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DOCTORAL THESIS

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**Phenolate-bridged dimeric macrocyclic
ligands**

Dimerní macrocyclické ligandy spojené fenolátovým můstkem

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Abstract

Copper is one of the most prevalent biogenic metals. It can be found in virtually all living organisms, fulfilling many roles critical for the functions of life. Many proteins, that utilize copper cations for their function, bear clusters of two or more metal ions. These active copper sites can be simulated and even exploited by synthesizing their artificial analogues where the structure of protein with coordinating amino acids is replaced with nitrogen containing macrocycles or multipodal ligands. In presented work, two bis(macrocyclic) ligands with two cyclam units connected by *p*-cresol or *p*-nitrophenol spacers were synthesized. From these, mono- and dicopper(II) complexes were prepared and studied by combination of spectral, diffraction and electrochemical methods, revealing that the phenolate (regardless of *para* substituent on the aromatic ring) coordinates central Cu^{II} only weakly or not at all. Although all obtained crystal structures show cyclam-Cu^{II} subunits in *trans*-III geometry, HPLC-MS, UV-Vis, polarography (DCP) and cyclic voltammetry (CV) indicate presence of more than one isomer in solutions with their relative abundances dependent on pH. Electrochemical behaviour shows irreversible 2 electron reduction of Cu^{II} cations which are decoordinated and amalgamated during reduction. During anodic CV scan, copper is re-oxidized and re-coordinated. Rate of this coordination is dependent on pH and can be observed during subsequent steps of CV as a ratio between free Cu^{II} cations and complex. Different ratio of isomers of complexes was also observed during subsequent cycles of CV, as they are formed at different reaction rates, and show different thermodynamic stabilities. The ligand containing nitro group shows 4 electron irreversible reduction that overlaps with irreversible reduction of Cu^{II} cations. Weak reversible oxidation-reduction couple was revealed in free form of *p*-cresol variant of free ligand, that disappears upon coordination to Cu^{II}. The ligands and complexes also slowly degrade when incubated in aqueous solution. This degradation progresses more rapidly in heated samples and composition of degradation products depends on the pH of the aqueous solution. These degradations processes are complex, but mostly follow "retro-Mannich" mechanism, where methylene bridge between phenol moiety and cyclam breaks, resulting in either formation of aldehyde or alcohol, or release of formaldehyde and formation of aminal from cyclam subunits.

Keywords: Macrocyclic ligands, copper(II) complexes, UV/VIS spectroscopy, electrochemistry, stability

Abstrakt

Měď je jedním z nejčastěji vyskytujících se biogenních kovů. Vyskytuje se v téměř všech živých organismech, kde plní funkce kritické pro jejich přežití. Mnoho proteinů obsahuje měď ve formě klastrů tvořených více ionty. Tato aktivní místa lze simulovat, případně i využívat, ve formě jejich syntetických analogů, ve kterých jsou koordinující aminokyseliny nahrazeny dusíkatými makrocyclickými ligandy. V této práci byly připraveny ligandy obsahující dvě cyklamové jednotky spojené *p*-kresolovým nebo *p*-nitrofenolovým můstkem. Z těchto ligandů byly připraveny mono- a diměďnaté komplexy, které byly studovány pomocí spektroskopických, difrakčních a elektrochemických metod. Tyto studie ukazují, že fenolát (ať *p*-kresolový či *p*-nitrofenolový) koordinuje měďnaté kationty pouze slabě nebo vůbec. I když všechny získané krystalové struktury obsahují cyklamové jednotky ve formě *trans*-III isomerů, HPLC/MS, UV-Vis, polarografické (DCP) a voltametrické (CV) experimenty ukazují na přítomnost většího množství izomerů v roztoku. Relativní zastoupení těchto izomerů závisí na pH. Elektrochemické chování měďnatých komplexů vykazuje dvouelektronovou ireverzibilní redukci měďnatých kationtů, spojenou s jejich dekoordinací a vytvoření amalgámu na pracovní elektrodě. Během anodického scanu je tato měď reoxidována a opětovně koordinována. Rychlost této koordinace je závislá na pH a může být pozorována jako poměr koncentrací volných měďnatých kationtů a komplexu v roztoku. Různý poměr izomerů komplexu byl také pozorován během následných cyklů CV, jelikož izomery vznikají s různými rychlostními konstantami a mají odlišné termodynamické stability. Ligand obsahující *p*-nitrofenolový můstek vykazuje čtyřelektronovou ireverzibilní redukci, která se překrývá s redukcí měďnatých kationtů. Slabý reverzibilní oxidačně-redukční děj byl pozorován u volné formy *p*-kresolového ligandu a mizí s koordinací měďnatých kationtů. Ligandy a komplexy pomalu degradují, pokud jsou ponechány ve vodných roztocích. Tento proces se výrazně zrychluje zahříváním, složení degradačních produktů závisí na pH. Degradační procesy představují komplexní soubor, ale většina procesů probíhá "retro-Manichovým" mechanismem. Methylenové můstky mezi aromatickým jádrem a cyklamovou jednotkou jsou rozloženy za vzniku alkoholů či aldehydů, ale může docházet též k uvolnění formaldehydu a vzniku aminů na cyklamových jednotkách.

Klíčová slova: Makrocyclické ligandy, měďnaté komplexy, UV/VIS spektroskopie, elektrochemie, stabilita

1 Overview

All known living organisms utilize (and are made from) proteins. Proteins are naturally occurring polymeric chains of amino acids. These molecules fulfil many roles in living organisms, from structural (like collagen or keratin) to enzymatic. Many enzymes (proteins that enable chemical reactions like catalysts) need cofactor for their proper function. These cofactor are often metallic ions from a group called biogenic metals. These include several *s* elements (Na, K, Mg, and Ca), some first row transition metals from *d* group (Mn, Fe, Co, Cu, Zn) and Mo[1]. These metals can be either coordinated by donor atoms from amino acids or enclosed in heme-like group, like iron in haemoglobin.

1.1 Role of copper in living organisms

Copper is an essential biogenic metal for all living organisms. Naturally occurring compounds contain copper mainly in its Cu^{II} oxidation state. This is the most stable oxidation state of copper and it is typical representative of d^9 electron configuration with its typical octahedral distortion (Jahn-Teller effect).

In proteins, copper acts mainly as redox mediator[2] because it can be easily reduced by one electron to Cu^{I} and back. To ensure that active copper center can stay active regardless of its oxidation state, it is coordinated by rigid structure created from amino acids, that can act as ligands. These amino acid are combination of histidine, cysteine, methionine and sometimes glutamate side chains. This coordination sphere has close connection to function of the active copper site. and it ensures that toxic Cu^{I} ions are not released from the protein, as they could generate harmful radicals.

Copper cations (or the active centre) contained in protein can be classified by the coordination(fig. 1.1) of Cu^{II} : in type I, copper is coordinated strongly by two methionine and one cysteine and more loosely by the fourth ligand in distorted tetrahedral coordination sphere. This type has typical blue color in oxidized state (giving name to blue copper proteins). Type II has four nitrogen or oxygen donors in square plane around the central atom. It lacks the color of type I. Type III is binuclear center and can be differentiated from the other two centres because it is EPR silent. This means that single electrons from the d^9 electron configuration are antiferromagnetically coupled[3].

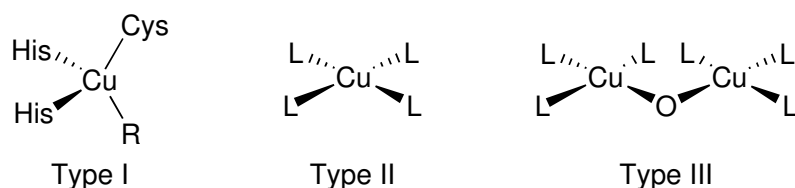


Figure 1.1 Three types of copper active sites in proteins. R = Met (typically), L = N or O ligands

Sometimes type II and type III combine into one trinuclear copper site that is then sometimes denoted as type IV site. Copper-bearing proteins are also classified by number of these active copper sites into single and multicopper proteins. Monocopper proteins with type I center are represented by proteins like azurin, rusticyanin or plastocyanin. These small single copper bearing proteins all share the same function: single electron transfer. These proteins act as intermediators in electron transport chains, e.g. plastocyanin transfers electrons from cytochrome f to photosystem I during photosynthesis.

An example of a protein with one active copper site that contains two Cu^{II} cations is hemocyanin, respiratory protein of molluscs and arthropods. In its deoxygenated form, the Cu^{I} ions are independent and coordinated in planar trigonal geometry. Upon oxygenation, the Cu^{I} cations are oxidized (the O_2 molecule is coordinated in peroxide form) and are closer together, forming a planar shape (see fig. 1.2) from the two Cu^{II} ions and the now coordinated O_2^{2-} . Histidine residues move only slightly from the original plane[4, 5, 6]. This coordination results in type III site.

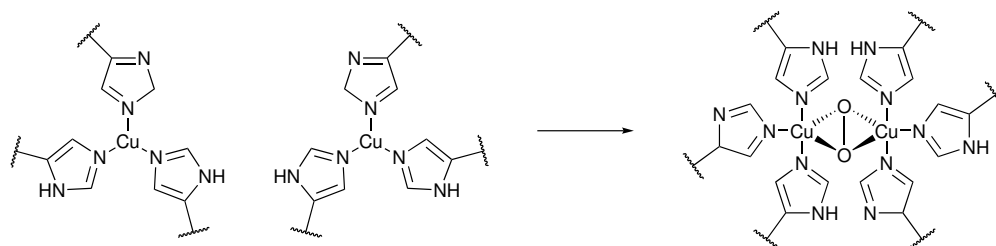


Figure 1.2 Oxygenation of active center of hemocyanin

As this process is favourable at $\text{pH} = 7$ the oxygen binds readily to the dicopper center of hemocyanin. Oxygenated hemocyanin is then transported through the organism. Upon arriving into oxygen-starved tissues oxygen is released in its molecular form because of the lower pH in oxygen-starved tissues (similarly to the function of hemoglobin, in accordance with Bohr effect[7]).

Structure of hemocyanin dicopper site is isostructural with active sites of catechol oxidase and tyrosinase, even though they fulfil different function in living organisms: instead of transport, they both facilitate oxidation of hydroxyl groups in

phenols and catechols. Catalytic activity of catechol oxidase (or its active dicopper site) is shown in fig. 1.3. These processes are closely connected to signalling pathways in mammals (dopamine pathway) and o-quinone synthesis. O-quinones fulfil various roles in various organisms, from melanin synthesis in humans to its antibacterial and antifungal activity in plants[8, 9].

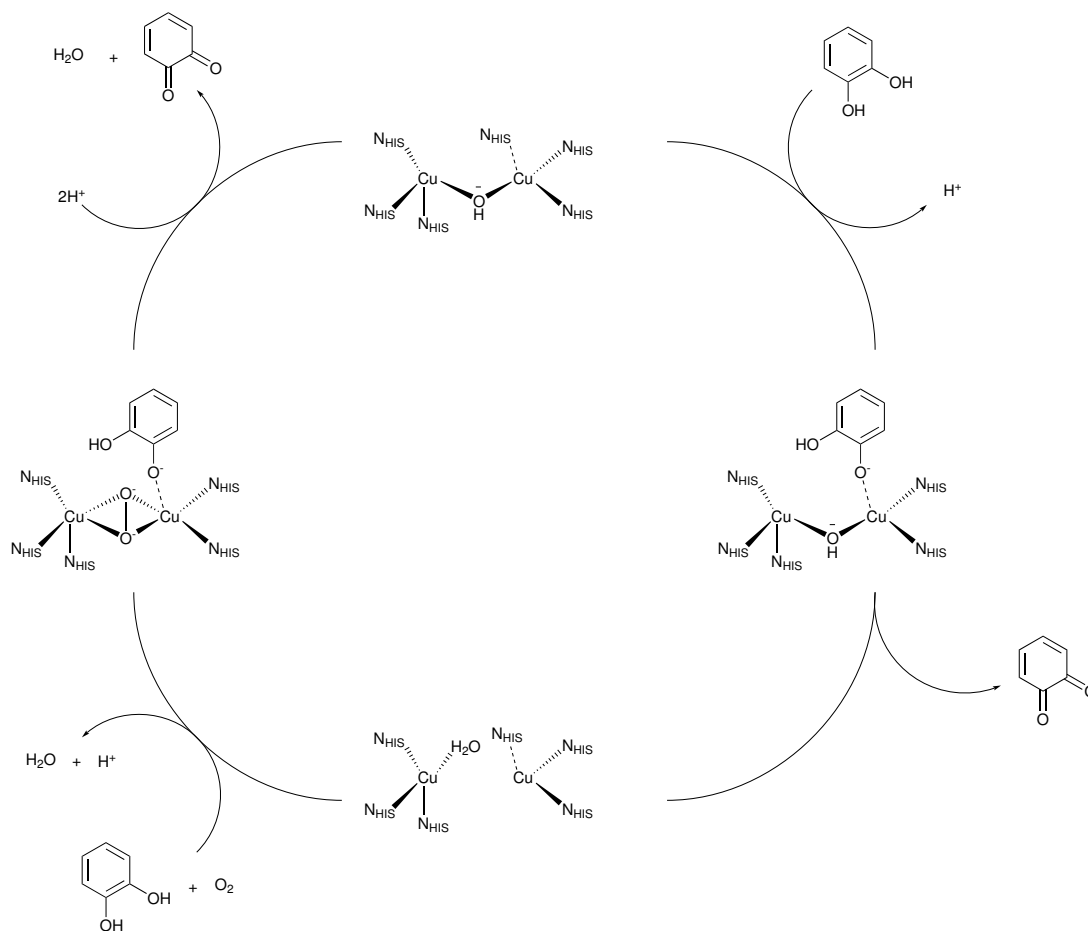


Figure 1.3 Enzymatic activity of copper center of catechol oxidase. Edited from [9], oxidation numbers of copper cations are omitted for clarity.

Proteins that contain multiple copper active sites usually combine several types of active copper centres in their structure. Large group of proteins called blue copper oxidases have at least one type I centre which serves as electron acceptor from oxidised substrate. An example of this process would be protein laccase[10], in which single electron oxidation leads to radical crosspolymerisation leading to lignin. Extracted electrons are then transported to the combined type II and type III center, in which oxygen is reduced to water. This process can be found in many different oxidases, e. g. protein-lysine 6-oxidase, dopamine- β -monooxygenase, monophenol monooxygenase and peptide- α -amidating monooxygenase[2].

The combination of type II and type III is not the only solution nature has found for the oxygen reduction. Alternative pathway is found in cytochrome c oxidase. This enzyme terminates respiratory electron transport chain. The protein consists of several subunits and contains two heme units and two copper centres (one type I and one type III). Conversely from oxidases, the type III center acts as receiver for electrons from cytochrome c. These electrons are then passed internally to binuclear site formed from one heme unit and the type I copper center, where oxygen is reduced to water. This process is also connected to the transport of protons across the mitochondrial membrane. Hydrogen potential across the membrane established in this way serves as an energy source for ATP synthase, and therefore synthesis of ATP which is the final goal of respiratory chain[11].

1.2 Complexes mimicking biological functions

Copper, and other transition metals, play various roles in chemical processes of living organisms. One can therefore expect that compounds of similar structures and functions were synthesized artificially, whether for the purpose of mechanistic studies or as biologically inspired catalyst, that can be used in various industries. There are many examples of artificial catalysts that are inspired or similar in structure to some naturally occurring proteins and some of these will be discussed below to illustrate inspiration for our work. These examples can be split along two lines: the number of metal ions present in the complex; or the nature of the ligands that bind this metal (or these metals). In natural conditions, many active centres of proteins contain more than one metal ion. Similar strategies were used in searching for their synthetic analogues. Probably because they are synthetically more accessible many of these enzyme mimicking complexes contain open chained ligands.

1.2.1 Open chained compounds

Mononuclear open chained transition metal complexes could fill pages upon pages of reviews. For example many older MRI contrast agents contained complex of Gd^{III} with linear ligands (Magnevist®, Optimark®). However, these compounds were banned in 2017 by European Medicine Agency [12] because of their questionable stability. This will be discussed more closely below.

There are of course many other applications for open chained ligands. For example there is an effort to simulate photosynthesis, because it is a process that, in short, effectively fixes solar energy into chemical bonds while generating oxygen. It is

therefore a process worth mimicking. Open chained Co^{II} complexes (fig. 1.4) were studied as cocatalysts for water oxidation by light. Photosensitizing $[\text{Ru}(\text{bpy})_3]^{2+}$ complex was used in these experiments mimicking, in a way, natural photosystems. The system of two complexes has shown photooxidation of water to oxygen but closer study has revealed that the catalytic activity came from nanoparticles of $\text{Co}(\text{OH})_x$. The complexes acted as precatalyst and the active particles resulted from their oxidation by sodium persulfate $\text{Na}_2\text{S}_2\text{O}_8$ present in the reaction as an electron acceptor[13].

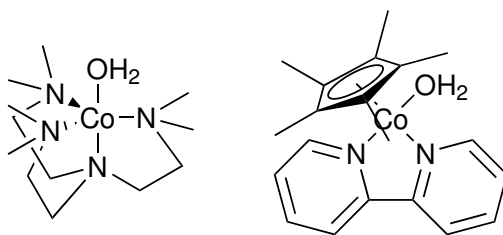


Figure 1.4 Cobalt complexes investigated for their water oxidation ability

1.2.2 Dinuclear open chained compounds

As was mentioned before, many naturally occurring catalysts employ more than one metal ion (like dicopper centre of catecholases or iron-copper centre of cytochrome c oxidase). There is therefore large number of dinuclear complexes that have some enzyme-like activity. What follows is a small window into large field of study, once again aiming to inform the reader about our motivation. Enzymes can employ metals other than copper. One open chained structure containing two Fe^{III} (see fig. 1.5) was synthesized in effort to mimic activity of catecholases[14]. Prepared dinuclear complex has indeed shown catecholase-like activity. In its structure, it contains two Fe^{III} cations captured each by tripodal ligand and bridged by phenolate. In the study, the complex was reduced during catalysis and reoxidised by molecular oxygen present in the solution. This activity is similar to some dinuclear Cu^{II} complexes, although the mechanism is distinct from catechol oxidase. Instead of water, the oxygen is reduced to hydrogen peroxide.

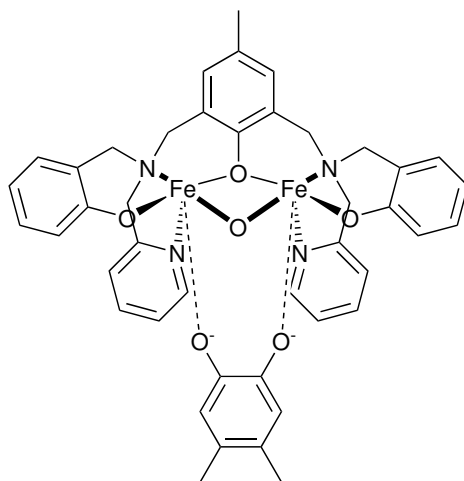


Figure 1.5 Binuclear Fe^{II} complex with catecholase-like activity. The picture is an active intermediate proposed by the authors[14].

Apart from catecholase-like activity, this complex has also shown DNA cleavage[14]. Nickel complexes of similar dinuclear open chain structure have also shown antibacterial activity[15].

Number of dicopper complexes with structure similar to that in fig. 1.5 is very large and the research was summarized in many reviews[16, 17]. The motif of two tripodal ligands connected by aromatic or aliphatic unit is repeated in literature many times. Large portion of this research is concerned with study of catecholase-like activity of these compounds. An example of two dinuclear compounds can be seen in fig. 1.6. Both of these complexes can act as catecholase and, in presence of H_2O_2 can oxidize 1,2-diphenols to catechols. There are even mononuclear copper complexes that can catalyse these oxidations, but current understating states that the particle active in catalysis is dimeric complex that is formed in solution from the mononuclear species.

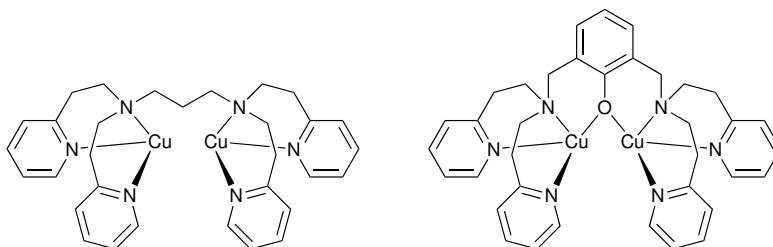


Figure 1.6 Dinuclear Cu^{II} complexes. Complex with phenolate bridge[18] is more active in oxidation of phenols than the complex with propylene linker [16, 19]

Some researchers have described different behaviour of dinuclear complexes: rather exotic example is CO_2 fixation and subsequent reduction to oxalate (fig. 1.7)[20]. From this example (and maybe the di-iron complex in fig. 1.5)

we can see that the wealth of possible configurations and activities of dinuclear complexes like those mentioned here is rather wide.

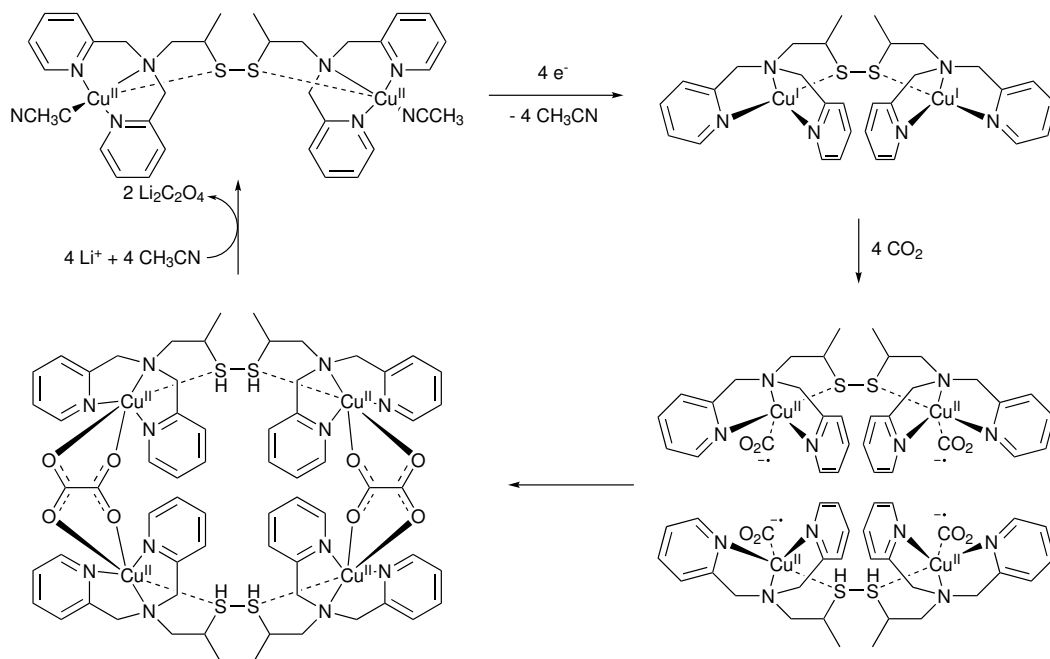


Figure 1.7 Catalytic cycle of binuclear copper complex that can bind and electrochemically reduce CO_2 to oxalate[20]

From the example at the beginning of the chapter and from the EMA resolution about contrast agents for MRI one can conclude that linear ligands are not exactly stable. Experiments done with dicopper complexes are overwhelmingly done in nonaqueous environment. Data about formation of the complexes and their thermodynamic and kinetic stability also aren't available. Majority of the complexes are not even soluble in water, therefore not exactly useful for current direction of green chemistry. This fact also prevents study of their behaviour in response to pH, which is important in study of natural processes. Proteins or enzymes do not suffer from these issues and can exist in solutions with high concentrations of competing metal cations. Their behaviour also heavily depends on changes in pH in living organisms (see hemocyanin). The way to copper complexes that are highly stable (thermodynamically and kinetically), soluble in water and even resistant to transmetalation lies in macromolecular ligands and macrocyclic effect.

1.2.3 Macrocyclic compounds

Macrocyclic compounds are defined as cyclic macromolecules. To act as ligands they also have to contain some functional groups that can create coordination bond. These two conditions lead to macrocyclic ligands. Their typical representa-

tives are alkane rings from nine members up with some heteroatoms in the ring structure, mainly (but not limited to) oxygen, nitrogen and sulphur. This trio gives rise to crown-ethers, azamacrocycles and thia-crown ethers.

Nature of these heteroatoms in the cyclic alkane derivative has defining effect on nature of these compounds as ligands. The heteroatoms play large role in selectivity of these ligands towards various metals, for example: crown ethers are essential part of organic synthesis as they can bind alkali metal ions and resulting complexes are usually soluble in organic solvents. Similarly, thia-crown ethers show high affinity to heavier metal ions like silver or mercury[21], with nitrogen atom being somewhere in the middle. The affinity that these compounds show towards different metal cations is governed by "soft" and "hard" metal-ligand theory[22], which states that smaller, highly charged, therefore less polarizable (hard) cations bond more readily with donors of similar properties (this is exemplified by complexes of crown ethers with alkali metal ions like K^+) and larger, less charged metal ions like Ag^+ or Hg^{2+} would prefer ligands with softer donating atoms or groups, like thioethers or phosphines.

All macrocyclic ligands act as chelators. Chelators are molecules that can create more than one coordination bond with central metal ion, usually by having more than one donating functional group (a group with free electron pair). This multidenticity leads to some differences when compared to formation of complex with monodentate ligands. This difference can be easily seen in formation of hexamine copper(II) cation ($[Cu(NH_3)_6]^{2+}$) and tris(ethylenediamine) copper(II) cation ($[Cu(en)_3]^{2+}$) in fig. 1.8. Both of these compounds are formed from hexaaqua copper(II) species found in aqueous conditions and the affinity of amine functional group to Cu^{II} should be similar to the ammonia. But there are differences between the two complexes.

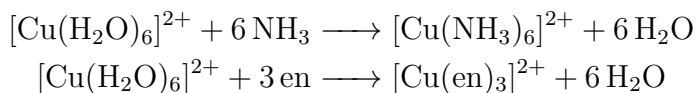


Figure 1.8 Reaction between ammonia and Cu^{II} and ethylenediamine and Cu^{II} in aqueous conditions. Note the change of the number of particles in both reactions: seven to seven in the case of ammonia but four to seven in case of ethylenediamine.

When hexaaqua metal ion reacts with multidentate ligand, the molecules of water are displaced by the donor groups of the ligand. This means that ligands with more than one donor group release more than one molecule of water from the starting aqua complex. This in turn leads to bigger number of particles on the right side of equation and therefore the entropy of the system grows (more particles means more randomness in the system).

