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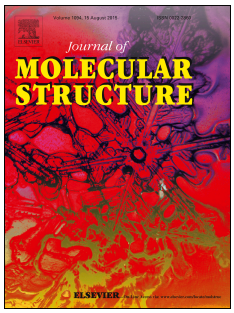

Synthesis, structure and stability of a chiral imine-based Schiff-based ligand derived from L-glutamic acid and its [Cu_4] complex

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PII: S0022-2860(16)30784-0

DOI: 10.1016/j.molstruc.2016.07.100

Reference: MOLSTR 22803

To appear in: *Journal of Molecular Structure*

Received Date: 19 June 2016

Revised Date: 23 July 2016

Accepted Date: 25 July 2016

Please cite this article as: S. Muche, I. Levacheva, O. Samsonova, A. Biernasiuk, A. Malm, R. Lonsdale, E. Popiołek, U. Bakowsky, M. Hołyńska, Synthesis, structure and stability of a chiral imine-based Schiff-based ligand derived from L-glutamic acid and its [Cu_4] complex, *Journal of Molecular Structure* (2016), doi: 10.1016/j.molstruc.2016.07.100.

Synthesis, structure and stability of a chiral imine-based Schiff-based ligand derived from L-glutamic acid and its [Cu_4] complex

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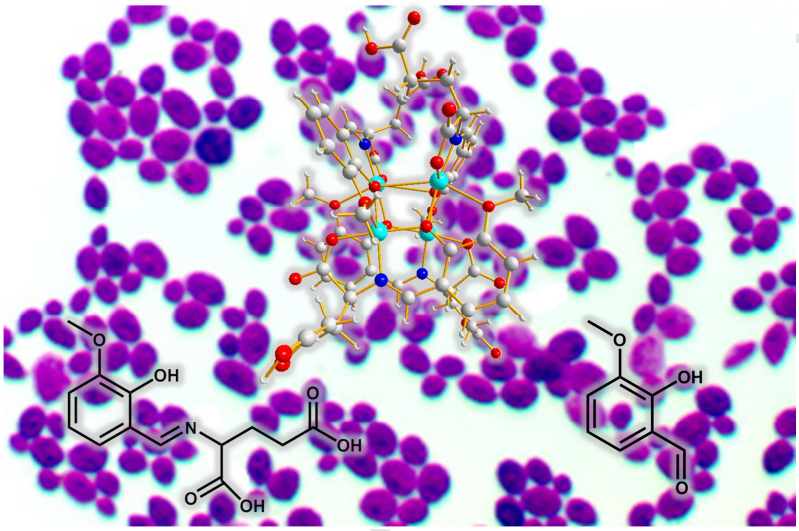
KEYWORDS copper (II) • Schiff-base • stability •crystal structure

ABSTRACT: Studies of the stability of a ligand derived from *L*-glutamic acid and *ortho*-vanillin and its new [Cu_4] complex are presented. The [Cu_4] complex contains a heterocubane [$Cu^{II}_4O_4$] core and pendant carboxylic groups increasing its solubility in water, also under basic conditions. The stability of the complex in different solvents is confirmed with ESI-MS studies and such experiments as successful recrystallization. The complex is stable also under physiological conditions whereas the ligand is partly decomposed to *L*-glutamic acid and *ortho*-vanillin.

Graphical Abstract



Synopsis: Structure and stability of a chiral Schiff-base ligand and its new [Cu_4] complex are presented.

Highlights

- Stability of a chiral Schiff-base ligand and its new [Cu_4] complex are reported
- The complex shows high solubility in polar solvents due to pendant carboxylic groups
- Initial studies of biological properties point out antifungal activity

