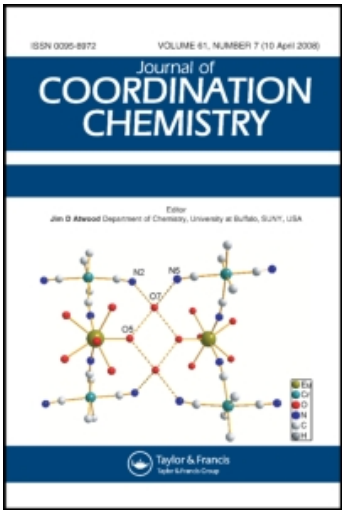

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Copper(II) complexes with hydroxyl-containing dipeptides glycyl-<i>_L-serine and <i>_L-seryl-<i>_L-tyrosine

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Copper(II) complexes with hydroxyl-containing dipeptides glycyl-$_L$-serine and $_L$-seryl-$_L$-tyrosine

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Dipeptides glycyl-$_L$-serine and $_L$-seryl–$_L$-tyrosine are tridentate ligands in coordination with Cu(II) through their NH_2–, N– (from deprotonated amide group) and O–atom (by COO- group), forming $[Cu^{II}(LH_{-1})H_2O]$. The forth position of square-planar geometry of Cu^{2+} is occupied by H_2O as terminal ligand. Solid-state linear dichroic IR-spectroscopy, UV-Vis, mass spectrometry with ESI and FAB, tandem mass spectrometry (HPLC-MS/MS), TGV and DSC methods, EPR and magnetochemistry data prove the formation of five-membered chelate rings with participation of Cu^{2+} both in solution and in solid state.

Keywords: Glycyl-$_L$-serine; $_L$-seryl–$_L$-tyrosine; Cu(II) complexes; Solid-state IR-LD; UV-Vis; ESI-MS; FAB-MS; HPLC-MS/MS; TGV; DSC; EPR

1. Introduction

In the course of our systematic spectroscopic and structural elucidation of coordination ability of peptides with essential biometals [1–6] the complexation between Cu^{2+} and $_L$-serine containing dipeptides glycyl-$_L$-serine (*H-Gly-Ser-OH*) and $_L$-seryl–$_L$-tyrosine (*H-Ser-Tyr-OH*) (scheme 1) are studied. Investigation in solution and solid state includes the application of linear-polarized IR- (IR-LD) spectroscopy, mass spectrometry with electrospray ionization (ESI) and fast atom bombardment (FAB), hybrid method high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS), UV-Vis spectroscopy, electron magnetic resonance (EPR), thermogravimetry (TGA) and difference scanning calorimetry (DSC). *In vitro* study of Cu(II) complexes with $_L$-serine containing peptides is of interest because Cu(II) is an essential biometal and important human proteins contain amino acid residue. A study of Cu-binding proteins in liver and kidney tissue has shown that the proteins are characterized with higher content of glutamic acid, serine, alanine and lysine [7]. The gly-ser fragment has been obtained in chymotrypsin, which is a prominent member

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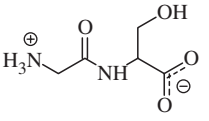
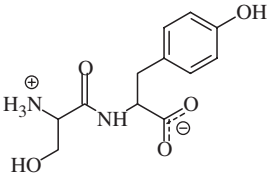
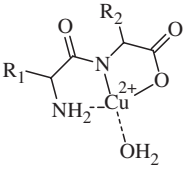
of the family of serine proteases [8]. Thus spectroscopic and structural elucidation of coordination with $_L$-seryl containing peptides and proteins are important [9–11].



H-Gly-Ser-OH



H-Ser-Tyr-OH



$R_1 = H, R_2 = CH_2OH$ (**1**)

$R_1 = CH_2OH, R_2 = CH_2(4\text{-}OH\text{–}C_6H_5)$ (**2**)

Scheme 1. Chemical diagram of peptides studied.

