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Copper Complexes of Di−, Tri− and Tetra-Peptides Containing Tryptophan, Histidine and Arginine

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ABSTRACT. Fifty seven copper complexes of di-, tri- and tetra-peptides containing tryptophan, histidine and arginine are studied spectrophotometrically. The λ_{max} and colour of the complex is dependent on the sequence of the amino acid in the dipeptide methyl esters of tryptophan and arginine; and independent on the sequence of dipeptides of histidine or in any of the tri- and tetra-peptides of histidine, arginine and tryptophan. The results achieved confirmed that the nitrogen atoms of the indole nucleus in tryptophan, imidazole ring in histidine and guanidino group in arginine do not participae in complex formation of all studied di-, tri- and tetra-peptides. However, the amide group and the hydrazide group of dipeptide amide and dipeptide hydrazides participate in complex formation.

Poddubnaya and El-Naggar (1966, 1967) have investigated the copper complexes of a series of di-, tri- and hexapeptides containing lysine, ornithine and serine. El-Naggar *et al.* (1973) studied a series of penta-, hexa- and heptapeptides and some hydrazides containing threonine, serine, lysine and diaminobutyric acid (Salem *et al.* 1974 and El-Naggar *et al.* 1976).

The present investigation involves studies of the copper complexes of some di-, tri- and tetrapeptides (I-LVII) containing tryptophan, histidine and arginine with different amino acid sequences.

Experimental Procedures

The synthesis of the di-, tri- and tetrapeptides (I-LVII) was conducted by the procedures described in references (El-Naggar *et al.* 1982, 1983).

Preparation of copper complexes

A 0.01 M Solution of the peptide in 1 N KOH in absolute methanol (4 ml) was introduced into 5 ml measuring flask. The mixture was brought to the mark by addition of a 0.04 M solution of copper acetate in water. The complex solution was centrifuged, filtered off and the clear solution examined using a spectrophotometer. The absorption curves of the peptides are given in Fig. 1, λ_{max} and the absorbances of the peptides are listed in Table 1.

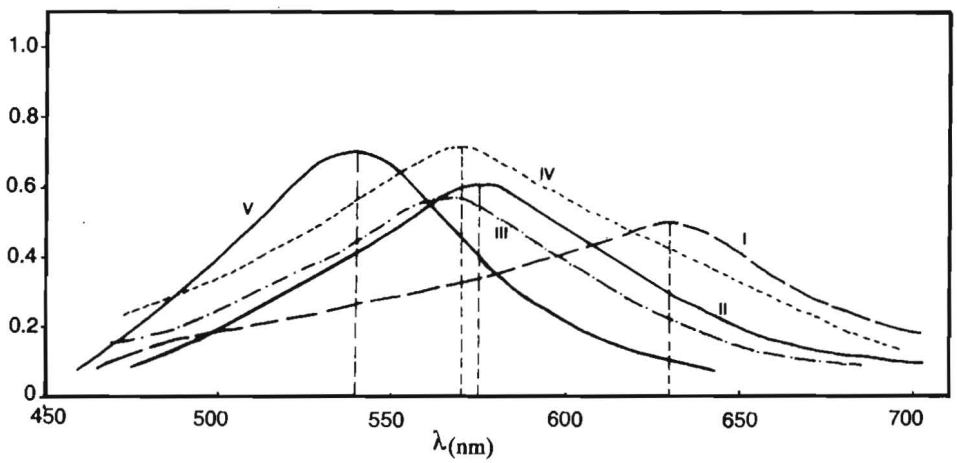


Fig. 1. Copper complexes of some di-, tri- and tetra peptides of:

1) Tos-Val-His-OMe
2) Tos-Val-His-NH_2
3) Tos-Val-His-N_2H_3
4) Tos-His-Gly-Gly-OMe
5) Tos-$(Gly)_3$-His-OMe

Determination of the Cu: peptide ratio

Isomolar solutions 25 ml of the peptide and the copper salt were prepared from 0.003 M solution of the peptide in absolute methanol and a 0.003 M solution of $CuCl_2$. $2H_2O$ in absolute methanol, which were mixed to give the following proportions of the components: 0.5:4.5; 1:4; 1.5:3.5; 2:3; 2.5:2.5; 3:2; 3.5:1.5; 4:1; 4.5:0.5. To each mixture 1 ml of aqueous KOH was added, the mixture was stirred and filtered and the absorbance measured at λ_{max}. From the results, diagrams were constructed showing the relation between absorbance and the Cu: peptide ratio (*cf.* Fig. 2 and Table 1). All spectrophotometric measurements were performed using a Unicam SP 8000 spectrophotometer.

