Interaction of the lysine side chain amino group with Cu^{II} in (glycyl-L-lysine^{2-})Cu

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(Received September 17, 1993)

Abstract

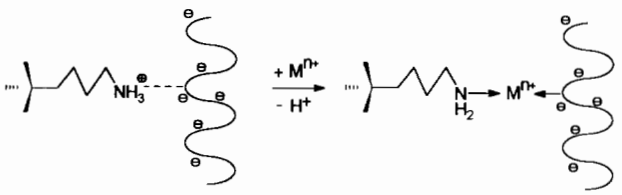
Two different modifications of the (gly-L-lys^{2-})Cu(II) complex (**1** and **2**) were determined by X-ray crystallography. The dipeptide forms a tridentate chelate via COO^- (lysine), NH_2 (glycine) and the deprotonated amide group. The amino group of the lysine side chain links copper units, thereby generating a one-dimensional polymeric structure. The two modifications differ in a coordinated water molecule at the Cu^{II} with **1** analyzing as $(C_8H_{15}N_3O_3)Cu(H_2O)$ and **2** as $(C_8H_{15}N_3O_3)Cu \cdot 2H_2O$.

Key words: Crystal structures; Copper complexes; Amino acid complexes

Introduction

As far as metal binding properties of amino acid side chains are concerned, that of the basic amino acid lysine is generally not considered a potent donor. This is primarily due to the fact that the NH_2 side chain ('lateral' amino group) is protonated at physiological pH ($pK_a \cong 10.5$). At the same time, this feature makes lysine a major component of the DNA binding histone proteins [1]. In principle, the H bonding interaction between lysine side chains and DNA and the charge neutralization between the cationic side chain and the DNA polyanion might also be accomplished by the action of a positively charged metal ion (Scheme 1). In order to probe such a possibility, we decided to start with a study on the metal binding ability of a simple dipeptide, glycyl-L-lysine (gly-L-lys; GK) toward Cu^{II}. The stability constant of this complex has already been reported [2]. Although we have been unable as yet to characterize any ternary (metal, peptide, nucleobase) complex in this system, we herewith report on our findings of two modifications of a gly-L-lys complex of Cu^{II}, both of which show clear evidence for intermolecular interactions between the lateral lysine amino group and the metal ion. Our findings further support conclusions of Kozlowski and co-workers [3] concerning the metal binding properties of the side chain amino group of lysine, which were based on X-ray structural data on a Cu^{II} complex of L-lysyl-L-tyrosine.

Studies of metal ion complexes with lysine entities are relatively scarce [2–7], especially with regard to structural data [3, 4]. An interesting variant of a chemical DNA nuclease, employing a $Cu(lys)_2$ motif, has recently been introduced [7].



Scheme 1.

Experimental

Syntheses

$CuCl_2 \cdot H_2O$ and glycyl-L-lysine (Bachem, Heidelberg) were mixed in a 1:1 ratio, dissolved in water ($c \cong 0.060$ mol/l), the pH adjusted to 8.7, and methanol (five-fold volume) was added. Upon standing at room temperature in sealed vials, deep blue crystals formed in several days. Two different modifications **1** and **2** were obtained in different experiments which were prepared in the same way. The crystal habits of **1** and **2** are rather similar and cannot readily be distinguished but crystals of **1** deteriorate more rapidly when kept in air. The

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SSDI 0020-1693(93)03740-2

complexes were characterized by IR spectroscopy, C, H, N analysis and X-ray analysis.

Spectra

^{1}H NMR spectra were reported on a Bruker AC200. The pK_a values were obtained from graphs of chemical shifts versus uncorrected pH (pH*). pD values were obtained by adding 0.4 to the pH meter reading. IR spectra were taken on a Perkin-Elmer model 580 B spectrophotometer as KBr pellets.