Another effect that comes with chelators is ring formation. Ethylenediamine forms 5-membered ring with central metal, very stable (energetically low) conformation. Of course, these are not alkane rings and their geometry and energy of tension is related to the geometry of the coordination bond. Macrocyclic compounds can take advantage of both of these effects by utilizing them multiple times. In this way, we can get from simple ethylenediamine to smallest macrocycle 1,4,7-tiazacyclononane, usually shortened to TACN.

TACN is 9-membered ring with three nitrogen atoms that can be thought of as three condensed ethylenediamine molecules. It was synthesized in 1978 by K. Wieghardt et al.[23]. There are many more macrocyclic compounds with similar structure (alkane ring with secondary amine functional groups interspersed within the enclosed ring), mainly cyclam and cyclene, which with TACN form a trio of probably most relevant nitrogen based macrocyclic compounds (see fig. 1.9), although many bigger and less symmetrical compounds were studied and synthesized throughout years.

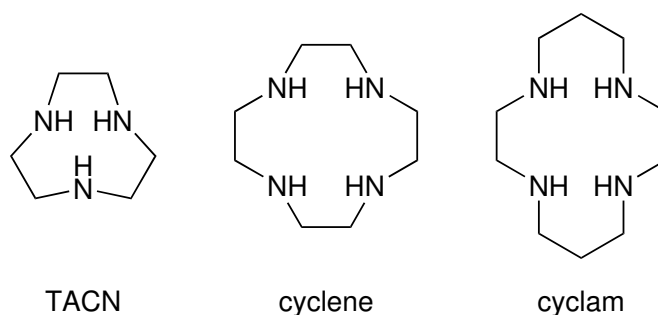


Figure 1.9 Trio of most studied azamacrocyclic compounds

The ring formation mentioned above plays an important role in matching of the ligand and the complex. Rather than bite angle of bidentate ligands like ethylenediamine, macrocyclic ligands are defined by the size of the cavity that is enclosed by the ring. Shape of coordination bond then depends on size of the metal and size of the cavity. Goodness of match between metal and ligand is closely related to stability constant describing formation of the complex. We can see these effects on the following copper complexes (compare stability constants of similar amino complexes of Cu^{2+} : $\log K_{[\text{Cu}(\text{en})_2]^{2+}} = 19.6$, $\log K_{[\text{Cu}(\text{cyclen})]^{2+}} = 23.29$ and $\log K_{[\text{Cu}(\text{cyclam})]^{2+}} = 27.2$)[24, 25].

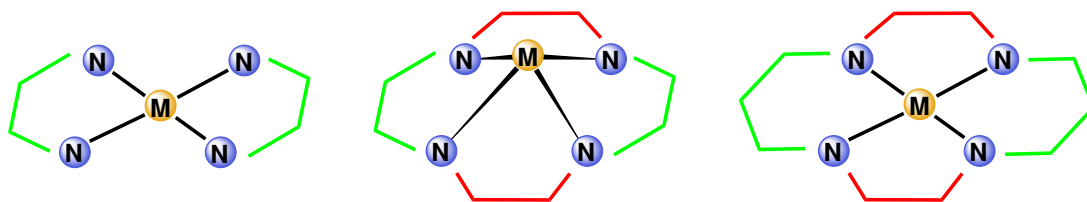


Figure 1.10 Comparison of geometry of nitrogen based copper complex cations, from left to right $[\text{Cu}(\text{en})_2]^{2+}$, $[\text{Cu}(\text{cyclen})]^{2+}$, $[\text{Cu}(\text{cyclam})]^{2+}$, axial ligands are omitted for clarity

Fig. 1.10 shows geometry of copper complex cations. Bis(ethylenediamine) complex is stabilized only by two 5-membered rings, being less stable than cyclen complex. The difference of stability constants between cyclen and cyclam complexes is caused by more favourable geometry of the latter one. In cyclam, copper cation fits inside the macrocyclic cavity, whereas in cyclen the copper cation cannot occupy same plane as ligand nitrogen atoms. This favourable compatibility leads not only to high thermodynamic stability (cyclen complex is also thermodynamically stable) but cyclam complex is also highly kinetically stable. That is important when we want to study enzymatic behaviour of any complex, because our model compound should be resistant to any changes during the enzymatic cycle. This ensures that the enzymatic activity comes from the complex and not from some intermediate that exists in small amount in the solution. Kinetic and thermodynamic stability is also important to applications of these potential catalysts, as it should ensure longer usability. Cu^{II} cyclam complex is one of the most stable complexes of copper (considering macrocyclic ligands without pendant arms), which is the reason why are these complexes of high interest. First row transition metal ions (at least in their more common oxidation states) bind six donating atoms (ligands) that occupy octahedral coordination sphere. Nitrogen atoms in azamacrocycles can be modified further by alkylation (by means of substitution of hydrogen atoms) and these pendant arms can interact further with central atom. Typical example of this modification would be ligand **H₂te2a**, cyclam-1,8-diacetic acid.

Whether the cyclam is modified by pendant arms or not, its complexes can be found in form of several isomers that stem from orientation of hydrogen atoms (or pendant arms) in combination with ethylene and propylene fragments. These five isomers are shown in fig. 1.11, for clarity hydrogen atoms or pendants arms are shown only as bonds going either above or below plane formed by nitrogen atoms. In the case of Cu^{II} , isomer *trans*-III is the most abundant[26] and it is easy to see why: the nitrogen atoms on the opposite sides of Cu^{II} cation carry the pendant acetates in opposite orientation, therefore they can occupy both axial positions of the deformed octahedral (square bipyramidal) coordination sphere

around the central atom.

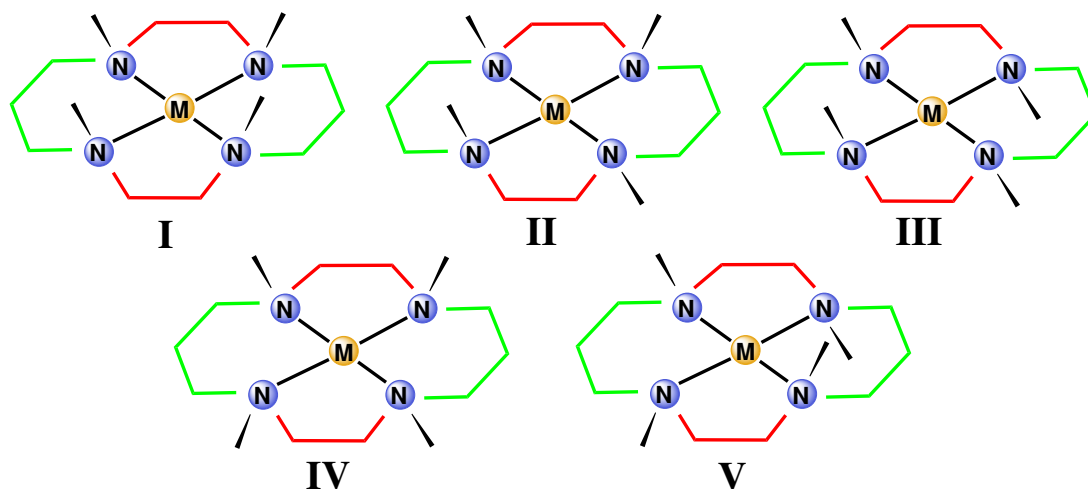


Figure 1.11 Isomerism of cyclam complexes

In the case of copper(II) complex with H_2teta , the stability constant is not significantly raised, most likely because of Jahn-Teller distortion and higher acidity of the ligand, but the selectivity of ligand toward copper(II) cation is raised significantly when compared with metal cations usually found in blood, like Ca^{2+} . Of course, pendant arms can introduce much more than just another donor atom for the coordination bond. The options are as far and wide as are possibilities of organic synthesis. Examples of these functionalities can range from bulky alkyl substituents that change ligand coordination properties[27], across functional groups that allow selective adsorption of the complex in human body (from bone[28] to cancerous growths[29]) back to the simple acetate in DOTAREM® (MRI contrast agent with Gd^{3+}).

One thing that connects many use-cases of macrocyclic compounds is their thermodynamic stability provided by macrocyclic effect. Macrocyclic ligands have many uses in many fields. In medicine, they can introduce toxic metal anions (like Ga^{3+} or Lu^{3+}) into living organisms without their pathological effects, because the ions are safely bound in the ligand cavity. In nuclear energy, they can be used for extraction of f-elements[30] due to easily tunable selectivity. This width of application is the reason for research of azamacrocyclic compounds.

1.2.4 Macrocyclic compounds modelling protein active centres

From view of coordination chemistry, copper ions are coordinated only loosely in enzymes. The active copper centres cannot fill the deformed octahedron typical

for Cu^{II} and histidine and glutamate amino acid residues can be considered too "hard" for Cu^{I} . Their stability is ensured by the protein that enfolds the active centre. This can be seen in hemocyanin: when it is partly denaturated, it starts to act like a catecholase. In its natural state, this cannot happen because the substrate can't fit through the channel of hemocyanin like oxygen does[31]. Other metals, like iron, cobalt or magnesium, can be found in macrocyclic compounds naturally like enterobactin and heme subunit[32, 33] (fig. 1.12). Both of these macrocycles bind iron. Enterobactin is so stable that the release in bacterial cytoplasm is facilitated by hydrolysis of amide bonds instead of decomplexation. Heme unit is found in many proteins, in hemoglobin it acts as a carrier of oxygen. Therefore when we are designing compounds that could act similarly to enzymes and have their stability, it is logical to turn to macrocycles.

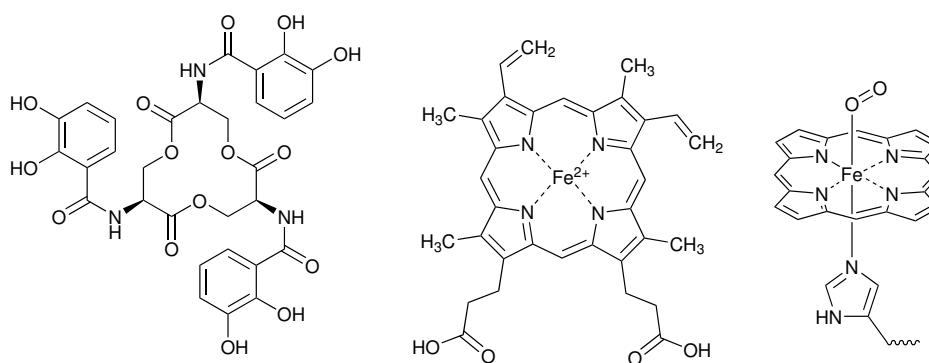


Figure 1.12 From left to right: Enterobactin, protein for transporting Fe^{III} into bacteria; Heme unit of human hemoglobin and its oxygenated form

Complexes with naturally occurring macrocycles like ZnPhc complex (fig. 1.13) were studied widely. ZnPhc can be used as an anticancer medicine: Coordinated oxygen can be excited to singlet state by IR light. Reactive oxygen species are then able to induce cell apoptosis in irradiated tissue. This process can be used as efficiently targeted anticancer therapy. Many other metals, including iron were tested with this ligand with variable results[34].

Not all enzymes that require iron for their function have heme subunit. These enzymes also usually activate oxygen, therefore are also of interest in catalysis. Macrocyclic compounds were synthesized as way to study these non-heme enzymes because they are usually unstable (especially their superoxo and hyperoxo forms involved in their catalytic activity) and the only way to study their spectroscopic and electronic properties is by synthesizing their analogues[35], like those in fig 1.13. Oxygen activation is a prominent challenge of today's chemistry, as it could provide a green path to many crucial chemical compounds.

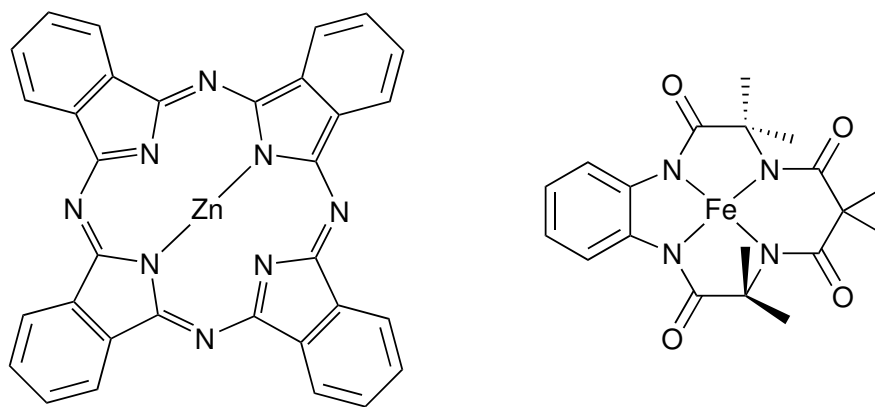


Figure 1.13 Left: ZnPhc complex; Right: [(TAML)Fe] complex that can activate oxygen and catalyse oxidation of benzaldehydes[35]

Cobalt coordinated by azamacrocycle is also often found in literature as a potential catalyst. Indeed, in [13] authors have described Co^{II} complex with cyclen and homocyclen ($[13]\text{aneN}_4$) that has shown lesser catalytic activity than open chain ligands. Lesser activity was most likely caused by higher stability of macrocyclic complexes and therefore worse $\text{Co}(\text{OH})_x$ (actual catalyst) generation. In a similar way, $[\text{Co}(\text{cyclam})\text{ClO}_4]\text{ClO}_4$ complex was used to create electrode coated with Co^{II} hydroxide/phosphate thin film. This electrode was then successfully used for electrochemical water oxidation with great yield[36].

Another closely studied process that has natural inspiration is CO_2 fixation and reduction. Here, cyclam and cyclam-like macrocycles with Co^{III} or Ni^{II} were closely studied. Although mechanism remains unclear, these complexes show strong coordination of CO_2 . This coordination can be followed by electroreduction resulting in CO. Should this process be effective enough, it can lead to transformation of excess CO_2 to fuel, either as through Fisher-Tropsch synthesis or as a precursor for methanol[37, 38].

1.2.5 Bis(macrocylic) complexes

Macrocylic ligands represent a stable site to employ metal ion in some chemical process. One can then expect that macrocylic ligands would be useful for binding more than one metal ion. The stability common in macrocylic complexes can even ensure geometrical relations between the metal ions. This spatial relation can be ensured by pendant arms, although bis(macrocylic) ligands connected by the substitution on alkane parts of the macrocycle are also known. Examples of both variants were studied for their antiviral activity. They are bicyclam and *p*-xylylene-*N,N*-bicyclam, sold under commercial name Plerixafor (fig. 1.14). Both these compounds show antiviral (mainly anti-HIV) properties, as well as cell immobilization. These effects are both connected to the bicyclam binding

to CXC4 site on cell surface. It is thought, that the actual active substance is dizinc complex and these bicyclic macrocycles act only as a prodrug. Many other bicyclics with different sizes of azamacrocycles show similar activity[39].

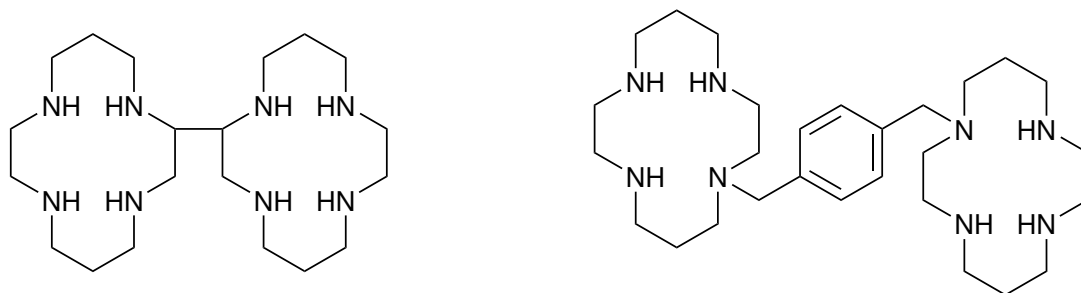


Figure 1.14 Left: bicyclam; Right: Plerixafor

Discovery of antiviral activity of bicyclam was more or less accidental, bicyclam was present in some batches of cyclam as a by-product of its synthesis. There are many more multimeric macrocycles. The desire for "more metal" per molecule is known from MRI contrast agents, where it is desirable to bind more than one Gd^{III} to one large ligand. The research in multimeric MRI is extensive and some examples are shown in fig. 1.15. Even though these complexes do not act like enzymes, they are good examples of possibilities that stem from close control of their coordination chemistry. The supramolecule connected with Fe^{II} complex is an example of massing gadolinium complexes, the DO3A ligands connected through EDTA-like linker has shown interaction with Ca^{2+} cations in human serum. This sensibility to Ca^{2+} ions is useful in some specialized areas, like MRI of brain, where fluctuating levels of Ca^{2+} are usually connected to abnormal neural activity. Both of these ligands represent a continuation of DOTAREM® contrast agent widely used in MRI[40, 41].

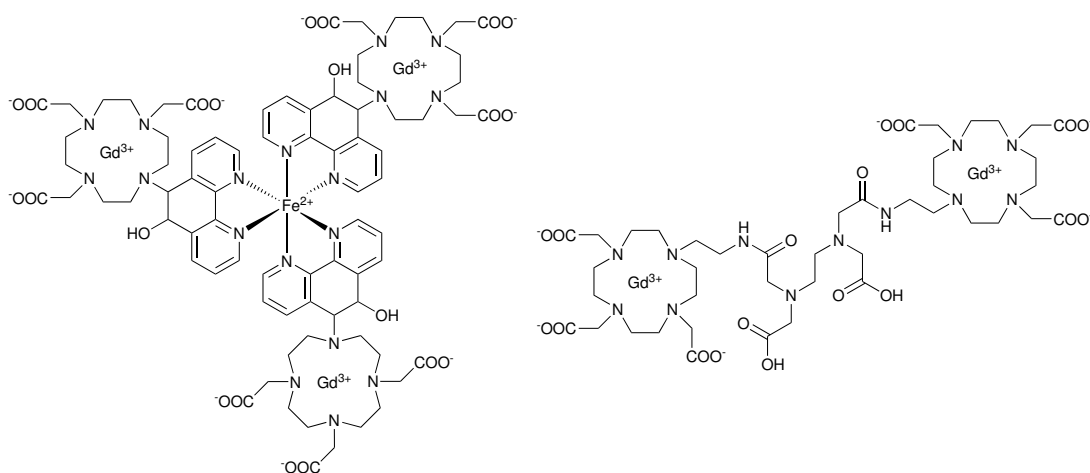


Figure 1.15 Left: MRI contrast agent with three Gd^{3+} centres; Right: Dinuclear MRI contrast agent that is activated by presence of Ca^{2+}

A rather large group of dinuclear macrocyclic ligands have a bridging ligand between the metal centres. This can not only lead to enzymatic activity, but also to some unexpected spectral properties. Luminescence of lanthanides can be enhanced by antenna effect. This is usually realized by some aromatic moiety bound to macrocycle that contains lanthanide. Such effect does not require coordination of antenna to the lanthanide and was observed in dieuropium complex of *p*-xylene-*N,N*-bis(DO3A), see fig 1.16. In this complex, Eu^{3+} emission is sensitized by the aromatic linker[42]. Similar sensitisation can be achieved in dinuclear complex where two Eu^{3+} are coordinated by one *p*-nitrophenol bridge[43] (fig. 1.16). Here, authors reported that the only luminescent species is the one, where *p*-nitrophenol acts as a bridging ligand. In mononuclear species, luminescence was not observed probably due to quenching of emissive state by ligand to metal charge transfer (fig. 1.16).

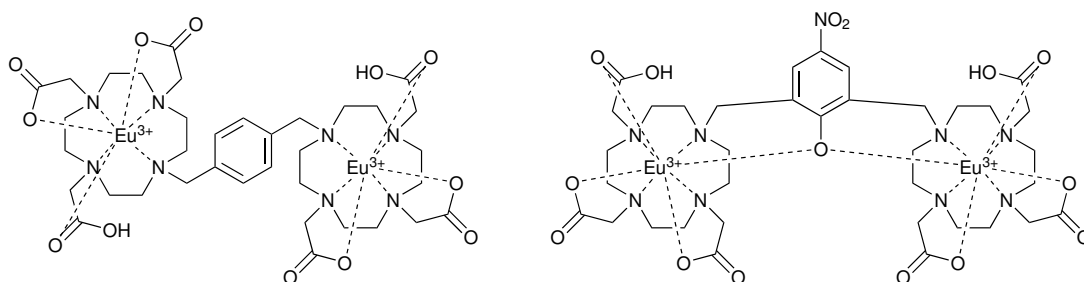


Figure 1.16 Luminescent dieuropium complexes, with and without bridging ligand

Coordination of both metal centres by phenolate also leads to biological activity, regardless of metal in macrocyclic cavities. Dinuclear complexes like these can often cleave DNA. Mechanism of this cleavage is shown on typical model compound bis(2,4-dinitrophenyl) phosphate, BDNPP in fig. 1.17. This compound is used as a model of phosphodiester bond, similar to phosphate bond of sugar phosphate backbone of DNA. BDNPP (and some other nitrophenolates) are used because the resulting dinitro aromatic compounds are easily detected and quantified by UV spectroscopy. The proposed mechanism of DNA cleavage starts with coordination of phosphate to one of the metal centres (no difference in mechanism has been reported between Cu, Fe and Zn), followed by nucleophilic attack of terminal hydroxo group. μ -OH group is a poorer nucleophile and stays coordinated[44]. Second method for studying DNA cleavage uses pUC19 as DNA model. pUC19 is a small (2686 base pairs) double stranded circular DNA. It has some endonuclease cleavage sites, and at these sites hydrolytic cleavage can occur. These cleavage products are identified and quantified by electrophoresis. This method is also very common, but it is not easy to compare observed rate constants from BDNPP and pUC19 methods.

Iron, copper and zinc complexes of the ligand have shown DNA (BDNPP) cleavage with similar rate constant ($k_{\text{obs}} \approx 5 \cdot 10^5$) in neutral pH. Rate constant of cleavage of these complexes differ in their response to pH: While iron complex has a maximum in neutral pH, both copper and zinc complexes cleave DNA more quickly in basic conditions. This difference is most likely caused by different pK_a of coordinated water molecule. The proposed mechanism relies on hydroxide ion coordinated to one of the metal ions[45, 46, 47].

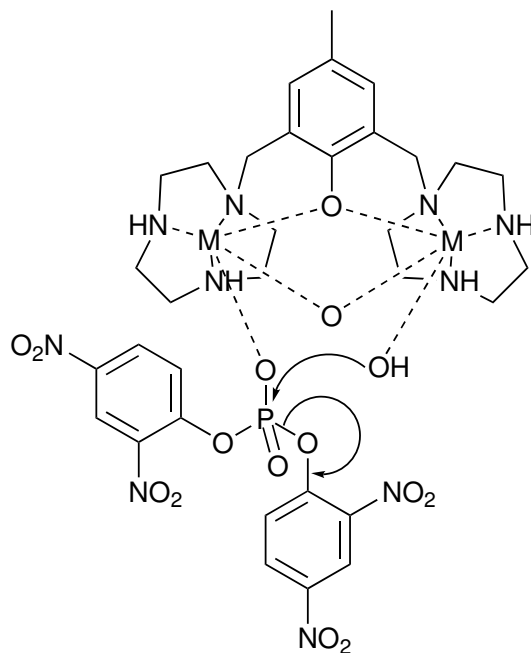


Figure 1.17 Proposed mechanism of DNA cleavage by dinuclear complexes. BDNPP serves as a model of phosphodiester bond present in DNA backbone. $M = \text{Fe}, \text{Cu}, \text{Zn}$

Replacing the two TACN subunits with cyclene (fig. 1.18) leads to different mechanism of DNA cleavage. The complex cleaves DNA slower than the TACN variant, however it is still active. This activity comes from participation of NH groups. The phosphate bond is destabilized and the nucleophilic attack comes from water molecule from the solution. The Cu^{II} is only pentacoordinated in solid phase, the situation in solution is less clear. There was an effort to pull cyclen subunits further apart by introducing amide bond instead of methylene bridge between aromatic centre and cyclen subunits. This leads to reintroduction of bridging $\mu\text{-OH}$ group into the complex. However hydrolytic DNA cleavage between amine and amide variant is virtually the same. Difference between these complexes can be observed in presence of hydrogen peroxide. While it has no effect on the amine variant, the amide shows oxidative DNA cleavage. Larger space between Cu^{II} can accommodate (or coordinate) peroxide, which is turn activated towards oxidative cleavage. Use of pUC19 as a DNA model is advantageous in

this case, because the products of hydrolytic cleavage can be differentiated from the oxidative cleavage products by electrophoresis. This activity of bis-cyclene dicopper complex with amide bonds simulates activity of purple acid phosphatases. These enzymes are common in many biological systems and are characterised by their dinuclear metal centres with bridging μ -OH group, but the metals are usually Fe^{III} or Mn^{II} . [48]

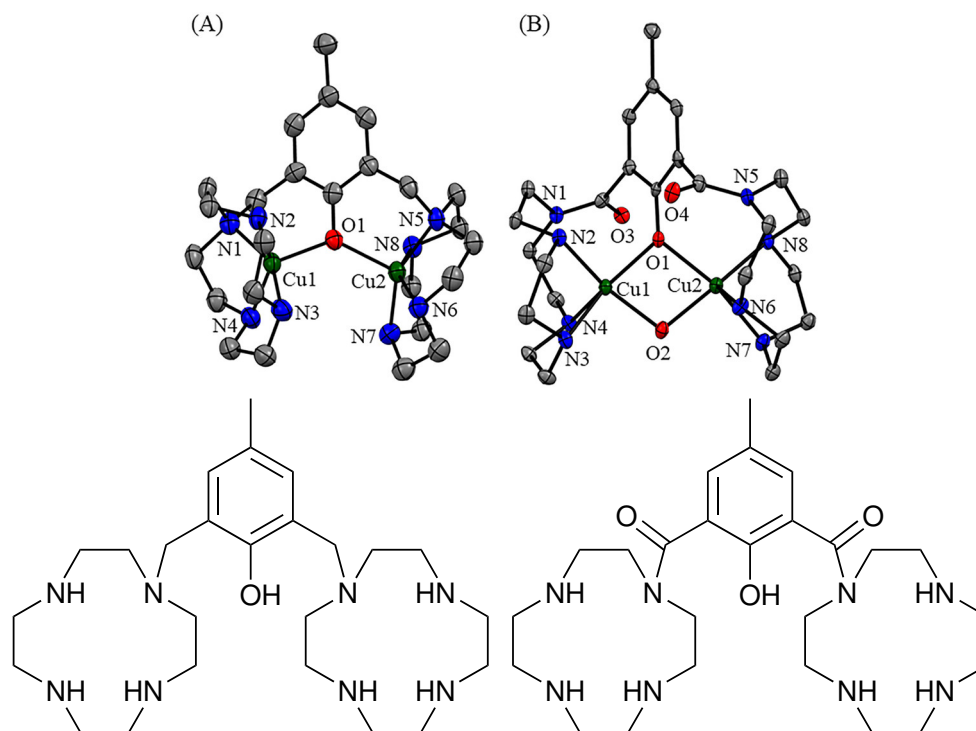


Figure 1.18 Copper complexes of phenolate ligands with cyclen subunits. Crystal structures taken from [48]

There are as of yet no data for complexes with similar structure that would connect two cyclam subunits. These should provide thermodynamic and kinetic stability of Cu^{II} complexes. Thermodynamic stability can then be used for synthesis of monocopper species for comparison studies. Kinetic stability should provide easier to interpret spectroscopic data. Question remains how will be these complexes influenced by isomerism of copper cyclam complexes (fig. 1.11).