Introduction

Oligonuclear metal complexes with Schiff-base ligands have been shown to often display antibacterial, antifungal, anti-inflammatory and antiviral activities.[1] In spite of these observations, attempts to rationalize them are scarcely available. For instance, Raman et al. [2a] studied Schiff bases derived from 1H-indole-2,3-dione (isatin) and 4-(2-aminoethyl)phenol (tyramine) and their Cu, Ni, Zn complexes interacting with the minor groove of CT-DNA. The increased antibacterial activity of the metal complexes was rationalized in terms of Tweedy's chelation theory on metal-ligand bonding enhancing the system's liphophilicity and, as a consequence, the ability to penetrate lipid membranes and to block the metal-binding sites of microbial enzymes. Moreover, the highest activity of the Cu(II) complex was explained in terms of the atomic radius / electronegativity of the Cu^{2+} ion leading to the conclusion that the antimicrobial properties are more promising for ions with higher electronegativities and large atomic radii. These considerations were completed with docking studies. Such theoretical reports rationalizing the interaction with DNA and proteasome inhibition of some Schiff-base complexes are available also for other systems. [2b-f]

In particular, fungal infections (candidiasis) are a serious problem to civilization, increasing the mortality of infants and individuals suffering from decreased immunity. The administration of therapeutic doses of the currently available antimicrobial agents to patients leads to multiple side effects.[3] The most common adverse effects are allergic reactions, shifts in microflora balance and the generation of resistance in pathogenic species. After misbalancing of the natural defense system in the body, so follows the increase in the host vulnerability towards external pathogens, leading to a more aggressive disease progression.[4,5] To find the "golden ratio" in the interplay of

host-drug-microbe means not only to improve the therapeutic efficacy but also to immensely improve the patients' life quality.

In this contribution we report on a sodium salt of a Schiff-base derived from *ortho*-vanillin and *L*-glutamic acid (**Na_2HL,** Scheme 1), as well as its [Cu_4] complex (**$[Cu_4(HL)_4]\cdot 4.6H_2O$**) featuring pendant carboxylic groups. It is shown that the ligand partly decomposes under physiological conditions. On the other hand, the copper(II) complex displays significant stability, even in its aqueous solution. This is part of our project on the coordination chemistry of Schiff-base ligands containing biologically relevant moieties which already yielded a biologically compatible wheel-like [Ni_{15}] complex, reported elsewhere.[6] Apart from that study, the title copper(II) complex is the second example of a metal complex with this ligand system.

Results and Discussion

2.1 Syntheses and stability

Although the reported ligand has been known for many decades,[7] no analytical data are available, except for the stability constants of its metal complexes.

Herein the ligand **H_3L** (Scheme 1) was synthesized in the form of a sodium salt **Na_2HL** (Figure 1) by modification of a method reported by Heinert and Martell [8] for the potassium salt of the vanillin analogue. Formation of a disodium salt of the organic ligand is consistent with the elemental analysis results (see SI).

Under ESI conditions the exact mass peaks were found. The ^{1}HNMR spectrum in MeOD (Figure S2) shows all of the expected signals, except for the proton from the phenolic –OH group, which

is due to a fast exchange with the solvent. In D_2O partial decomposition takes place as evidenced by the presence of a new peak at 9.97 pm, attributed to the aldehyde H atom of *ortho*-vanillin.

Attempts to crystallize the ligand in its neutral form **H_3L** were unsuccessful and usually led to yellow oily phases.

The corresponding complex **$[Cu_4(HL)_4]\cdot 4.6H_2O$** was crystallized from a system where the organic ligand was generated *in situ* (see Scheme 2 for the ligand coordination mode). Subsequent protonation of the side chains is essential for crystal growth of the **$[Cu_4(HL)_4]\cdot 4.6H_2O$** complex. Addition of hydrochloric acid to the reaction mixture from the beginning hampers crystallization.

$[Cu_4(HL)_4]\cdot 4.6H_2O$ is soluble in polar solvents, e.g. water, ethanol, methanol and isopropanol and slightly soluble in dichloromethane. Compound **Na_2HL** dissolves immediately with partial decomposition in water. Furthermore, **Na_2HL** is soluble in methanol and DMSO and sparingly in ethanol.

$[Cu_4(HL)_4]\cdot 4.6H_2O$ displays molecular peaks under ESI-MS conditions in its aqueous and dichloromethane solutions. On drying in vacuum for 6 hours neither the color nor the solubility changes. The vacuum-dried and air-dried samples of **$[Cu_4(HL)_4]\cdot 4.6H_2O$** show identical IR spectra (see SI). These observations confirm a reasonable stability of both **$[Cu_4(HL)_4]\cdot 4.6H_2O$** and **$Na_2HL$** in solution and in solid state.