X-ray structure determinations

Crystal data and data collection parameters are summarized in Table 1. Data were collected for **1** and **2** on a Nicolet R3m/V diffractometer at room temperature using graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). **1** crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 8.759(3)$, $b = 9.365(3)$, $c = 13.398(4)$ Å. Cell dimensions were refined by least-squares fitting of the θ values of 23 reflections. In the second modification (**2**) the cell dimensions were determined from 18 centered reflections. This structure was solved in the monoclinic space group $P2_1$ ($a = 8.674(6)$, $b = 8.164(7)$, $c = 10.449(7)$ Å, $\beta = 92.37(5)°$). The intensities were corrected for absorption effects for both **1** and **2**. Friedel pairs were collected for both complexes and employed in the subsequent refinements. The structures were solved by Patterson methods and difference Fourier syntheses, using the SHELXTL-PLUS program [8]. Full-matrix least-squares refinement (on F) was performed with anisotropic thermal parameters for all non-H atoms with the exception of C3 in structure **1**. Hydrogen atoms were fixed at calculated positions for **1**. The quality of structure **2** was not good enough to justify addition of H atoms. The two water molecules of this structure are disordered over four positions (O1: 60/40%; O2: 50/50%). The scattering factors for the atoms are those given in the SHELXTL-PLUS program. Final positional and equivalent isotropic thermal parameters for non-H atoms of **1** and **2** are listed in Table 2.

Results and discussion

The composition of glycyl-L-lysine (gly-L-lys; GK) at pH 4–7 is given in Scheme 2. Its ^{1}H NMR spectrum in D_2O consists of a singlet due to the α-CH_2 protons of the glycyl residue (3.82 ppm, pD 7.3) and four multiplets due to the lysine protons (α', 4.17; β' and δ', 1.61–1.85; γ', 1.43; ϵ', 2.99 ppm; pD 7.3 each). The assignment of the resonances is made on the basis of 2D-NMR spectroscopy and is in agreement with results of Wüthrich who examined the free acids [9]. ^{1}H NMR

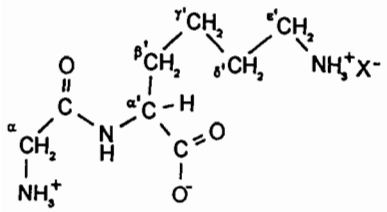
TABLE 1. Crystal data and data collection parameters

	1	**2**
Empirical formula	$CuC_8H_{17}N_3O_4$	$CuC_8H_{19}N_3O_5$
Formula weight (g/mol)	282.8	300.8
Crystal size (mm)	0.224×0.16×0 048	1.376×0.32×0.064
Crystal system	orthorhombic	monoclinic
Space group	$P2_12_12_1$	$P2_1$
a (Å)	8.759(3)	8.674(6)
b (Å)	9.365(3)	8.164(7)
c (Å)	13.398(4)	10.449(7)
β (°)		92.37(5)
Volume (Å^3)	1099.0(6)	739.4(9)
D_{calc} (g cm^{-3})	1.697	2.703
μ (mm^{-1})	5.751	2.942
F(000)	780	628
Diffractometer	Nicolet R3m/V	Nicolet R3m/V
Radiation (Å)	Mo Kα, 0.71073	Mo Kα, 0.71073
2θ Range (°)	2.0–50.0	2.0–50.0
Scan type	2θ/ω	2θ/ω
Index ranges	(−10, 0, 0) to (10, 11, 15)	(0, −9, −12) to (10, 9, 12)
Reflections collected	2180	2507
Independent reflections	1953 ($R_{int} = 2.53\%$)	2337 ($R_{int} = 5.79\%$)
Observed reflections	815 ($F \geqslant 4.0\sigma(F)$)	2199 ($F \geqslant 4.0\sigma(F)$)
Absorption correction	semi empirical	semi empirical
No. parameters	141	172
Weighting scheme	$w^{-1} = \sigma^2(F) + 0.0080F^2$	$w^{-1} = \sigma^2(F) + 0.0050F^2$
R (%)	4.09	6.45
R_w (%)	5.14	8.72
Largest and mean Δ/σ	0.000, 0.000	0.000, 0.001

TABLE 2. Atomic coordinates and equivalent isotropic displacement coefficients ($Å^2 \times 10^3$)

