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Conformational and coordination properties of a peptide containing the novel α,α-bis(2-pyridyl)glycine amino acid †

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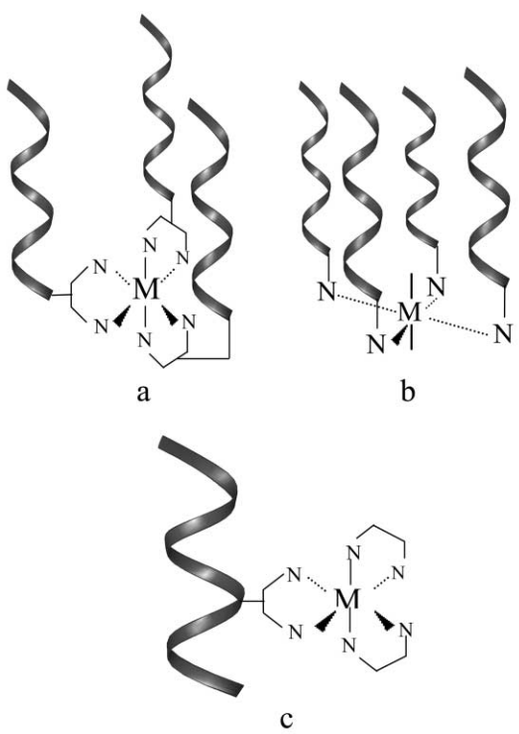
A fully protected tripeptide containing a novel $C^{\alpha,\alpha}$-disubstituted glycine—α,α-di(2-pyridyl)glycine (2Dpy)—in the central position and $C^{\alpha,\alpha}$-dimethylglycine (Aib) in positions 1 and 3 was synthesized and structurally characterized by single crystal X-ray analysis. The 2Dpy residue bears two 2-pyridyl moieties, which may coordinate metal ions, and promote self-assembly of peptide units. The tripeptide Z-Aib^1-2Dpy^2-Aib^3-OCH_3 (Z: benzyloxycarbonyl) adopts in the solid state a folded type III (III′) β-turn conformation (with Aib^1-2Dpy^2 as corner residues), stabilized by an i ⟵ i + 3 hydrogen bond between the Aib^3 NH and the urethane CO group. The peptide was able to self-assemble in the presence of Cu(II) ions, giving rise to an octahedral complex with a 2 : 1 peptide/Cu(II) stoichiometry, which has been structurally characterized. The metal ion, arranged on a crystallographic symmetry centre, is coordinated by two symmetry related 2Dpy residues, which act as tridentate ligands: each 2Dpy residue donates to the Cu(II) the N atoms of both pyridyl moieties in the equatorial positions, and the carbonyl oxygen atom in the axial positions. A very similar folded conformation characterizes the peptide in both the free and metal-bound states. This indicates that the overall peptide structure is ready to coordinate the metal ion without substantial conformational changes. Metal binding of the tri-peptide toward copper ions was analyzed by UV-visible spectroscopy performed in methanol solution. The experimental data agree with an equilibrium between two species in solution, one in the ratio Cu/peptide 1 : 1, the other 1 : 2. The formation constants are 9.7×10^3 M^{-1} and 3.5×10^2 M^{-1}, respectively. The molar extinction coefficients are 66 ± 3 M^{-1} cm^{-1} (590 nm) for the 2 : 1 complex and 17 ± 2 M^{-1} cm^{-1} (690 nm) for the other. All the data obtained in the present work suggest that the 2Dpy residue is well suited to be accommodated into folded conformations. The spontaneous formation of an ordered structure by the 2Dpy containing tri-peptide and copper ions may guide toward the design of more elaborate self-assembling systems.