To investigate the behavior in solution and, at the same time, the potential of **Na_2HL** and **$[Cu_4(HL)_4]\cdot 4.6H_2O$** for application in biological systems, their stability under physiological conditions was investigated. Due to the paramagnetic nature of the complex, measurement by NMR was not possible. Therefore the stability for **$[Cu_4(HL)_4]\cdot 4.6H_2O$** was investigated mainly with IR spectroscopy. Fig. 1 shows the IR spectra in the relevant range from 1800 cm^{-1} to 400

cm^{-1} for the complex as-synthesized and the remaining dried solids after various time spans of shaking the dissolved compound in phosphate-buffered saline (PBS) at 37 °C. All spectra show no significant discrepancy compared to the spectra of the as synthesized **$[Cu_4(HL)_4]\cdot 4.6H_2O$** (black line). These results are conform with our unsuccessful efforts to explore the reactivity of the carboxylic groups of **$[Cu_4(HL)_4]\cdot 4.6H_2O$**. Furthermore, we could recrystallize the complex from a sample prepared in the same manner like for the stability test without any structural changes. Crystal growth on the inner face of the tube was observed at 4 °C within 2 weeks. Cell constants / full X-ray diffraction measurements confirmed that these crystals structures are identical as for the copper complex.

The same experimental setting as for **$[Cu_4(HL)_4]\cdot 4.6H_2O$** was also performed for **Na_2HL**. The spectra of the remaining solid after drying showed significant differences compared to the spectra of HL as-synthesized. Furthermore, the color of the solution changed to brown after around 20 h. Based on these results and the fact, that the NMR spectra recorded in D_2O show a decomposition of **Na_2HL** to *ortho*-vanillin, we performed short- and long-term NMR measurement to determine the stability of **Na_2HL** under physiological conditions. Fig. 2 shows the relevant signals for *ortho*-vanillin (10.17 ppm, CO*H*) and the **Na_2HL** (8.44 ppm, *H*C=N) in the recorded spectra. The *ortho*-vanillin/ligand ratio was determined by the integrals, which should give 1 in total. The spectrum A was recorded immediately after mixing **Na_2HL** with the NMR solvent (t_0). The spectra B-F were recorded in steps of 2 min. The spectra G and H were recorded 2 h and 24 h after t_0. All spectra show decomposition of **Na_2HL** in PBS/D_2O into *ortho*-vanillin. At t_0 15% of compound **Na_2HL** is decomposed into *ortho*-vanillin. This value increases up to 23% during the next 10 min, where it remains stable. After 24 h the value persists at 23%. Furthermore, after 24 h the solution turned to light brown and a new signal appears at 8.59 ppm.

Identification of the new signal was not possible. However, the spectrum F resembles the other spectra.

2.2 Structural aspects

$[Cu_4(HL)_4]\cdot 4.6H_2O$ was obtained in crystalline form and found suitable for single crystal X-ray diffraction studies. The compound crystallizes in the $P2_1$ space group type (see Experimental and SI for the details of the crystal structure determination). The complex molecules lie in general positions and comprise 4 doubly deprotonated ligands and four Cu^{2+} ions (Figure 3). A central distorted-cubane $[Cu_4O_4]$ motif is created by the involvement of phenoxo O, imino N and carboxylate O atoms from each ligand. Each ligand thus chelates two Cu^{2+} ions in three modes: O,O and 2(O,N). Such a motif is common in Cu(II) chemistry. Selected parameters of the complex core are listed in Table S2. In each organic ligand the second carboxylic group remains protonated, pointing outside the complex core. Two of these groups are disordered in two positions. Unexpectedly, only the carboxylic O atom (O55) is involved in O-H…O hydrogen bonds as donor. The acceptor is the symmetry-related coordinated O33 carboxylate atom. Thus hydrogen-bonded chains are formed along [010] (Figure 4). Water molecules are located between the chains in a layered-like arrangement.

3. Conclusions

The title compound **Na_2HL** derived from *L*-glutamic acid and *ortho*-vanillin isolated as a disodium salt was shown to undergo partial decomposition under physiological conditions. Initial studies show its potential for applications as an antifungal agent (see SI).