Atom	x	y	z	U_{eq} [a]
Compound **1**				
Cu(1)	0.7969(2)	−0.2414(2)	0.3842(1)	25(1)
O(1)	1.0085(10)	−0.1634(9)	0.4029(5)	26(3)
O(2)	1.1763(8)	−0 113(10)	0.3393(6)	30(3)
O(3)	0.6470(10)	0.0094(10)	0.1613(6)	37(4)
O(1w)	0.6842(11)	−0.1310(8)	0.5211(6)	35(3)
N(1)	0.6194(13)	−0.3153(11)	0.3028(9)	39(4)
N(4)	0.8353(13)	0.5791(9)	0.4663(7)	28(4)
N(2)	0.7858(14)	−0.0835(9)	0.2923(7)	24(3)
C(2)	0.6671(14)	−0.0801(13)	0.2301(10)	23(5)
C(3)	0.9154(12)	0.0071(13)	0.2835(9)	20(3)
C(1)	0.5535(16)	−0.1944(13)	0.2464(10)	31(5)
C(4)	1.0452(13)	−0.0593(13)	0.3457(9)	20(4)
C(5)	0.8862(15)	0.1626(13)	0.3195(9)	28(5)
C(6)	0.8332(19)	0.1698(14)	0.4295(9)	32(5)
C(7)	0.8634(18)	0.3158(13)	0.4736(10)	34(5)
C(8)	0.7861(17)	0.4436(12)	0.4251(8)	27(4)
Compound **2**				
Cu(1)	0.97602(9)	1.00000(0)	0.83331(7)	37(3)
N(2)	1.0511(8)	0.7837(9)	0.8322(6)	41(2)
O(1)	1.1912(7)	1.0562(8)	0.7936(6)	46(2)
N(1)	0.7744(9)	0.889(1)	0.8771(8)	48(2)
C(2)	0.972(1)	0.668(1)	0.8882(7)	40(3)
C(1)	0.806(1)	0.714(1)	0.9133(8)	52(3)
O(3)	1.0206(7)	0.5297(7)	0.9238(6)	49(2)
O(2)	1.4240(8)	0.9450(9)	0.7694(7)	62(3)
C(3)	1.213(1)	0.760(1)	0.7981(8)	41(3)
C(4)	1.277(1)	0.935(1)	0.7861(7)	40(3)
N(4)	1.1053(8)	0.7243(9)	0.1923(6)	38(2)
C(7)	1.157(1)	0.682(1)	0.4278(8)	48(3)
C(5)	1.224(1)	0.668(1)	0.6686(8)	46(3)
C(8)	1.082(1)	0.791(1)	0.3232(8)	52(3)
C(6)	1.143(1)	0.766(1)	0.5574(9)	55(3)
O(1wa)	1.5585(9)	0.217(1)	0.858(1)	54(4)
O(1wb)	1.488(1)	0.368(2)	0.851(2)	59(6)
O(2wa)	0.636(2)	0.712(3)	0.511(1)	108(10)
O(2wb)	0.365(2)	0.266(4)	0.381(2)	147(13)

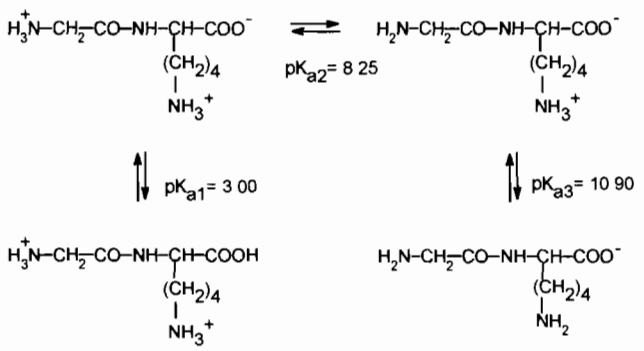
[a]Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.



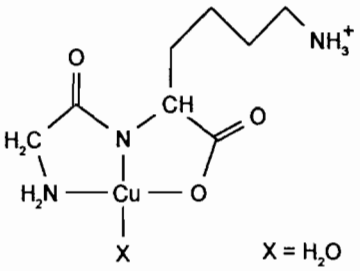
Scheme 2.

spectra, which were recorded in the pH* range 1–13, showed a dependence of chemical shifts of methine and methylene protons consistent with the acid/base equilibria shown in Scheme 3.

The three pK_a values were taken from chemical shift dependence of the α' proton of lysine (pK_{a1}), the α



Scheme 3.



Scheme 4.

protons of glycyl (pK_{a2}), and the ϵ' protons of lysine (pK_{a3}). Values for pK_{a1} and pK_{a2} agree well with data established by potentiometry [2].