Results and Discussion

Previous studies (Poddubnaya and El-Naggar 1966, 1967 and El-Naggar *et al.* 1973, 1976 and Zewail *et al.* 1974) revealed that the λ_{max} of the copper complexes of the di-, tri-, penta- and hexapeptide methyl esters depends on the order and structure of the amino acids in the peptide.

Our experimental data showed that proteced N-tosyldipeptide methyl esters containing tryptophan residues (I-VI), histidine residues (XIV-XX) and arginine residues (XXIII-XXVI) have maximum absorption characteristic of normal N-tosyldipeptide methyl esters of alanine, glycine, serine (Poddubnaya and El-Naggar 1966, 1967) and valine (VII) at λ_{max} 610-640 nm (*cf.* Fig. 1 and Table 1). Moreover, it was found that the position of λ_{max} depends on the order of the amino acids in the dipeptide methyl esters. When the C-terminal amino acid in a dipeptide methyl esters is tryptophan or arginine and the N-terminal is glycine, alanine, valine, leucine, tryptophan or arginine, they form normal blue copper (II) complexes with λ_{max} 610-640 nm. When the N-terminal amino acid is tryptophan or arginine and the C-terminal is glycine, valine, serine or phenylalanine, the dipeptide methyl esters did not form Cu (II) complexes (*cf.* Table 1), Compounds VIII - XIII and XXVII - XXXI). However, all N-tosyldipeptide methyl esters containing histidyl and histidine residues (XIV-XX) form blue copper (II) complexes, λ_{max} 615-640 nm, characteristic of normal dipeptide methyl esters of valine (*cf.* Table 1).

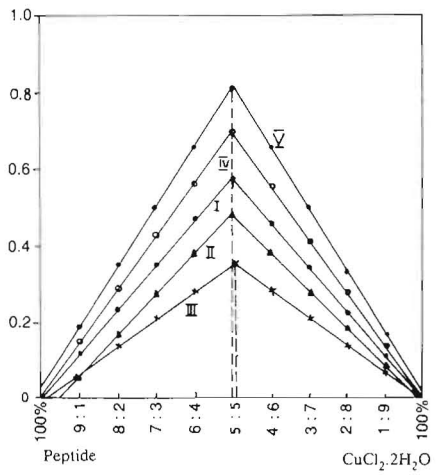


Fig. 2. Diagram of Cu: peptide ratios.

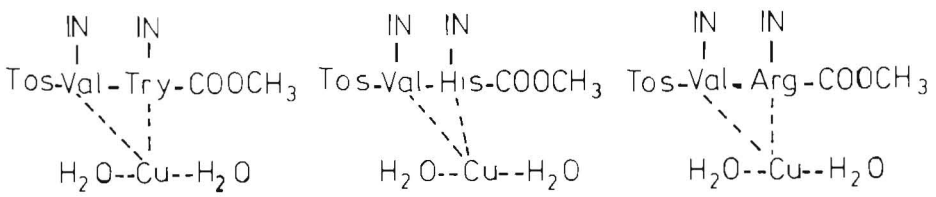
I. Tos- L-Val-L-Try-OMe at λmax 610 nm;
II. Tos- L-Val-L-Try-NH_2 at λmax 575 nm;
III. Tos- L-Val-L-L-His-N_2H_3 at λmax 565 nm;
IV. Tos- L-Val-L-Val-L-Try-OME at λmax 580 nm;
V. Tos- Gly-Gly-Gly-L-Try-OME at λmax 540 nm.

Determination of the compositions of the complexes by the Ostromysslenski (1911) - Job (1934) method showed that in all dipeptide methyl esters investigated the Cu:peptide ratio was 1:1 (*cf.* Fig. 2 and Table 1). Hence, the suggestion of the participation of several copper atoms or peptide molecules in the complex-formation had to be abondoned.