Na_2HL can be used as a precursor of a tetranuclear copper(II) complex **$[Cu_4(HL)_4]\cdot 4.6H_2O$** with pendant carboxylic groups and lower antibacterial/antifungal activity but increased cytotoxicity, as shown by the first tests (see SI). The carboxylic functional groups of **$[Cu_4(HL)_4]\cdot 4.6H_2O$** allow to tune its solubility and our studies confirm its high stability in solution. Inclusion of biologically relevant moieties in **$[Cu_4(HL)_4]\cdot 4.6H_2O$** and **$Na_2HL$** seems to increase their biological compatibility as confirmed with studies of cytotoxicity.

4. Experimental Section

4.1 Syntheses

The ligand **Na_2HL**: 320 mg (8 mmol) of NaOH was dissolved in 60 mL of warm methanol. 588 mg (4 mmol) of *L*-glutamic acid was added and stirred until the solid was dissolved completely. 608 mg (4 mmol) of *ortho*-vanillin was added and the yellow solution was stirred for 1 h at room temperature. Addition of 150 mL of diethyl ether led to precipitation of the yellow product **Na_2HL**. The solid was filtered off, washed with diethyl ether and dried under air for 12 h. The resulting product was dried for 2 h under high vacuum.

IR bands for **Na_2HL** (cm^{-1}): 471.9 (vw), 512.7 (vw), 559.2 (vw), 576.4 (m), 605.6 (vw), 638.6 (vw), 664.0 (w), 735.6 (s), 776.5 (m), 794.0 (w), 844.6 (s), 930.1 (w), 973.1 (m), 1012.4 (vw),

1074.2 (m), 1170.3 (w), 1254.1 (s), 1280.01 (w), 1316.4 (vw), 1336.6 (w), 1413.6 (s), 1455.4 (s), 1575.4 (vs), 1632.5 (m)

^{1}HNMR (ppm, MeOD-d_4):8.31 (s, 1H), 6.86 (d, 2H, 3J = 8.27 Hz), 6.48 (t, 1H, , 3J = 7.73 Hz; 8.03 Hz), 4.06-4.11 (m, 1H), 3.81 (s, 1H), 2.10-2.39 (m, 4H)

Elemental analysis for the substance dried under vacuum for 2 h, analysed as $C_{13}H_{13}NO_6Na_2$ (see SI for details): Calcd (found): C 48.01 (47.24), H 4.03 (4.00), N 4.31 (4.23), O 29.52 (29.76).

ESI-MS: Molecular peak was found at *m/z* = 282.1 (ESI+; $[M+H]^+$) and 280.1 (ESI-; $[M-H]^-$) in methanol.

The complex **$[Cu_4(HL)_4]\cdot 4.6H_2O$**: 147 mg (1.0 mmol) of *L*-glutamic acid and 408 mg (3.0 mmol) of sodium acetate was dissolved in 3.5 mL of water and 5 mL of methanol and heated to 80 °C. When the solid part was completely dissolved, 152 mg (1.0 mmol) of *ortho*-vanillin was added to the stirred *L*-glutamic acid/sodium acetate solution. To the resulting yellow solution 250 mg (1 mmol) of $Cu(SO_4)\bullet 5H_2O$ was added. The solution color changed to green immediately. The solution was stirred for 30 min. at 80 °C and afterwards combined with a solution containing 11.5 mL of water, 6 mL of methanol and 0.6 mL of 2M hydrochloric acid. The solution was stirred for one minute and transferred into a 30 mL vial for crystallization by slow evaporation. Green crystals in form of plates were obtained within one day. Crystallization is finished after few days depending on evaporation rate. Yield: average 147 mg per sample.

IR bands for **$[Cu_4(HL)_4]\cdot 4.6H_2O$** ($cm^{-1}$): 463.38 (w), 534.03 (w), 651.98 (w), 740.59 (m), 785.20 (w), 812.55 (w), 846.58 (w), 966.57 (m), 1083.14 (w), 1085.77 (m), 1216.71 (s), 1251.56 (s), 1348.50 (m), 1453.65 (m), 1565.89 (w), 1602.37 (s), 1634.46 (s), 1694.58 (m), 2335.63 (vw), 2362.63 (vw), 2842.87 (vw), 2908.44 (vw), 2947.02 (vw), 2985.59 (vw).