2 Aims of the thesis

The goal of this thesis was synthesis of novel cyclam-based ligands that were missing from the logical progression of azamacrocycles. Dinuclear complexes of TACN and cyclen connected with some form of phenolate bridge were synthesized before, while cyclam was still missing. This can be surprising because cyclam forms most stable complexes with Cu^{II} . The interest in copper may come from two different areas.

First of these areas is inspired by naturally occurring enzymes. The motif of two copper cations closely coordinated together is heavily represented in proteins that are critical to oxidative and reductive processes (tyrosinases), termination of electron transport chains (cytochrome c oxidase) and oxygen transportation or activation (hemocyanin, catechol oxidase). Since the mentioned enzymes coordinate copper only with amino acids, there is an interest in simulating these centres with synthetic analogues that could provide similar catalytic activity. Using water as an oxygen source without the need for direct electrolysis or mild and selective oxidation of various substrates are both of high interest in currently promoted green chemistry and ecological and renewable industry.

Second area also finds its inspiration in natural processes: proteins known as purple acid phosphatases. These enzymes employ Fe^{III} and Mn^{II} , also usually coordinated closely in dinuclear active centres. These complexes can catalyse a dissociation of phosphodiesteres. Some dinuclear copper complexes show similar activity as these enzymes. Cleavage of these esters is usually connected to DNA cleavage in various natural processes. Complexes that can induce cleavage of DNA find their use in medicine. If targeted, they can induce apoptosis in cancerous cells. They can also be used as a surface compound that prevents growth of bacterial films.

Copper in cyclam complex should provide more stable complexes than those that were previously reported. Multinuclear complexes with different metals are currently being investigated as MRI, SPECT or PET contrast agent with either added reporting functions or as combination of diagnostic and therapeutic compounds. Copper has isotopes that can be used in some of these methods. The phenolate bridge is an established moiety that can introduce various functional groups that can be used for subsequent functionalization of the complexes. The functional group in *para* position from the coordinating hydroxyl group has an effect on $\text{p}K_{\text{a}}$ of the phenolate. Taking control of the deprotonation of the phenolate can play crucial role in the copper (or other metal) complex formation, their stability and even the shape of the coordination complex.

Although many dinuclear complexes were studied by electrochemical methods, there is scarcely anything known about their electrochemical behaviour in aqueous solutions. While different members of our research group have analysed coordination and electrochemical behaviour of various mononuclear Cu^{II} -cyclam, we have aimed to study dinuclear complexes.

By designing two dinuclear ligands with difference in $\text{p}K_{\text{a}}$ we have aimed to study the coordination situation in dinuclear complex and their electrochemical behaviour, especially subsequent reduction of two copper cations.

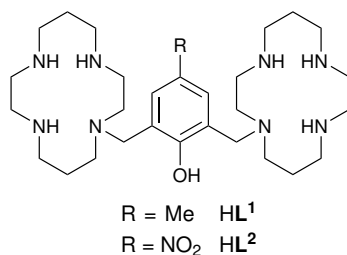


Figure 2.1 Ligand discussed in this work

3 Results and discussion

3.1 Ligand synthesis

Synthetic route to both **HL**¹ and **HL**² begins with thrice protected cyclam. Two of these molecule then react with alkylating reagent prepared from parent *p*-substituted phenol. Final step is removal of the protecting Boc groups. These steps are discussed below, beginning with synthesis of alkylating reagents.

Preparation of alkylating agents consist of hydroxomethylation of phenols (fig. 3.1) and their subsequent chlorination. Hydroxomethylation is known from the beginning of 20th century as the first step in synthesis of phenol-formaldehyde resins[49]. Compound **8** was prepared according to literature[50]. Subsequent chlorination with HCl to **2** according to [51] proceeded as expected. However when SOCl₂ was used we have obtained product of cyclotrimerization **2b** in high (~70%) yield after recrystallization. Similar reaction was previously reported with similar compounds[52, 53] but not with **2**. This trimer has crystallized readily and could be identified by X-ray diffraction(fig. 3.1).

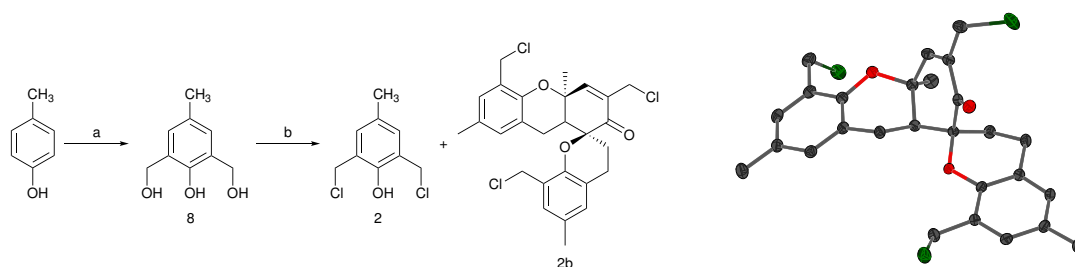


Figure 3.1 Left: synthesis of **2**. a) 37% formaldehyde, aq. NaOH, b) conc. HCl, DCM; Right: The molecular structure of compound **2b** found in solid state. Hydrogen atoms are not shown for the sake of clarity.

During cyclotrimerization, one aromatic ring is fused with two other aromatic centres. The aromaticity of this middle aromatic ring is broken: double bond is formed between oxygen of former hydroxy group and interestingly methylene bridge is formed on *m*-carbon. Previously prepared bromide was obtained by elimination of HBr from bromide monomer by strong base. In our case the cyclotrimerization happened when DCM solution was mixed with aq. K₂CO₃. While *p*-cresol can be activated towards hydroxomethylation by deprotonization of phenol and is hydroxomethylated completely at RT, the *p*-nitrophenol is less reactive and the reaction requires heating and highly acidic conditions. Even in

these harsh conditions, we were able to observe product **10b**(fig. 3.2).

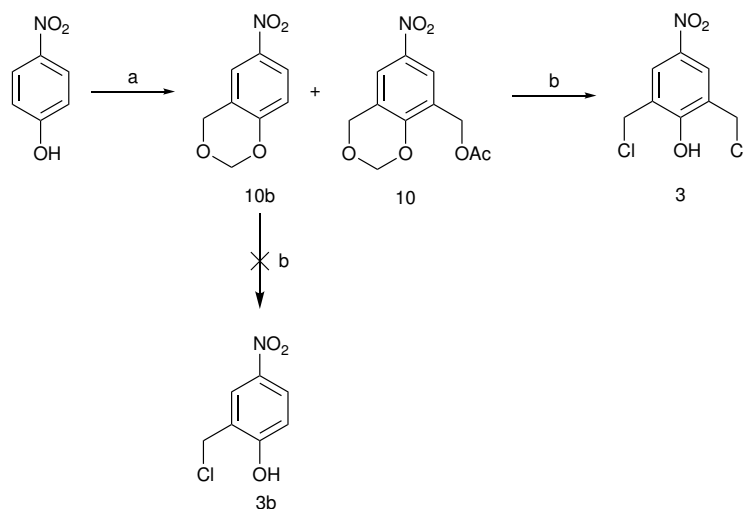


Figure 3.2 Hydroxomethylation of *p*-nitrophenol and chlorination of **10**.
a) $(\text{CH}_2\text{O})_n$, H_2SO_4 , AcOH , $55\text{ }^\circ\text{C}$ b) conc. HCl , $100\text{ }^\circ\text{C}$

Side product **10b** was isolated because its chlorinated form **3b** is an interesting alkylating reagent for monomeric copper complexes with phenolate pendant arms. However, we have discovered that submitting **10b** to same conditions as **10** leads to mixture of insoluble oligomers. **3b** can be prepared according to [54] by introduction of hydrogen chloride into the mixture of *p*-nitrophenol, paraformaldehyde and ZnCl_2 in hydrochloric acid.

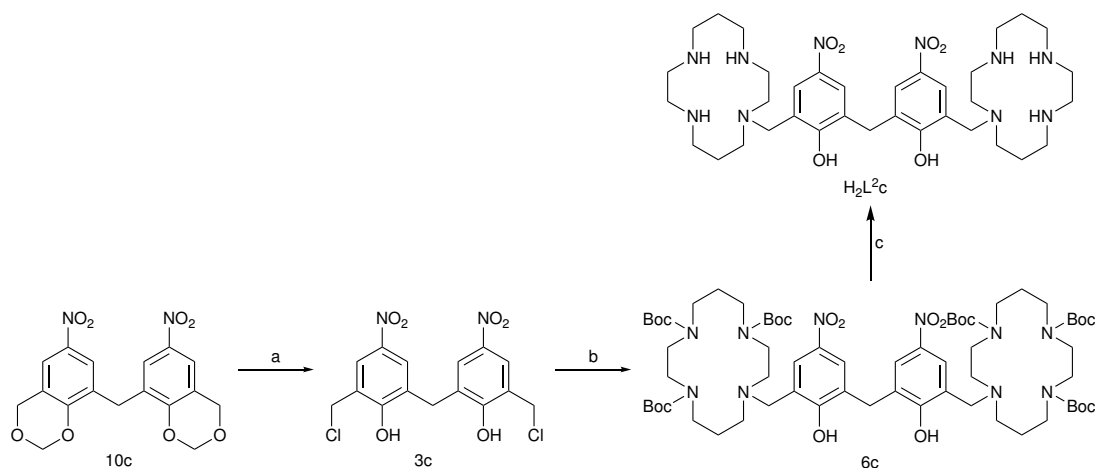


Figure 3.3 Side products of hydroxomethylation of *p*-nitrophenol, a) conc. HCl , $100\text{ }^\circ\text{C}$ b) **1**, K_2CO_3 , $0\text{ }^\circ\text{C}$ c) TFA , DCM

Harsh conditions also lead to polymerization. In some runs of the hydroxomethylation reaction, dimeric compound **10c** was observed(fig. 3.3). As it cannot

be removed by recrystallization, it contaminated the alkylating reagent **3** and ultimately lead to isolation of H_2L^2c . The separation of H_2L^2c and HL^2 was achieved by RP chromatography in the last step (after removal of Boc protecting groups), because separation of protected ligands **6** and **6c** is impossible. It is easier to separate these compounds in the last step of synthesis during final purification of HL^2 . The compound H_2L^2c was identified only by HPLC-MS and wasn't studied further.

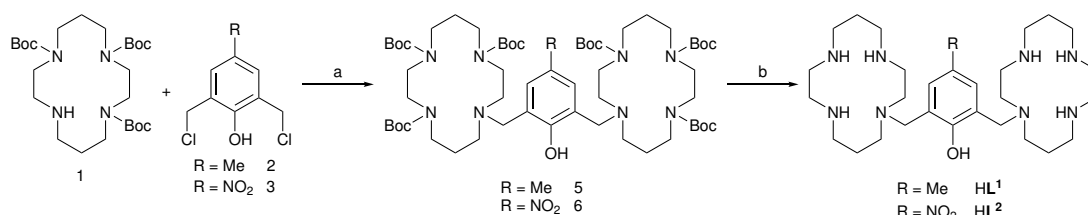


Figure 3.4 Syntheses of studied ligands: a) DCM, K₂CO₃, 0 °C b) DCM, TFA, 0 °C

Tri-Boc-cyclam was prepared according to literature[55]. Alkylation with **2** yielded compound **5** (fig.3.4) in yields of over 80 %, the losses come most likely from chromatography purification. Usually, some fractions couldn't be used because they were contaminated by unreacted **1**, which had to be used in excess to ensure full conversion of alkylating reagents. Alkylation with **3** had worse results, around 60 %. This was caused by worse purity of alkylating reagent, as we have not observed side products (apart from **6c**). Worse quality of **3** when compared to **2** is caused by solubility issues: while **2** is almost pure after evaporation and crystallizes readily from hexane, **3** is obtained as reddish brown mud-like precipitate from hydrochloric acid and the recrystallization leads to powder rather than large, well defined crystal like in the case of **2**.

Removal of Boc protecting group is facilitated by protons (in acidic conditions). There are two variants of this deprotection as it can be done either in aqueous solution by hydrochloric acid or in non-aqueous condition, usually in dichloromethane with catalytic amount of TFA. In both cases, carbamate is destabilized by protonation, *tert*-butyl cation is released and CO₂ is eliminated from the amine. The *tert*-butyl cation then goes through elimination and leaves reaction mixture as isobutene gas. Regardless of chosen way of deprotection the amine is protonated and exists as a salt of respective acid. In case of hydrochloric acid, it can be precipitated from the solution by addition of EtOH or acetone. Usually, number of hydrochloride ions equates number of amines in the molecule and the hydrochloride is reasonably well defined. Trifluoroacetates on the other hand form brown nonstoichiometric oils. These are usually soluble in dichloromethane but we have observed that in larger runs of the deprotection $L^x \cdot n CF_3COOH$ separates from the solution. This can seem to be less desirable result than the well

defined hydrochloride but TFA route leads to smaller amount of decomposition of products. This decomposition leads to free cyclam in the mixture and is discussed below. Because this decomposition is unavoidable, TFA route is beneficial for another reason: The hydrochloride cannot be purified by RP chromatography because it elutes with dead volume. The trifluoroacetate is purified easily on RP column and anions can be removed by ion exchange chromatography (DOWEX 1, OH^- cycle). It is also possible to lyophilize fractions from chromatography directly resulting in pure hygroscopic *n*-trifluoroacetates in the form of usable powder.

Synthesis of unsymmetrical bis(macrocyclic) compounds

Further, we have studied preparation of compounds that contain two different macrocycles connected with phenolate bridge. These unsymmetrical bis(macrocyclic) ligands could be useful for coordination of two different transition metals that are also present in some enzymes.

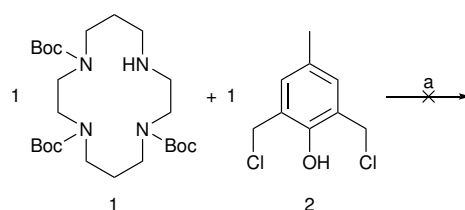


Figure 3.5 First approach toward monosubstituted phenolate bridge, stoichiometrically controlled reaction of **1** and **2** a) DCM, K_2CO_3 , 0 °C

The first approach was reaction of **2** and **1** in 1:1 ratio (fig. 3.5). Desired product has been observed in small amount by MS in the reaction mixture but after evaporation, none was isolated, or indeed found. We hypothesize that heating during evaporation leads to formation of ammonium salts by further alkylation.

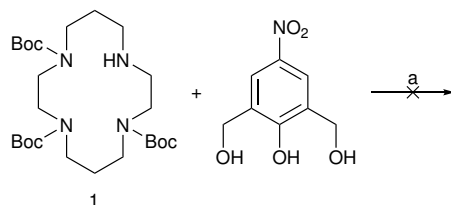


Figure 3.6 Second approach toward monosubstituted phenolate bridge, Mitsunobu reaction of alcohol and secondary amine a) PPh_3 , DIAD, DCM

The second approach was using even less active alkylating reagent. We have turned to Mitsunobu reaction (fig. 3.6). It was reported in [56] that these condi-

tions (triphenyl phosphine and azodicarboxylate like DIAD) can lead to alkylation of secondary amines by alcohols. However, we have once again observed only small amounts of desired products and according to HPLC-MS analysis of the reaction mixture, the alcohol has reacted with hydrazine-containing residue from DIAD rather than with **1**. Coupled with high cost of DIAD, difficult removal of $\text{Ph}_3\text{P}=\text{O}$ from the reaction mixture and complicated separation of products this approach was also judged infeasible.

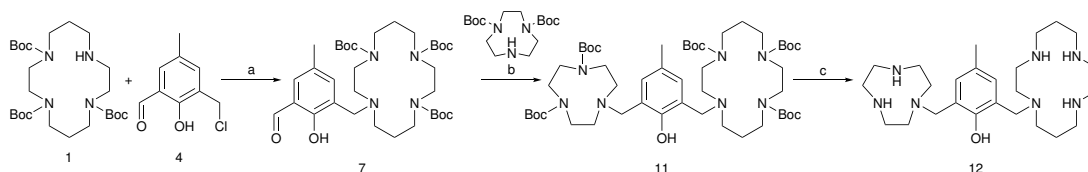


Figure 3.7 Successful synthetic approach toward **12**, bis(macrocyclic) ligand with asymmetrically substituted phenolate bridge a) DCM, K_2CO_3 , 0 °C b) $\text{NaBH}(\text{OAc})_3$, Na_2SO_4 , DCM c) DCM, TFA, 0 °C

Because experiments with symmetrical alkylating reagents were unsuccessful, the third approach used an unsymmetrical reagent **4** (fig. 3.7). That was already known from literature [57] for synthesis of unsymmetrical open chained dinuclear complexes. However, to our knowledge, macrocycles do not react with aldehydes like linear amines by formation of iminium salts. Benzaldehyde reacts with TACN and cyclam yielding aminals rather than iminium salts. Because the iminium salts are not formed, classical approach to reductive amination cannot work because instead of iminium reduction to tertiary amines, aldehydes are reduced to alcohols. In some cases, this can be solved by use of weaker reducing agent like $\text{NaBH}(\text{OAc}_4)$ [58]. We have then successfully prepared compounds **11** and **12**.

3.2 Ligand protonation constants

The acid-base properties of the ligands are crucial for the evaluation of their coordination behaviour. Thus, the protonation constants of HL^1 and HL^2 were determined by potentiometry. We were able to determine 6 protonation constants from the possible 9 (two cyclam subunits and phenolate). Because potentiometry carries no information about the protonation site, UV titration was used to determine which protonation constant reflects deprotonation of the phenol. Protonation constants are summarized in table 3.1 and compared with cyclam and *p*-XBC. Cyclam has two protonation constants (11.4 and 10.28) in alkaline pH and two are really low in the acidic region (2.1 and 1.6). *p*-XCB follows

this trend in kind with two sets of four constant close to cyclam. Behaviour of studied ligands in alkaline conditions is comparable to both cyclam and *p*-XBC as we can observe four protonation constants that correspond to two protonations on each cyclam subunit that occur in alkaline pH. While negatively charged deprotonated phenolate increases basicity of $\mathbf{L}^1$, this effect is compensated by electron withdrawing effect of nitro group and $\mathbf{L}^2$ is comparable to *p*-XBC. In case of $\mathbf{L}^1$, protonation of phenolate occurs, as determined by UV titration at 6.85. Protonation constant of nitrophenol is (as expected) much lower: it was found at 3.32. In both cases, the protonation of phenol is observable by great change in absorption spectra (fig. 3.8).

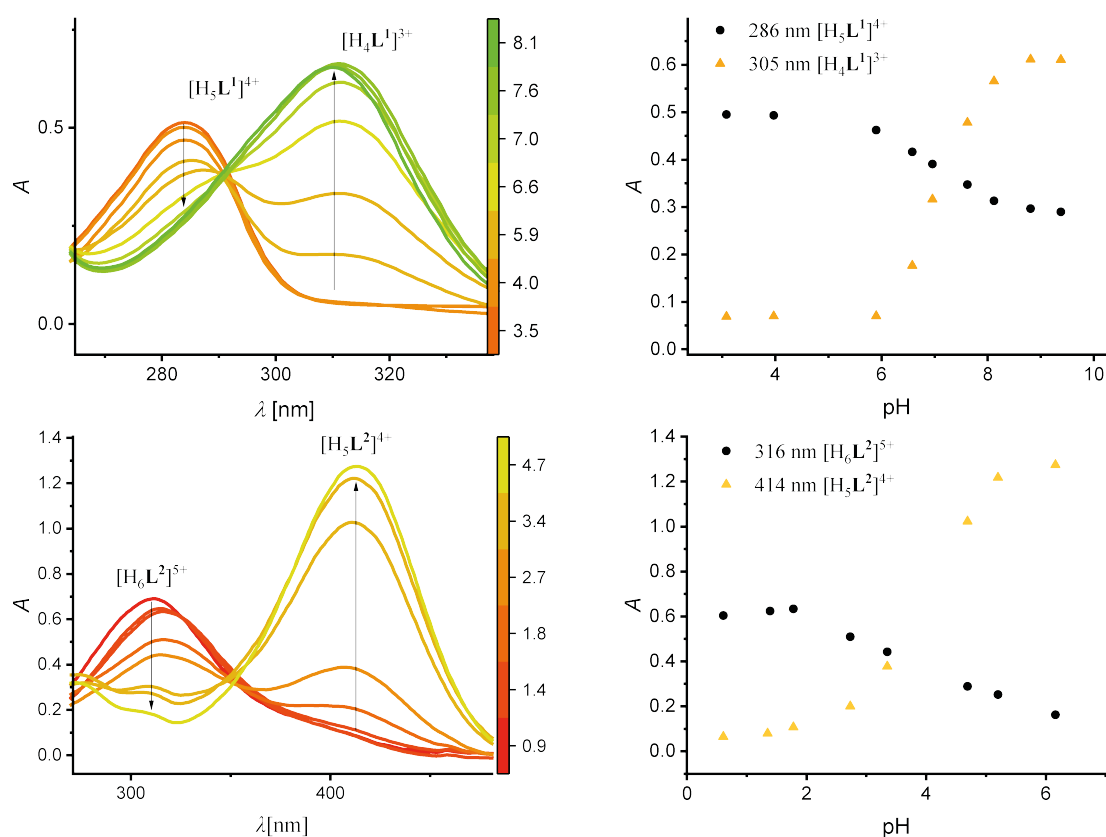


Figure 3.8 Determination of protonation constants of phenolates. Above: deprotonation of *p*-cresol of $\mathbf{L}^1$; below: deprotonation of *p*-nitrophenol of $\mathbf{L}^2$

Difference in protonation of phenol leads to great difference in the first protonation under acidic conditions: the first cyclam subunit of $\mathbf{L}^1$ is protonated to 3+ cation at 2.38 similarly to *p*-XCB, $\mathbf{L}^2$ gains the same proton 4.92. This is most likely caused by stabilization of the protonated macrocycle via hydrogen bond with deprotonated nitrophenolate. At this pH *p*-cresol is already protonated.

Table 3.1 Overall ($\log \beta$) and stepwise ($\log K$) protonation constants of studied and discussed ligands (25 °C, I = 0.1 M NMe₄Cl)

Species	L¹		L²		cyclam	p-XBC
	$\log \beta$	$\log K$	$\log \beta$	$\log K$	$\log K$	$\log K$
HL	12.65(5)	12.65	11.51(1)	11.51	11.4	11.1
H ₂ L	23.89(4)	11.24	21.83(1)	10.32	10.28	10.5
H ₃ L	34.23(5)	10.35	30.82(1)	8.99	1.6	9.44
H ₄ L	43.50(5)	9.27	37.31(2)	6.49	2.1	8.75
H ₅ L	50.34(6)	6.85 ^a 7.00(5) ^b	42.23(2)	4.92	—	2.45
H ₆ L	52.72(6)	2.38	45.55(2)	3.32 ^a 3.65(5) ^c	—	—

^a The constant corresponds to the phenolate protonation. ^b Determined from the absorbance at 305 nm. ^c Determined from the absorbance at 414 nm.

3.3 Preparation of copper complexes

Even though cyclam can form complexes with many transition metals, our main interest was copper. Preparation of both dinuclear complexes [Cu₂(HL¹)](SO₄)₂ and [Cu₂(HL²)]Cl₄ was done in similar ways: reaction of freshly prepared base (trifluoroacetate or hydrochloride of L^x passed through ion exchange resin and concentrated *in vacuo*) with excess of either Cu(SO₄)₂ or CuCl₂ · 2 H₂O. If needed the pH of reaction was adjusted with same acid as the copper salt used or with LiOH. Since the addition of LiOH can complicate identification of compounds in MS, we have tried to avoid it when possible. To ensure existence of thermodynamic products, we usually stirred the mixture overnight, but the observation suggest that the reaction was complete after an hour at pH≈5. After that the mixture was purified on Al₂O₃ eluting with mixture of ethanol and water 1:1 which removes excess copper salts as they are retained on the column. The eluent can be lyophilized affording powdered complex (the sulphate was prepared in this way). Separation of [Cu₂(HL¹)]Cl₄ on RP column is also viable if the mobile phase contains TFA. Passing of this eluent through ion exchange resin (DOWEX 1 in OH[−] cycle) removes only trifluoroacetic acid while the chloride anions coelute with the complex.

[Cu₂(HL²)]Cl₄ could not be purified on Al₂O₃ as the retention is stronger than

for $\mathbf{L}^1$ complexes, so we had to use RP column and same way of TFA removal. Chloride anions were then removed by addition of Ag_2O and X-ray diffraction analysis (see below) confirms removal of chlorides.

Preparation of $[\text{Cu}(\text{HL}^1)](\text{SO}_4)$ was more complicated: we haven't observed any selectivity toward monocopper complex dependent on pH, temperature or stoichiometry of the reaction. HPLC analysis, always showed statistical mixture of HL^1 , $[\text{Cu}(\text{HL}^1)]^{2+}$ and $[\text{Cu}_2(\text{HL}^1)]^{4+}$. We were able to separate monocopper complex by FLASH chromatography on Al_2O_3 with mobile phase $\text{iPrOH}:\text{ammonia}:\text{H}_2\text{O}$ 7:3:3. This MF stops dicopper complex, most likely by deprotonization, while the monocopper complex is still eluted. Free ligand elutes with dead volume of the column and is easily separated. This eluent was concentrated *in vacuo* to oil and redissolved in H_2O several times to remove ammonia. All solvents were removed by lyophilization affording powder that quickly melts. The samples were kept in tightly closed vials under N_2 atmosphere. Our efforts to prepare monocopper complex of $\mathbf{L}^2$ were unsuccessful, as we haven't found any chromatographic system that would separate the statistical mixture.

