2$ (2): A solution of copper(II) acetate (0.39 g, 2 mmol) in water (15 mL) was added to a solution of (pyrazol-1-yl)acetic acid (**1**, 0.50 g, 4 mmol) in water (25 mL) with stirring. The blue solution was filtered and the solvent was evaporated slowly. Large, blue, X-ray quality crystals of the complex **2** deposited. Yield: 0.39 g (56 %). $C_{10}H_{14}CuN_4O_6$ (349.79): calcd. C 34.34, H 4.03, N 16.02; found C 34.11, H 3.98, N 15.86. UV/Vis (H_2O): λ = 14700 cm^{-1} (32 M^{-1}cm^{-1}). IR (KBr): $\tilde{\nu}$ = 3466, 1655, 1415, 1369, 1338, 1284, 1076, 926, 809, 755, 722, 614, 577 cm^{-1}.

(*S*)-[1-(Benzyloxycarbonylmethylcarbamoyl)-2-(phenylethyl)]ammonium Trifluoroacetate (TFA·H–Phe−Gly−OBn, 4): The dipeptide **3** (2.51 g 6.09 mmol) was dissolved in dichloromethane (10 mL) to which was added trifluoroacetic acid (3 mL). The mixture was stirred in an open flask for 2–3 h, and then the solvent and excess trifluoroacetic acid were evaporated in vacuo. The resulting oil was triturated thoroughly with diethyl ether/toluene to give **4** as a white solid. Yield: 2.26 g (87 %). ^{1}H NMR (400 MHz, $CDCl_3$/[D_6]DMSO): δ = 3.18 (ABX, $^2J_{H,H}$ = 13.9, $^3J_{H,H}$ = 6.2, 6.8 Hz, 2 H, $PhCH_2$), 3.98 (d, $^3J_{H,H}$ = 5.6 Hz, 2 H, CH_2NH), 4.31 (br. t, $^3J_{H,H}$ ≈ 6.7 Hz, 1 H, C*H*CH_2), 5.10 (s, 2 H, CH_2O), 7.21–7.47 (m, 10 H, PhH), 8.80 (br. t, $^3J_{H,H}$ ≈ 5.6 Hz, 1 H, NH) ppm.

Benzyl {(*S*)-3-Phenyl-2-[(2-pyrazol-1-yl)acetylamino]propionylamino}acetate ($PzCH_2CO$–Phe−Gly−OBn, 5): A sample of dipeptide **3** (3.0 g, 7.3 mmol) was deprotected as above and the resulting crude oil dissolved in dry dimethylformamide (50 mL). Next (pyrazol-1-yl)acetic acid (**1**, 1.08 g, 8.0 mmol) hydroxybenzotriazole (1.18 g, 8.8 mmol) and diisopropylethylamine (1.52 mL, 9.6 mmol) were added to the solution followed by 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.54 g, 8.8 mmol). After stirring at room temperature overnight the dimethylformamide was removed by rotary evaporation under high vacuum and the oil dissolved in dichloromethane/water. After separation, the dichloromethane layer was washed with water, dried with anhydrous sodium sulfate, filtered then the solvent removed in vacuo. The resulting buff-coloured solid was dissolved in the minimum volume of hot ethyl acetate, and upon cooling an amorphous white solid precipitated. Filtration and thorough drying (to remove large amounts of ethyl acetate associated with the solid) under vacuum gave **5**. Yield: 2.34 g (76 %). $C_{23}H_{24}N_4O_4$ (420.18): calcd. C 65.70, H 5.75, N 13.33; found C 65.50, H 5.85, N 13.31. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.02 (ABX, $^2J_{H,H}$ = 13.9, $^3J_{H,H}$ = 6.6, 7.3 Hz, 2 H, $PhCH_2$), 3.98 (ABX, $^2J_{H,H}$ = 18.3, $^3J_{H,H}$ = 5.2, 5.6 Hz, 2 H, CH_2NH), 4.68 (br. q, $^3J_{H,H}$ ≈ 7.3 Hz, 1 H, C*H*CH_2), 4.75 (AB, $^2J_{H,H}$ = 16.8 Hz, 2 H, CH_2Pz), 5.14 (s, 2 H, CH_2O), 6.30 (t, $^3J_{H,H}$ ≈ 2 Hz, 1 H, PzH), 6.63 (br. t, $^3J_{H,H}$ ≈ 5.6 Hz, 1 H, NH), 6.77 (br. d, $^3J_{H,H}$ ≈ 7.8 Hz, 1 H, NH), 7.04–7.06 (m, 2 H, PhH), 7.17–7.26 (m, 3 H, PhH), 7.32–7.39 (m, 6 H, PhH + PzH), 7.58 (d, $^3J_{H,H}$ ≈ 2 Hz, 1 H, PzH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 37.7, 41.3, 54.3, 54.7, 67.2, 106.8, 127.0, 128.4, 128.5(7), 128.6(3), 128.6(5), 129.2, 131.1, 135.1, 136.1, 141.3, 167.5, 169.2, 170.5 ppm. MS (EI): *m*/*z* (%) = 420 (1) [M$^+$]. HRMS found: [M]$^+$ 420.1798; $C_{23}H_{24}N_4O_4$ requires [M]$^+$ 420.1798.