Introduction

Metal ion coordination has been shown to be able to promote and control the assembly of suitable ligands into well-ordered architectures. A large variety of coordination compound arrays have been reported with several kinds of ligands.[1–4] Today, the construction of peptide-based supramolecules, capable of performing novel functions, represents a great challenge. In a few cases, metal ion-assisted self-organizing processes have been described, which lead to peptide assembly into topologically predetermined protein tertiary structures.[5–11] For example, either bidentate or monodentate ligands, appended to one end of the peptide helix, have been exploited to bring together the helices through coordination to the metallic site, with a metal ion assisted self-organizing molecular process. Similarly, three-helix (Scheme 1a) and four-helix bundles (Scheme 1b) have been reported to be formed in solution through coordination of N-donor ligands (appended to an α-helix C-terminal) to a metal center.[12–14] Another promising approach consists of the formation of the metal donor sites within the peptide chain, through insertion of unnatural amino acids. Suitable build-in of either a 2,2′-bipyridyl or a 1,10-phenanthrolyl side chain into a β-hairpin motif makes the resulting coordinated protein able to tune the metal binding affinity.[15] A pyridyl based amino acid has been incorporated into an α-helix and shown to



Scheme 1

† Electronic supplementary information (ESI) available: Figs. 1S, 2S. See http://www.rsc.org/suppdata/dt/b2/b209199b/

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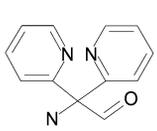
Table 1 Crystal data of Z-Aib[1]-2Dpy[2]-Aib[3]-OCH_3 and Cu(II)-(Z-Aib[1]-2Dpy[2]-Aib[3]-OCH_3)$_2$

	Z-Aib[1]-2Dpy[2]-Aib[3]-OCH_3	Cu(II)-(Z-Aib[1]-2Dpy[2]-Aib[3]-OCH_3)$_2$
Molecular formula	$C_{29}H_{33}N_5O_6$	$(CuC_{58}H_{66}N_{10}O_{12})(ClO_4)_2$
M_r	547.60	1357.65
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/n$
Z	4	2
a/Å	11.634(3)	13.673(3)
b/Å	18.948(3)	12.096(3)
c/Å	13.398(2)	18.691(2)
β/°	94.24(2)	96.55(2)
V/Å^3	2945.4(10)	3071.1(11)
ρ_{calcd}/g cm^{-3}	1.235	1.468
Radiation (λ/Å)	Mo-Kα (0.71073)	Mo-Kα (0.71073)
T/K	293(2)	293(2)
Absorption coefficient/mm^{-1}	0.088	0.572527
θ range/°	2–26	2–24
Reflections collected/unique	6025/5775	6553/4809
Unique reflections $I > 2\sigma(I)$	2680	1816
Data/restraints/parameters	5775/0/367	4809/20/215
Goodness-of-fit on F^2	0.993	1.014
$R1$ ($I > 2\sigma(I)$)	0.0555	0.0933
$WR2$ ($I > 2\sigma(I)$)	0.1447	0.2973

coordinate a Ru(II)(bipyridyl)$_2$ fragment in solution (Scheme 1c).[16] The azacrown-functionalized amino acids, which coordinate transition metals, have been incorporated into small peptides rich in C$^\alpha$-tetra-substituted amino acids. The resulting dinuclear zinc complexes have been shown to be good catalysts of the cleavage of RNA and DNA model substrates.[17] Furthermore, a template consisting of a tris(aminoethyl) (tren) platform and three 3_{10} helices of these peptides, containing azacrown amino acid, has been shown to coordinate four Zn^{2+} ions, one in the tren site and three in the aza sites.[18] Therefore, peptides containing such unnatural amino acids are candidates to provide functionality for metal-assisted supramolecular array formation, as well as a protein recognition site. In order to construct such complex devices we began from the synthesis and structural characterization of a rigid pyridyl functionalized small peptide containing the novel amino acidic residue, namely α,α-di(2-pyridyl)glycine, 2Dpy (Scheme 2). This amino acid bears two 2-pyridyl moieties on Cα, which may act as potential ligands to metal ions. Furthermore, C$^{\alpha,\alpha}$-disubstituted amino acids provide an excellent tool for the construction of conformationally rigid peptides.[19–23] With the aim of shedding light on the preferred conformational and coordination properties of 2Dpy, we report the synthesis and structural characterization of the fully protected tripeptide containing the 2Dpy residue in the central position: Z-Aib[1]-2Dpy[2]-Aib[3]-OCH_3 (Aib: C$^{\alpha,\alpha}$-dimethylglycine), in both the metal-free and copper-bound states. The binding properties of the peptide to copper in solution have been studied by UV-visible spectroscopy. Two species with 1 : 1 and 2 : 1 peptide/Cu ratios have been identified and their formation constants determined.