Elemental analysis: Elemental analysis for the substance dried under vacuum for 6 h, analysed as $C_{52}H_{52}N4O_{24}Cu_4$•4 H_2O: Calcd (found): C 43.28 (43.37), H 4.18 (4.19), N 3.88 (3.86).

ESI-MS: Molecular peak was found at *m/z* = 1394 (ESI+;dichloromethane; $[M+Na]^+$) and 1372 (ESI+;water and small amount of sodium acetate trihydrate; $[M+H]^+$).

4.2 Physicochemical measurements

Elemental analyses were carried out on an Elementar Vario Microcube elemtal analyzer in CHNS mode. Oxygen content analysis was carried out on an Elementa rapid OXY Cube elemental analyzer.

IR spectra were recorded using a Bruker Alpha-P Infrared-spectrometer equipped with a Platinum-ATR with a diamond crystal.

Optical absorption spectra were recorded with a Varian Cary 5000 spectrometer. Samples prepared as solutions in methanol and water with small amount of sodium acetate trihydrate were used. **1** displays three absorption maxima in both, methanol and a slightly basic aqueous solution. For methanol as solvent the maxima are at 382 nm, 275 nm and 238 nm. The maxima for the slightly basic aqueous solution are at 371 nm, 277 nm and 237 nm.

^{1}H NMR spectra for **Na_2HL** were recorded in methanol-d4 with a Bruker DRX 300 MHz spectrometer at room temperature. Chemical shifts were quoted in ppm relative to the residual protons of deuterated solvents.

Electrospray ionization mass spectrometry (ESI-MS) was performed on a Finnigan LTQ-FT spectrometer by Thermo Fischer Scientific in the positive and negative ion mode with solvent as carrier gas.

X-ray diffraction studies

Single crystal of **$[Cu_4(HL)_4]\cdot 4.6H_2O$** was mounted on an IPDS2 diffractometer equipped with an image plate detector and graphite-monochromated MoK_α radiation. Problems with weak X-ray scattering and solvent loss had to be faced.

4.3 Refinement details

Two $(CH_2)_2(COO)$ moieties were found to be disordered in two positions. The positions were refined with fixed half-occupancies setting restraints on C-C (1.54(5) Å), C-O (1.25(5) Å) bond lengths and O…O distances (2.25(5) Å). EADP constraints were applied to the corresponding atoms displacement factors. Anti-bumping restraints were applied to the H10F…H96B, H13A…H54A at -x+1, y-0.5, -z (1.90(5) Å) and O63…O1W (2.60(1) Å) distances. The C11-phenyl ring was treated as a rigid hexagon. 6 water molecules of solvation were localized. The occupancies refined to 1.0 for O1W, O3W, to 0.8 for O2W, O4W, O5W, to 0.2 for O6W and were subsequently fixed. The remaining solvent molecules were heavily disordered and required application of a SQUEEZE procedure[9] removing 0.6-6 water molecules from four voids of 31-195 $Å^3$ volume. On the difference Fourier map the highest maximum of 0.86 e/$Å^3$ was found at 2.15 Å from the H13B atom, attributable to additional solvent molecules of low occupancy which could not be assigned a reasonable model.

4.4 Stability tests

The stability of the complex **$[Cu_4(HL)_4]\cdot 4.6H_2O$** under physiological conditions was determined by IR spectroscopy. 144 mg (0.1 mmol) of the copper complex was dissolved in 10 mL PBS. The green solution was aliquoted (1 mL) and transferred to micro tubes. With a thermal shaker the tubes were heated to 37 °C and shook at 300 rpm. After 0.5 h, 2 h, 6 h, 20 h, 24 h and 48 h the particular tube was removed from the shaker, the solution was dried *in vacuo* to dryness and the remaining green solid was analyzed by IR spectroscopy.

The stability of the ligand **Na_2HL** under physiological conditions was determined by NMR spectroscopy. 10 mg of the ligand was dissolved in a NMR tube in 0.7 mL PBS/D2O (90:10) preheated to 37 °C and the measurement was started immediately. Further measurements were done in 2 min-steps for 10 minutes, after 2 h and 24 h. After 24 h a color change of the solution to brown was observed. The *ortho*-vanillin/ligand ratio was determined from the integrals of the particular signals. Baseline and phase corrections were done with Bruker TopSpin 3.5 pl2. Integration and images were done with MestReNova 6.0.2-5475.