Benzyl {(*S*)-3-Phenyl-2-[(2-pyridin-2-yl)acetylamino]propionylamino}acetate (2-$PyCH_2CO$–Phe−Gly−OBn, 6): A sample of the dipeptide salt **4** (5.0 g, 12.0 mmol) was coupled to (pyridin-2-yl) acetic acid hydrochloride (2.04 g, 12.0 mmol) as described above to give the title compound as a white solid. Yield: 3.40 g (67 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.10 (ABX, $^2J_{H,H}$ = 14.0, $^3J_{H,H}$ = 5.9, 7.9 Hz, 2 H, $PhCH_2$), 3.66 (AB, $^2J_{H,H}$ = 15.7 Hz, 2 H, CH_2Py), 4.01 (ABX, $^2J_{H,H}$ = 18.2, $^3J_{H,H}$ = 5.0, 5.9 Hz, 2 H, CH_2NH), 4.77 (br. q, $^3J_{H,H}$ ≈ 8.0 Hz, 1 H, C*H*CH_2), 5.14 (s, 2 H, CH_2O), 7.09–7.20 (m, 8 H, ArH), 7.30–7.38 (m, 5 H, ArH), 7.60 (td, $^3J_{H,H}$ = 7.6, $^4J_{H,H}$ = 1.7 Hz, 1 H, ArH), 7.76 (br. d, $^3J_{H,H}$ ≈ 7.9 Hz, 1 H, NH), 8.41 (br. d, $^3J_{H,H}$ ≈ 4.8 Hz, 1 H, ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 37.4, 41.4, 44.7, 54.4, 67.1, 122.1, 124.0, 126.7, 128.3, 128.5 (two peaks), 128.6, 129.2, 135.2, 136.6, 137.2, 149.0, 155.1, 169.4, 169.6, 171.3 ppm. MS (EI): *m*/*z* (%) = 431 (2) [M$^+$], 340 (20), 239 (45), 120 (100).

{(*S*)-3-Phenyl-2-[(2-pyrazol-1-yl)acetylamino]propionylamino}acetic Acid (H_3L^2, 7): The modified tripeptide **5** (1.0 g, 2.38 mmol) was dissolved in H_2O/ethanol (1:1, 8.0 mL), and to this solution was added 1 M NaOH (7.2 mL, 7.2 mmol). The mixture was stirred at room temperature overnight, then carefully acidified to pH ≈ 2–3 with 2 M HCl causing precipitation of a white fluffy solid. After removal by filtration the solid obtained was dried in vacuo to yield **7**. Yield: 0.546 g (69 %). $C_{16}H_{18}N_4O_4$ (330.34): calcd. C 58.17, H 5.49, N 16.96; found C 58.15, H 5.53, N 16.74. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 2.90 (ABX, $^2J_{H,H}$ = 13.7, $^3J_{H,H}$ = 4.3, 9.5 Hz, 2 H, $PhCH_2$), 3.77 (d, $^3J_{H,H}$ = 6.1 Hz, 2 H, CH_2NH), 4.57 (br. dt, $^3J_{H,H}$ ≈ 9, 4.2 Hz, 1 H, C*H*CH_2), 4.75 (AB, $^2J_{H,H}$ = 16.0 Hz, 2 H, CH_2Pz), 6.20 (br. t, $^3J_{H,H}$ ≈ 2 Hz, 1 H, PzH), 7.16–7.27 (m, 5 H, PhH), 7.39 (d, $^3J_{H,H}$ = 1.7 Hz, 1 H, PzH), 7.55 (d, $^3J_{H,H}$ = 2.5 Hz, 1 H, PzH), 8.32 (br. d, $^3J_{H,H}$ ≈ 8.5 Hz, 1 H, NH), 8.49 (br. t, $^3J_{H,H}$ ≈ 5.8 Hz, 1 H, NH) ppm. ^{13}C NMR (100.6 MHz, [D_6]DMSO): δ = 37.9, 40.9, 53.6(8), 53.7(1), 106.3, 126.3, 128.1, 129.2, 131.2, 137.5, 138.8, 166.4 (2 peaks), 171.0 ppm. MS (EI): *m*/*z* (%) = 330 (2) [M$^+$].