Scheme 2

Experimental

Synthesis of Z-Aib-2Dpy-Aib-OMe

A solution of Z-Aib 1.44 g (6.06 mmol), di(2-pyridyl)methanimine 1.10 g (6.00 mmol; prepared from di(2-pyridyl)ketone and NH_3 according to the Verardo method[24]) and methyl 2-isocyano-2-methylpropionate[25] 0.72 g (5.66 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 3 weeks. The solvent was removed under vacuum, and the residue was dissolved in $CHCl_3$ (50 mL). This solution was washed with 1 M HCl (20 mL × 4), H_2O (20 mL), 1 M $NaHCO_3$ (20 mL × 4) and NaCl saturated H_2O (20 mL × 2) successively, and dried (Na_2SO_4). The solvent was evaporated and the crude product was purified by re-crystallisation from EtOAc. Yield 12.8%. mp 165–166 °C.

MALDI-TOF MS for $C_{29}H_{33}N_5O_6$: 548.2468 (M + H^+, 548.2509), 570.2298 (M + Na^+, 570.2328), 586.2028 (M + K^+, 586.2068). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.50 (s, 6H, Aib[3]-CH_3), 1.55 (s, 6H, Aib[1]-CH_3), 3.55 (s, 3H, OCH_3), 5.14 (s, 2H, Z-CH_2), 5.47 (s, 1H, Aib[1]-NH), 7.16 (dd, 2H, J = 7.5 Hz, J = 5.0 Hz, Py-5H), 7.26–7.29 (m, 3H, ϕ-*m*H, *p*H), 7.35 (m, 2H, ϕ-*o*H), 7.63 (t, 2H, J = 7.5 Hz, Py-4H), 7.75 (d, 2H, J = 7.5 Hz, Py-3H), 8.43 (d, 2H, J = 5.0 Hz, Py-6H), 9.07 (br-s, 1H, Aib[3]-NH), 9.70 (s, 1H, 2Dpy-NH). ^{13}C NMR ($CDCl_3$): δ (ppm) = 24.7 (Aib[3]-βC), 25.2 (Aib[1]-βC), 52.0 (OCH_3), 56.5 (Aib[3]-αC), 57.2 (Aib[1]-αC), 66.7 (Z-CH_2), 68.8 (2Dpy-αC), 122.5 (2Dpy-C5), 123.9 (2Dpy-C3), 127.7 (ϕ-*o*C), 128.1 (ϕ-*p*C), 128.5 (ϕ-*m*C), 136.4 (ϕ-*ipso*C), 136.6 (2Dpy-C4), 147.6 (2Dpy-C6), 155.4 (Z-C=O), 158.1 (2Dpy-C1), 168.4 (2Dpy-C=O), 172.8 (Aib[1]-C=O), 174.8 (Aib[3]-C=O).

Preparation of the single crystals and structure determination of 1 and 2

The single crystals of the Z-Aib-2Dpy-Aib-OCH_3 peptide (**1**) were grown by slow evaporation of a methanol solution at 4 °C.

Single crystals of the copper(II) complex of Z-Aib-2Dpy-Aib-OCH_3 (**2**) were obtained at 4 °C by addition of 1.3×10^{-2} mmol of **1** to 1.5 mL of a 8.6 mM methanol solution of $Cu(ClO_4)_2$. Fairly good single crystals were obtained after one week.

Data collection were performed with Mo-Kα radiation (λ = 0.7107 Å) on an Enraf-Nonius CAD4 single-crystal diffractometer equipped with a graphite monochromator. Cell constants were obtained from least-squares refinement using the setting angles of 25 reflections in the range $14 < \theta < 18°$. The data were collected at room temperature using the $\omega/2\theta$ scan mode. Intensities were corrected for Lorentz-polarization effects and extinction. An anomalous dispersion correction was applied, and the values for f' and f'' and the atomic scattering factors were taken from International Tables.[26] Crystal data and some details of the data collection and refinements are summarised in Table 1.