2. Experimental

2.1. *Materials and methods*

Dipeptides were purchased from Bachem and $CuCl_2 \cdot 2H_2O$ from Acros Organics. The *conventional* (KBr pellets) and *IR-LD spectra* were recorded between 4000 cm^{-1} and 400 cm^{-1} on a Bomem–Michelson 100 FTIR spectrometer equipped with a Perkin Elmer wire-grid polarizer. 150 scans were performed for each spectrum with a resolution of 4 cm^{-1}. A 4-cyano-4′-alkylbicyclohexyl mixture (ZLI-1695 in the Merck notation) was used for orientation of the solid samples as a nematic liquid crystal colloid suspension [12–15].

Elemental analyses were performed according to the classical methods: C and H as CO_2 and H_2O, N through the Duma's method, chlorine by titration with $Hg(NO_3)_2$ after a wet digestion of the sample. The Cu^{2+} is obtained after ignition of a sample, dissolving of the residue in dilute HNO_3 and titration with standard solution of EDTA using murexide at pH 8 (ammonia buffer) as an indicator.

The *molecule weight* was determined using FAB mass spectra, measured on a Fusion VG Autospect instrument employing 3-nitrobenzyl alcohol as a matrix.

The *thermogravimetric* (*TGA*) *study* was performed using Perkin-Elmer TGS2 apparatus. The *calorimetric* (*DSC*) *analyses* are performed on DSC-2C Perkin Elmer equipment in argon.

The *UV-spectra* ($2.5.10^{-4}$ M aqueous solutions, 1 cm quartz cell) were obtained on a Perkin Elmer Lambda 15 UV-VIS spectrophotometer.

The *EPR* data are obtained in X-band on a Bruker ER 420 spectrometer.

HPLC-MS/MS analysis. The analyses of the samples were performed with a Thermo Finnigan surveyor LC-Pump. Compounds were separated on a Luna C18 column (150 × 2 mm, 4 μm particle size) from Phenomenex (Torrance, CA, USA). The mobile

Table 1. HPLC-MS/MS conditions.

	t [min]	*A* [%]	*B* [%]	Flow rate [μL min^{-1}]
0	0.00	100	0	200
1	3.00	100	0	200
2	8.00	65	35	200
3	9.00	0	100	200
4	14.00	0	100	200
5	14.50	100	0	200
6	20.00	100	0	200

phase consisted of water +0.1% HCOOH (A) and acetonitrile +0.1% HCOOH (B) using a gradient program presented in table 1. The compound was detected via UV and a TSQ 7000 (Thermo Electron Corporation, Dreieich, Germany) mass spectrometer. The spectra were obtained using the TSQ 7000 equipped with an ESI ion source and operated with the following conditions: capillary temperature 180 °C; sheath gas 60 psi and spray voltage 4.5 kV. 1 mg mL^{-1} of the sample was dissolved in acetonitrile and injected into the ion source by an auto sampler (Finnigan Surveyor). Excalibur 1.4 software processed the data obtained.

The *magnetic* measurements were carried out in argon by a Faraday balance.

2.2. *Synthesis*

The Cu(II) complexes with dipeptides *H-Gly-Ser-OH* (**1**) and *H-Ser-Tyr-OH* (**2**) were synthesized by common procedure: 5 mL 0.1 M solution of $CuCl_2 \cdot 2H_2O$ (170.4 mg) in solvent mixture H_2O:CH_3OH 1 : 1 was added to 5 mL 0.1 M solution of *H-Gly-Ser-OH* (162.2 mg) or *H-Ser-Tyr-OH* (268.2 mg) in the same solvent mixture. The molar ratio of metal to ligand is 1 : 1 in both cases. The blue precipitates formed after about 20 days were filtered off, washed with methanol and dried in air at 298 K. Yields: 72% for **1** and 63% for **2**, respectively. Found%: C, 24.9; H, 4.2; N, 11.7; Cu, 26.4. Calcd for [($C_5H_{10}N_2O_5$)Cu] (**1**): C, 24.8; H, 4.1; N, 11.5; Cu, 26.2. Found%: C, 41.4; H, 4.5; N, 8.1; Cu, 18.4. Calcd for [($C_{12}H_{16}N_2O_6$)Cu] (**2**): C, 41.4; H, 4.6; N, 8.0; Cu, 18.2.