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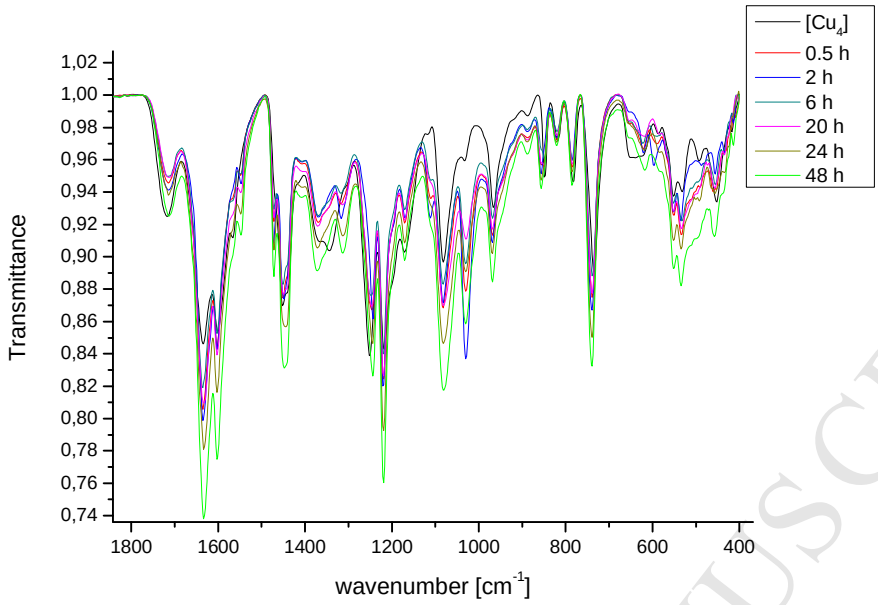


Figure 1. IR spectra of **$[Cu_4(HL)_4]\cdot 4.6H_2O$** as synthesized (black line) and after various time spans.