Mononuclear complex $[\text{Cu}(\text{HL}^1)]^{2+}$ gives major peak in HPLC with MS spectrum that corresponds to adduct with trifluoroacetate. Both $[\text{Cu}_2(\text{HL}^1)]^{4+}$ and $[\text{Cu}_2(\mathbf{L}^2)]^{3+}$ have two coeluting peaks with similar MS spectra. This suggests two isomers that cannot be attained by the monocopper complex. It is also unlikely that these two species are different TFA adducts. Although large scale separation of these isomers is not possible, small amount of pure isomer was obtained by collecting small fractions during FLASH chromatography (fig. 3.9, red line). The complex was dissolved in water and HPLC-MS of the solution was measured over time. Stacked HPLC traces show that while the second chromatogram (just after separation) is almost pure, the mixture returns to equilibrium state after 30 minutes. Our mobile phase is quite acidic ($\text{pH} \approx 2.5$) so the isomerisation is slow. Neutralizing of the fraction has lead to equilibrium state immediately (or fast enough so it couldn't be observed via HPLC).

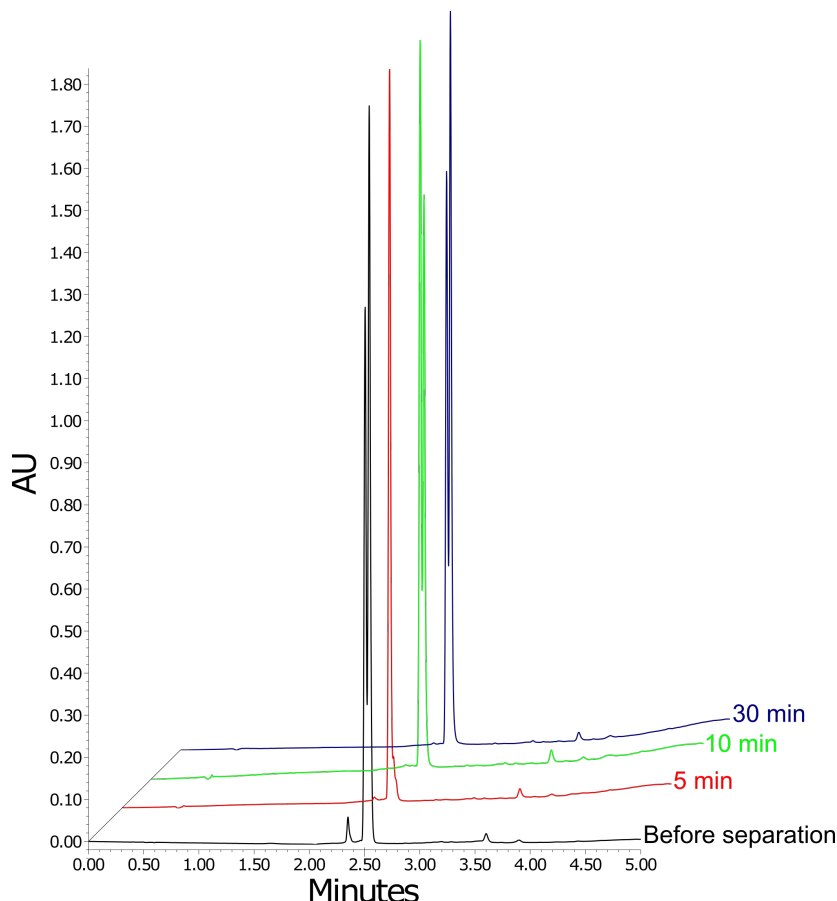


Figure 3.9 Chromatograms of the isomeric mixture (black) of $[\text{Cu}_2(\text{HL}^1)]^{4+}$ and changes of the chromatogram of the purified isomer 5 (red), 10 (green) and 30 (blue) min after isolation (pH = 2.5, RT)

3.4 Structure of complexes in solid state

Although the complexes evaporate into oils and have to be lyophilized in order to obtain them in usable form, few single crystals were grown. Three different structures were obtained for $[\text{Cu}_2(\text{HL}^1)]^{4+}$: $[\text{Cu}_2(\text{HL}^1)(\text{SO}_4)(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 8 \text{H}_2\text{O}$ (**X1**) was obtained when H_2SO_4 was used to neutralize alkaline solution of the complex. Both $[\text{Cu}_2(\text{HL}^1)\text{Cl}_2]\text{Cl}_2 \cdot 5 \text{H}_2\text{O}$ (**X2**) and $[\text{Cu}_2(\text{HL}^1)\text{Cl}_2(\text{H}_2\text{O})_2][\text{Cu}_2(\text{HL}^1)\text{Cl}_4]\text{Cl}_2 \cdot 18 \text{H}_2\text{O}$ (**X3**) were obtained from one vial when H_3PO_4 was used for neutralization of the same solution. The fourth structure $[\text{Cu}_2(\text{HL}^2)(\text{ClO}_4)_4] \cdot 4.5 \text{H}_2\text{O}$ (**X4**) was obtained by removing chloride anions from the solution of complex and using HClO_4 for neutralization, pH of the solution was ≈ 1 . No crystals were obtained from solution with higher pH. As was discussed in the first chapter, cyclam complexes form several isomers with first-row transition metals. These isomers (noted I-V) differ in orientation of pendant arms or hydrogen atoms on the nitrogen atoms. Type of isomer, size of metal cation and ligand size control whether hexa- or pentacoordinated complex is formed. In the case of hexacoor-

minated complexes, two additional ligands can be bound in *cis* or *trans* geometry. All cyclam subunits in all obtained structures attain *trans*-**III** conformation. The Cu^{II} ions are in the center of the plane formed by four nitrogen atoms. Bond lengths are also consistent in all structures ($d_{\text{Cu-N}} = 1.99 - 2.09 \text{ \AA}$, fig. 3.2). This coordination behaviour is not surprising, as this is the most stable conformation and it dominates structural databases[26].

While coordination of cyclam subunits is similar in all observed crystals, we can observe some differences in coordination of phenol. Following figures contain representation of obtained crystals as thermal ellipsoid plot (probability 50 %) and are shown without carbon-bound hydrogen atoms for clarity. Some molecules of water and some chloride ions are also omitted.

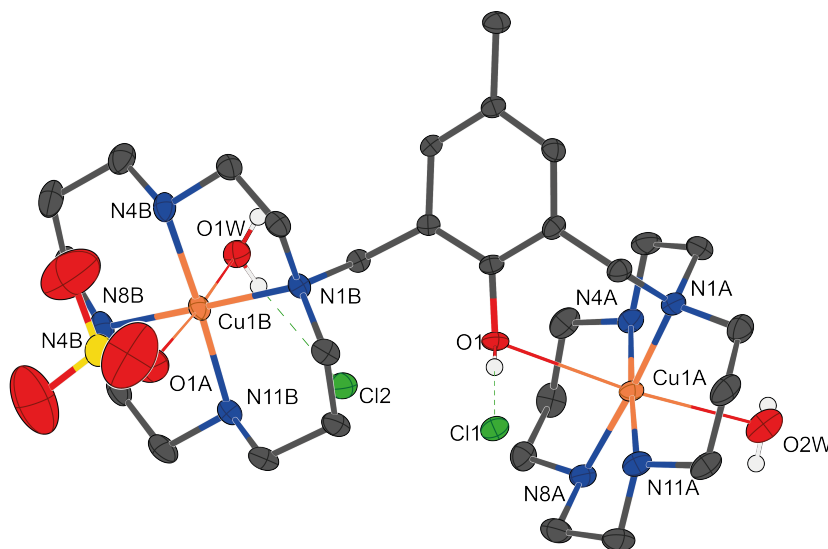


Figure 3.10 Structure of **X1**, only the more abundant position of the disordered sulphate anion is shown.

We can observe rather long coordination bond between Cu^{II} and the hydroxy group of phenol in structure **X1**. Bond length $d_{\text{Cu-O}} = 2.70 \text{ \AA}$ is longer than that reported for dicopper complex with cyclen subunits ($d_{\text{Cu-O}} = 2.1 \text{ \AA}$)[48] and we have not observed formation of $\mu\text{-OH}$ bridge. This suggests that the cavity of cyclam is not optimal for strong phenol coordination.

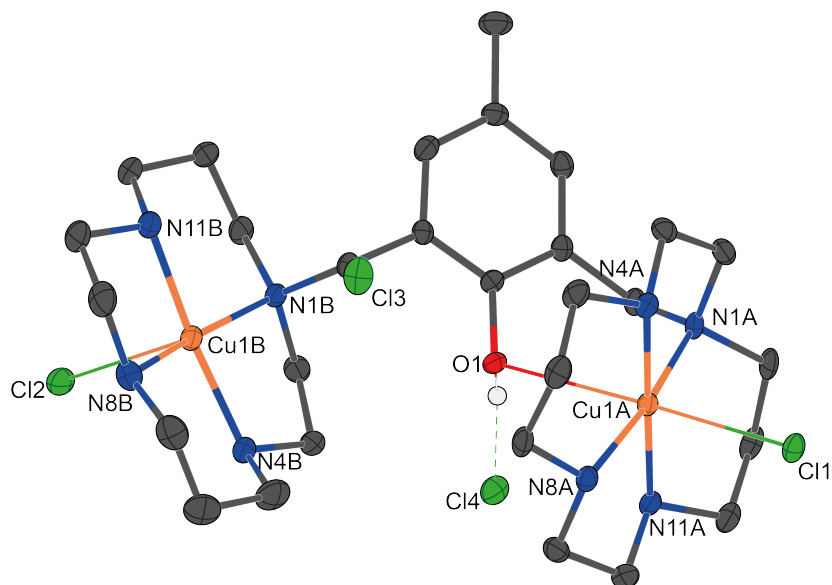


Figure 3.11 Structure of **X2** Chloride ion Cl3 is semi-coordinated

Structure of **X2** is very similar to **X1**. Only difference is semi-coordination of axial chloride ion Cl3, but it fills one of axial positions of the Cu^{II} so the structures **X1** and **X2** are virtually the same.

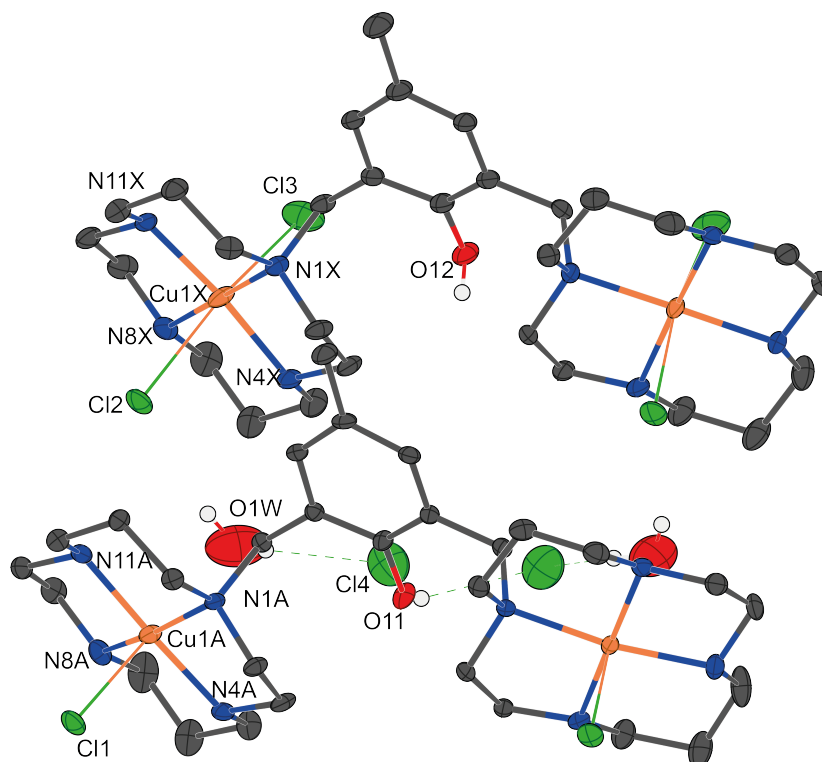


Figure 3.12 Structure of **X3**, Cu1A is pentacoordinated

Structure of **X3** consists of two complex cations $[\text{Cu}_2(\text{HL}^1)]^{4+}$. Both share plane of symmetry perpendicular to the aromatic rings and both have no bond between Cu^{II} ion and the phenol. Cu^{II} ions in the first complex are hexacoordi-

nated. The axial positions are filled with chloride ions. Both are rather distant, but show only small disorder. The second complex cation contains only one axial chloride ion. The second axial position is filled with disordered and distant water molecule.

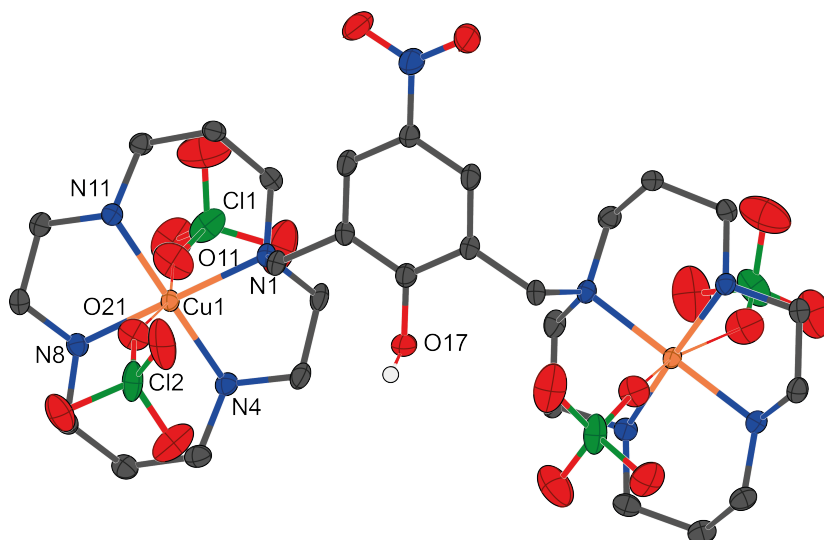


Figure 3.13 Structure of **X4**, only the more abundant position of the disordered perchlorate anion (Cl2) is shown

Structure **X4** is the only structure that was obtained for **HL**². Like other structures, the phenol is protonated and, similarly to **X3** the structure has plane of symmetry and no bond between phenol and Cu^{II} ion. While the ClO₄⁻ anions are disordered, we can observe shorter bond lengths and closer coordination than those for chloride axial ions. Perchlorate is coordinated similarly to sulphate in **X1**.

Bridging mode of phenolate that was reported in dicopper complexes with phenolate bridge between TACN or cyclen subunits was not observed[48, 59]. All structures also contain noncoordinated water molecules, structures **X1**, **X2** and **X3** also contain noncoordinated chloride anions. Chloride anions and water molecules form complex net of hydrogen bonds with cyclam subunits and the phenol.

Table 3.2 Coordination bond lengths found in the reported CuII complexes.

Distance	X1	X2	X3 ^a	X4 ^b
Cu1A				
–N1A	2.049(3)	2.051(3)	2.073(4) ^a	2.080(3) ^b
–N4A	2.000(3)	2.027(3)	2.018(4) ^a	2.008(3) ^b
–N8A	2.027(3)	2.011(3)	2.033(4) ^a	2.021(3) ^b
–N11A	2.009(3)	2.027(3)	2.003(4) ^a	2.006(3) ^b
...Ax1A ^c	2.590(4)	2.691(1)	2.619(1)	2.511(4)
	(H ₂ O) ^c	(Cl [–]) ^c	(Cl [–]) ^{a,c}	(ClO ₄ [–]) ^{b,c}
...Ax2A ^c	2.702(3)	2.760(2)	3.095(9)	2.574(3)
	(Ar–OH) ^c	(Ar–OH) ^c	(H ₂ O) ^{a,c}	(ClO ₄ [–]) ^{b,c}
Cu1B				
–N1B	2.085(3)	2.053(3)	2.087(4) ^a	– ^b
–N4B	2.005(3)	2.025(3)	2.007(4) ^a	– ^b
–N8B	2.031(3)	2.040(3)	2.028(4) ^a	– ^b
–N11B	2.017(3)	1.991(3)	2.008(4) ^a	– ^b
...Ax1B ^c	2.450(6)	2.682(1)	2.826(2)	– ^b
	(SO ₄ ^{2–}) ^c	(Cl [–]) ^c	(Cl [–]) ^{a,c}	
...Ax2B ^c	2.699(3)	–	2.916(1)	– ^b
	(H ₂ O) ^c		(Cl) ^{a,c}	

^a The structure contains two independent complex molecules with a plane of symmetry; Cu1A corresponds to one molecule, whereas Cu1B to another one. ^b The structure contains one independent complex molecule with a plane of symmetry. ^c Semi-coordinated axial ligand.

3.5 Spectral characterization of Cu^{II} complexes

Because we have observed the phenol group only in protonated state in crystal structures, UV-Vis spectra of isolated mono- and dicopper complexes of **L**¹ were recorded in aqueous solution in pH range 2-12 (fig. 3.14 and 3.15). As we have observed by HPLC it wasn't feasible to try and analyse purified isomers, we have worked with the mixture. Similarly to free **L**¹, UV band at 250 nm shows significant change around pH = 6 for both mono and dicopper complexes of **HL**¹. This change corresponds to deprotonation of the phenol. The absorption band is HOMO-LUMO absorption of the aromatic ring. The deprotonation reflects also in the intensity of the band at 400 nm. This absorption band has same maximum for both mono- and dicopper complex and most likely arises from charge transfer (CT) between phenolate and the Cu^{II} ion. This similarity between mono- and dicopper complex suggests that only one bond between Cu^{II} and phenolate is present in both molecules.

Deprotonation in both complexes is shifted to lower pH in comparison with free ligand. This is caused by coordination of copper. The difference in pK_a is considerable: 7.00 for free ligand, 5.9 ± 0.2 and 5.0 ± 0.3 for mono and dicopper complex respectively. Difference between two complexes is on the other hand less significant and also suggests that there is no bridging in the dicopper complex. This is also consistent with the fact that complexes that form these bridges (with TACN an cyclen subunits) are not found in their protonated form and the phenol is deprotonated during the complexation[47]. In the case of $[\text{Cu}_2(\text{HL}^1)]^{4+}$ complex, the intensity of the band centred at 400 nm is decreased at $\text{pH} > 8$, which might be related to the replacement of the phenolate by hydroxide anion and/or to changes in the coordination geometry (isomerism) upon hydroxide coordination.

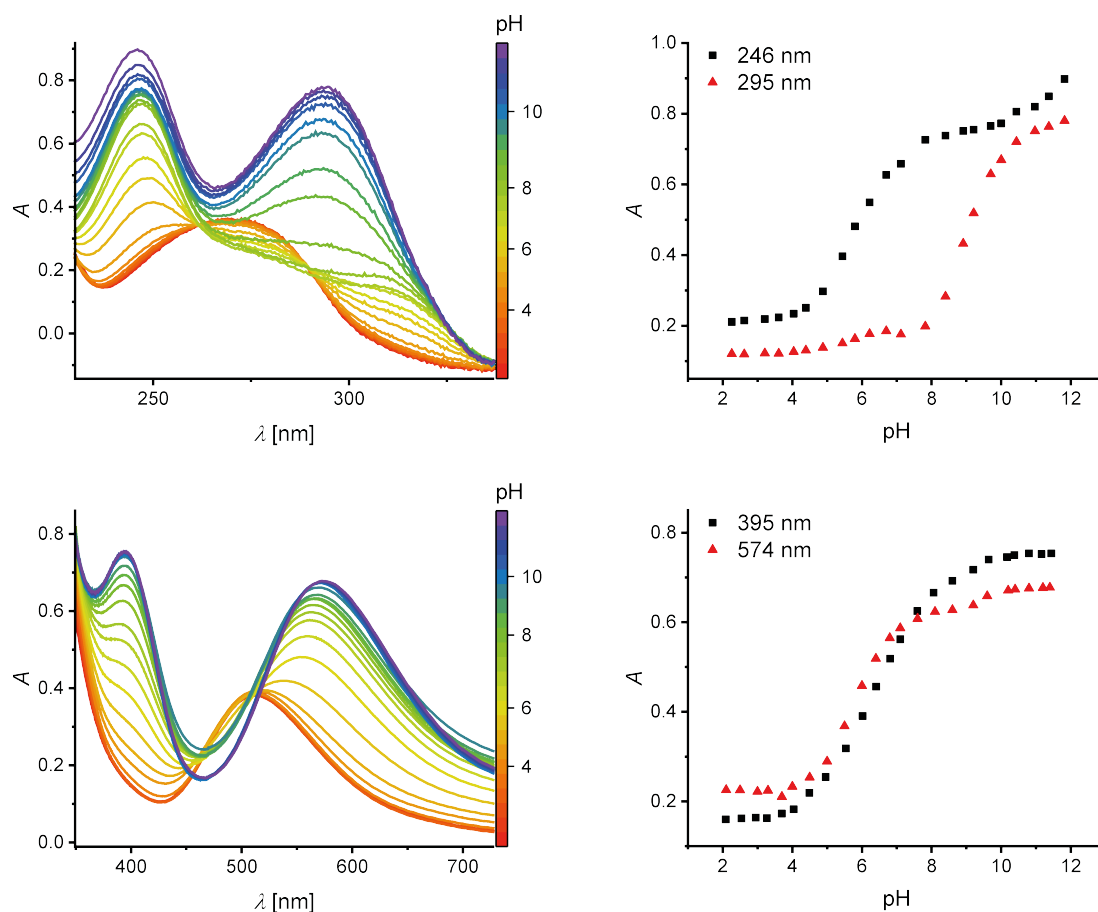


Figure 3.14 UV (top, $c = 0.04$ mM) and Vis (bottom, $c = 4$ mM) spectra and intensities of absorption bands $[\text{Cu}(\text{HL}^1)]^{2+} / [\text{Cu}(\text{L}^1)]^+$ as a function of pH (25 °C, $I = 0.1\text{M}$ NMe_4Cl).

Another band is observed at 300 nm in spectra of both complexes at $\text{pH} = 8$. pK_a associated with these processes is 9.4 ± 0.2 for monocopper complex and 9.1 ± 0.1 for dicopper complex. We can ascribe this band to CT from coordinated hydroxide molecule. Because ligand HL^1 cannot completely fill the metal

coordination sphere, we can observe deprotonation of coordinated water molecules.

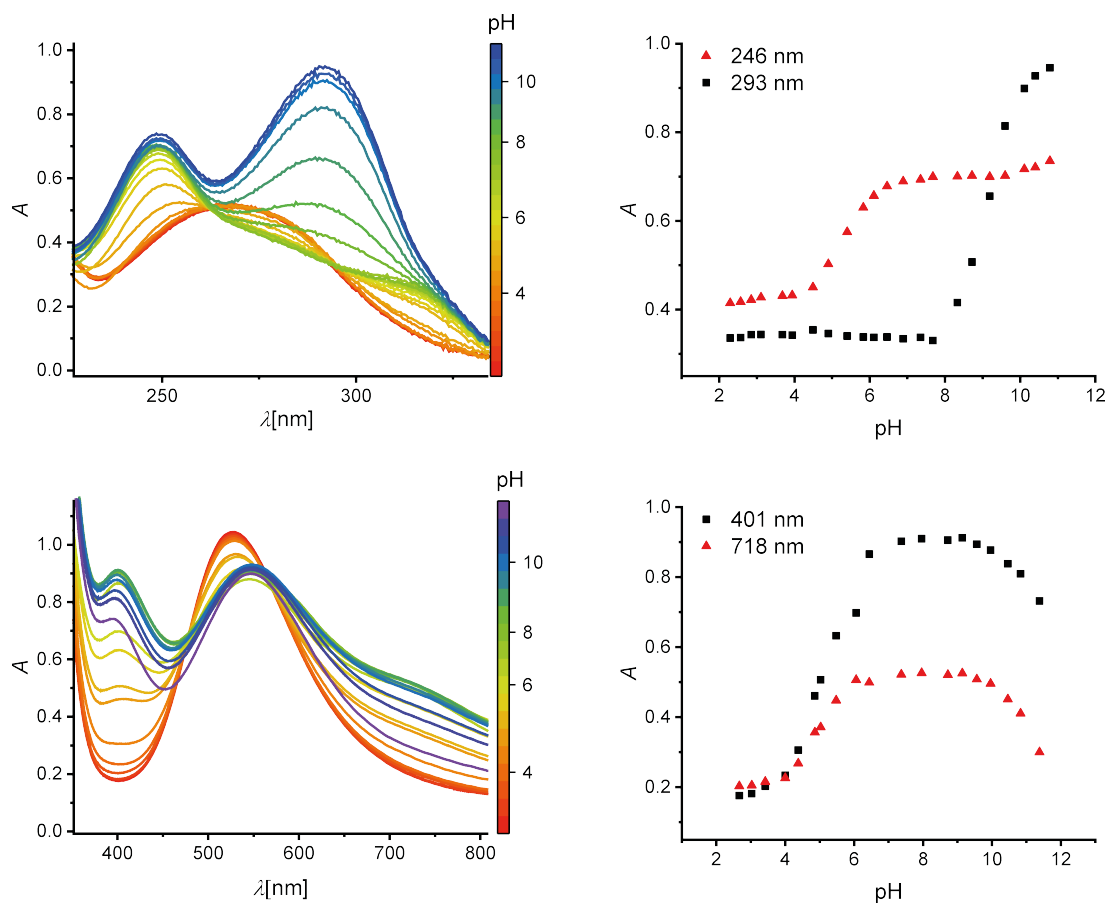


Figure 3.15 UV (top, $c = 0.04$ mM) and Vis (bottom, $c = 4$ mM) spectra and intensities of absorption bands $[\text{Cu}_2(\text{HL}^1)]^{4+} / [\text{Cu}_2(\text{L}^1)]^{3+}$ as a function of pH (25 °C, $I = 0.1\text{M}$ NMe_4Cl).

Absorption band at 500 nm, typical for copper-cyclam complexes also reflects deprotonation of the phenolate. In monocopper complex we can observe shift of the $d-d$ absorption maximum from 500 nm to 600 nm with corresponding $\text{p}K_{\text{a}}$. This change can be observed even without instrumentation as the colour of the solution shifts from violet to blue. In dicopper complex, this change isn't so significant and the complex is violet in alkaline conditions. However, shape of the $d-d$ absorption band is noticeable: It starts narrow like in monocopper complex but then gets wider when the phenol is deprotonated but only one of the Cu^{II} changes its absorption behaviour. The noncoordinated Cu^{II} stays unchanged and the apparent widening of the absorption band is caused by superposition of two different metal centres. Also the absence of clear isosbestic point in $d-d$ transition indicates presence of more isomeric species. We have also observed this isomerism in synthesis of the dicopper complex. Some fractions of the eluate from Al_2O_3 appeared as grey and changed their color to typical violet upon standing (during

5 to 10 minutes).

HL² and its dicopper complex were studied in the same way. UV-Vis spectra are simpler: because the *p*-nitrophenol group is much more acidic than *p*-cresol, the proton in $[\text{Cu}_2(\text{HL}^2)]^{4+}$ is cleaved at very low pH and $[\text{Cu}_2(\text{L}^2)]^{3+}$ is present over the whole pH range that was studied. Because of this, spectra show little change with pH. The absorption of deprotonated *p*-nitrophenolate dominates UV area with strong absorption at 400 nm. Similarly to other complexes the second absorption band appears in alkaline region at 350 nm. This absorption is caused by CT between hydroxide anion and Cu^{II} . *d-d* absorption in visible region does not change over the studied pH range.