Addition of trace amounts of Cu^{II} to a weakly acidic (pD 5.5) solution of gly-L-lys in D_2O causes paramagnetic broadening of the α' proton of lysine and of the α protons of the glycyl residue, while the protons of the lysine side chain are affected at higher Cu^{II} concentration only. This behavior clearly rules against metal binding at the lysine side chain under these conditions and rather points to Cu^{II} binding via the carboxylate group of lysine and/or the NH_2 group of glycyl. Even if the pD is raised to 7.3, we have no evidence for lysine side chain binding of Cu^{II}. On the other hand, deprotonation of the peptide amide group and Cu^{II} coordination is expected to take the place at this pH [10–12]. From comparison with the behavior of Cu^{II} with other dipeptides containing no coordinating side chains [10–12], we assume that at neutral pH the Cu^{II} chelate shown in Scheme 4 is formed to a substantial degree.

The two types of crystals **1** and **2** isolated from weakly basic solutions (pH 8.7) containing $CuCl_2$ and gly-L-lys revealed polymeric composition with the deprotonated amino group of the lysine side chain acting as an intermolecular donor group for Cu^{II} (see below). IR spectroscopy proved unsuitable to differentiate between the two species **1** and **2**, although it suggested binding via the lysine carboxylate (shift of $\nu_{as}COO^-$ from 1670 to 1610 cm^{-1}) and the amide group (loss of δNH at 1510 cm^{-1} upon Cu^{II} binding).

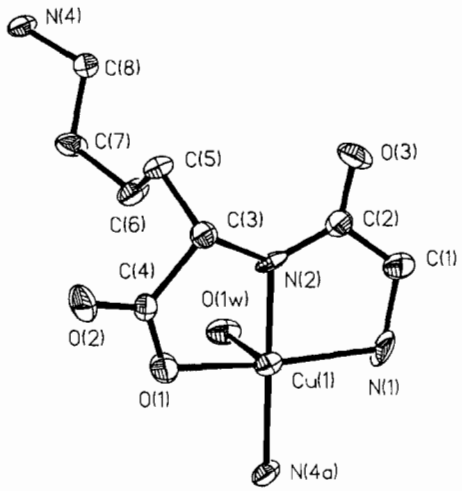


Fig. 1. Molecular unit of (gly-L-lys^{2-})Cu(H_2O) (**1**) with atom numbering scheme (50% thermal ellipsoids).

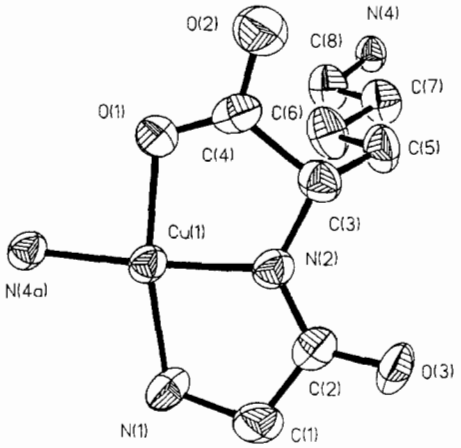


Fig. 2. Molecular unit of (gly-L-lys^{2-})Cu (**2**) with atom numbering scheme (50% thermal ellipsoids).

In Figs. 1 and 2, monomeric entities of (gly-L-lys^{2-})Cu(H_2O) (**1**) and (gly-L-lys^{2-})Cu (**2**) are shown. Selected interatomic distances and angles are listed in Tables 3 and 4. In both compounds three coordination sites are occupied by NH_2 of the glycyl entity, N of the deprotonated amide group, and O of the carboxyl group of the lysine in a chelating fashion, with NH_2 of the side chain occupying a fourth coordination site in a bridging fashion. In **1**, a water molecule (O1w) serves as a fifth ligand, thereby generating a distorted trigonal-bipyramidal coordination sphere about the Cu, with NH_2 (glycine), O (lysine), and the water molecule roughly in the plane, and the amide nitrogen and the bridging lateral lysine amino group approximately in the apical positions. On the basis of considerations of angles about Cu, this description seems to be more appropriate than that of a square-pyramid with the water molecule O1w at the top. In contrast, angles about Cu^{II} in **2** suggest a distorted square-plane about the Cu with N1, N2, O1 and N4a being the donor atoms. If a long contact to an adjacent dipeptide carbonyl oxygen is included, Cu^{II} in **2** becomes five-coordinated (O3$\cdots$Cu, 2.549(6) Å) with the coordination geometry of Cu^{II} then best described as a square-pyramid.