3. Results and discussion

3.1. *Conventional and linear-polarized IR-data*

Investigation of Cu^{2+}-complexation processes with dipeptides requires detailed IR band assignment of ligand and corresponding changes in the IR-spectra of the complexes isolated. For these reasons the IR-LD analysis started with interpretation of polarized spectra of pure dipeptides *H-Gly-Ser-OH* and *H-Ser-Tyr-OH*. The detail IR-spectroscopic assignment of *H-Gly-Ser-OH* was done [4], where the coordination ability with Au(III) was studied [4]. For *H-Ser-Tyr-OH* the IR-LD characterization is presented in this article with IR-characteristic bands of both ligands and corresponding complexes presented in table 2.

Table 2. Solid-state IR-spectral data of dipeptides *H-Gly-Ser-OH*, *H-Ser-Tyr-OH* and their Cu^{2+}-complexes **1** and **2**.

	ν [cm^{-1}]			
Assignment*	*H-Gly-Ser-OH* [5]	**1**	*H-Ser-Tyr-OH*	**2**
ν_{OH}	3170	3477	3214	3444
ν_{H_2O}	–	3551	–	3473
ν_{NH_3+}-stretches	3100–2300 broad	–	3300–2700	–
ν_{NH}	3315	–	3336	–
$\nu^{as}{}_{NH_2}$, $\nu^{s}{}_{NH_2}$	–	3340, 3206, 3100	–	3355, 3215, 3150
$\nu_{C=O}$	–	1710	–	1716
$\delta^{as}{}_{NH_{3+}}$, $\delta^{as'}{}_{NH_{3+}}$	1693, 1623	–	1681, 1633	–
δ_{H_2O}	–	1636	–	1639
δ_{NH_2}	–	1681	–	1683
$\nu_{C=O}$ (Amide I)	1660	1642	1662	1650
8a, **8b**	–	–	1610, 1575	1611, 1575
8b	–	–	1588	1590
$\delta^{s}{}_{NH_3+}$	1590	–	1587	–
δ_{NH} (Amide II)	1555	–	1545	–
$\nu^{as}{}_{COO-}$	1515	–	1556	–
19b	–	–	1467	1469
$\nu^{s}{}_{COO-}$	1405	–	1411	–
11-γ_{CH}	–	–	842	848
$\delta_{C=O}$ (Amide IV)	721	724	742	750
γ_{NH} (Amide V)	668	–	665	–
$\gamma_{C=O}$ (Amide VI)	624	674	630	644

*The aromatic in-plane and out-of-plane modes are assigned using Wilson's notation [21].

The non-polarized IR-spectrum of *H-Ser-Tyr-OH* contains a broad maximum at 3300–2700 cm^{-1}, assigned to $\nu_{NH_{3+}}$ asymmetric and symmetric stretching modes. The intensive peaks at 3336 and 3214 cm^{-1} (table 2) belong to ν_{NH} and ν_{OH} modes. The low frequency shift of ν_{OH} is explained by participation of OH in the serine side chain in strong intermolecular interactions. The 1700–1400 cm^{-1} IR-spectral region contains maxima characteristic of $-NH_3^+$, COO^- and amide groups and in-plane modes of *p*-disubstituted phenyl ring in the tyrosine side chain (table 2). Confirmation is obtained by the following IR-LD analysis. Simultaneous reduction of 3315 cm^{-1} and 1662 cm^{-1} peaks in the reduced IR-LD spectrum of *H-Ser-Tyr-OH* indicated their character as ν_{NH} and $\nu_{C=O}$ (Amide I) modes as well as a *transoid* configuration of the amide fragment. A reduction of both Amide V and Amide VI peaks (table 2) in the same dichroic ratio confirms their assignment as γ_{NH} and $\gamma_{C=O}$ out-of-plane frequencies.