It is evident that the specific features of the complex formation of the dipeptide methyl esters of polyfunctional amino acids are associated with the internal structure of the complex and not its composition. For example, in the case of Tos - Gly - Try - OMe the dipeptide contains three nitrogen atoms capable of complex formation, Tos - Gly - His - OMe contain four nitrogen atoms. Tos - Gly - Arg - OMe contains five nitrogen atoms, and each contains a considerable number of oxygen containing groups. Hence, on the basis of the data available in the literature (Babko 1955 and Plekhan 1961), there are several possibilities for the formation of copper complexes with different structures:

1. The blue complex as of a typical dipeptide methyl ester with the participation of two nitrogen atoms only;
2. The violet complex as of a typical tripeptide methyl ester with the participation of three nitrogen atoms only;
3. The red complex as of a typical tetra- and higher peptides with the participation of four nitrogen atoms.

As stated above the dipeptide methyl esters containing C-terminal tryptophan or arginine residues and N- or C- terminal histidine residues form normal blue complexes with a Cu-peptide ratio 1:1, identical with the complexes of Tos - Gly - Gly - OMe and Tos - Val - Val - OMe. These results led to the conclusion that these complexes will have the schematic structures 1-3, with the participation of α - NH groups. Moreover, the presence of the additional nitrogen atoms of the indole nucleus in tryptophan, imidazole ring in histidine and guanidino group in arginine did not affect the colour, nature and composition of these complexes.



(Structure 1) (Structure 2) (Structure 3)

(Blue Cu (II) complexes, λ_{max} 610-640 nm, Cu: peptide 1:1)

All dipeptide methyl esters containing 2-aminobutyryl or, 3,4-dihydroxyphenlalanyl residues as the N-terminal amino acid in the dipeptide methyl ester and the C-terminal amino acid is tryptophan, arginine or histidine did not form copper (II) complexes (*cf.* Table 1, Compounds XII, XIII, XXII, XXIII, XXXI and XXXII).

The abnormal properties of some dipeptide methyl esters containing N-terminal 2-aminobutyryl-, 3,4-dihydroxyphenylalanyl-, arginyl- and tryptophyl residues may be due to steric effects. Complex dimensions and stability should also be taken into consideration. Similar observations were reported in the case of some hippuryl-, trityl- and 2-aminobutyryl peptides (El-Naggar *et al.* 1977 and 1978).

Studies of the copper complexes of the dipeptide amides and dipeptide hydrazides of histidine, arginine and tryptophan show certain peculiarities when compared with the dipeptide methyl esters. For example: the copper complexes of Tos-Val-Try-amide, Tos-Val-Arg-amide and Tos-Val-His-amide and their corresponding hydrazides show absorption maxima characteristic of higher glycine and valine peptides (tri-) at λ_{max} 555-590 nm (violet biuret reaction) (Plekhan 1952, 1961). However, determination of the composition of the complexes by the Ostromysslenski (1911)- Job (1934) method showed that in all the dipeptide amide and dipeptide hydrazide complexes investigated the Cu: peptide ratio was 1:1 (*cf.* Fig. 2 and Table 1. Complexes XXXII-XLIV). Note that the dipeptides (Tos-Val-Try-NH_2, Tos-Val-His-NH_2 and Tos-Val-Arg-NH_2 contain four, five and six nitrogen atoms respectively and each of their corresponding hydrazides contains one more nitrogen atom. Since all these nitrogen atoms are capable of complex formation, there are several possibilities for formation of copper complexes with different structures:

1. The violet complex as of a typical tripeptide methyl ester with the participation of three atoms of nitrogen (2 α - NH and one NH of indole or imidazole or guanidino group);
2. All four or five or six nitrogen atoms may take part in complex formation with Cu: peptide ratio 2:1 (blue or violet complexes);
3. Four nitrogen atoms may participate with a Cu: peptide ratio 1:1 (red complex); and
4. Participation of the hydrazide group ($-CONHNH_2$) or the amide group ($-CONH_2$) in the complex (a violet complex as of a typical tripeptide methyl ester with the participation of 2 α $-NH$ groups and one NH of the amide ($-CONH_2$) or the hydrazide group ($-CONHNH_2$).