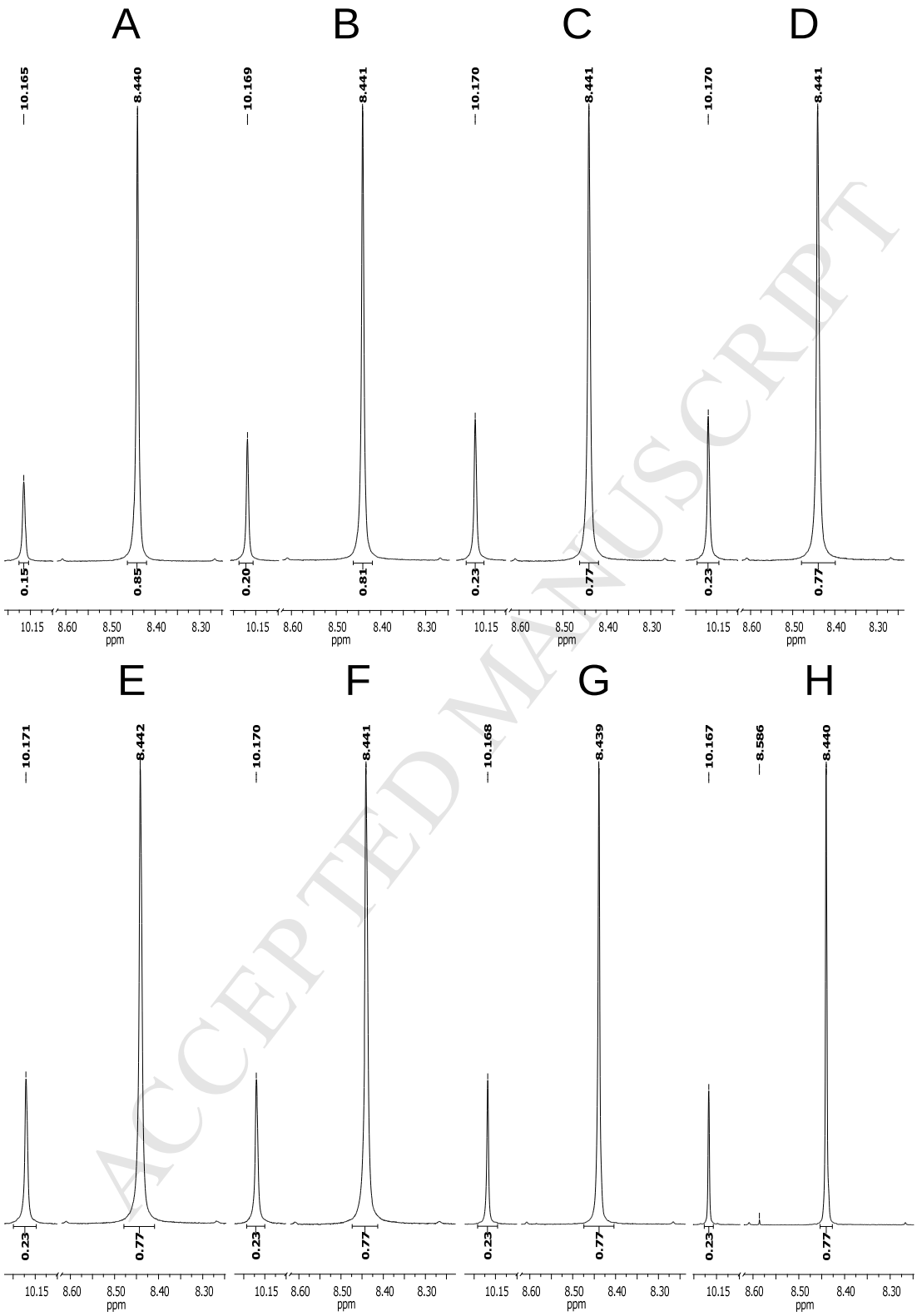


Figure 2. NMR spectra of **Na_2HL** dissolved in PBS/D_2O (90:10) recorded after various time spans (see text). The relevant signals and integrals are shown only for the ligand and *ortho*-vanillin.

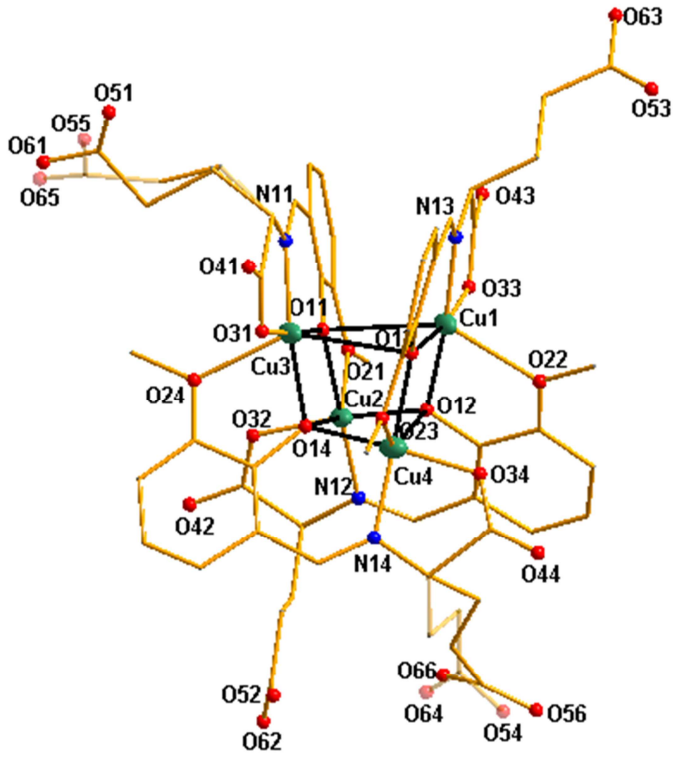


Figure 3. The molecular structure of **$[Cu_4(HL)_4]\cdot 4.6H_2O$** with atom labelling scheme (thermal ellipsoids of the non-C/H atoms are plotted at 30% probability level). C atoms are shown as sticks and H atoms are omitted for clarity. Disorder components are distinguished with different transparencies.