Comparing the IR-spectral data of ligands and their Cu^{2+}-complexes the following main differences are determined (table 2): (i) A disappearance of ν_{NH} bands about 3330 cm^{-1} indicates Cu^{2+} coordination through N-amide supported by a preliminary deprotonation process in the ligands; (ii) Observation of new peaks (table 2) in the complex typical for $\nu^{s}_{NH_2}$ and Fermi-resonance split $\nu^{s}{}_{NH_2}$ of primary NH_2-group, when the asymmetric HNH ↑ X intermolecular interactions are established; (iii) 1700–1400 cm^{-1} region indicated disappearance of all bending (δ_{NH3+}) maxima of NH_3^+ and observation of δ_{NH_2} about 1680 cm^{-1}, typical for complexes where NH_2^- group is included in coordination. The fact that $\nu_{C=O}$ (Amide I) is significantly lower frequency and that δ_{NH} (Amide II) disappears in the IR-spectrum of the complex means that the metal ion is coordinated through N-amide (scheme 1); (iv) The observation of a new maximum of $\nu_{C=O}$ higher than 1700 cm^{-1} with disappearance of $\nu^{as}{}_{COO-}$ and

ν^{s}_{COO-} in the IR-spectra of complexes indicates coordination of metal with COO^{-} in the peptide distorting the C–O bond lengths and reversing C=O double and C–O single bonds in the complex (scheme 1); (v) As expected deprotonation of –C(=O)–NH-amide in the complex caused disappearance of the γ_{NH} mode and high frequency shift of $\delta_{C=O}$ and $\gamma_{C=O}$ (table 2); (vi) In both complexes new bands belonging to stretching and bending vibrations of H_2O are observed (table 2).

Elimination of δ_{NH_2} in the IR-LD spectra of Cu^{2+}-complexes caused disappearance of corresponding ones assigned to δ_{NH_2} and Fermi-resonance split $\nu^{s}_{NH_2}$, where transition moments are co-linear in the frame of one NH_2-fragment (scheme 1, table 2). These data correlate with previously reported assignment of corresponding isolated amino acids and small peptides [16, 17].

3.2. *ESI-MS, FAB-MS and HPLC-MS-MS data*

The HPLC ESI-MS/MS spectra of Cu(II) complexes with *H-Gly-Ser-OH* and *H-Ser-Tyr-OH* are shown in figure S1A and S1B. The ESI-MS spectrum of **1** has peaks at *m/z* 259.02, corresponding to $[[C_5H_{11}O_5N_2Cu]^+ + NH_4^+]$ adduct with a molecule weight of 259.2 (figure S1A). It is well known that the Na^+ and NH_4^+ adducts are typical for ESI-MS in the analysis of peptides and their derivatives [18–20]. The peak at *m/z* 217.97 corresponds to species with fragmentated metal ion. FAB-MS spectrum of **1** show a peak at *m/z* 241.0 assigned to $[C_5H_{11}O_5N_2Cu]^+$ with molecule weight of 241.69. The fragmentation of metal ion leads to an observation of *m/z* signal at 176.0 of $[C_5H_{11}O_5N_2]^+$ ion. The ESI-MS data of **2** (figure S1B) show an *m/z* at 364.5 corresponding again to NH_4^+ adduct of **2** with molecule weight of 365.2 ($[C_{12}H_{16}O_6N_2Cu]^+ + NH_4^+$]). The fragmentation of metal ion leads to *m/z* of 266.9 of $[C_{12}H_{16}O_6N_2Cu]^+$. FAB-MS spectrum of **2** show a peak at *m/z* 351.1 assigned to $[C_{12}H_{16}O_6N_2Cu]^+$ ion.