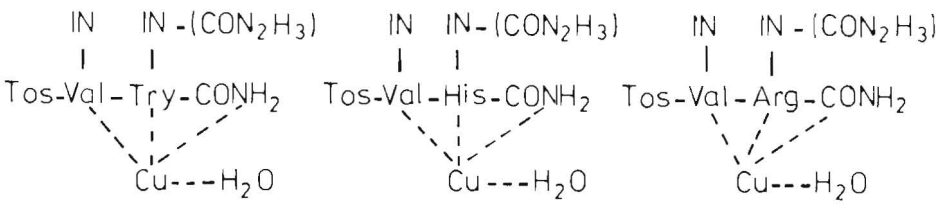
As stated above violet copper complexes are formed with Cu: peptide ratio 1:1 and λ_{max} 555-590 nm. It was found that when the second amino acid (C-terminal) (Try, His or Arg) in the dipeptide amide Tos-Val-Try-NH_2 or Tos-Val-His-NH_2 or Tos-Val-Arg-NH_2 or in the dipeptide hydrazide Tos-Val-Try-N_2H_3 is replaced by valine (*i.e.* Tos-Val-Val-NH_2 or Tos-Val-Val-N_2H_3) no shift in λ_{max} was observed

(*cf.* Table 1, complexes XXXII - XLIV). Hence, the side chain N-atoms in Tos-Val-Try-amide, Tos-Val-His-amide and Tos-Val-Arg-amide (or their corresponding hydrazides), do not appear to participate in complex formation and it is almost certain that in these complexes with λ_{max} 550-590 nm and Cu: peptide ratio 1:1, three nitrogen atoms take part in complex formation: two α-NH amino groups and one N-atom of the amide group ($-CONH_2$) or the hydrazide group ($-CONHNH_2$) of the C-terminal amino acid.

Comparative studies of the copper complexes of different dipeptide methyl esters and their corresponding dipeptide amides and hydrazides support the same conclusion (*cf.* Table 1, complexes I-XLIV).

From these results it is evident that the indole nitrogen of tryptophan, imidazole nucleus of histidine and guanidino group of arginine do not participate in complex formation in any of the dipeptide derivatives studied.

The dipeptide amide and hydrazide copper (II) complexes are suggested to have the schematic structures 4-6.



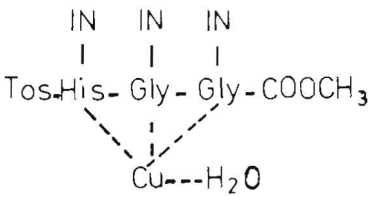
(Structure 4) (Structure 5) (Structure 6)

(Violet copper (II) complexes, λ_{max} 555-590 nm, Cu: peptide = 1:1).

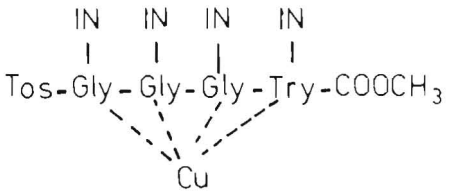
Studies of the copper complexes of different tripeptides containing N- or C-terminal and intermediate Try or Arg or His- residues show that all tripeptide methyl esters form normal violet copper (II) complexes, λ_{max} 560-580 nm, Cu: peptide ratio 1:1 (*cf.* Figs. 1,2 and Table 1, complexes XLV-LIV). For example: the N-tosyl tripeptide methyl esters of Val-Val-Try, Val-Try-Try, Try-Val-Try, Val-Try-Val, Try-Val-Val, Try-Try-Try, and Try-Try-Val gave Cu (II) complexes with the same λ_{max} and Cu: peptide ratio as normal tripeptides of glycine (Gly-Gly-Gly), valine and alanine (Plekhan 1952, 1961).

In previous papers (Poddubnaya and El-Naggar 1966, 1967 and El-Naggar *et al.* 1976, 1978), the participation of the N-terminal lysine or ornithine residues in tri- and hexapeptide complexes was confirmed and some violet or reddish - violet complexes were formed with Cu: peptide ratio 2:1.

All the tetrapeptides investigated form red copper complexes with λ_{max} 530-550 nm, and their Cu: peptide ratio is 1:1 (*cf.* Table 1, complexes LV-LVII and Figs. 1,2). These data show that once again the indole nucleus of tryptophan, imidazole ring of histidine and guanidino group of arginine did not participate in complex formation with di-, tri- and tetrapeptides even when Try-, Arg- or His-residues were N- or C-terminal. The tri- and tetrapeptide methyl ester Cu (II) complexes appear to have the schematic structures 7,8.