{(*S*)-3-Phenyl-2-[(2-pyridin-2-yl)acetylamino]propionylamino}acetic Acid (H_3L^3, 8): The modified tripeptide ester **6** (3.0 g, 6.96 mmol) was hydrolysed by the method described for **7** to give three crops of a white solid. Total yield: 1.57 g (66 %). ^{1}H NMR (400 MHz, [D_6]DMSO + $CDCl_3$): δ = 2.77 (dd, $^2J_{H,H}$ = 13.9, $^3J_{H,H}$ = 10.4 Hz, 1 H, PhCH*H*CH), 3.10 (dd, $^2J_{H,H}$ = 13.9, $^3J_{H,H}$ = 3.9 Hz, 1 H, PhCH*H*CH), 3.59 (AB, $^2J_{H,H}$ = 14.6 Hz,, 2 H, CH_2Py), 3.80 (ABX, $^2J_{H,H}$ = 13.9, $^3J_{H,H}$ = 14.6, 14.6 Hz, 2 H, CH_2NH), 4.58 (br. dt, $^3J_{H,H}$ = 8.7, 4.2 Hz, 1 H, C*H*CH_2), 7.07 (d, $^3J_{H,H}$ = 7.9 Hz, 1 H, ArH), 7.15–7.24 (m, 6 H, ArH), 7.62 (td, $^3J_{H,H}$ = 7.6, $^4J_{H,H}$ = 1.7 Hz, 1 H, ArH), 8.42–8.46 (m, 2 H, NH + ArH), 8.52 (br. t, $^3J_{H,H}$ ≈ 5.9 Hz, 1 H, NH), 12.6 (br. s, 1 H, CO_2 H) ppm. ^{13}C NMR (100.6 MHz, [D_6]DMSO + $CDCl_3$): δ = 37.5, 40.7, 44.6, 53.8, 121.6, 123.4, 126.1, 127.9, 129.1, 136.4, 137.8, 148.6, 156.1, 168.8, 171.0, 171.4 ppm. MS (EI): *m*/*z* (%) = 341 (1) [M$^+$], 250 (15), 239 (20), 120 (90).

Table 3. Crystal data and structure refinement for **1**, **2** and **9**.

Compound	**1**	**2**	**9**
Empirical formula	$C_5H_6N_2O_2$	$C_{10}H_{14}CuN_4O_6$	$C_{32}H_{51}CuN_5O_4$
M_r [g mol^{-1}]	126.12	349.79	633.32
Crystal system	orthorhombic	monoclinic	orthorhombic
Space group	$P2_12_12_1$	$C2/c$	$P2_12_12_1$
a [Å]	4.9166(4)	12.4705(16)	10.2652(3)
b [Å]	7.7232(9)	10.2272(10)	17.4034(6)
c [Å]	15.8610(15)	10.3266(12)	18.8660(8)
β [°]	90	99.298(10)	90
V [Å^3]	602.27(10)	1299.7(3)	3370.4(2)
Z, $D_{calcd.}$ [g cm^{-3}]	4, 1.391	4, 1.788	4, 1.248
μ (Mo-K$_\alpha$) [mm^{-1}]	0.110	1.716	0.689
$F(_{ooo})$	264	716	1356
Crystal dimensions [mm^3]	0.60 × 0.20 × 0.20	0.30×0.20×0.20	0.50×0.40×0.30
$\theta_{min.}$, $\theta_{max.}$ [°]	2.57, 34.81	2.59, 30.00	2.46, 34.77
h, k, l ranges	–7/6, –12/10, –25/25	–17/17, –12/14, –14/14	–16/14, –27/27, –30/30
Collected/unique reflections	8870/1525	6426/1862	50653/14515
Restraints, parameters	0, 87	0, 106	0, 380
$T_{min.}$, $T_{max.}$	–	0.6075, 0.7546	0.6962, 0.8495
R_{int}	0.0389	0.0195	0.0380
Goodness-of-fit on F^2	1.088	1.025	1.085
R indices [$I > 2\sigma(I)$]	R_1 = 0.0429, wR_2 = 0.1082	R_1 = 0.0212, wR_2 = 0.0563	R_1 = 0.0392, wR_2 = 0.0961
R indices (all data)	R_1 = 0.0529, wR_2 = 0.1148	R_1 = 0.0260, wR_2 = 0.0571	R_1 = 0.0475, wR_2 = 0.1029
Flack parameter	not determined	–	0.001(7)
Larg. diff. peak/hole [e·Å^{-3}]	0.219/–0.249	0.427/–0.362	0.696/–0.732