3.3. *TGA and DSC data*

TGA data of **1** and **2** in the temperature range 300–600 K show a weight loss of 7.4% and 5.1% in **1** and **2**, respectively, corresponding to one H_2O in the Cu^{2+} complexes. The DSC data are shown in figure 1 and an enthalpy effect of 10.3 kcal mol^{-1} **1** and 8.9 kcal mol^{-1} **2** is observed, also confirming the inclusion of one solvent molecule in the coordination compounds obtained. The enthalpy effect is observed at the temperature about 125°C in both complexes, supposing that the solvent molecule is included in the coordination sphere.

3.4. *UV-Vis spectra*

The coordination of dipeptides (scheme 1) with Cu^{2+} causes hypsochromic shifts of the absorption maxima of phenyl chromophor in **2** less than 5 nm in UV-diapason (figure 2). New maxima both in **1** and **2** are observed at 588 nm ($\varepsilon = 88$ l mol^{-1} cm^{-1}) and 580 nm ($\varepsilon = 90$ l mol^{-1} cm^{-1}), respectively (figure 2). A low intensity shoulder about 600 nm (ε within 86–88 l mol^{-1} cm^{-1}) is observed in both complexes. These maxima

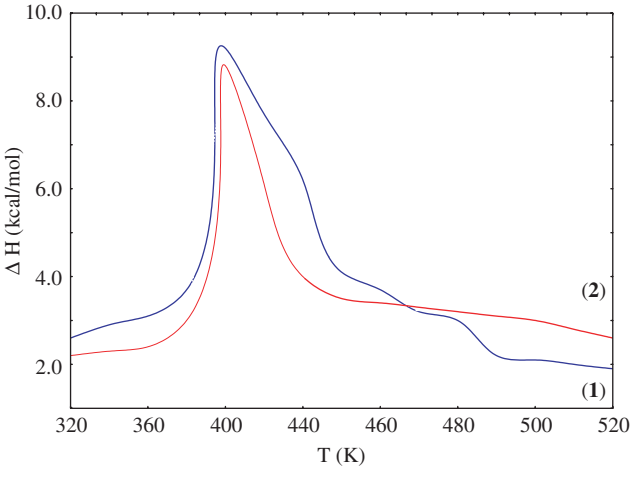


Figure 1. DSC data of **1** and **2** between 300–600 K.

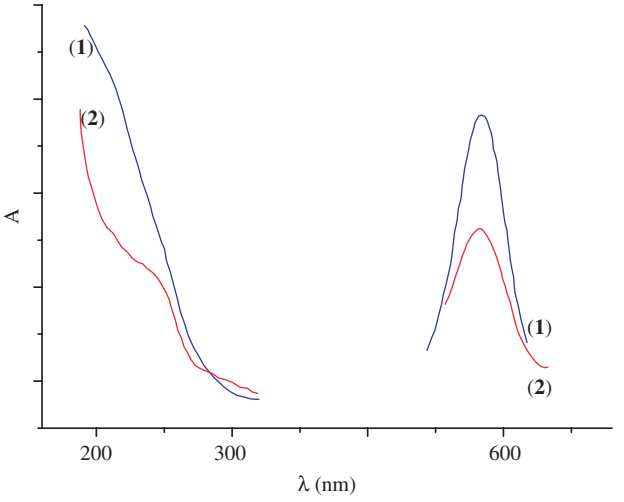


Figure 2. UV-Vis spectrum of Cu^{2+}-complexes of dipeptides studied in water/methanol solvent mixture.

correspond to d–d transitions as a result of the presence of the Cu–O and Cu–N bonds providing direct evidence about the complex formation.