(Structure 7)



(Structure 8)

(Structure 7, violet complex, λ_{max} 570 nm, Cu: peptide = 1:1)

(Structure 8, red complex, λ_{max} 530 nm, Cu: peptide = 1:1)

Table 1. Copper complexes of di-, tri- and tetrapeptides containing tryptophan, histidine and arginine.

Compd. No.	Name of the peptide*	Colour of complex	λ_{max} (nm)	Absorbance	Cu: peptide ratio
I	Tos-Gly-L-Try-OMe	Blue	620	0.320	1:1
II	Tos-L-Ala-L-Try-OMe	Blue	620	0.460	1:1
III	Tos-β-Ala-L-Try-OMe	Blue	610	0.125	—
IV	Tos-L-Val-L-Try-OMe	Blue	610	0.385	1:1
V	Tos-L-Leu-L-Try-OMe	Blue	615	0.287	1:1
VI	Tos-L-Try-L-Try-OMe	Blue	610	0.245	1:1
VII	Tos-L-Val-L-Val-OMe	Blue	625	0.420	1:1
VIII	Tos-L-Try-Gly-OMe	-ve	-ve	—	—
IX	Tos-L-Try-L-Val-OMe	-ve	-ve	—	—
X	Tos-L-Try-DL-Ser-OMe	-ve	-ve	—	—
XI	Tos-L-Try-DL-Phe-OMe	-ve	-ve	—	—
XII	Tos-2-Aba-L-Try-OMe	-ve	-ve	—	—
XIII	Tos-3,4 Di(OH)-Phe-L-Try-OMe	-ve	-ve	—	—
XIV	Tos-Gly-L-His-OMe	Blue	640	0.730	1:1

* Abbreviations are those proposed by IUPAC-IUB Commission on Biochemical Nomenclature., *J. Biol. Chem.*, **250,** 3215 (1975); 2-Aba = 2-aminobutyryl, 3,4-Di(OH)-Phe = 3,4-dihydroxyphenylalanyl, N^{im} = imidazole nitrogen, N^{G} = guanidino group, Tos = p-tosyl, -OMe = methyl ester, $-NH_2$ = amide and $-N_2H_3$ = hydrazide.

Table 1. (Continued)

Copper complexes of di-, tri- and tetrapeptides containing tryptophan, histidine and arginine.