Bond distances about the Cu^{II} in both compounds are in agreement with literature data [13].

TABLE 3. Selected bond distances (Å) and bond angles (°) for **1**

Cu(1)–O(1)	2.009(10)	Cu(1)–O(1w)	2.308(11)
Cu(1)–N(1)	2.020(14)	Cu(1)–N(2)	1.927(11)
Cu(1)–N(4a)	2.040(11)	O(1)–C(4)	1.279(17)
O(2)–C(4)	1.236(16)	O(3)–C(2)	1.260(18)
N(1)–C(1)	1.479(20)	N(4)–C(8)	1.443(18)
N(2)–C(2)	1 332(20)	N(2)–C(3)	1.422(19)
C(2)–C(1)	1.471(21)	C(3)–C(4)	1.539(20)
C(3)–C(5)	1.553(21)	C(5)–C(6)	1.546(22)
C(6)–C(7)	1.510(23)	C(7)–C(8)	1.517(22)
O(1)–Cu(1)–O(1w)	98.0(4)	O(1)–Cu(1)–N(1)	154.4(5)
O(1w)–Cu(1)–N(1)	104.3(5)	O(1)–Cu(1)–N(2)	81.2(5)
O(1w)–Cu(1)–N(2)	99 0(4)	N(1)–Cu(1)–N(2)	83 1(6)
O(1)–Cu(1)–N(4a)	94.7(5)	O(1w)-Cu(1)-N(4a)	89 8(4)
N(1)–Cu(1)–N(4a)	97.7(5)	N(2)–Cu(1)–N(4a)	170.7(5)
Cu(1)–O(1)–C(4)	115.8(9)	Cu(1)–N(1)–C(1)	108.3(9)
C(8)–N(4)–Cu(1a)	118.1(9)	Cu(1)–N(2)–C(2)	117.0(10)
Cu(1)–N(2)–C(3)	117.9(10)	C(2)–N(2)–C(3)	124.2(12)
O(3)–C(2)–N(2)	125.4(13)	O(3)–C(2)–C(1)	119.7(13)
N(2)–C(2)–C(1)	114.9(13)	N(2)–C(3)–C(4)	107.8(12)
N(2)–C(3)–C(5)	113.7(12)	C(4)–C(3)–C(5)	109.8(12)
N(1)–C(1)–C(2)	111.6(13)	O(1)–C(4)–O(2)	123.7(13)
O(1)–C(4)–C(3)	116.4(12)	O(2)–C(4)–C(3)	120.0(13)
C(3)–C(5)–C(6)	112.5(12)	C(5)–C(6)–C(7)	111.3(14)
C(6)–C(7)–C(8)	118.2(14)	N(4)–C(8)–C(7)	113.5(13)

TABLE 4. Selected bond distances (Å) and bond angles (°) for **2**

Cu(1)–N(2)	1.882(8)	Cu(1)–O(1)	1.983(6)
Cu(1)–N(4a)	1.977(7)	Cu(1)–N(1)	2.039(8)
N(2)–C(2)	1.32(1)	O(1)–C(4)	1.24(1)
N(2)–C(3)	1.48(1)	C(2)–C(1)	1.52(1)
N(1)–C(1)	1.50(1)	O(2)–C(4)	1.30(1)
C(2)–O(3)	1.26(1)	C(3)–C(5)	1.55(1)
C(3)–C(4)	1.54(1)	C(7)–C(8)	1.53(1)
N(4)–C(8)	1.49(1)	C(5)–C(6)	1.55(1)
C(7)–C(6)	1.53(1)		
O(1)–Cu(1)–N(1)	166 8(3)	N(2)–Cu(1)–N(1)	83.4(3)
N(2)–Cu(1)–O(1)	83.5(3)	N(2)–Cu(1)–N(4a)	171.6(4)
N(1)–Cu(1)–N(4a)	98.1(4)	O(1)–Cu(1)–N(4a)	98.1(4)
Cu(1)–N(2)–C(3)	117.1(6)	C(2)–N(2)–C(3)	122.2(8)
Cu(1)–N(2)–C(2)	118.8(6)	Cu(1)–N(1)–C(1)	109.4(6)
Cu(1)–O(1)–C(4)	113.7(6)	N(2)–C(2)–C(1)	114.7(8)
N(2)–C(2)–O(3)	127.0(8)	N(1)–C(1)–C(2)	110.5(8)
C(1)–C(2)–O(3)	118.3(8)	N(2)–C(3)–C(4)	104.3(7)
N(2)–C(3)–C(5)	111.2(7)	O(2)–C(4)–C(3)	115.1(8)
C(4)–C(3)–C(5)	110.2(7)	O(1)–C(4)–O(2)	123.9(9)
O(1)–C(4)–C(3)	121.0(8)	C(7)–C(6)–C(5)	112.4(8)
N(4)–C(8)–C(7)	112.0(8)		