3.5. *EPR data*

In methanol solution at room temperature isotropic EPR-spectra of the investigated mononuclear complexes with a resolved hyperfine structure for $^{63,65}Cu$ and parameters $g_{iso} = 2.11$, $A_{iso} = 73.11 \times 10^{-4}\,cm^{-1}$ for **1** and $g_{iso} = 2.09$, $A_{iso} = 69.11 \times 10^{-4}$ for **2** are obtained. Frozen solutions at 77 K give anisotropic signals with additionally resolved SHFS from ^{14}N in the perpendicular region of

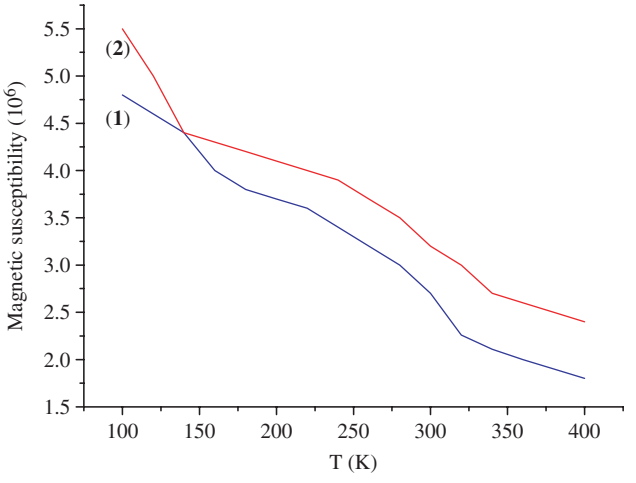


Figure 3. Magnetic susceptibility *vs.* T for **1** and **2**.

the magnetic field. The obtained parameters are: $g_{II} = 2.19$ ($A_{II} = 189.10 \times 10^{-4}\,cm^{-1}$), $g_{\perp} = 2.02$ ($A_{\perp} = 48.50 \times 10^{-4}\,cm^{-1}$) for **1** and $g_{II} = 2.17$ ($A_{II} = 191.10 \times 10^{-4}\,cm^{-1}$), $g_{\perp} = 2.03$ ($A_{\perp} = 50 \times 10^{-4}\,cm^{-1}$) for **2**. In both cases the A_N is 14 and indicates formation of Cu–N bonds. The complexes are also EPR active in the solid state both at 77 K and 298 K, indicating formation of mononuclear Cu^{2+} complexes with dipeptides.

3.6. *Magnetochemistry data*

The temperature dependence of the magnetic susceptibility for both complexes are typical for a mononuclear paramagnetic complex (figure 3), without any measurable exchange interaction between Cu^{2+} centers. The Curie–Weiss law is not obeyed in the temperature range studied and in such conditions the magnetic moment was calculated using the equation $\mu_{eff} = 2.828\ (k_M T - \theta)^{1/2}$; $\theta = -80$. At low temperatures, the magnetic moment μ is temperature dependent and corresponds to a tetrahedral coordination of Cu^{2+} (figure 4), but at higher temperatures the μ-value differs from the theoretical one. Most probably the deviation observed is due to a deformation of the tetrahedral structure to a nearly square-planar one, a fact supported by the obtained EPR data.

4. Conclusions

By physical methods such as solid-state IR-LD spectroscopy based on orientation technique of samples as nematic liquid crystal suspensions, mass spectrometry (ESI and FAB), hybrid methods (HPLC MS/MS), UV-Vis, EPR, TGS and DSC analysis, the structures of Cu^{2+}-complexes with *H-Gly-Ser-OH* and *H-Ser-Tyr-OH* have been determined. The metal ion is coordinated through N- and O-atoms from NH_{2-} and $-COO^{-}$-groups. The joining of Cu^{2+} ion with N-amide, accompanied with

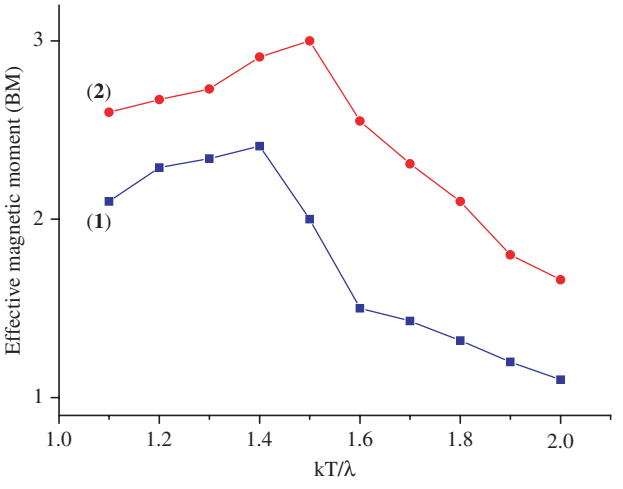


Figure 4. Graphs μ_{eff} *vs.* *T* for **1** and **2**.