The structures were solved by direct methods using SHELXS-86.[27] The structure parameters were refined against

$|F^2|$ by the full-matrix least-squares method using SHELXL-97.[28] The H atoms were located at the calculated positions and their coordinates were refined as riding, including free torsion of the methyl groups. The isotropic thermal parameters U for H atoms were set to 1.2 U_{eq} of the bonded atom. All the non-H atoms in **1** and only the Cu and Cl atoms in **2** were refined anisotropically in order to obtain a suitable observable/parameters ratio (Table 1). The ClO_4^- anion in **2** was found disordered over two orientations of the O atoms with occupancy factors of 0.6 and 0.4, respectively. The two sites of the anion were refined using geometry restraints and the partially occupied O sites were refined using the same anisotropic thermal factors. In the last stage of refinement the weighting scheme was $w = 1.0/[\sigma(F_o^2) - 0.1276|F_o|^2]$ for **1** and $w = 1.0/[\sigma(F_o^2) - 0.31|F_o| + 0.0580|F_o|^2]$ for **2**, where $\sigma(F_o^2)$ is the standard deviation of the intensities based on the counting statistics. The final difference density map showed all the positive peaks in the range 0.28–0.15 e Å^{-1} for **1** and 0.50–0.32 e Å^{-3} for **2**.

CCDC reference numbers 165344 and 165345 for **1** and **2**.

See http://www.rsc.org/suppdata/dt/b2/b209199b/ for crystallographic data in CIF or other electronic format.

Determination of equilibrium constants

UV-Vis spectra in methanol solution were recorded on a Perkin-Elmer Lambda 7 UV spectrophotometer using quartz cells of 2 cm path length. Wavelength scans were performed at room temperature, from 400 to 850 nm, with a 60 nm min^{-1} scan speed. $Cu(ClO_4)_2$ methanol solution, in appropriate molar ratio, was added to 5.74 mM peptide solution in methanol.

Experimental data indicated that the following equilibria were present:

$$P + M \Leftrightarrow PM;\ PM + P \Leftrightarrow P_2M$$

with equilibrium constants:

$$K_1 = [PM]/[P]_f[M]_f;\ K_2 = [P_2M]/[PM][P]_f$$

where $[P]_f$ and $[M]_f$ are the concentrations of the free-peptide and the free-copper ion whereas [PM] and $[P_2M]$ are the concentration of the species with Cu/peptide ratios of 1 : 1 and 1 : 2, respectively. The total peptide concentration $[P]_t$ is:

$$[P]_t = [P]_f + [PM] + 2[P_2M]$$

The total copper concentration $[M]_t$ at each titration point is:

$$[M]_t = [M]_f + [PM] + [P_2M]$$

The absorbance A at a given wavelength is related to the metal complex concentrations through the Lambert–Beer equation:

$$A = \varepsilon_1[PM]l + \varepsilon_2[P_2M]l$$

where ε_1 and ε_2 are the molar extinction coefficients (M^{-1} cm^{-1}) of the mononuclear and dinuclear complexes, respectively and l is the path length (cm). The observed absorbance intensities were fitted (Kaleidagraph[29]) to estimate the formation constants K_1 and K_2 through the following equations:

$$K_1K_2[P]_f^3 + (K_1 - K_1K_2[P]_t + 2K_1K_2[M]_t)[P]_f^2 + (1 + K_1[M]_t - K_1[P]_t)[P]_f - [P]_t = 0 \quad (1)$$

$$(AK_1K_2 - \varepsilon_2K_1K_2[M]_t)[P]_f^2 + (AK_1 - \varepsilon_1K_1[M]_t)[P]_f + A = 0 \quad (2)$$

Table 2 Torsion angles for Z-Aib^1-2Dpy^2-Aib^3-OMe in the free (**1**) and Cu(II)-bound state (**2**) (e.s.d.'s are given in parentheses)

		ϕ_i/°	ψ_i/°	ω_i/°
1	Aib^1	58.9(4)	34.8(4)	172.7(2)
	2Dpy^2	61.3(3)	29.5(3)	175.7(2)
	Aib^3	−48.1(3)	−41.0(3)	−177.8(3)
2	Aib^1	56.1(12)	33.6(12)	175.6(8)
	2Dpy^2	52.8(11)	31.6(11)	−169.3(8)
	Aib^3	−51.7(12)	126.3(10)	−176.4(10)

Φ_i, Ψ_i and ω_i are the C'_{i-1}–N_i–$C\alpha_i$–C_i', C'_{i-1}–N_i–$C\alpha_i$–C_i' and $C\alpha_i$–C_i'–N_{i+1}–$C\alpha_{i+1}$ torsion angles, respectively.