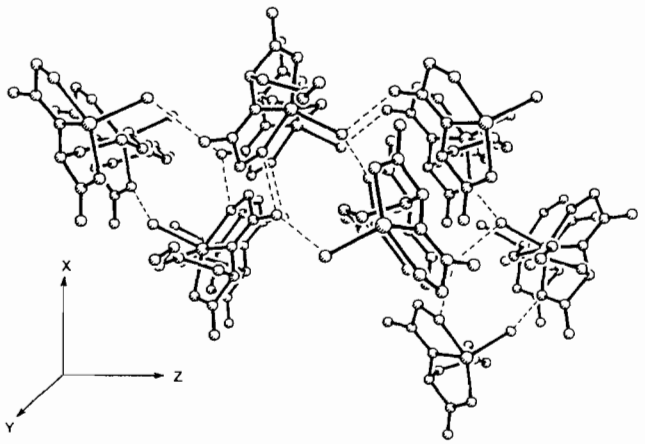


Fig. 3. Packing of strands of **1** with H bonding interactions indicated. The view is along the *y* axis.

In the crystal lattice, **1** forms infinite, antiparallel strands which are connected via H bonds (Figs. 3 and 4). Of these H bonds (Table 5), two are quite short, 2.64(1) Å and 2.66(1) Å: they are between the water molecule O1w and a carboxyl oxygen (O1) and a peptide carbonyl oxygen (O3) of two different neighboring strands. Within each strand, Cu centers are 9.4 Å apart.

The absence of the water molecule in **2** changes the packing pattern quite dramatically (Figs. 5 and 6). In contrast to the slim strands formed by **1**, the infinite strands in **2** are compressed, thereby becoming wider with intermolecular Cu···Cu distances along the axis becoming shorter (8.2 Å). Of the short contacts formed between adjacent strands (Table 5), that between Cu

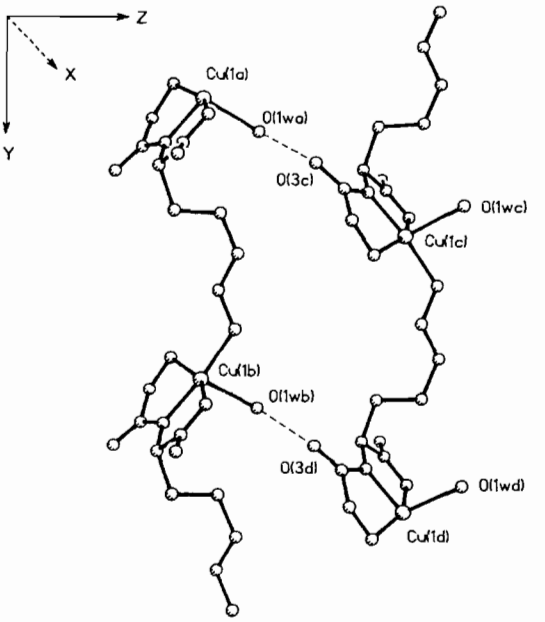


Fig. 4. Section of the packing of **1** with two antiparallel strands and the short H bonds 2.66(1) Å between O1w and O3 of an adjacent strand.