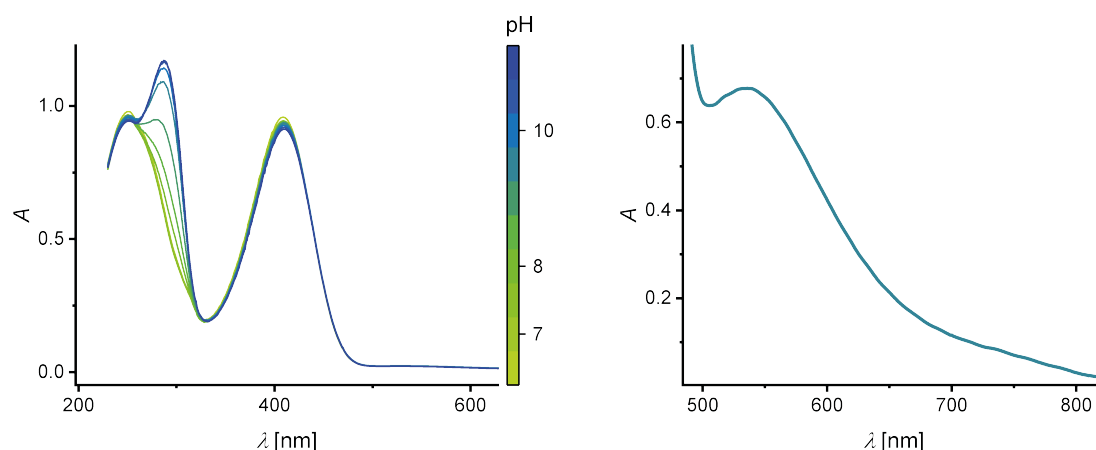


Figure 3.16 UV-Vis spectra of $[\text{Cu}_2(\text{L}^2)]^{3+}$, left: $c = 0.04$ mM (UV), right: $c = 4$ mM, pH = 9.6 (Vis), (25 °C, $I = 0.1\text{M}$ NMe_4Cl). No changes in the UV spectra were observed in the acidic region, the Vis spectra were identical along the whole studied pH range (3–11)

We have also tested whether we can observe some preference toward mono-copper complexes by titrating ligands **HL¹** and **HL²** with solution of CuSO_4 . Titration of **L¹** was performed at pH = 4.0 and has shown steady rise of the absorption at 500 nm with no break around 1 equivalent of added Cu^{II} (fig. 3.17). For **L²**, the titration was done at pH ≈ 2 in diluted hydrochloric acid so we could observe deprotonation of the ligand. Because the mixture is statistical, free **HL²** is fully consumed (and deprotonated) only after addition of two equivalents of Cu^{II} . Plotting absorption maximum at 400 nm reflects this situation.

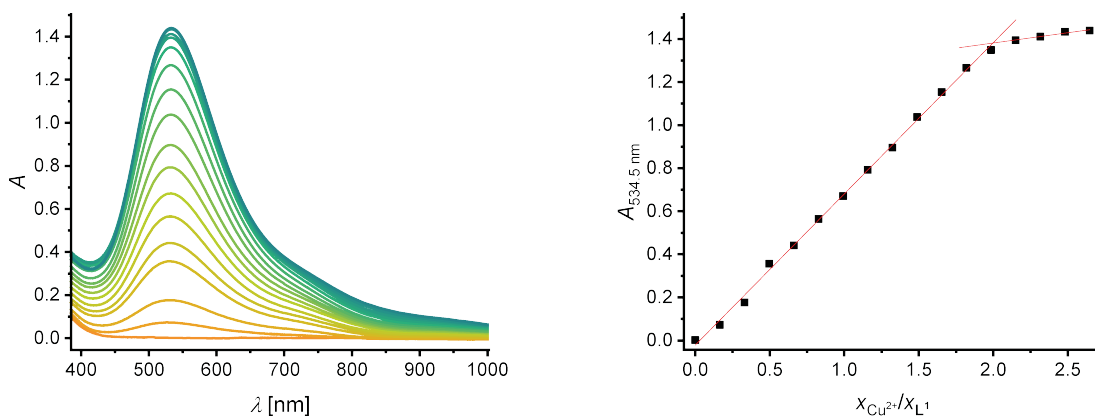


Figure 3.17 Titration of HL¹ with Cu^{II} ions, *d-d* absorption is shown on the left, the absorbance in the maximum of the absorption band as function of stoichiometry on the right (25 °C, pH = 4.0).

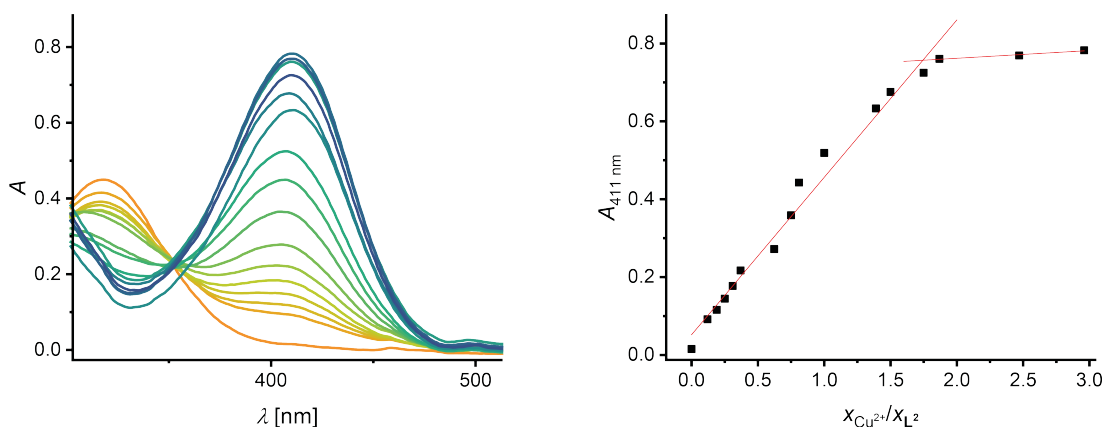


Figure 3.18 Titration of HL² with Cu^{II} ions, aromatic ring absorption is shown on the left, the absorbance in the maximum of the absorption band as function of stoichiometry on the right (25 °C, pH ≈ 2).

3.6 Electrochemical study

The electrochemical behaviour of the studied ligands and their Cu^{II} complexes was investigated by polarography (fig. A.38) and cyclic voltammetry. The experiments were performed in the aqueous solutions; pH was maintained by Britton-Robinson buffers, which span a large region of pH values. Because we were able to obtain both [Cu(L¹)]⁺ and [Cu₂(L¹)]³⁺ complexes, we studied differences in these two systems. Because of the nature of complexation reaction (statistical mixtures of mono and dicopper complexes) [Cu₂(L²)] was studied only as the isolated dicopper complex.

3.6.1 Ligand HL¹ and its Cu^{II} complexes

Ligand HL¹ is inactive in polarography measurement window (from 0.1 to –1.5 V vs. SCE) along whole pH range (2–12). In cyclic voltammetry weak reversible couple near 0.1 V (fig. 3.19) was observed. According to the literature[60] this process corresponds to reversible generation of phenoxy radical. It can be observed only in alkaline solution (after deprotonization of phenol). Similar reversible couple was observed in HL³ at $E^\circ = -0.06$ V also only in alkaline solutions. This peak disappears after addition of Cu^{II} ions as result of coordination.

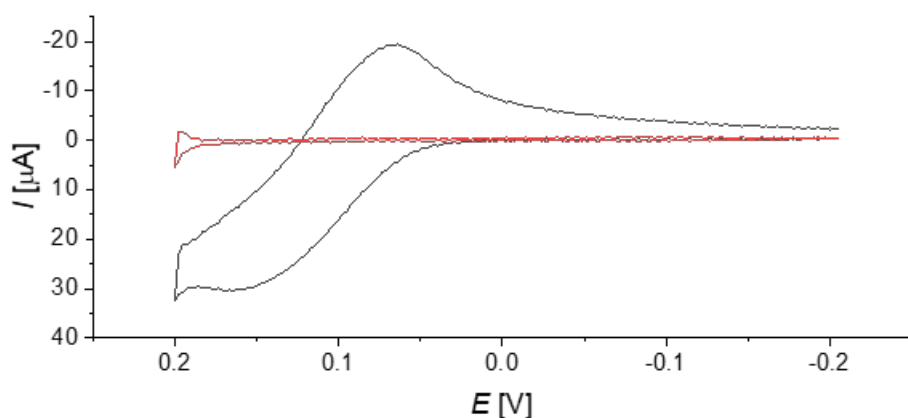


Figure 3.19 Reversible oxidation-reduction couple in the cyclic voltammogram of HL¹ (black, $c_L = 0.3$ mM, 25 °C). The curve after the Cu^{II} addition is shown for comparison (red, $c_L = 0.3$ mM, $c_{Cu} = 0.3$ mM, 25 °C).

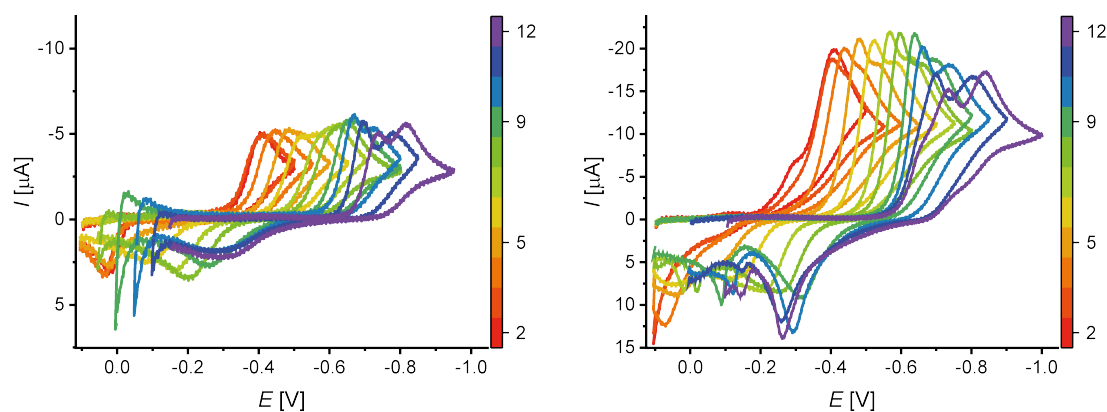


Figure 3.20 Cyclic voltammograms of the isolated complexes [Cu(L¹)]⁺ (left) and [Cu₂(L¹)]³⁺ (right) at various pH ($c = 0.3$ mM, 25 °C).

Isolated monocopper and dicopper complexes show irreversible two- or four-electron reduction. Two-electron reduction was already reported for [Cu(cyclam)]²⁺ complex[61]. Despite more complex shape of reductive peak of the dicopper [Cu₂(L¹)]³⁺ complex, overall the two systems are very similar both in shape and potential. They differ in total current: Total current of dicopper complex is twice

that of monocopper complex because it contains twice the amount of Cu^{II} ions. Reductive potential of both systems shifts linearly with pH (40-44 mV/pH) and towards alkaline pH split into two irreversible reduction steps. In monocopper complex this splitting is apparent from pH 4 to pH 12. The shape of CV of dicopper complex shows splitting into more peaks in whole studied pH range. In acidic condition shoulder with more positive potential is present. Two peaks like the case of monocopper complex are present from pH 3 to 12.

In acidic solutions peaks with less negative potential are dominant. With growing pH more negative peaks become more significant. Because this behaviour is present in both mono- and dicopper complexes, these two steps cannot be ascribed to two subsequent reductions (one Cu^{II} coordinated by phenolate and one coordinated only by cyclam subunit). These two steps must therefore be ascribed to presence of two isomers in the solution differing in the mutual orientation of the substituents on cyclam nitrogen atoms. The same behavior was recently reported for monomacrocyclic cyclam complexes with phenolate pendant arms.[61]

Subsequent cycles of CV were also recorded (fig 3.21). In the case of monocopper complex during the first cycle, Cu^{II} centres of both isomers are reduced and the complex decomposes. Metallic copper is amalgamated on the mercury electrode. It is then reoxidised during the reversal scan and the complexes are regenerated. In acidic conditions it is possible to observe reversible couple of free Cu^{II} cations near 0 V vs. SCE. This observation is possible because of the slow complexation at low pH. The complex is only partially restored and its reductive peak is lower than in the first scan. At higher pH the complex is restored completely and no reduction of free Cu^{II} can be observed. Rate of complexation dependent on pH is typical for cyclam complexes because the rate-determining step is deprotonation of the macrocycle. At pH = 5.55 we can observe that ratio of the two reduction peaks is different for subsequent cycles of CV. This supports the theory of multiple isomers in solution, because one of them is formed faster than the other. In alkaline solutions the complex is reformed completely and the mixture of isomers is same in the subsequent cycles of CV.

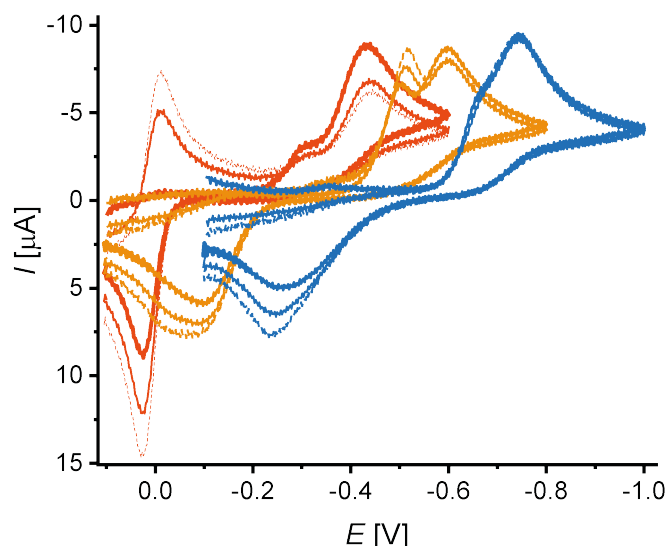


Figure 3.21 Repeated scans in the CV of the isolated $[\text{Cu}(\text{L}^1)]^+$ at various pH (1.89 red, 5.55 orange, 9.63 blue) ($c = 0.3 \text{ mM}$, 25°C) – three cycles.

Like with UV-Vis experiment, titration of L^1 with Cu^{II} was performed with similar result: Two species $[\text{Cu}(\text{L}^1)]^+$ and $[\text{Cu}(\text{L}^1)]^{3+}$ could not be identified as separate peaks and no break in the current/stoichiometry graph can be seen around 1 equivalent. The peak grows consistently until 2 equivalents of Cu^{II} are added. After this point free Cu^{II} appears around 0 V, similarly to experiments with subsequent cycles of CV (fig. 3.22). These observations are consistent with data from synthetic and UV-Vis experiments.

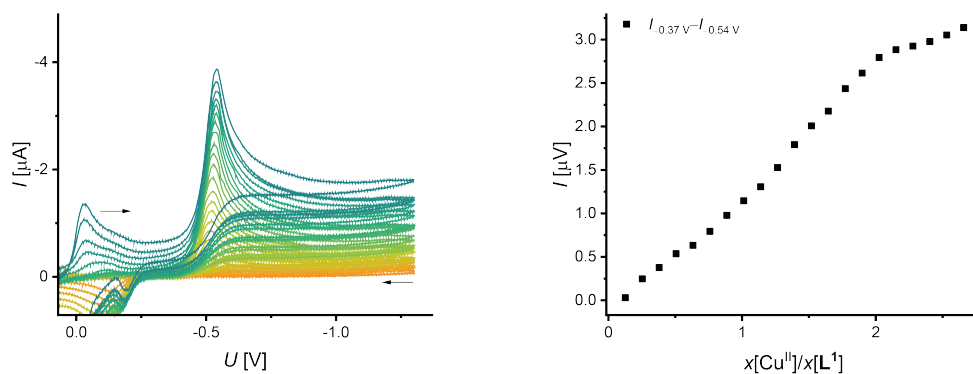


Figure 3.22 Cyclic voltammograms obtained in the course of HL^1 titration with Cu^{II} ions.

All our observations point to the fact that metal centres in dicopper complex act as independent units and that there is a negligible intramolecular electronic communication. Conversely to previously reported bismacrocylic copper complexes[47, 59] we haven't observed formation of phenolate bridge that would

coordinate both metal centres. Similarity of electrochemical behaviour of mono- and dicopper complexes can be explained in this way: reduction of one of the Cu^{II} ions in dicopper complex generates metallic copper which is immediately released from the complex and the remaining monocopper complex acts in the same way as the one synthesized and isolated.

Similarity of all obtained data can be further explained by the weak phenolate coordination: If there are two isomers, one with one Cu^{II} coordinated to the phenolate and one with no phenolate interaction and they are in equilibrium mixture, the noncoordinated Cu^{II} will be reduced first due to less negative potential and decreased stability. After this reduction, those Cu^{II} ions that are coordinated by phenolate are reduced regardless of the original molecule.

In conclusion, the electrochemical data indicate that the geometry and electronic properties of our phenol-bridged cyclam „dimer“ does not allow formation of the symmetrical dinuclear complexes with the short distance (and electronic communication) between the Cu^{II} ions, but the phenolate coordinates only weakly to one Cu^{II} ion.

3.6.2 Ligand HL^2 and its Cu^{II} complexes

Ligand HL^2 contains the redox-active nitro group on the central aromatic ring. Conversely from the HL^1 it is electrochemically active even in its free form. In aqueous conditions the nitro group undergoes four-electron irreversible reduction observable on both polarography and CV (fig. 3.23). It also behaves predictable in response to pH: the reductive potential shifts linearly with slope of 66 mV/pH over entire studied range (2-12). Deprotonation of phenolate happens too low to be observable in this way. The reduction limiting current is also consistent and corresponds to consumption of four electrons. Cathodic peak behaves in the same way.

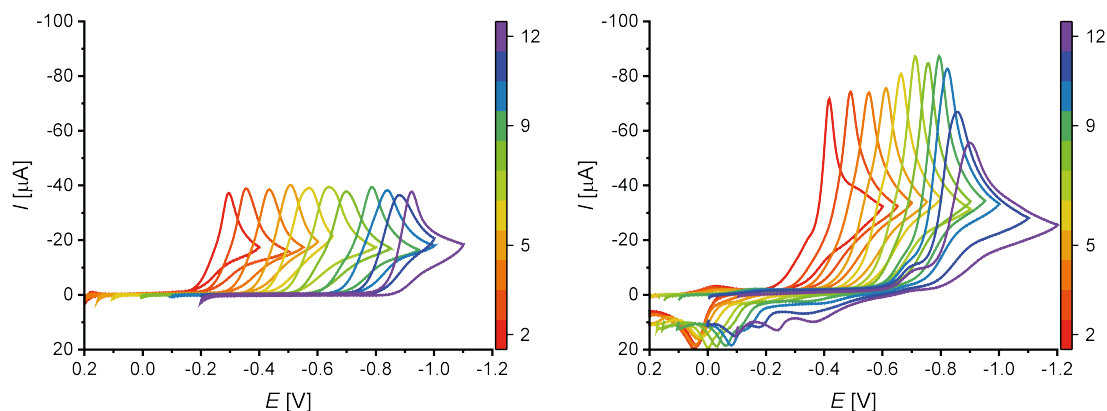


Figure 3.23 Cyclic voltammograms of HL^2 (left) and $[\text{Cu}_2(\text{L}^2)]^{3+}$ (right) at various pH ($c = 0.3 \text{ mM}$, $25 \text{ }^\circ\text{C}$) (only the first cycles)

When Cu^{II} are introduced into this system and $[\text{Cu}_2(\text{L}^2)]^{3+}$ is formed, there is little apparent change as the CV still contains one dominating peak and polarogram shows one main wave. However, these peaks and waves are significantly higher than those of free ligand. Reduction of nitro group and Cu^{II} are overlapped, imperceptibly in polarograms but more visibly in CV. Limiting current in DCV and height of CV peaks are about twice as high for complex than for HL^2 , confirming the hypothesis. Reduction of the dicopper

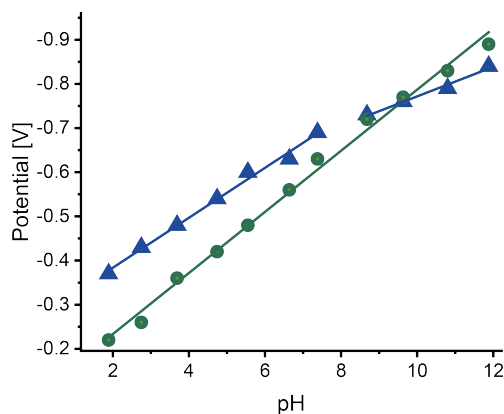


Figure 3.24 Cyclic voltammograms of HL^2 (left) and $[\text{Cu}_2(\text{L}^2)]^{3+}$ (right) at various pH ($c = 0.3 \text{ mM}$, 25°C) (only the first cycles)

complex consumes 8 electron (4 for nitro group and 2×2 for two Cu^{II} ions). Both reduction centres are reduced with similar potentials. Comparison of free ligand and the complex shows shift towards more negative potentials. There are minor shoulders present in CV of the complex, similarly to the situation in HL^1 complexes, they are most likely also caused by presence of isomers.

pH dependence of reductive potential of the complex $[\text{Cu}_2(\text{L}^2)]^{3+}$ is the same as that of free ligand from pH 2 to 8. After this point the slope changes to $\sim 30 \text{ mV/pH}$ (fig/ 3.24). Because the *p*-nitrophenol group is already fully deprotonated, this change may be associated with replacement of the phenolate by hydroxide anion. This is consistent with general weakness of this bond and observations from UV spectra.

3.7 Stability of ligand backbone

Although we have expected the phenol-macrocycle motif to be stable (it has never been reported otherwise), some mono-macrocyclic species appeared in the last step of ligand synthesis (removal of protecting Boc groups). Retrospectively, this was also reason why we have obtained crystals of $[\text{Zn}(\text{cyclam})](\text{CF}_3\text{COO})_2$ after prolonged heating of $[\text{Zn}_2\text{L}^1]^{3+}$ complex. The third sign of this instability was appearance of reduction peak at very negative potential in CV in some samples of isolated monocopper complex of L^1 and in samples that were heated between CV measurements overnight (this was done in order to detect possible thermodynamic complexes).

To investigate stability of the ligand backbone and rate of degradation, series of samples (HL^1 , HL^2 and their dicopper complexes) were incubated at RT and

at 90 °C in aqueous solutions with pH $\approx$ 2, 7 and 10. These solution were periodically analysed by HPLC-MS. The results show that both, free ligands and their complexes, undergo decomposition. The compounds decompose in the range of days at room temperature and in the range of hours at 90 °C (table 3.3).

Table 3.3 Decomposition degree of the studied ligands and their dinuclear complexes. Protonation states and charges of species are omitted.

Compound	Temperature	pH 2	pH 7	pH 10
HL ¹	25 °C	$\sim$ 35%	$\sim$ 20%	$\sim$ 35%
		after 300h	after 300h	after 300h
	90 °C	$\sim$ 100%	$\sim$ 100%	$\sim$ 100%
		after 2h	after 24h	after 2h
HL ²	25 °C	stable	stable	stable
		over 300h	over 300h	over 300h
	90 °C	$\sim$ 100%	$\sim$ 50%	$\sim$ 100%
		after 4h	after 50h	after 50h
[Cu ₂ (HL ¹)]	25 °C	$<$ 10%	stable	$<$ 10%
		after 300h	over 300h	after 300h
	90 °C	$\sim$ 100%	$\sim$ 100%	$\sim$ 10%
		after 24h	after 100h	after 24h
[Cu ₂ (HL ²)]	25 °C	stable	stable	stable
		over 50h	over 50h	over 50h
	90 °C	stable	stable	$\sim$ 50%
		over 50h	over 50h	after 50h

From decomposition data obtained in this way, few observations can be made: HL¹ is less stable then HL², which could be correlated with harshness of conditions necessary for hydroxomethylation of their parent compounds. Both extremes of pH scale also destabilize these compounds and several routes of decomposition were found: In the case of HL¹ decomposition leads to breaking of bond between cyclam and the methylene bridge releasing cyclam ($m/z = 201$). In acidic solution hydroxomethylene is formed and was identified by HPLC-MS ($m/z = 351$, fig. 3.25). In alkaline conditions, aldehyde is formed instead of alcohol ($m/z = 349$).

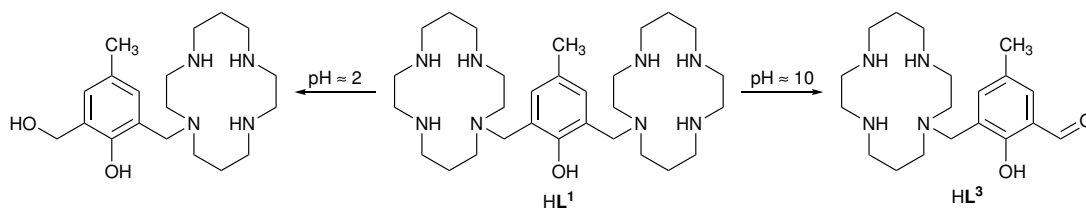


Figure 3.25 Structures of alcohol and aldehyde formed by decomposition of HL¹

Example of stacked chromatograms showing decomposition is shown in fig. 3.26. Identification of major peaks was performed using MS spectra. There were other minor products present in the mixture, but these were not identified.