Results and discussion

Molecular structures

The torsion angles along the chain, given in Table 2, lead to the folded conformation shown in Fig. 1. The peptide secondary structure can be classified as a type III′ β turn with Aib^1 and 2Dpy^2 as corner residues, with the φ_i/ψ_i torsion angles for Aib^1 and 2Dpy^2 of 58.9(4)°/34.8(4)° and 61.3(3)°/29.5(3)°, respectively. The conformation is stabilized by an $i \longleftarrow i + 3$ intramolecular H-bond [N3–H (0.860 Å), H ··· O5 (2.259 Å), N3 ··· O5 3.119(3) Å] between Aib^3–NH and the urethane –CO of the Z group. This folding is quite similar to that observed in the analogous peptides, in which the 2Dpy residue is replaced either by a Dph[19] (Dph = $C^{\alpha,\alpha}$-diphenylglycine) or an Afc[20] (Afc = 9-aminofluorenecarboxylic acid) residue. An additional intramolecular H-bond of 2.575 Å between N2γ of 2Dpy^2 and N2 (N2γ ··· H–N2 of 112°) is present in **1** and dictates the orientation of one pyridyl substituent. In fact, the torsional angle N2–C2α–C2β–N2γ ($\chi^{1,2}$) is −1.5(3)°, whereas the torsion angle N2–C2α–C1β–N1γ ($\chi^{1,1}$) for the other pyridyl substituent is −76.6(3)°. The interplanar angle between the pyridyl groups is 79.7(1)°, similar to that of 74.0° between the two phenyl groups in the Dph analogue. The geometries of Z and OMe are normal.[30]

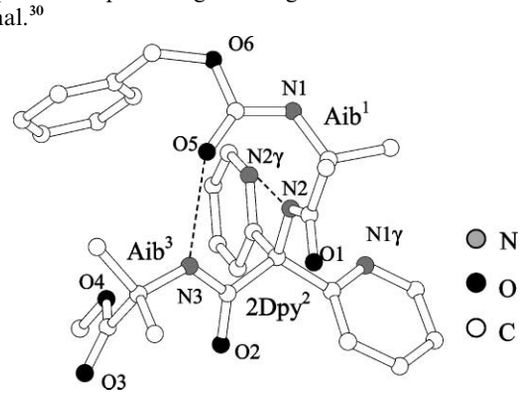


Fig. 1 Molecular structure of **1** with the atom-labelling scheme. Intramolecular H-bonds are indicated by dashed lines.

The bond lengths and angles of the 2Dpy moiety in **1** are shown in Fig. 1S (ESI †). The bond lengths involving Cα of the 2Dpy residue shows no significant variation with respect to the Dph analogue.[19] Comparison of bond angles at Cα indicates a narrowing of about 4° closing of the σ angle (C1β–C2α–C2β, in Fig. 1S) in the 2Dpy peptide with respect to the Dph analogue. This variation can be ascribed either to the electron-withdrawing ability of the pyridyls being greater than that of the phenyl groups or to steric requirements for the formation of the intra-molecular H-bond between N2γ and N2. The other angles do not differ by more than 2°.

It is interesting to note the enormous difference of about 13° between the C2α–C1β–C1γ and C2α–C1β–N1γ angles (Fig. 1S) as compared with the analogous difference of about 4° observed for the angles involving the C2β pyridyl atom.

The crystal packing of **1**, viewed along the *z* axis, is depicted in Fig. 2S (ESI †). The molecules are held together in rows along the *z* axis by intermolecular H-bonds N ··· H (0.860 Å), H ··· O (2.032 Å), N ··· O (2.892 Å) (Aib^1N–H ··· O= C′Dpy). These rows are packed together by van der Waals forces.