TABLE 5. Short contacts (Å) in **1** and **2**

Compound **1**	
O(1)–O(1w)[a] = 2.64(1)	O(3)–O(1w)[b] = 2.66(1)
O(3)–N(1)[c] = 2.89(2)	
[a] = $x-0.5, -y+0.5+2, -z+1$; [b] = $-x+0.5, -y+2, z+0.5$; [c] = $-x+1, y-0.5, -z+0.5+1$.	
Compound **2**	
O(1)–N(4)[a] = 2.924(9)	O(3)–N(1)[b] = 2.916(10)
O(3)–Cu(1)[b] = 2.549(6)	O(3)–N(4)[c] = 2.962(9)
O(1wa)–O(2)[d] = 2.661(13)	O(2wb)–O(2)[c] = 2.859(24)
[a] = $-x+2, y+0.5, -z+1$. [b] = $-x, y+0.5, -z$. [c] = $-x+2, y-0.5, -z+1$. [d] = $x, y-1, z$.	

and the peptide carbonyl group O3 is the most significant one.

Supplementary material

Tables of anisotropic displacement coefficients of **1** and **2**, H atom coordinates of **1**, structure factors of **1** and **2** can be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany, under CSD 400327 and CSD 400328 on request. Requests should be accompanied by the complete literature citation.

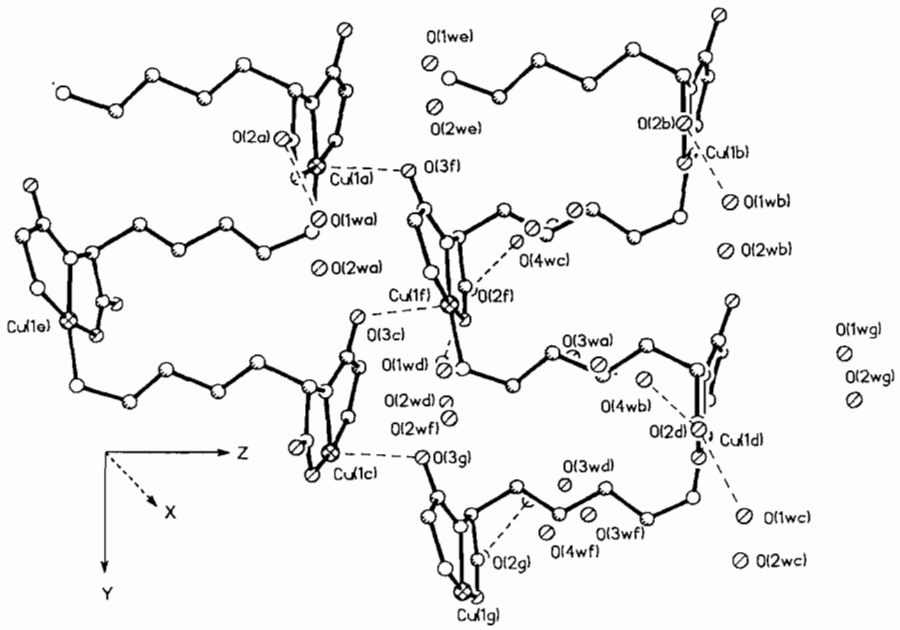


Fig. 5. Section of the packing of **2**. Adjacent lysine side chains run antiparallel *within* a single polymeric chain.

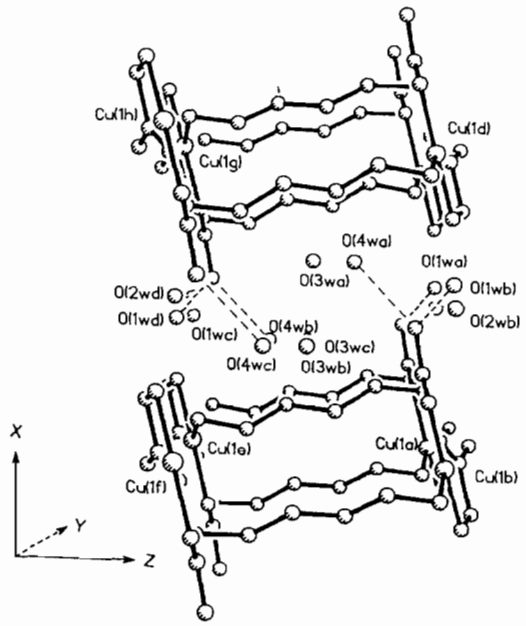


Fig. 6. Packing of strands of **2**. The view is along the *y* axis.

Acknowledgements

This work has been supported by the Deutsche Forschungsgemeinschaft (DFG) and the Fonds der Chemischen Industrie (FCI).

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