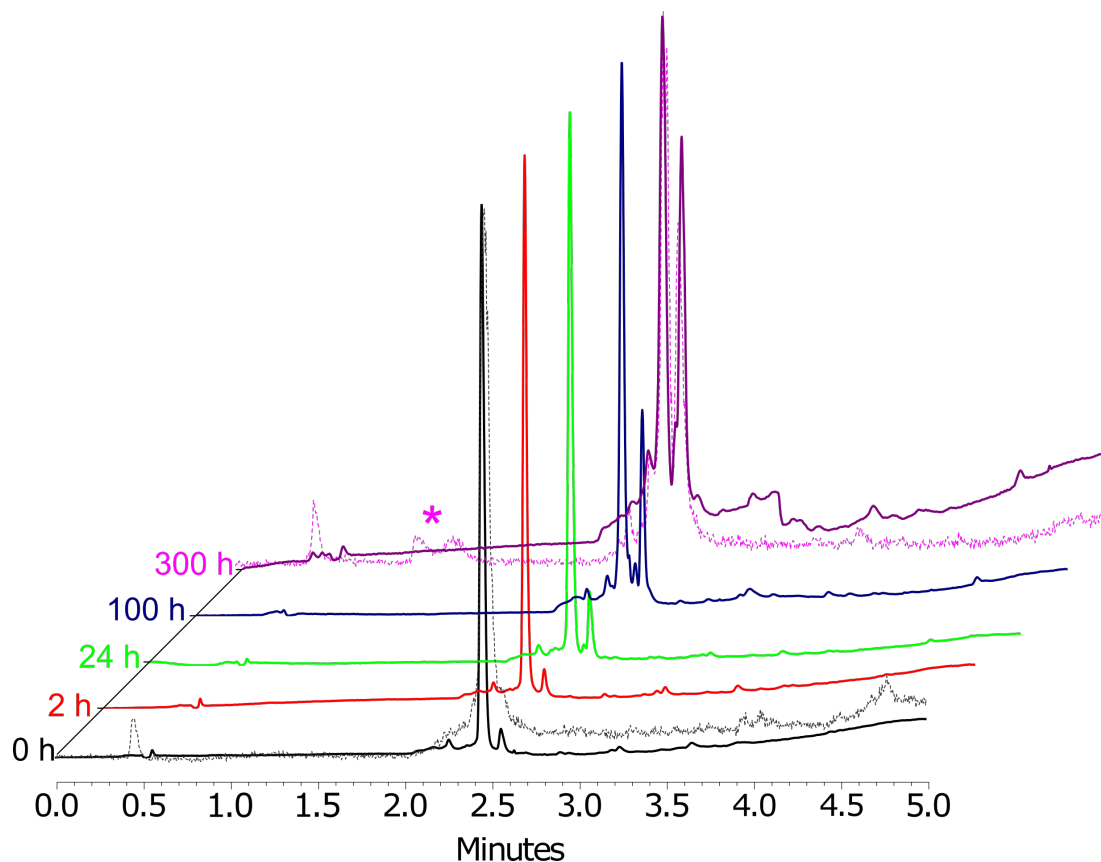


Figure 3.26 Chromatograms gathered in the course of $\mathbf{L}^1$ decomposition experiment ($\text{pH} = 2.4$, $c = 0.02 \text{ M}$, RT), full lines: UV detection at 254 nm, dashed lines MS-TIC detection. Asterisk indicates peaks of cyclam, method A.

As mentioned before, products of this decomposition were observed as separate reduction peak in CV of $[\text{Cu}(\text{HL}^1)]^{2+}$ samples that were obtained from alkaline reaction mixture (fig. 3.27). For this reason, we have synthesized compound HL^3 and studied its electrochemical behaviour. CV revealed reductive peak similar to the one observed in decomposed samples of $[\text{CuHL}^1]^{2+}$. Comparison between $\mathbf{L}^3$ and *in situ* prepared Cu^{II} complex shows that the copper is once again reductively decomplexed and reduction of aldehyde group at $E^\circ \approx -1.4 \text{ V}$ vs. SCE is same for complex and free ligands. This proves that copper is completely removed from the complex. Another proof of this hypothesis came from electrolysis of the sample contaminated with decomposition products: HPLC-MS analysis of product of electrolysis at -1.0 V showed aldehyde product but electrolysis at 1.5 V revealed that the aldehyde is indeed reduced to alcohol. As expected, this reduction is irreversible.

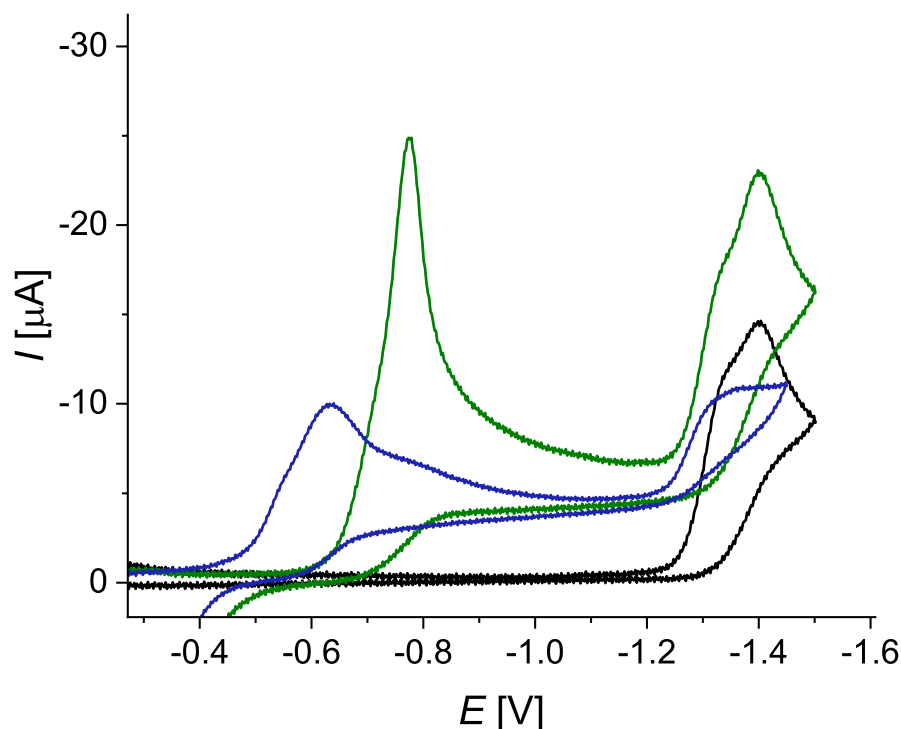


Figure 3.27 Cyclic voltammogram of $[\text{CuL}^1]^+$ after long standing showing the reduction peak of aldehyde (at -1.4 V) in the decomposition product (blue), cyclic voltammogram of the model compound HL^3 (black) and *in situ* formed $[\text{Cu}(\text{L}^3)]^+$ complex (green)

Decomposition of HL^2 is, first of all, slower than decomposition of HL^1 . Dicopper complex of L^2 can be called stable with the exception of heating in alkaline conditions. The decomposition of ligand has shown different decomposition route than HL^1 : instead of formation of alcohol or aldehyde, formation of free cyclam, amina HL^2_{am} and bis(aminal) HL^2_{am2} (fig. 3.28) was detected by HPLC-MS ($m/z = 574, 588$).

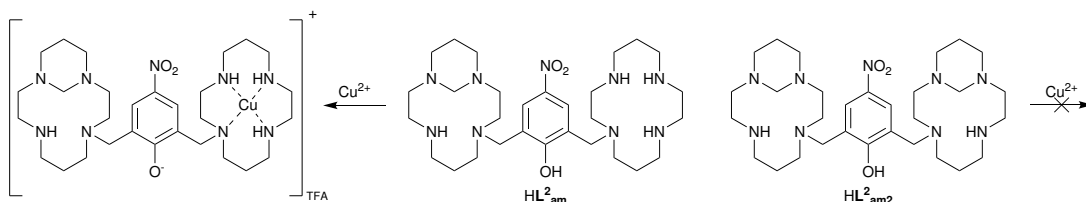


Figure 3.28 Structures of amina and bis(aminal) formed by decomposition of L^2

Since we have not detected free *p*-nitrophenol, mechanism of this decomposition is uncertain. Apart from mass spectra, we have confirmed existence of amins by $^{13}\text{C}\{^1\text{H}\}$ NMR measurement of sample that contained both amins: Two signals at 75-80 ppm can be observed. These signals are typical for formaldehyde amins

of cyclam. Existence of amins was further proved by *in situ* complexation with Cu^{II} . Excess of CuSO_4 was added to the sample that contained both amins and only trace amount of HL^2 . After 20 minutes, HPLC-MS was measured again. Peak associated with HL^2_{am2} , $m/z = 588$ was unchanged. Conversely peak associated with HL^2_{am} disappeared and was replaced with peak that contained compound with $m/z = 751$ with isotope pattern of compound containing one copper atom. This ion corresponds to $[\text{Cu}(\text{HL}^2_{\text{am}})]\text{TFA}$ adduct. Since the cyclam modified with formaldehyde cannot coordinate copper, only HL^2_{am} can form the complex.

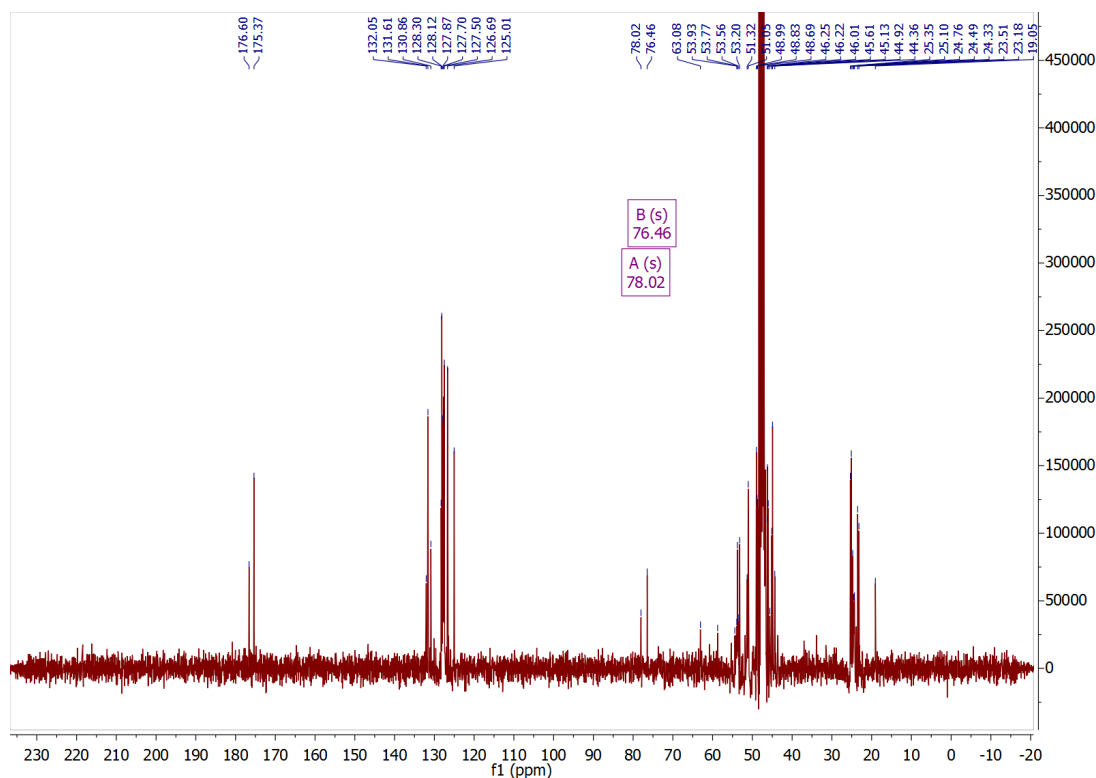


Figure 3.29 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of decomposition products of L^2 , containing two different aminal carbon atoms at 76.5 and 78.0 ppm

4 Conclusion

Two novel phenolate bridged bis(cyclam) ligands were prepared and characterized. Both form mono- and dinuclear complexes with Cu^{II} . For ligand **HL**¹ containing *p*-cresol, both complexes were isolated. In the case of **HL**² containing *p*-nitrophenol only dicopper complex was isolated. The complexes exist in solution as mixture of isomers. These isomers differ either in orientation of the substituents on cyclam subunits or in coordination of phenol/phenolate pendant arm. Both pendant arms are coordinated only weakly and do not form bridge between Cu^{II} ions. Likely cause of this behaviour is weakening of axial bonds of Cu^{II} -cyclam subunit by Jahn-Teller effect combined with longer distance between hydroxyl group and plane formed by Cu^{II} and nitrogen atoms. Instead of position above the plane of macrocycle (like in TACN and cyclen previously reported), Cu^{II} ions lies between the nitrogen atoms and geometry of the ligand does not force stronger bond between phenolate and metal. Cyclam units therefore behave mostly independently. In solid state structures Cu^{II} -cyclam subunits adopt *trans*-**III** geometry, which can be expected as it is the most stable of possible geometries. Electrochemically, these subunits also act independently: Cu^{II} ions undergo irreversible two-electron reduction to Cu^0 resulting in formation of amalgam at mercury electrode and dissociation of the complex.

We have also observed previously unreported instability of ligands and their complexes. This decomposition prevented studies of enzyme-like activity of complexes. In a sense, the kinetic stability of Cu^{II} -cyclam complexes that inspired this research was offset by the instability of pendant arms.

Last but not least usage of $\text{NaBH}(\text{OAc})_3$ with aromatic phenol-aldehyde has been found for alkylation of macrocycles protected by bulky groups, which has not been reported previously. Alkylation of macrocycles with phenol-aldehydes had to proceed through formation of aminal on unprotected macrocycle[62].

5 Experimental procedures

General

All commercially available starting materials were used without further purification. NMR spectra were acquired at 25 °C on spectrometers Varian VNMRs300, Bruker 400 Avance, or Bruker Avance III 600 MHz. For ^1H and ^{13}C NMR in D_2O measurements, the methyl signal of *t*-BuOH was used as an internal standard ($\delta = 1.2$ and 31.2 ppm for ^1H and ^{13}C NMR, respectively), for measurements in CDCl_3 , TMS was used as an internal standard ($\delta = 0.0$ ppm for both ^1H and ^{13}C NMR). In other solvents, the solvent itself was used as a reference according to published data[63]. All values of chemical shifts are given in ppm. Mass spectra were measured on Waters ACQUITY QDa spectrometer. Merck aluminum foils with silica gel 60 F254 were used for TLC.

Ligand stability study

0.05 M aqueous solutions of ligands HL^1 or HL^2 or their isolated Cu^{II} complexes were prepared at pH = 2, 7 and 10 adjusted with 1M HCl or 2M LiOH. The samples were stirred at RT or 90 °C and analysed by HPLC.

HPLC

The analyses were carried out on Waters ACQUITY QDa spectrometer (MS: electrospray ionization at atmospheric pressure, m/z +100–1250; UV-Vis: $\delta = 210 - 800$ nm diode array) equipped with RP column (Cortecs C18 2.7 μm , 4.6 $\times$ 50 mm) using mixture of 0.1 % TFA in H_2O and 0.1 % TFA in acetonitrile (MeCN) according to these gradients: Method A: 0.0 min 0% MeCN, 0.5 min 0% MeCN, 3.5 min 100% MeCN, 4.7 min 100% MeCN. Method B: 0.0 min 25% MeCN, 1.0 min 25% MeCN, 2.0 min 100% MeCN, 4.7 min 100% MeCN.

High resolution mass spectrometry

HR-MS spectra were acquired on the Bruker QqTOF compact instrument operated using Compass otofControl 4.0 (Bruker Daltonics, Bremen, Germany) software. ESI/MS data were collected in the positive ion mode at a scan range m/z 50–1000. The temperature of the drying gas was set to 220 °C at 4.0 L·min flow rate. Cone voltage was 2000 V. Samples were injected into the nebulizer with a syringe pump (Cole Parmer, USA) at a flow rate 3 $\mu\text{L} \cdot \text{min}^{-1}$.

FLASH chromatography

The separations were performed on ECOM ECS28P0X (UV-Vis Diode array detector $\delta = 200\text{--}800\text{ nm}$) with the following stationary phases: SiO_2 : NORMASIL 60 (40-63 μm) VWR chemicals; Al_2O_3 : Aluminium oxide (Activated, neutral, Brockman I) Honeywell-Fluka. Classical stationary phases were used in various reusable empty columns and those are specified in experiments as well as mobile phases. The reverse phase FLASH chromatography was performed using 40g columns prefilled with C18 stationary phase (YMC-Dispocack AT, ODS-25, Lot: 13578). The universal gradient of MeCN in H_2O (both solvents with 0.1 % of TFA) with a flow of 50 ml/min was used: 0.0 min 0% MeCN, 2.0 min 0% MeCN, 7.0 min 100% MeCN, 10.0 min 100% MeCN. After each use, the column was also washed with 150 ml of MeOH and was equilibrated with 100 ml of H_2O .

X-ray diffraction study

The single crystals of the complexes were grown by a slow diffusion of acetone over the gas phase to the aqueous solution of the particular di-copper(II) complex. Perchlorate anion in $[\text{Cu}_2(\text{HL}_2)(\text{ClO}_4)_4] \cdot 4.5\text{ H}_2\text{O}$ originates from perchloric acid which was used for acidification of the complex solution to pH 1. Diffraction data were collected at 120 K (Cryostream Cooler, Oxford Cryosystem) on a Bruker D8 VENTURE Kappa Duo PHOTON100 diffractometer with a micro-focus-sealed tube using $\text{Mo-K}\alpha$ ($\lambda = 0.71073\text{ \AA}$) radiation. Data were analysed using the SAINT (Bruker AXS Inc.) software package and subsequently corrected for absorption effects using the numerical method (SADABS). The structure was solved using direct methods (SHELXT2018)[64] and refined with full-matrix least-squares techniques (SHELXL2014)[65] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were found in the difference density map. However, hydrogen atoms bound to carbon atoms were fixed in theoretical positions using $U_{\text{eq}}(\text{H}) = 1.2 U_{\text{eq}}(\text{C})$ to keep the number of parameters low and only hydrogen atoms bound to nitrogen and oxygen atoms were fully refined. In the crystal structure of $[\text{Cu}_2(\text{HL}^1)(\text{SO}_4)(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 8\text{ H}_2\text{O}$, one formula unit forms the crystallographically independent part. The sulphate anion was found disordered in two positions with a relative occupancy of 82:18%. A large disorder was found also in water molecules of crystallization, thus, the corresponding electron density was squeezed using Platon.[66] In the crystal structure of $[\text{Cu}_2(\text{HL}^1)\text{Cl}_2]\text{Cl}_2 \cdot 5\text{ H}_2\text{O}$, one formula unit forms the crystallographically independent part. In the crystal structure of $[\text{Cu}_2(\text{HL}_1)\text{Cl}_2(\text{H}_2\text{O})_2][\text{Cu}_2(\text{HL}_1)\text{Cl}_4]\text{Cl}_2 \cdot 18\text{ H}_2\text{O}$, both complex molecules pose a plane of symmetry, so the structurally independent unit is a half of the formula unit. The crystal quality was rather low, as reflected by

high R and wR indices. However, all atoms (including the water molecules of crystallization) have reliable thermal factors, and the high value of R indices is caused probably by a large mosaicity, as reflected by high electron maxima in the difference map located very close to the central CuII ions. In the crystal structure of $[\text{Cu}_2(\text{HL}^2)(\text{ClO}_4)_4] \cdot 4.5 \text{H}_2\text{O}$, the structurally independent unit is half of the formula unit as the complex poses a plane of symmetry. A large disorder in water molecules of crystallization was found and, thus, it was treated using Platon SQUEEZE.[66] One of the perchlorate anions was split into two positions with a relative occupancy of 67:33%. Deposition Number(s) 2354224 (for $[\text{Cu}_2(\text{HL}^1)\text{Cl}_2]\text{Cl}_2 \cdot 5 \text{H}_2\text{O}$), 2354225 ($[\text{Cu}_2(\text{HL}^2)(\text{ClO}_4)_4] \cdot 4.5 \text{H}_2\text{O}$), 2354226 ($[\text{Cu}_2(\text{HL}^1)(\text{SO}_4)(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 8 \text{H}_2\text{O}$), 2354227(2b), 2354228($[\text{Cu}_2(\text{HL}^1)\text{Cl}_2(\text{H}_2\text{O})_2][\text{Cu}_2(\text{HL}^1)\text{Cl}_4]\text{Cl}_2 \cdot 18 \text{H}_2\text{O}$) contain(s) the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service.

Potentiometry

The methodology of the potentiometric titrations and the processing of the experimental data were analogous to those previously reported[67]. The titrations were carried out at $c_L = 0.004 \text{ M}$ in a vessel thermostated at $25 \pm 0.1 \text{ }^\circ\text{C}$ at ionic strength $I = 0.1 \text{ M}$ (NMe_4Cl). The water ion product, $\text{p}K_w = 13.81$, was taken from ref. [68] The overall protonation constants β_n are concentration constants and are defined by $\beta_n = [\text{H}_n \text{L}] / ([\text{H}]_n \cdot [\text{L}])$. The stepwise protonation constants are defined as $\log K_1 = \log \beta_1$; $\log K_n = \log \beta_n - \log \beta_{n-1}$. The constants (with their standard deviations) were calculated with the program package OPIUM[69].

UV-Vis spectroscopy

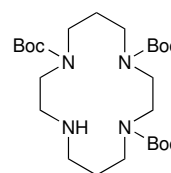
The measurements were carried out on the spectrometer Specord 50 Plus (Analytic Jena) in a quartz glass cell with an optical path of 1.00 cm. The cell was held in the temperature- controlled holder ($t = 25.0 \pm 0.2 \text{ }^\circ\text{C}$). Ligands and complexes were studied at $c = 4 \text{ mM}$ in the 360–1000 nm range and at $c = 0.04 \text{ mM}$ in the 210–350 nm range. The pH-dependent experiments were carried out in a 0.1M solution of NMe_4Cl using solutions of NMe_4OH and HCl in various concentrations to adjust pH. The complexation studies were carried out in buffers (acetate for $\text{pH} = 4$ and HEPES for $\text{pH} = 8$, both at 0.1M concentration). The $\text{p}K_a$ values were calculated from the absorbance at the maximum of the particular band as a function of pH by GEMS software[70].

Electrochemistry

The solutions were prepared by dissolving ligand or complex in 0.1M Britton-Robinson buffers within the pH range 2-12 at the analyte concentration of 0.3 mM. All voltammetric measurements were performed in a three-electrode setup in an undivided electrochemical cell. For DC polarography, a dropping mercury electrode (DME) with a drop time of 1 s was used. For cyclic voltammetry (CV), a hanging mercury drop electrode (HMDE) was used. The potentials were measured versus a saturated calomel reference electrode (SCE) which was separated by a salt bridge filled with buffer; the potentials are uncorrected. A platinum foil served as an auxiliary electrode. The polarographic cathodic scans were recorded at the scan rate of $10 \text{ mV} \cdot \text{s}^{-1}$. The CVs were recorded at the scan rates 50-1000 $\text{mV} \cdot \text{s}^{-1}$ using variable potential ranges and rates. For polarography, the analogic potentiostat PA3 and XY recorder 4103 (both Laboratorní přístroje, Prague, CZ) were used. The cyclic voltammetry was carried out using Static mercury drop electrode SMDE 1 (Laboratorní přístroje, Prague, CZ) and Autolab potentiostat PGSTAT 101 (Metrohm, Switzerland) driven by Nova 1.11 software. Argon was used as an inert gas to remove oxygen from the studied solutions. The number of electrons transferred in the course of redox processes were determined from polarography by comparison the substrate wave height with standard, which was CuSO_4 solution of known concentration.

Tri-tert-butyl-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (**1**)

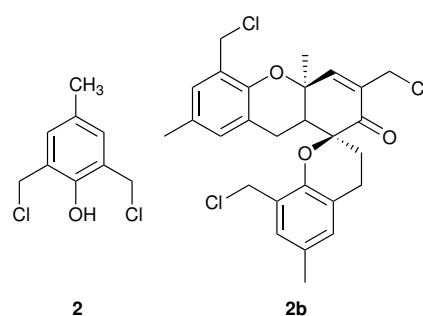
The compound was prepared by modified procedure [55]. Cyclam (2.00 g, 10 mmol) was dissolved in dichloromethane (400 mL) in a round-bottom flask which was then immersed in an ice bath. Solution of Boc_2O (6.54 g, 30 mmol) in dichloromethane (150 mL) was added dropwise during 2 h under stirring and the stirring continued at RT overnight. The volatiles were evaporated *in vacuo*, the oily residue was dissolved in hexane (250 mL), solution was filtered through S4 glass frit and solvent was evaporated. The resulting mixture of tetra-Boc cyclam and **1** was purified by FLASH chromatography (120 g SiO_2 , EtOAc $\longrightarrow$ 1 % EtOH in EtOAc, fraction volume 40 mL). Fractions were analysed by TLC (SiO_2 , EtOAc, Dragendorff reagent, tetra-Boc $\text{RF} = 0.9$, **1** $\text{RF} = 0.3$ with strong tailing) or by HPLC-MS (method B). Pure fractions were combined and evaporated *in vacuo*, yielding compound **1** (3.20 g, 64 %) as a white foam. ^1H NMR (400 MHz, MeOD) δ 3.53–3.30 (m, macrocyclic $-\text{CH}_2-$ next to carbamate, 12H), 2.82 (m, $-\text{N}-\text{CH}_2-$ ethylene, 2H), 2.66 (m, $-\text{N}-\text{CH}_2-$ propylene, 2H), 2.02 (p,



$^3J_{\text{HH}} = 6.03$ Hz, $\text{BocN}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NBoc}$, 2H), 1.76 (p, $^3J_{\text{HH}} = 6.11$ Hz, $\text{HN}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NBoc}$, 2H), 1.50 (m, CH_3 , 27H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, CDCl_3) δ 157.8, 157.3 and 157.2 (s, $-\text{C}(\text{O})-\text{O}-$, 3C), 81.0 (bs, $-\text{C}(\text{CH}_3)_3$, 3C), 51.1 (s, $-\text{HN}-\text{CH}_2-$ ethylene, 1C), 48.7, 47.9 and 45.6 (bs, macrocyclic CH_2 next to carbamate, 6C), 46.8 (s, $-\text{HN}-\text{CH}_2-$ propylene, 1C), 31.1 (bs, $\text{BocN}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NBoc}$, 1C), 30.2 (bs, $\text{HN}-\text{CH}_2-\text{CH}_2-\text{CH}_2\text{NBoc}$, 1C), 28.8 (m, CH_3 , 9C).

2,6-Bis(chloromethyl)-p-cresol (**2**)

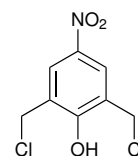
The compound was prepared by modified procedure [51]. The compound **8** (2.00 g, 12 mmol) was dissolved in the mixture of dichloromethane (40 mL) and concentrated aqueous HCl (80 mL) in a round-bottom flask. The mixture was stirred overnight at RT, the organic phase was separated, and the aqueous phase was reextracted with dichloromethane (2×50 mL). The organic phases were combined,



dried with Na_2SO_4 and the solvent was evaporated *in vacuo*. The crude product was recrystallized from hot hexane, affording yellowish crystals (1.75 g, 72 %). ^1H NMR (400 MHz, DMSO): δ 7.13 (s, ArH , 2H), 4.76 (s, CH_2Cl , 4H), 2.20 (s, CH_3 , 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO) δ 151.21 ($\text{ArC}-\text{OH}$, 1C), 132.08 (ArCH , 2C), 128.47 ($\text{ArC}-\text{CH}_3$, 1C), 125.17 ($\text{ArC}-\text{CH}_2\text{Cl}$, 2C), 42.36 (CH_2Cl , 2C), 19.81 (CH_3 , 1C). During one run of this procedure, a product of trimerization **2b** was also obtained, similar to one reported before in [52, 53]. The structure of this product was determined by X-ray crystallography; the single crystals were obtained from dichloromethane.