The crystals of **2** are built up by Cu(Z-Aib-Dpy-Aib-OMe)$_2^{2+}$ cations and perchlorate anions held together by electrostatic interactions and by H-bonds between N1 and O9 (2.98(3) Å) and between N2 and the O8 disordered atom (2.96(3) Å) of the perchlorate anion. The cation (Fig. 2) lies on a crystallographic symmetry center and Cu displays an octahedral coordination with Jahn–Teller distortion. The two symmetrically equivalent tridentate peptide ligands are in a *fac* arrangement coordinating Cu through the pyridyl N donors in the equatorial positions and the carbonyl O2 atom in the axial position. In spite of some conformational changes, due to the Cu coordination (see below), the secondary structure of the Aib^1-$2Dpy^2$-Aib^3 moiety observed in **1** is still present. The intramolecular H-bond between N3 and O5 (3.119(3) Å) is shortened by about 0.2 Å with respect to that observed in **1**. Comparison with the free ligand shows that no significant changes in the torsion angles along the backbone occur upon coordination (Table 2), likely because of the geometrical requirements needed to form the H-bond between O5 and N3. Superimposition of the free and coordinated ligands is shown in Fig. 3. The most significant conformational changes are detected in the two external Z and OMe groupings and, as expected, in the orientations of the pyridyl side groups which coordinate Cu with consequent cleavage of the intramolecular N2–N2γ H-bond, observed in the free peptide. It is apparent that coordination of **1** to Cu occurs through a rotation of about 180° in the $\chi^{1,4}$ angle and of about 100° in $\chi^{1,2}$ angle, whereas O2 remains essentially unchanged. The torsion angles around the C^{α}–py bonds $\chi^{1,2}$ and $\chi^{1,4}$ are very similar, being 179.8(8)° and 173.1(8)°, respectively and the interplanar angle between the two groups is 60.9(3)°. The bond lengths and angles in the coordinated and free 2Dpy show no significant variation within the experimental errors.

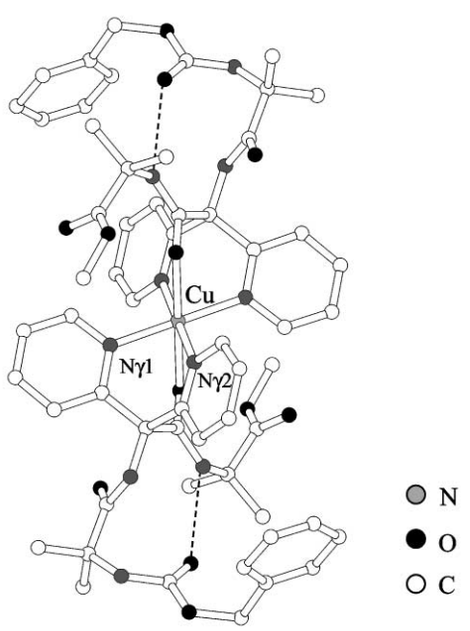


Fig. 2 Molecular structure of **2**. Intramolecular H-bonds are indicated by dashed lines.

Coordination bond lengths and angles are given in Table 3. The N donors of the four pyridyl residues coordinate Cu in the equatorial positions with two slightly different distances, Cu–N1γ and Cu–N2γ of 1.964(9) Å and 2.000(7) Å, respectively.

Table 3 Comparison of relevant bond distances and angles in Cu-(Z-Aib^1-$2Dpy^2$-Aib^3-OCH_3)$_2$ (**2**) and Cu(II)-bis[2-methyl-1,1-di(2-pyridyl)-2-propanol] (**3**)

2	Distances/Å	**3**	Distances/Å
Cu–N1γ	1.964(9)	Cu–N1	2.016(2)
Cu–N2γ	2.000(7)	Cu–N2	2.026(2)
Cu–O	2.335(7)	Cu–O	2.294(2)
2	Angle/°	**3**	Angle/°
N2γ–Cu1–N1γ	86.4(3)	N1–Cu–N2	86.7(8)
N1γ–Cu–O	79.6(3)	N2–Cu–O	84.8(6)
N2γ–Cu–O	91.2(3)	N1–Cu–O	85.6(6)

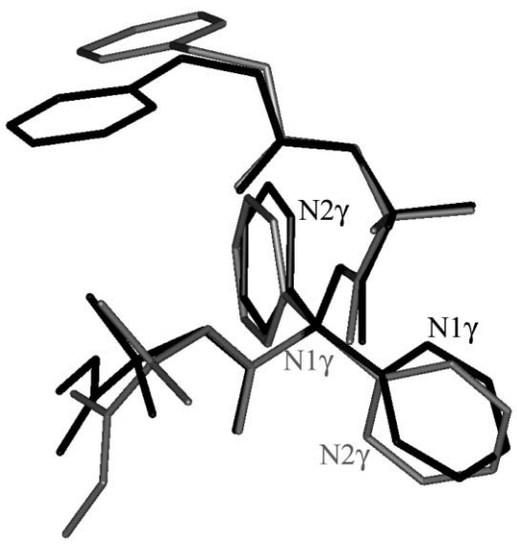


Fig. 3 Superimposition of the free (grey color) and Cu-coordinated (black color) peptide.