Compd. No.	Name of the peptide	Colour of complex	λ_{max} (nm)	Absorbance	Cu: peptide ratio
XV	Tos-L-Val-L-His-OMe	Blue	630	0.145	1:1
XVI	Tos-L-Leu-L-His-OMe	Blue	630	0.125	1:1
XVII	N^{α},N^{im}-Di Tos-L-His-Gly-OMe	Blue	610	0.185	1:1
XVIII	N^{α},N^{im}-Di Tos-L-His-L-Val-OMe	Blue	630	0.150	1:1
XIX	N^{α},N^{im}-Di Tos-L-His-L-Leu-OMe	Blue	615	0.230	—
XX	N^{α},N^{im}-Di Tos-L-His-L-His-OMe	Blue	620	0.720	1:1
XXI	Tos-2-Aba-L-His-OMe	-ve	-ve	—	—
XXII	Tos-3,4-Di(OH)-Phe-L-His-OMe	-ve	-ve	—	—
XXIII	Tos-Gly-N^{G}-nitro-L-Arg-OMe	Blue	625	0.420	1:1
XXIV	Tos-L-Val-N^{G}-nitro-L-Arg-OMe	Blue	625	0.370	1:1
XXV	Tos-L-Leu-N^{G}-nitro-L-Arg-OMe	Blue	620	0.425	1:1
XXVI	N^{α},N^{G}-Di Tos-N^{G}-nitro-L-Arg-N^{G}-nitro-L-Arg-OMe	Blue	625	0.530	1:1
XXVII	N^{α},N^{G}-Di Tos-N^{G}-nitro-L-Arg-Gly-OMe	-ve	-ve	—	—
XXVIII	N^{α},N^{G}-Di Tos-N^{G}-nitro-L-Arg-L-Val-OMe	-ve	-ve	—	—
XXIX	N^{α},N^{G}-Di Tos-N^{G}-nitro-L-Arg-L-Leu-OMe	-ve	-ve	—	—
XXX	Tos-2-Aba-N^{G}-nitro-L-Arg-OMe	-ve	-ve	—	—
XXXI	Tos-3,4-Di(OH)−Phe-N^{G}-nitro-L-Arg-OMe	-ve	-ve	—	—
XXXII	Tos-L-Ala-L-Try-NH_2	Violet	555	0.542	1:1
XXXIII	Tos-L-Val-L-Try-NH_2	Violet	575	0.350	1:1
XXXIV	Tos-L-Leu-L-Try-NH_2	Violet	560	0.159	1:1
XXXV	Tos-L-Try-L-Try-NH_2	Violet	555	0.750	1:1
XXXVI	Tos-L-Val-L-His-NH_2	Violet	575	0.560	1:1
XXXVII	Tos-L-Leu-L-His-NH_2	Violet	575	0.850	1:1
XXXVIII	Tos-L-Val-N^{G}-nitro-L-Arg-NH_2	Violet	560	0.360	1:1
XXXIX	Tos-L-Leu-N^{G}-nitro-L-Arg-NH_2	Violet	590	0.400	1:1
XL	Tos-L-Val-L-Val-NH_2	Violet	565	0.450	1:1
XLI	Tos-L-Val-L-Val-N_2H_3	Violet	570	0.310	1:1
XLII	Tos-L-Val-L-His-N_2H_3	Violet	570	0.350	1:1
XLIII	Tos-Gly-L-His-N_2H_3	Violet	565	0.250	1:1
XLIV	Tos-Gly-N^{G}-nitro-L-Arg-N_2H_3	Violet	570	0.450	1:1
XLV	N^{α},N^{im}-Di-(Tos-Gly-Gly)-L-His-OMe	Violet	590	0.620	1:1
XLVI	Tos-L-Val-L-Val-L-Try-OMe	Violet	580	0.350	1:1
XLVII	Tos-L-Try-L-Val-L-Try-OMe	Violet	585	0.440	1:1
XLVIII	Tos-L-Val-L-Try-L-Try-OMe	Violet	580	0.310	1:1
XLIX	Tos-L-Val-L-Try-L-Val-OMe	Violet	585	0.480	1:1
L	Tos-L-Try-L-Val-L-Val-OMe	Violet	590	0.630	1:1
LI	Tos-L-Try-L-Try-L-Try-OMe	Violet	585	0.450	1:1
LII	Tos-L-Try-L-Try-L-Val-OMe	Violet	590	0.650	1:1
LIII	N^{α},N^{im}-Di Tos-L-His-Gly-Gly-OMe	Violet	570	0.390	1:1
LIV	Tos-Gly-Gly-N^{G}-nitro-L-Arg-OMe	Violet	590	0.470	1:1
LV	Tos-Gly-Gly-Gly-L-Try-OMe	Red	540	0.640	1:1
LVI	Tos-L-Try-Gly-Gly-Gly-OMe	Red	535	0.550	1:1
LVII	Tos-Gly-Gly-Gly-L-His-OMe	Red	540	0.460	1:1

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مـتراكبـات النحـاس للببتيـدات الثنـائيـة والثلاثية والرباعية المحتـويـة عـلى الـتربتـوفـان والهستيـدين والأرجينين

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تضمن البحث دراسة سبكتروفوتومترية لسبعة وخمسين من متراكبات النحاس للببتيدات الثنائية والثلاثية والرباعية المحتوية على التربتوفان والهستيدين والأرجينين.

واتضح من الدراسة أن طول الموجة القصوى ولون المتراكب يعتمد على ترتيب الأحماض الأمينية في الاسترات الميثيلية للببتيدات الثنائية المحتوية على التربتوفان والأرجينين وبينما لا يعتمد على ترتيب الأحماض الأمينية في الببتيدات الثنائية المحتوية على الهستيدين أو في أي من الببتيدات الثلاثية والرباعية المحتوية على الهستيدين والأرجنين والتربتوفان.

وأوضحت النتائج أن ذرات النيتروجين في مجموعة الأندول في الأرجنين أو في مجموعة الأميدازول في الهستيدين أو في مجموعة الجوانيدين في الأرجنين لا تشارك في تكوين المتراكبات للببتيدات الثنائية والثلاثية والرباعية التي تمت دراستها مع النحاس ـ وبينما ثبت أن مجموعة الأميد في أميدات الببتيدات الثنائية ومجموعة الهيدرازيد في هيدرازيدات الببتيدات الثنائية تشارك في تكوين متراكبات هذه الببتيدات مع النحاس.