2,6-Bis(chloromethyl)-4-nitrophenol (**3**)

The compound was prepared by modified procedure [71]. The compound **10** (1.00 g, 3.9 mmol) dissolved in concentrated aqueous hydrochloric acid (30 mL) in a round-bottom flask and refluxed overnight. After cooling, the solid formed was filtered and washed with cold water. The resulting powder was recrystallized from hot toluene and **3**

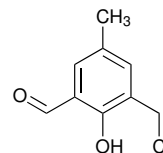


(0.76 g 82 %) was obtained in the form of pale-yellow crystals. ^1H NMR (400 MHz, CDCl_3): δ 8.25 (s, ArCH , 2H), 4.73 (s, CH_2Cl , 4H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, CDCl_3) δ 158.5 ($\text{ArC}-\text{OH}$, 1C), 141.1 ($\text{ArC}-\text{NO}_2$, 1C), 126.6 ($\text{ArC}-\text{CH}_2\text{Cl}$, 2C),

125.5 (*Ar*CH, 2C), 41.6 ($-\text{CH}_2\text{Cl}$, 2C).

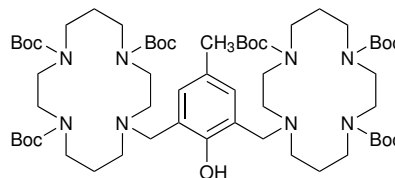
3-(Chloromethyl)-2-hydroxy-5-methylbenzaldehyde (**4**)

The compound was prepared by the modified procedure [57]. The compound **9** (2.15 g, 12.9 mmol) was dissolved in dichloromethane (30 mL) in a pear-shaped flask with a magnetic stirrer and a latex septum. The system was flushed with nitrogen and equipped with the nitrogen-filled balloon. The flask was then submerged in an ice bath and SOCl_2 (2.3 mL, 32 mmol) in dichloromethane (10 mL) was added with a syringe. Then, the mixture was stirred at RT for 16 hours. The solvents were evaporated in vacuo. The red residue was dissolved in the minimal volume of dichloromethane:hexane 1:1 mixture and red crystals were obtained by slow evaporation. The crystals were dissolved in dichloromethane (20 mL) and the solution was passed through a silica plug removing red colour. Silica was washed with additional dichloromethane (40 mL). The resulting clear solutions were combined and the solvent was evaporated in vacuo yielding **4** (2.00 g, 83 %) as a yellowish powder. ^1H NMR (300 MHz, CDCl_3): δ 11.23 (s, *Ar*-OH, 1H), 9.84 (s, $\text{CH}=\text{O}$, 1H), 7.44 (bs, *Ar*H, 1H), 7.32 (bs, *Ar*H, 1H), 4.65 (s, CH_2 , 2H), 2.34 (s, CH_3 , 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 196.45 ($\text{CH}=\text{O}$, 1C), 157.36 (*Ar*C-OH, 1C), 138.60 (*Ar*CH methylenechloride side, 1C), 134.02 (*Ar*CH aldehyde side, 1C), 129.23 (*Ar*C- CH_3 , 1C), 125.75 (*Ar*C- CH_2Cl , 1C), 120.42 (*Ar*C-CHO, 1C), 39.84 ($-\text{CH}_2-\text{Cl}$, 1C), 20.23 (CH_3 , 1C).



(2-Hydroxy-5-methyl-*m*-xylylene)-bis(tri-Boc cyclam) (**5**)

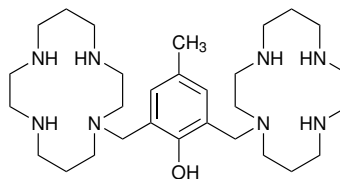
The compound **1** (1.92 g, 3.84 mmol) was dissolved in acetonitrile (100 mL) in a round-bottom flask and anhydrous K_2CO_3 (2.0 g, 14.5 mmol) was added. A dropping funnel was attached, and the apparatus was flushed with nitrogen. The flask was immersed in an ice bath and compound **2** (0.37 g, 1.8 mmol) dissolved in acetonitrile (20 mL) was added dropwise while stirring under the nitrogen atmosphere. The mixture was stirred overnight at RT. Then, the solids were filtered off on a S3 glass frit and the volatiles were evaporated *in vacuo*. The resulting foamy solid was dissolved in dichloromethane (10 mL) and purified by FLASH chromatography (120 g SiO_2 , 1% MeOH in dichloromethane $\rightarrow$ 2% MeOH in dichloromethane, fraction size 40 mL). The fractions containing pure product were combined and



the solvents were evaporated affording **5** (1.63 g, 80 %). ^1H NMR (600 MHz, MeOD) δ 6.87 (s, *Ar*H, 2H), 3.63 (s, *Ar*-CH₂-N, 4H), 3.42–3.35 (m, macrocyclic CH₂ next to carbamate, 24H), 2.68 (m, N-CH₂ ethylene, 4H), 2.50 (bs, N-CH₂ propylene, 4H), 2.33 (s, *Ar*-CH₃, 3H), 1.91 (bs, *Boc*N-CH₂-CH₂-CH₂-N*Boc*, 4H), 1.77 (bs, *Ar*-CH₂-N-CH₂-CH₂-CH₂-N*Boc*, 4H), 1.48 (s, *Boc*CH₃, 18H), 1.46 (s, *Boc*CH₃, 18H), 1.34 (bs, *Boc*CH₃, 18H); $^{13}\text{C}\{^1\text{H}\}$ NMR (150.93, MeOD) δ 156.0 (m, *Boc*C(O)O, 3C), 153.3 (*Ar*C-OH, 1C), 129.5 (bs, *Ar*C-CH₃, 1C), 127.4 (*Ar*CH, 2C), 123.0 (*Ar*C-CH₂, 2C), 79.8 (m, *Boc*C(CH₃)₃, 3C), 55.3 (bs, *Ar*-CH₂-N, 2C), 52.8 (bs, N-CH₂ ethylene, 2C), 51.5 (bs, N-CH₂ propylene, 2C), 46.6–46.2 (macrocyclicN-CH₂ next to carbamate, 12C), 28.4 (bs, *Boc*N-CH₂-CH₂-CH₂-N*Boc*, 2C), 27.4 (m, *Boc*C(CH₃)₃, 18C), 26.1 (bs, *Ar*-CH₂-N-CH₂-CH₂-CH₂-N*Boc*, 2C), 19.4 (*Ar*-CH₃, 1C): MS *m/z* 1134.16 (M + H, calc. 1134.78).

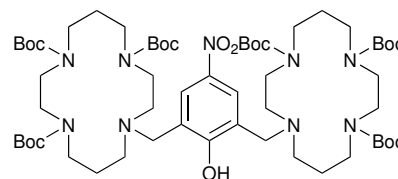
(2-Hydroxy-5-methyl-*m*-xylylene)-bis(cyclam) (**HL**¹)

The compound **5** (1.60 g, 1.43 mmol) was dissolved in dichloromethane (50 mL). Trifluoroacetic acid (10 mL) was slowly added dropwise while stirring. The mixture was stirred under nitrogen at RT overnight. The volatiles were evaporated *in vacuo*. The resulting brownish oil was purified by FLASH chromatography (40 g C18, 0.1 % TFA in H₂O → 0.1 % TFA in acetonitrile, fraction size 40 mL). The fractions containing product were combined and acetonitrile was removed *in vacuo* at 20 °C. The remaining aqueous solution was purified on the ion-exchange resin (DOWEX 1 in OH[−] cycle, elution with water). The fractions containing product were combined and lyophilized yielded **HL**¹ (0.57 g, 75 %) in the form of a white hygroscopic foam that quickly melts into a yellowish oil. ¹H NMR (600 MHz, MeOD) δ 6.94 (s, *Ar*H, 2H), 3.60 (s, *Ar*–CH₂–N, 4H), 2.80–2.77 (m, macrocyclic CH₂ next to secondary amine, 8H), 2.72–2.64 (m, macrocyclic CH₂ next to secondary amine, 16H), 2.61 (m, N–CH₂ ethylene, 4H), 2.54 (m, N–CH₂ propylene, 4H), 2.24 (s, CH₃, 3H), 1.86 (bs, *Ar*–CH₂–N–CH₂–CH₂–NH, 4H), 1.72 (m, HN–CH₂–CH₂–CH₂–NH, 4H); ¹³C{¹H} NMR (150.93 MHz, MeOD) δ 154.9 (*Ar*C–OH, 1C), 130.9 (*Ar*CH, 2C), 128.7 (s, *Ar*C–CH₃, 1C), 125.4 (*Ar*C–CH₂, 2C), 55.0 (s, N–CH₂ ethylene, 2C), 54.9 (s, *Ar*–CH₂–N, 2C), 53.9 (s, N–CH₂ propylene, 2C), 50.8 49.5, 48.9, 48.5 48.2 48.0 (macrocyclic N–CH₂ next to secondary amine, 12C), 29.0 (s, *Ar*–CH₂–N–CH₂–CH₂–CH₂–NH, 2C), 26.9 (bs, HN–CH₂–CH₂–CH₂–NH, 2C), 20.9 (CH₃, 1C); MS *m/z* 533.63 (M + H, calcd. 533.47); HR-MS *m/z* 533.4642 calcd. 533.4650. An alternative deprotection procedure: The compound **5** (1.00 g, 0.90 mmol) was suspended 6M aqueous HCl (35 mL) and the mixture was stirred overnight. The clear yellowish solution was layered with EtOH (50 mL), and the white solid precipitated after 4 h. The solid was filtered on a S4 glass frit, washed with cold EtOH, and dried in vacuum over P₂O₅ and SiO₂. **HL**¹·8HCl (0.7 g, 96 %) was obtained in the form of white powder. The product obtained using the alternative procedure shows lower purity (~95 %) due to the higher extent of the benzylic bonds degradation.



(2-Hydroxy-5-nitro-*m*-xylylene)-bis(tri-Boc cyclam) (6)

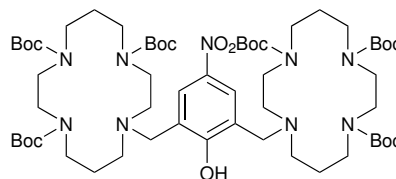
The compound **1** (1.33 g, 2.65 mmol) was dissolved in a round-bottom flask in acetonitrile (100 mL) and anhydrous K₂CO₃ (2.0 g) was added. A dropping funnel was attached, and the apparatus was flushed with nitrogen.



The flask was immersed in an ice bath and the solution of compound **3** (0.30 g, 1.8 mmol) in dichloromethane (20 mL) was added dropwise while stirring under the nitrogen atmosphere. The mixture was stirred overnight at RT. Then, the solids were filtered off on a S3 glass frit and the volatiles were evaporated *in vacuo*. The resulting foamy solid was dissolved in EtOAc (10 mL) and purified by FLASH chromatography (120 g SiO₂, 1 % MeOH in dichloromethane → 5 % MeOH in dichloromethane, fraction size 40 mL). The fractions containing pure product were combined and the solvents were evaporated affording **6** (0.94 g, 64 %) as a yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 8.03 (s, *Ar*H, 2H), 3.74 (s, *Ar*-CH₂-N, 4H), 3.40–3.28 (m, macrocyclic CH₂ next to carbamate, 24H), 2.73 (bs, N-CH₂ ethylene, 4H), 2.52 (bs, N-CH₂ propylene, 4H), 1.90 (bs, BocN-CH₂-CH₂-CH₂-NBoc, 4H), 1.78 (bs, *Ar*-CH₂-N-CH₂-CH₂-CH₂-NBoc, 4H) 1.48 (s, BocCH₃, 18H), 1.45 (s, BocCH₃, 18H), 1.37 (bs, BocCH₃, 18H); ¹³C{¹H} NMR (150.93, CDCl₃) δ 162.2 (*Ar*C-OH, 1C), 155.7 (m, BocC(O)O, 3C), 139.7.5 (*Ar*C-NO₂, 1C), 124.0 (bs, *Ar*C-CH₂ and *Ar*CH, overlapped, 4C), 79.9 (m, BocC(CH₃)₃, 3C), 55.1 (bs, *Ar*-CH₂-N, 2C), 52.0 (bs, N-CH₂ ethylene and N-CH₂ propylene, overlapped, 4C), 48.1–46.5 (m, macrocyclic N-CH₂ next to carbamate, 12C), 29.12 (bs, BocN-CH₂-CH₂-CH₂-NBoc, 2C), 28.5, (bs, BocC(CH₃)₃, 24C), 28.41 (bs, BocC(CH₃)₃, 12C), 26.1 (bs, *Ar*-CH₂-N-CH₂-CH₂-CH₂-NBoc, 2C), MS *m/z*: 1165.13 (M + H, calcd. 1164.75).

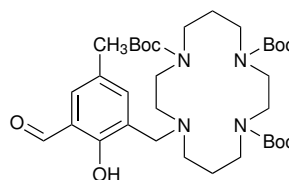
(2-Hydroxy-5-nitro-m-xylylene)-bis(cyclam) (HL²)

The compound **6** (0.20 g 1.4 mmol) was dissolved in dichloromethane (10 mL) and the solution was cooled in an ice bath. Trifluoroacetic acid (1 mL) was slowly added dropwise. The mixture was stirred under nitrogen at RT overnight. After evaporation of the volatiles *in vacuo*, the resulting brownish oil was purified by FLASH chromatography (40 g C18, 0.1 % TFA in H₂O → 0.1 % TFA in acetonitrile, fraction size 40 mL). The fractions containing pure product were combined and acetonitrile was removed *in vacuo* at 20 °C. The resulting aqueous solution was purified on ion-exchange resin (DOWEX 1 in OH⁻ cycle, 50–100 mesh, elution with water). The fractions containing the product were combined and the lyophilization yielded HL² (0.072 g, 74 %) as a yellow fluffy solid, that quickly melts into yellow oil. ¹H NMR (600 MHz, D₂O, pD = 9.4) δ 8.07 (s, *Ar*H, 2H), 3.58 (s, *Ar*-CH₂-N, 4H), 2.80–2.77 (m, macrocyclic CH₂ next to secondary amine, 8H), 2.72–2.64 (m, macrocyclic CH₂ next to secondary amine, 16H), 2.61 (m, N-CH₂ ethylene, 4H), 2.54 (m, N-CH₂ propylene, 4H) 1.86 (bs, *Ar*-CH₂-N-CH₂-CH₂-NH, 4H), 1.72 (m, HN-CH₂-CH₂-CH₂-NH, 4H); ¹³C{¹H} NMR (150.93 MHz, D₂O, pD = 9.4) δ 176.8 (*Ar*C-OH, 1C), 131.4 (*Ar*C-NO₂, 1C), 127.3 (*Ar*C-CH₂, 2C), 126.8 (s, *Ar*CH, 2C), 54.5 (s, N-CH₂ ethylene, 2C), 52.0 (s, *Ar*-CH₂-N, 2C), 51.9 (s, N-CH₂ propylene, 2C), 49.6, 48.9, 47.6 46.8, 45.8, 45.3 (macrocyclic N-CH₂ next to secondary amine, 12C), 24.9 (s, *Ar*-CH₂-N-CH₂-CH₂-CH₂-NH, 2C), 23.8 (bs, HN-CH₂-CH₂-CH₂-NH, 2C); MS *m/z*: 564.65 (M + H, calc. 564.80); HR-MS *m/z*: 564.4340 calcd. 564.4344.



(2-Hydroxy-5-methyl-3-methylene-(tri-Boc cyclam))-benzaldehyde (7)

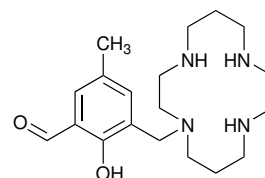
The compound **1** (1.5 g, 3 mmol) was dissolved in the suspension of anhydrous K₂CO₃ (1.6 g) in acetonitrile (100 mL) in a round-bottom flask. The flask was immersed in an ice bath. The solution of **4** (0.60 g, 3.2 mmol) in acetonitrile (50 mL) was added dropwise over 2 hours and the mixture was stirred at RT overnight. The solids were filtered off on a S3 glass frit and the solvents were evaporated *in vacuo*. The resulting foam was dissolved in dichloromethane and purified by FLASH chromatography (120 g SiO₂, 1 % MeOH in dichloromethane → 2 % MeOH in dichloromethane, fraction size 40 mL, only fractions with absorption at 210 nm or 260 nm were collected).



The fractions containing the pure product (according to HPLC-MS, method A) were combined and evaporation of the solvents *in vacuo* gave **7** (0.80 g, 41 %) as a yellowish off-white foam. ^1H NMR (400 MHz, MeOD) δ 10.01 (s, CH=O, 1H), 7.42 (d, $^4J_{\text{HH}} = 1.70$ Hz, ArH aldehyde side, 1H), 7.35 (d, $^4J_{\text{HH}} = 1.70$ Hz, ArH macrocycle side, 1H), 3.64 (s, Ar-CH₂-N, 2H), 3.44–3.33 (m, macrocyclic CH₂ next to carbamate, 12H), 2.66 (bs, N-CH₂ ethylene, 2H), 2.51 (bs, N-CH₂ propylene, 2H), 2.32 (s, Ar-CH₃, 3H), 1.87 (bs, BocN-CH₂-CH₂-CH₂-NBoc, 2H), 1.79 (bs, Ar-CH₂-N-CH₂-CH₂-CH₂-NBoc, 2H), 1.48 (s, BocCH₃, 9H), 1.45 (s, BocCH₃, 9H), 1.37 (bs, BocCH₃, 9H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.62, CDCl₃) δ 194.57 (CH=O, 1C), 158.64 (ArC-OH, 1C), 155.74 (m, BocC(O)O-, 3C), 138.3 (ArCH macrocycle side, 1C), 130.7 (ArCH aldehyde side, 1C), 128.6 (ArC-CH₃, 1C), 125.6 (ArC-CH₂-N, 1C), 121.3 (ArC-CH=O, 1C), 79.9 (m, BocC(CH₃)₃, 3C), 54.3 (bs, Ar-CH₂-N, 1C), 53.4 (bs, N-CH₂ ethylene, 1C), 51.8 (bs, N-CH₂ propylene, 1C), 47.9–46.3 (m, macrocyclic N-CH₂ next to carbamate, 6C), 29.0 (bs, BocN-CH₂-CH₂-CH₂-NBoc, 2C), 28.6 (s, BocC(CH₃)₃, 9C), 28.5 (s, BocC(CH₃)₃, 9C), 26.7 (bs, Ar-CH₂-N-CH₂-CH₂-CH₂-NBoc, 1C), 20.45 (Ar-CH₃, 1C); MS *m/z*: 649.64 (M + H, calc. 649.85).

(2-Hydroxy-5-methyl-3-methylene(cyclam))benzaldehyde (**HL³**)

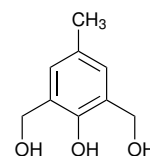
The compound **7** (0.75 g, 1.15 mmol) was dissolved in dichloromethane (20 mL) in and the flask was immersed in an ice bath. Trifluoroacetic acid (1 mL) was added slowly and the mixture was stirred overnight at RT. The reaction mixture (containing an yellowish oil separated from the dichloromethane solution) was washed with water (3×10 mL). The aqueous solutions were combined and purified by FLASH chromatography (40 g C18, 0.1 % TFA in H₂O → 0.1 % TFA in acetonitrile, fraction size 40 mL). The fractions containing the product were combined and the lyophilization yielded **L³·xTFA·yH₂O** (0.98 g) as a highly hygroscopic powder containing 0.351 g of **HL³** (87 % theor.) according to the titration with Cu^{II} ions. ^1H NMR (600 MHz, D₂O, pD = 3.2) δ 9.71 (s, -CH=O, 1H), 7.56 (d, $^4J_{\text{HH}} = 2.25$ Hz, ArH *o*-aldehyde, 1H), 7.42 (d, $^4J_{\text{HH}} = 2.25$ Hz, ArH *o*-methylenecyclam, 1H), 4.35 (s, Ar-CH₂-N, 2H), 3.58–3.50 (m, macrocyclic CH₂, 4H), 3.46 (bs, macrocyclic CH₂, 4H), 3.34–3.31 (m, macrocyclic CH₂, 2H), 3.26–3.20 (m, macrocyclic CH₂, 6H), 2.19 (s, Ar-CH₃, 3H), 2.09–2.02 (m, N-CH₂-CH₂-CH₂-NH, 4H); $^{13}\text{C}\{^1\text{H}\}$ NMR (150.93 MHz, D₂O, pD = 3.2) δ 198.6 (s, -CH=O, 1H), 156.9 (ArC-OH, 1C), 140.6 (ArCH



o-methylenecyclam, 1C), 137.2 (*Ar*CH, *o*-aldehyde, 1C), 130.9 (*Ar*C-CH₃, 1C), 120.7 (s, *Ar*C-CH=O, 1C), 116.2 (s, *Ar*C-CH₂-N, 1C), 53.7 (s, *Ar*-CH₂-N, 1C), 47.6 (s, N-CH₂ propylene, 1C), 44.1 (s, N-CH₂ ethylene, 1C), 41.3, 40.6, 40.6, 37.1, 37.0 36.6, (macrocyclic N-CH₂ next to secondary amine, 6C), 19.05 (s, *Ar*-CH₃, 1C), 18.1, 17.6 (s, HN-CH₂-CH₂-CH₂-NH, 2C); HR-MS *m/z* 349.2591 calcd. 349.2585.

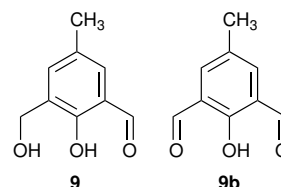
Bis(hydroxymethyl)-*p*-cresol (**8**)

The compound was prepared by modified procedure[50]. NaOH (9.48 g, 0.24 mol) was dissolved in water (36 mL). 37% aqueous formaldehyde (38 mL, 0.47 mol) and *p*-cresol (20.0 g, 0.185 mol) were added and the mixture was stirred at RT overnight. The formed solid sodium salt of **8** was filtered on a glass frit. The salt was dissolved in water (300 mL) and solution pH was adjusted to 7 with acetic acid. The mixture was cooled in a refrigerator for 30 min and **8** (18.5 g) in the form of white crystals was filtered on a glass frit and dried in a desiccator under vacuum. The filtrate obtained after isolation of the sodium salt was neutralized in similar manner to yield another portion of the crude product. The crude compound was recrystallized from hot EtOAc to yield the second fraction of **8** (8.2 g). Overall yield was 26.7 g (86 %). ¹H NMR (400 MHz, DMSO): δ 6.93 (s, *Ar*H, 2H), 4.51 (s, -CH₂OH, 4H), 2.19 (s, -CH₃, 3H) ¹³C{¹H} NMR (126 MHz, DMSO) δ 152.03 (*Ar*C-OH, 1C), 130.60 (*Ar*C-CH₃, 1C), 129.84 (*Ar*CH, 2C), 128.98 (*Ar*C-CH₂OH, 2C), 61.94 (-CH₂OH, 2C), 23.08 (-CH₃, 1C)



2-Hydroxy-3-(hydroxymethyl)-5-methylbenzaldehyde (**9**)

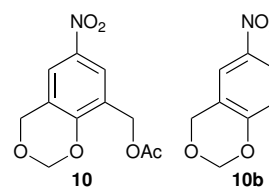
The compound was prepared by the modified procedure[72]. The compound **8** (4.0 g, 24 mmol) was dissolved in acetone (40 mL) in a round-bottom flask. MnO₂ (23.45 g, 270 mmol) was added and the mixture was stirred at RT overnight. The mixture was filtered on a S4 glass frit. The solid was extracted with acetone (40 mL) using ultrasound and filtered again. The filtrates were combined, and the volatiles were evaporated *in vacuo*. The resulting solid was purified by FLASH chromatography (90 g SiO₂, 1 % MeOH in dichloromethane → 2 % MeOH in dichloromethane, fraction volume 50 mL). The fractions were analysed by TLC (SiO₂, 1 % MeOH in DCM). The fractions containing pure product were combined and evaporation of solvents *in vacuo* yielded **9** (2.15 g, 57 %). The early fractions



of the chromatography yielded the dialdehyde **9b** (0.51 g, 13 %). **9**: ^1H NMR (300 MHz, CDCl_3): δ 11.16 (s, Ar-OH , 1H), 9.84 (s, CH=O , 1H), 7.38 (d, $^4J_{\text{HH}} = 2.24$ Hz, Ar-H , 1H), 7.27 (d, $^4J_{\text{HH}} = 2.24$ Hz, Ar-H , 1H), 4.71 (s, $-\text{CH}_2-\text{OH}$, 2H), 2.32 (s, CH_3 , 3H). **9b**: ^1H NMR (300 MHz, CDCl_3): δ 11.43 (s, Ar-OH , 1H), 10.19 (s, $-\text{CH=O}$, 2H), 7.74 (s, Ar-H , 2H), 2.36 (s, CH_3 , 3H).

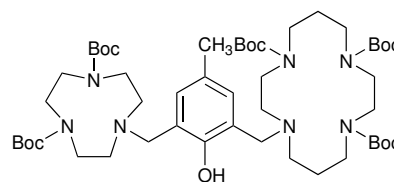
8-Acetoxymethyl-6-nitro-1,3-benzodioxene (**10**)

The compound was prepared by the modified procedure[71]. *p*-nitrophenol (13.19 g, 0.10 mol) was suspended in the mixture of 96% H_2SO_4 (20 mL) and acetic acid (100 mL) in a round-bottom flask. The mixture was heated to 55 °C and paraformaldehyde (24 g, 0.8 mol) was added. The mixture was stirred at the same temperature for 16 h. As the reaction was incomplete (according to ^1H NMR showing ~40 % **10b**), another paraformaldehyde portion (24 g) was added, and the mixture was heated and stirred for additional 20 hours. The reaction mixture was then transferred into a 1L beaker, cooled in an ice bath, diluted with water (100 mL) and neutralized with K_2CO_3 to $\text{pH} \approx 7$ (Caution: the neutralization produces copious amounts of foam). The solids were filtered and repeatedly recrystallized from hot EtOH to yield **10** (~90% purity, 23.2 g, 81% yield). ^1H NMR (400 MHz, DMSO): δ 8.10 (d, $^4J_{\text{HH}} = 2.80$ Hz, ArCH , 1H), 8.08 (d, $^4J_{\text{HH}} = 2.68$ Hz, ArCH , 1H), 5.45 (s, $-\text{CH}_2-\text{OAc}$, 2H), 5.09 (s, $\text{Ar-CH}_2-\text{O}$, 2H), 5.00 (s, $\text{O-CH}_2-\text{O}$, 2H), 2.09 (s, CH_3 , 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO) δ 170.2 (C=O , 1C), 155.6 (ArC-O , 1C), 140.3 (ArC-NO_2 , 1C), 124.7 ($\text{ArC-CH}_2-\text{OAc}$, 1C), 123.3 (ArCH , 1C), 122.4 ($\text{ArC-CH}_2-\text{O}$, 1C), 121.9 (ArCH , 1C), 92.0 ($\text{O-CH}_2-\text{O}$, 1C), 65.4 ($\text{Ar-CH}_2-\text{O}$), 59.5 ($\text{Ar-CH}_2-\text{OAc}$, 1C), 20.6 (CH_3 , 1C).