The O2 atoms of the two carbonyl groups occupy the axial positions at a long distance of 2.335(7) Å. The coordination to Cu is very similar to that found in bis[2-methyl-1,1-di-(2-pyridyl)-2-propanol] copper(II) cation (**3**),[31] where the tridentate ligand coordinates copper through two pyridyl groups and one alcoholic oxygen atom in a *fac* arrangement. The complex **3** is also found to be arranged on a crystallographic symmetry center. Selected bond lengths and angles in **3** are given in Table 3. The coordination geometries of the metal centers in **2** and **3** are almost identical.

The complex cations are packed in such a way that the –NH amide groups of Aib^1 and Aib^2 form a hydrogen bond with the perchlorate O atoms.

Metal binding

The binding properties of the tripeptide Z-Aib^1-$2Dpy^2$-Aib^3—OCH_3 towards Cu(II) ion were analysed through UV-visible spectroscopy. The spectral changes in the 400–850 nm region upon addition of $Cu(ClO_4)_2$ are due to the formation of the metal complexes (Fig. 4). The binding affinity of Cu(II) to the tripeptide Z-Aib^1-$2Dpy^2$-Aib^3-OCH_3 was measured by spectrophotometric titration (Fig. 5). The best fitting of equations (1) and (2) was obtained at 650 nm where $\varepsilon_1 \sim 1/2\ \varepsilon_2$ and gave $K_1 = (9.7 \pm 0.5) \times 10^3\ M^{-1}$ and $K_2 = (3.5 \pm 0.3) \times 10^2\ M^{-1}$.[31] The species distribution formed upon Cu(II) addition is shown in the inset of Fig. 5. The absorption maximum of the P_2M complex at 590 nm shows a molar extinction coefficient of 66 ± 3 M^{-1} cm^{-1}, while the PM complex at 690 nm shows a molar extinction coefficient of 17 ± 2 M^{-1} cm^{-1}. Since the data of Fig. 4 and 5 could also be consistent with the formation of 1 : 3 and 1 : 2 metal/peptide complexes, we have also computed the

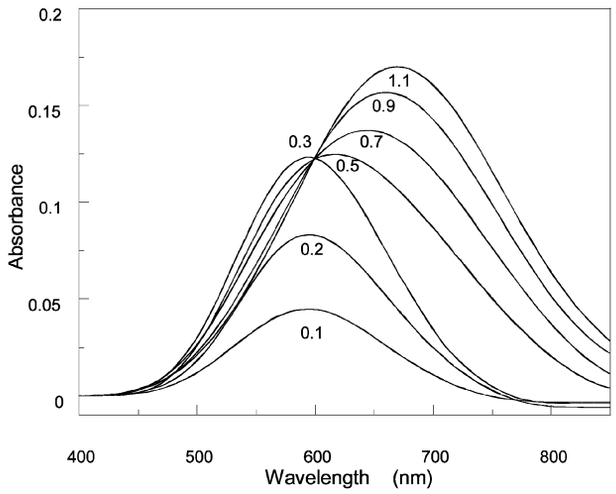


Fig. 4 UV-visible analysis of the interaction between Cu(II) and Z-Aib[1]-2Dpy[2]-Aib[3]-OCH_3 peptide at a fixed concentration (5.74 mM in methanol). Influence of increasing Cu(II)/peptide molar ratio (R) on the UV-visible spectrum.