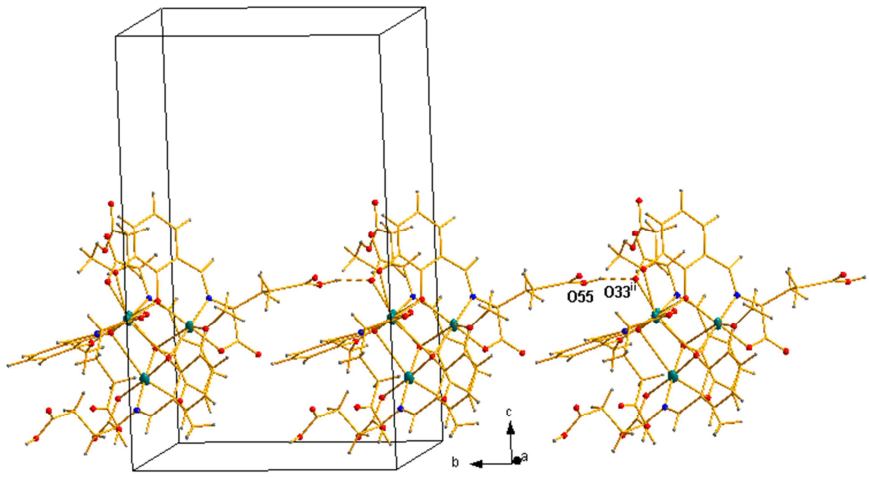
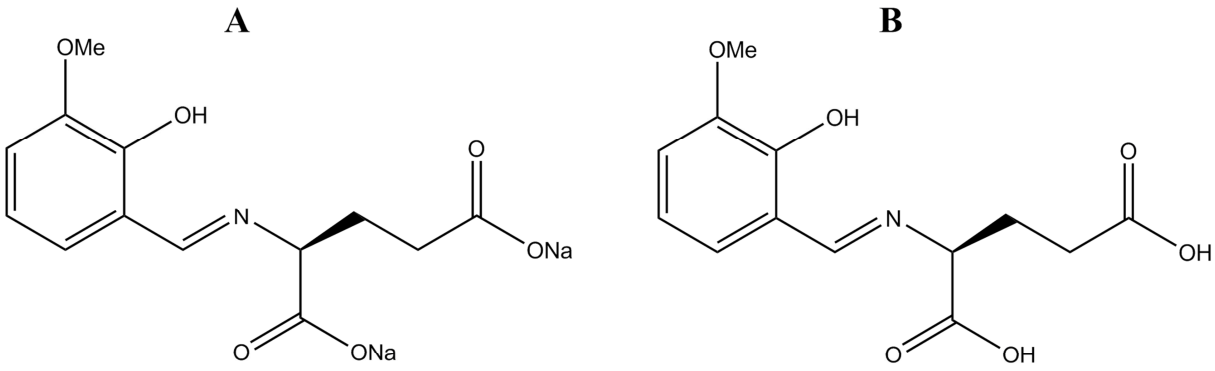


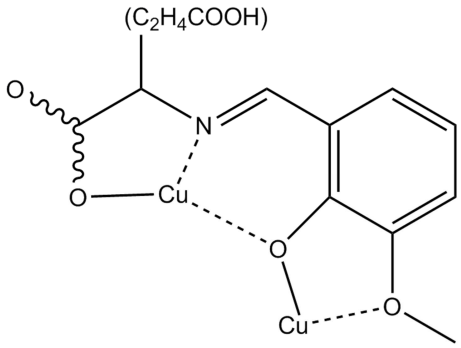
Figure 4. Hydrogen-bonded chains along [010] in **$[Cu_4(HL)_4]\cdot 4.6H_2O$**.



Chemical Formula: $C_{13}H_{13}NNa_2O_6$

Chemical Formula: $C_{13}H_{15}NO_6$

Scheme 1. Structural and chemical formula of the ligand as disodium salt (A; abbrev. **Na_2HL**) and its neutral form (B; abbrev. **H_3L**).



Scheme 2. Coordination mode of **HL** in **$[Cu_4(HL)_4]\cdot 4.6H_2O$** (stereochemistry not shown).