Synthesis of the Copper(II) Complex [Bu$_4$N][Cu(L^2)] (9): The modified tripeptide **7** (200 mg, 0.606 mmol) was added to potassium hydroxide (102.1 mg, 1.808 mmol) in water (2 mL) to give a pale yellow solution. To this was added dropwise a solution of cupric acetate monohydrate (120.7 mg, 0.606 mmol) in water (2 mL) and then the resulting royal blue solution was filtered through a cotton wool plug. (Tetra-*n*-butyl)ammonium bromide (194.3 mg, 0.606 mmol) was added and the solution evaporated to dryness giving a dark blue solid. Addition of chloroform (10 mL) resulted in the formation of a cherry red solution and white solid, the latter being removed by filtration. The red solution was evaporated and thoroughly dried in vacuo before crystallization from dichloromethane/ethyl acetate (1:6). Yield: 300 mg (78 %). Growth of crystals suitable for single-crystal X-ray diffraction was achieved by slow evaporation of dichloromethane from a dichloromethane/ethyl acetate solution, resulting in the isolation of red needle-like crystals. $C_{32}H_{51}CuN_5O_4$ (633.33): calcd. C 60.69, H 8.12, N 11.06; found C 60.48, H 7.84, N 11.05. MS (APCI): *m*/*z* (%) = 631.3 [M – H]$^+$. UV/Vis (CH_2Cl_2): λ = 19500 cm^{-1} (135 M^{-1} cm^{-1}); (H_2O): 18000 (110). IR (KBr): $\tilde{\nu}$ = 3090 (w), 2964 (m), 2935 (w), 2875 (m), 1623 (m), 1598 (s), 1387 (m), 1338 (m), 1081 (w), 1073 (w), 756 (w), 709 (w), 536 (w), 500 (w) cm^{-1}. EPR: See Table 1 and Figure 4. These spectra are available as Supporting Information (see also the footnote on the first page of this article).

Synthesis of the Copper(II) Complex [Bu$_4$N][Cu(L^3)] (10): The preparation of the copper(II) complex of the modified tripeptide **8** (200 mg, 0.606 mmol) was attempted using the method described above for **9**. Neither a pure sample nor X-ray quality crystals of **10** could be obtained due to the slow decomposition of the complex in solution over several days to give a pale green solution. The EPR and electronic absorption spectra were determined for freshly prepared solutions and the extinction coefficients were calculated with the assumption that all the ligand was successfully complexed to copper(II). UV/Vis [CH_2Cl_2/methanol (3:1)]: λ = 19200 cm^{-1} (125 M^{-1} cm^{-1}); (H_2O): 18000 (110). MS (negative ion electrospray) [CH_2Cl_2]: *m*/*z* (%) = 401.0 (100) ([M]$^-$ Cu^{63}), 403.0 (45) ([M]$^-$, Cu^{65}). EPR: see Figure 4 for the near identical spectra of complexes **9** and **10**. This data is available as Supporting information.

Crystal Structure Determinations: Data for **1**, **2** and **9** were collected at 150(2) K with graphite-monochromated Mo-K_α radiation (wavelength of 0.71073 Å) with a Stoe IPDS II image plate diffractometer (Table 3). Space groups were determined by an examination of the systematic absences in the data, and their correct identification was confirmed by the successful solution and refinement of the structures. Solutions were provided via direct methods using SHELXS-97 and refined by full-matrix least-squares on F^2 using SHELXL-97.[21] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms on carbon were placed in calculated positions with U_{iso} set at 1.2 times the U_{eq} of the parent atom, and the positional and isotropic displacement parameters of all O–H groups were freely refined. Numerical absorption corrections were applied to **2** and **9** using X-RED/X-SHAPE.[22] The analysis of the structures was carried out with PLATON[23] and WinGX.[24]

Supplementary data for **1**, **2** and **9** (CCDC-239525, -239527 and -239526, respectively) are available free of charge from the Cambridge Crystallographic Data Centre (CCDC) via www.ccdc.cam.ac.uk/data_request/cif.

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