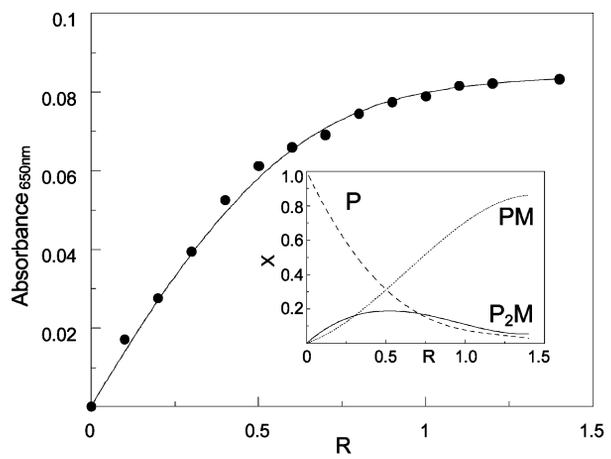


Fig. 5 Spectrophotometric titration of Z-Aib[1]-2Dpy[2]-Aib[3]-OCH_3 peptide with Cu(II). The absorbance at 650 nm is plotted against the concentration ratio, $[Cu]_t/[P]_t$. Solid line shows the best fit of data points. Inset: species distribution diagram as a function of the concentration ratio, $[Cu]_t/[P]_t$.

stoichiometry by the use of the program Hyperquad 2000.[32] Several possible solutions were attempted, including the latter. Still, the most reasonable stoichiometry is the one proposed here.

Conclusion

The tripeptide adopts a folded type III (III′) β-turn conformation, (with Aib[1]-2Dpy[2] as corner residues) stabilized by an $i \longleftarrow i + 3$ hydrogen bond between the Aib[3] NH and the urethane CO group. The peptide is able to self-assemble in the presence of Cu(II) ions, giving rise to an octahedral complex with a 2 : 1 peptide/Cu(II) stoichiometry. The metal ion is coordinated by two symmetry related 2Dpy residues, which act as tridentate ligands: each 2Dpy residue donates to the Cu(II) the N atoms of both pyridyl moieties in the equatorial positions, and the carbonyl oxygen atom in the axial positions. A very similar folded conformation characterizes the peptide in both the free and metal-bound states, thus indicating that the metal ion coordination does not affect the overall peptide structure. UV-visible spectrometric measurements have allowed us to establish that the species with 1 : 1 and 1 : 2 Cu(II)/peptide ratios are present in solution. The formation constant of the 1 : 1 species (K_1) is about 30 times larger than that of the other species (K_2). This study has shown that the 2Dpy unit acts as a tridentate ligand in the presence of metals with octahedral environments. The absence of chiral amino acids in the tripeptide studied leads to the formation of a centrosymmetric complex (Fig. 2) where the two β-turns have specular helicity. Insertion of 2Dpy into the peptide sequence should allow the construction of specific, structurally ordered synthetic receptors. However, molecular modeling of an octahedral complex with two polypeptide chains in α-helix conformation, that resembles the structurally characterized Cu(II)-(Z-Aib[1]-2Dpy[2]-Aib[3]-OCH_3)$_2$ complex, suggests that the potential helix aggregation through metal coordination should bend the secondary structure, as shown in Fig. 6. This deformation arises from the steric hindrance between one of the two pyridyl side groups of 2Dpy (in a generic position i) and the oxygen of the carbonyl group of the amino acid in position $i - 4$. In fact, the coordination geometry and α-helix conformation of the backbone force one pyridyl group to be coplanar to the $i/i - 1$ peptide plane, as shown in the crystal structure of the copper–peptide complex.

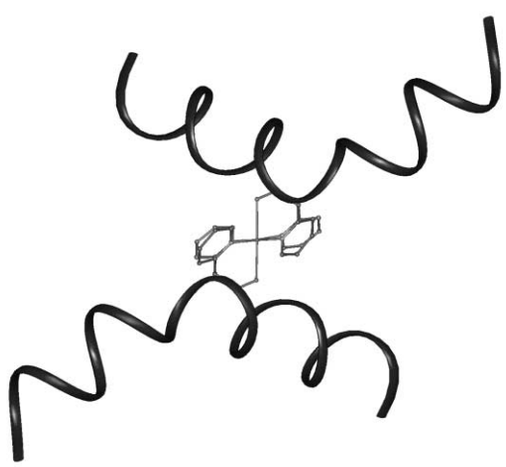


Fig. 6 Ribbon drawing of the molecular model of the helix assembly obtained using the coordination environment found in **2**.

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