deprotonation of corresponding –C=(O)–NH amide group, is also established. The fourth position of the square-planar geometry is a H_2O molecule as a terminal ligand.

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References

[1] B.B. Ivanova, M. Mitewa. *J. Coord. Chem.*, **57**, 217 (2004).
[2] B.B. Ivanova. *J. Coord. Chem.*, **58**, 587 (2005).
[3] B.B. Ivanova, M.G. Arnaudov, St.T. Todorov. *J. Coord. Chem.*, **59**, 1749 (2006).
[4] T. Kolev, B.B. Ivanova, S.Y. Zareva. *J. Coord. Chem.*, **60**, 109 (2007).
[5] Ts. Kolev, S.Y. Zareva, B.B. Koleva, M. Spiteller. *Inorg. Chim. Acta.*, **359**, 4367 (2006).
[6] B.B. Koleva, Ts. Kolev, M. Spiteller., *Inorg. Chim. Acta* (2006): http://dx.doi.org/10.1016/j.ica.2006.11.002.
[7] A.E. Port, D.M. Hunt. *Biochem. J.*, **183**, 721 (1979).
[8] N. Singh, T. Jabeen, S. Sharma, I. Roy, M. Gupta, S. Bilgrami, R. Somvanshi, K. Dey, M. Perbandt, C. Betzel, A. Srinivasan, T. Singh. *FEBS Journal*, **272**, 562 (2005).
[9] G. Zou, G.L. Boyer. 223*rd ACS National Meeting*, Orlando, FL, United States, April 7–11, 2002, ANYL-036.
[10] M.A. Zoroddu, T. Kowalik-Jankowska, H. Kozlowski, K. Salnikow, M. Costa. *J. Inorg. Biochem.*, **84**, 47 (2001).
[11] R. Liang, S. Senturker, X. Shi, W. Bal, M. Dizdaroglu, K.S. Kasprzak. *Carcinogenesis*, **20**, 893 (1999).
[12] B.B. Ivanova, M.G. Arnaudov, P.R. Bontchev. *Spectrochim. Acta*, **60(A)**, 855 (2004).
[13] B.B. Ivanova, D.L.Tsalev, M.G. Arnaudov. *Talanta*, **69**, 822 (2006).
[14] B.B. Ivanova, V.D. Simeonov, M.G. Arnaudov, D.L. Tsalev. *Spectrochim. Acta*, **67(A)**, 66 (2007).
[15] B.B. Koleva, T. Kolev, V. Simeonov, T. Spassov, M. Spiteller. *Adv. Colloid. Int. Sci.*, submitted (2007).
[16] T. Norio, H. Masahiko, T. Kiyoshi, S. Tokura, A. Tsutsumi. *Pept. Chem.*, 245 (1985).

[17] M. Tsuboi, Y. Ezaki, M. Aida, M. Suzuki, A. Yimit, K. Ushizawa, T. Ueda. *Biospectroscopy*, **4**, 61 (1998).
[18] D.G. Morgan, M.M. Bursey. *J. Mass Spectrom.*, **30**, 473 (1995).
[19] Y. Wang, F. Ke, K.W.M. Siu, R.J. Guevremont. *J. Mass Spectrom.*, **31**, 45 (1996).
[20] D.G. Morgan, M.M. Bursey. *J. Mass Spectrom.*, **30**, 290 (1995).
[21] G. Varsanyi, *Vibrational Spectra of Benzene Derivatives*, Akademiai kiado, Budapest (1969).