(2-Hydroxy-5-methyl-*m*-xylylene)-(di-Boc TACN)(tri-Boc cyclam)(**11**)

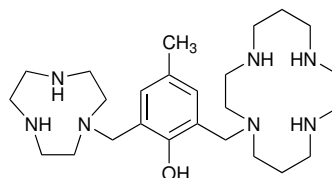
The compound **7** (0.23 g, 0.35 mmol) was dissolved in round-bottom flask in 20 ml of dichloromethane. Into this mixture 0.5 g of Na_2SO_4 and 0.12 g of $\text{NaBH}(\text{OAc})_3$ was added and the mixture was cooled to 0 °C. Via dropping funnel 0.127 g of di-Boc TACN (prepared according to [73] dissolved in 10 ml of dichloromethane was added. After overnight stirring at RT the mixture was analysed using TLC (5 % MeOH in DCM, detection by UV and Dragendorff reagent). Traces of **7** were detected and additional



di-Boc TACN (0.025 g) and NaBH(OAc)₃ (0.05 g) were added. After another night, traces of **7** could be detected only by HPLC-MS. The mixture was filtrated through S3 glass frit. Filtrate was washed twice with sat. aq. NaHCO₃ and dried *in vacuo*. Resulting foam was purified by FLASH chromatography (120 g SiO₂, 5 % MeOH in dichloromethane, fraction size 40 mL). Fractions containing **11** were dried *in vacuo* resulting in yellow foam of **11** (0.22 g 64 % teor.) The product was characterized only by MS, as it was immediately used for next step. MS:*m/z*: 962.94 (M + H, calc. 962.65).

(2-Hydroxy-5-methyl-*m*-xylylene)-TACN-cyclam)(**12**)

The compound **11** (0.1 g, 0.15 mmol) was dissolved in 10 mL 6M HCl and stirred at RT overnight. After it was layered with EtOH and after 2 hours, white precipitate was filtered. The filtrate was dried over mixture of SiO₂ and P₂O₅ in vacuum desiccator, affording



white powder. As the product was not titrated to ascertain number of chloride ions, yield was not calculated. ¹H NMR (600 MHz, D₂O, pD = 3.2): δ 7.36 (d, ⁴J_{HH} = 2.20 Hz, *Ar*CH, 1H), 7.31 (d, ⁴J_{HH} = 2.20 Hz, *Ar*CH, 1H), 4.53 (s, *Ar*-CH₂-N cyclene, 2H), 3.95 (s, *Ar*-CH₂-N TACN, 2H), 3.80–3.66 (m, CH₂N macrocyclic, 12H), 3.53–3.41 (m, CH₂N macrocyclic, 8H), 3.24 (m, CH₂N macrocyclic TACN, 4H), 3.05 (m, CH₂N macrocyclic TACN, 4H), 2.31 (s, *Ar*-CH₃, 3H), 2.24 (m, N-CH₂-CH₂-CH₂-NH, 4H). ¹³C{¹H}NMR (150.93 MHz, D₂O) δ 153.1, 136.9, 134.7, 133.7, 126.4, 119.6 (*Ar*C, 6C), 57.1 (*Ar*-CH₂-N cyclene, 1C), 55.9 (*Ar*-CH₂-N TACN, 1C), 50.0, 49.1, 46.0, 45.9, 45.9, 44.3, 43.0, 42.4, 42.3, 38.7, 38.4 (N-CH₂, 14C), 21.0 (*Ar*-CH₃, 1C), 20.0 (N-CH₂-CH₂-CH₂-NH 1C), 19.5 (N-CH₂-CH₂-CH₂-NH, 1C). MS:*m/z*: 462.58 (M + H, calc. 462.39).

[Cu(HL₁)](SO₄)

HL¹ (0.125 g, 0.23 mmol) was dissolved in water (5 mL) and solution of CuSO₄ · 5 H₂O (0.058 g, 0.23 mmol) was added. pH was adjusted to 5.5 by the slow addition of 1M H₂SO₄. The dark solution was stirred at RT overnight. The solvent was evaporated *in vacuo* at 40 °C, and the complex was purified by FLASH chromatography (alumina, iPrOH:ammonia:H₂O 7:3:3, collecting fractions with absorption at 260 nm). The pale green fractions were combined. The volatiles were repeatedly evaporated *in vacuo* and the residue redissolved in water to remove traces of ammonia. This process was accompanied by the color change from green to blue. [Cu(HL¹)]SO₄ (40 mg, 24 %) was obtained by lyophilization from the

aqueous solution. HPLC-MS m/z : 708.61 $\{[\text{Cu}(\text{L}^1)](\text{CF}_3\text{COO})\}^+$, calcd. 708.37), 822.61 $\{[\text{Cu}(\text{HL}^1)](\text{CF}_3\text{COO})_2\}^+$ calcd. 822.37).

$[\text{Cu}_2(\text{HL}^1)](\text{SO}_4)_2$

HL^1 (0.125 g, 0.23 mmol) was dissolved in water (5 mL) and the solution of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ (0.234 g, 0.94 mmol) was added. The violet solution was stirred overnight in a closed vial, pH at the end of the complexation was 3.5. The solution was filtered through a 0.22 μm PVDF microfilter and purified on alumina (elution with $\text{H}_2\text{O}:\text{EtOH}$ 1:1 until the collected fractions were colourless). The violet fractions were combined and lyophilized yielding $[\text{Cu}_2(\text{HL}^1)](\text{SO}_4)_2$ (0.20 g, 100 %) as a grey fluffy powder. HPLC-MS m/z : 885.46 ($\{[\text{Cu}_2(\text{L}^1)](\text{CF}_3\text{COO})_2\}^+$ calc. 885.23).

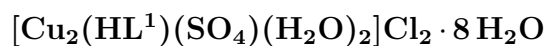
$[\text{Cu}_2(\text{L}^2)]\text{Cl}_3$

HL^2 (0.186 g, 0.33 mmol) was dissolved in water (10 mL) and $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$ (0.225 g, 1.32 mmol) added. pH was adjusted to 5.0 with LiOH and the solution was stirred at RT overnight. The complex was purified by HPLC (RP-C18, elution with 0.1 % TFA in water $\rightarrow$ 0.1 % TFA in acetonitrile, fraction size 20 mL). The fractions that contained complex were combined and acetonitrile was removed *in vacuo* at RT. The resulting aqueous solution was purified on anion exchanger (DOWEX 1 in OH^- cycle, elution with water). The colored fractions were combined and evaporation *in vacuo* yielded $[\text{Cu}_2(\text{L}^2)]\text{Cl}_3$ (0.213, 77 %) in the form of the deep brown solid. HPLC-MS m/z : 914.57 ($\{[\text{Cu}_2(\text{L}^2)](\text{CF}_3\text{COO})_2\}^+$ calc. 914.50).

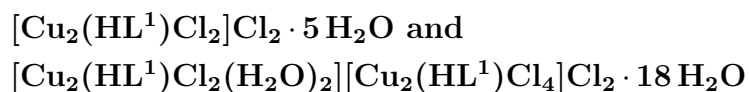
Preparation of single crystals for X-ray analysis

Stock solution of $[\text{Cu}_2(\text{L}^1)]$

HL^1 (0.025 g, 0.04 mmol) was dissolved in water (10 mL) and was adjusted with 1M solution of LiOH to pH = 5. $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$ (30 mg, 0.17 mmol) in water (10 mL) was added and solution was readjusted to pH = 5 with solid LiOH. The solution was stirred at RT for 2 hours. The excess of Cu^{II} ions was removed by column chromatography on Al_2O_3 (elution with water). The colored fractions were combined and purified on DOWEX 1 (OH^- cycle, elution with water). The resulting solution (pH $\approx$ 9) was used for crystallization experiments.



The $[\text{Cu}_2(\text{L}^1)]$ stock solution (0.5 mL) was acidified to $\text{pH} \approx 5$ with 1M aqueous H_2SO_4 . The deep violet needles were grown by the slow effusion of acetone into the solution.



The $[\text{Cu}_2(\text{L}^1)]$ stock solution (0.5 mL) was acidified to $\text{pH} \approx 5$ with 1M aqueous H_3PO_4 . A mixture of violet needles and violet crystals was obtained by the slow effusion of acetone into the solution. The two crystals of different shapes (needles and plates) were selected using a microscope.



$[\text{Cu}_2(\text{L}^2)]\text{Cl}_3$ (20 mg) was dissolved in water (5 mL) and Ag_2O was added. The mixture was stirred for 15 min, and the solids were removed by centrifugation. The solution was acidified to $\text{pH} = 1.25$ with 1M HClO_4 . The crystals were grown by the slow effusion of acetone into the solution.

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List of Abbreviations

HPLC-MS	High performance liquid chromatography - mass spectrometry
UV-Vis	Ultra violet and visible light spectroscopy
DCP	Direct current polarography
CV	Cyclic voltammetry
EPR	Electron paramagnetic resonance
His	Histidine
ATP	Adenosine triphosphate
MRI	Magnetic resonance imaging
bpy	2,2'-Bipyridine
DNA	Deoxyribonucleic acid
EMA	European medicine association
IR	Infra red
EDTA	Ethylenediamine tetraacetate
DO3A	1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid
BDNPP	bis(2,4-dinitrophenyl) phosphate
SPECT	Single photon emission computed tomography
PET	Positron emission tomography
TFA	Trifluoroacetic acid
DCM	Dichloromethane
Boc	<i>tert</i> -butyloxycarbonyl
RP	Reversed phase
DIAD	Diisopropyl azodicarboxylate
<i>p</i> -XCB	1,4-Bis[(1,4,8,11-tetraazacyclotetradecan-1-yl)methyl]benzene
MF	Mobile phase
HOMO-LUMO	Highest occupied molecular orbital-lowest unoccupied molecular orbital
NMR	Nuclear magnetic resonance
TMS	Tetramethyl silane
RT	Room temperature
MeCN	Acetonitrile
HR-MS	High resolution mass spectrometry
ESI/MS	Electrospray ionization mass spectrometry
DME	Dropping mercury electrode
HMDE	Hanging mercury drop electrode
SCE	Saturated calomel electrode

A Supplementary information for experiments

A.1 NMR spectra and HPLC-MS chromatograms of prepared compounds

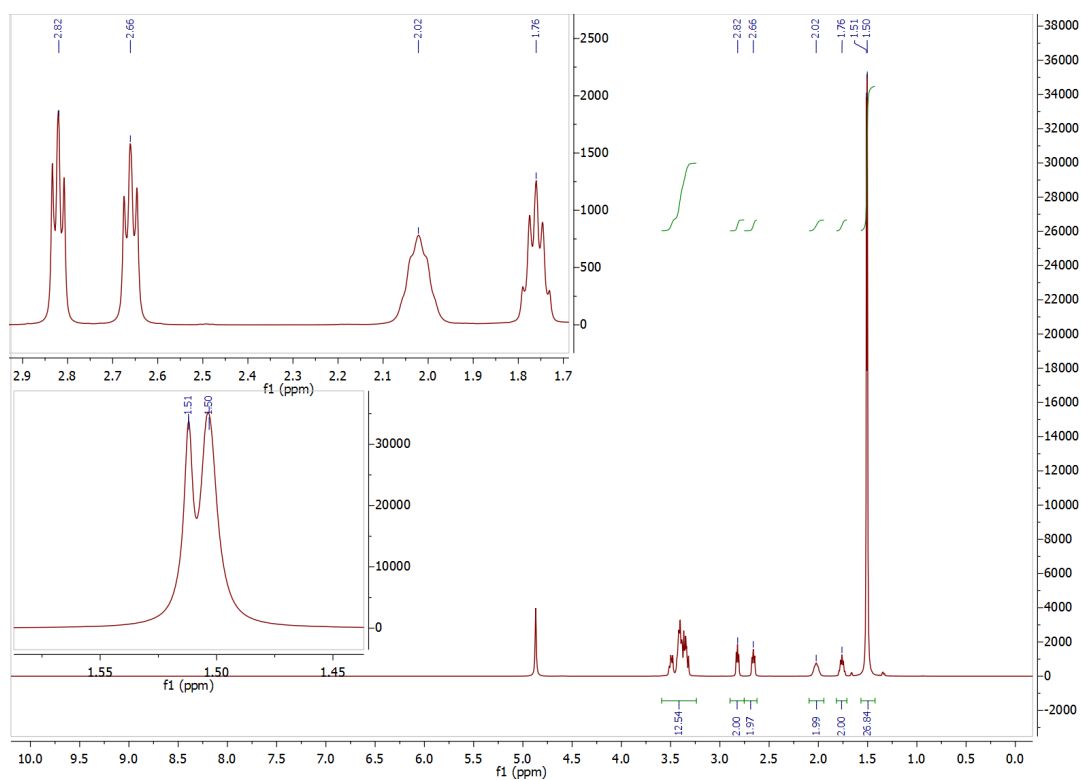


Figure A.1 ^1H NMR spectrum of compound 1

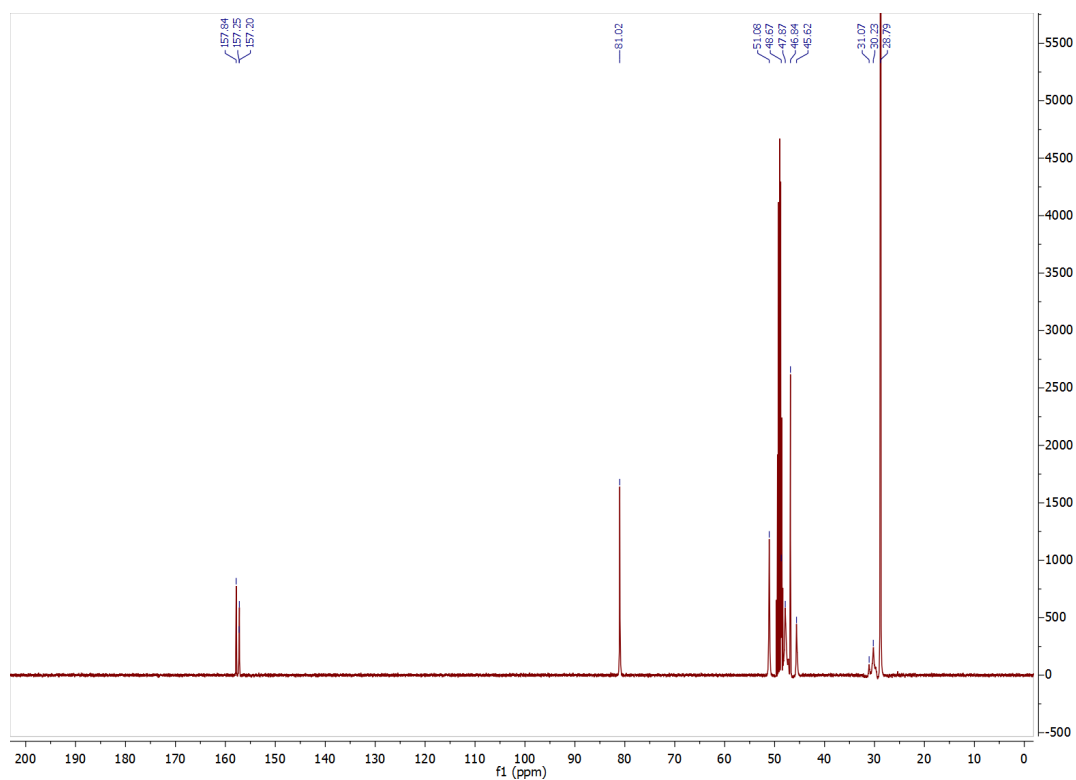


Figure A.2 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound **1**

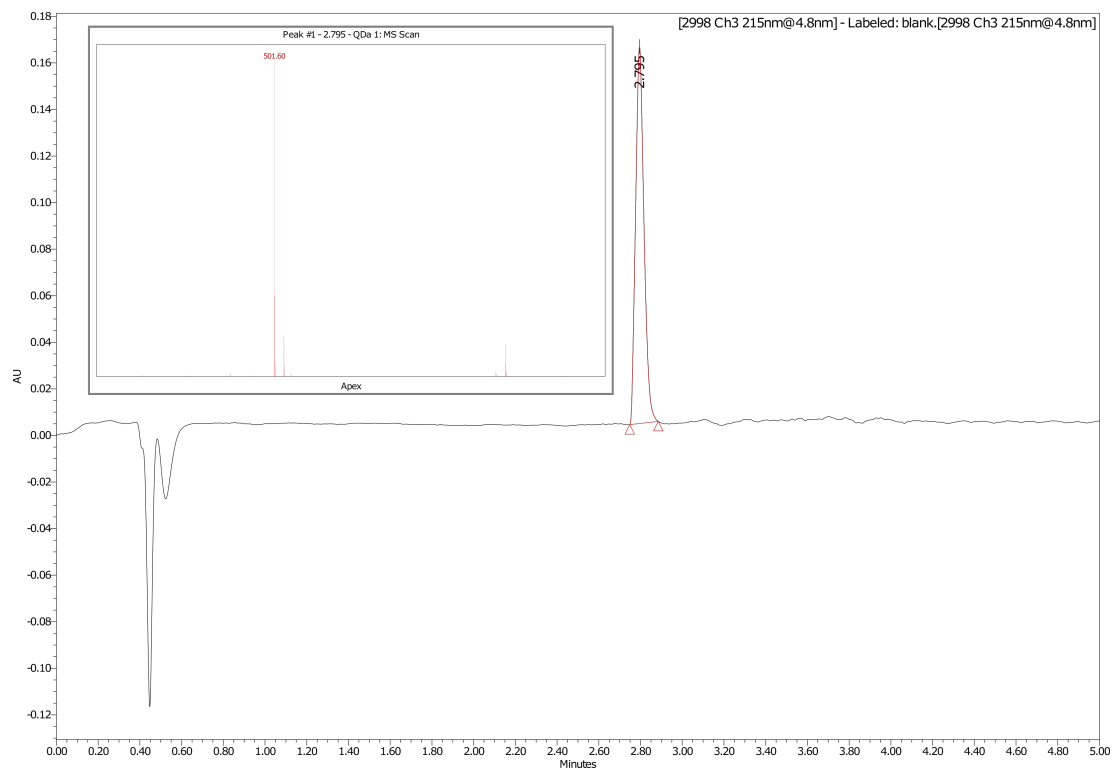


Figure A.3 HPLC-MS of compound **1**, method B.

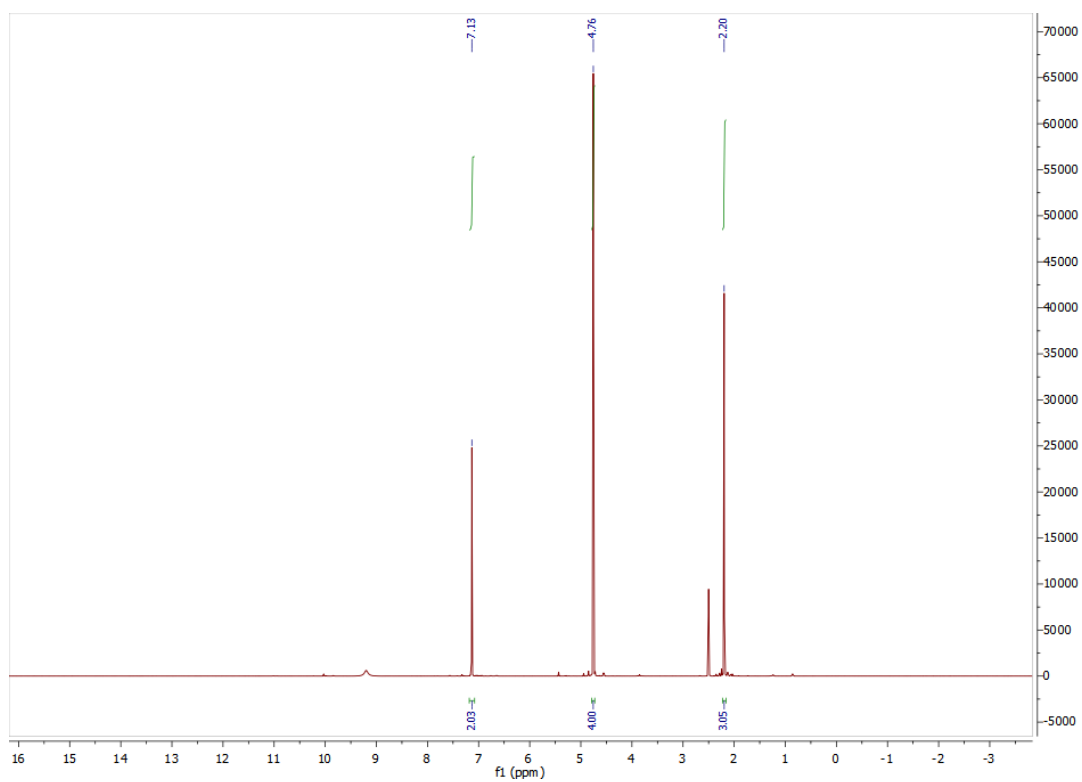


Figure A.4 ^1H NMR spectrum of compound **2**

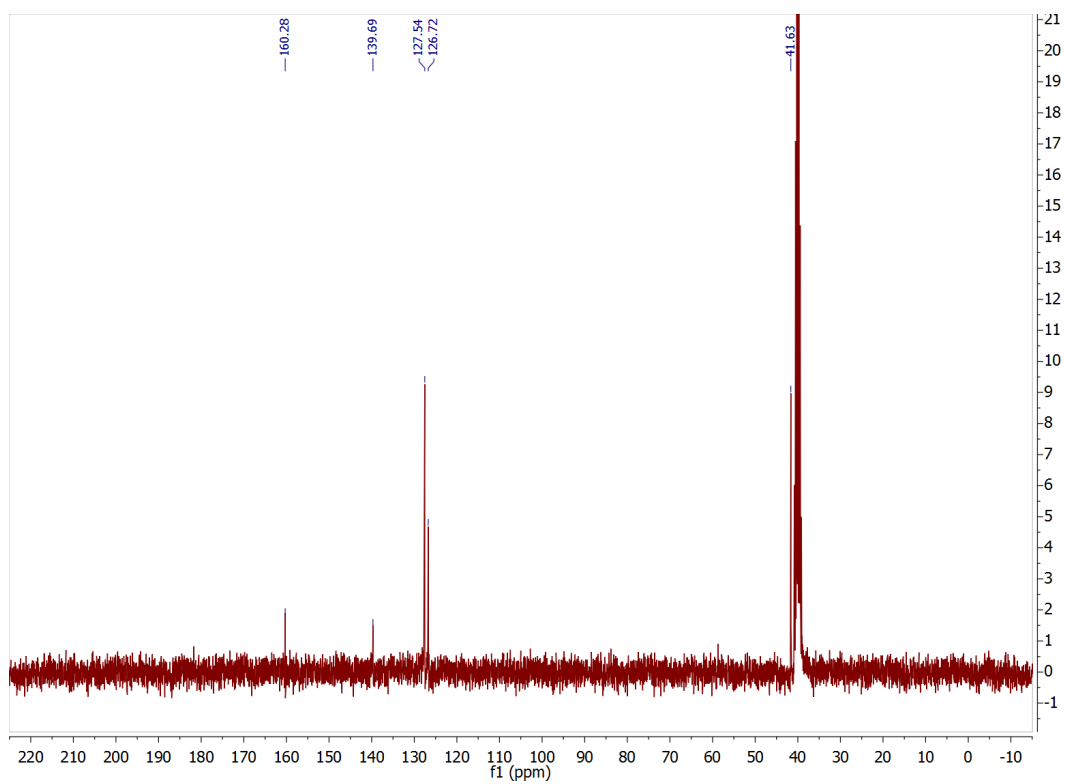


Figure A.5 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound **2**

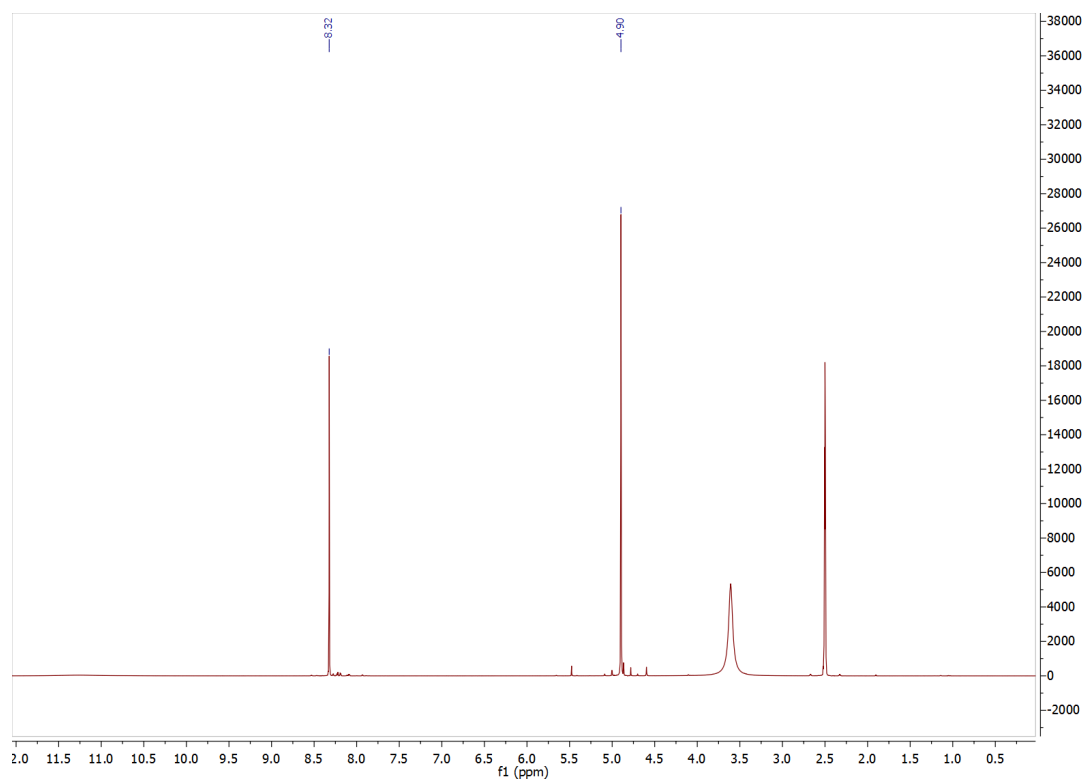


Figure A.6 ^1H NMR spectrum of compound **3**

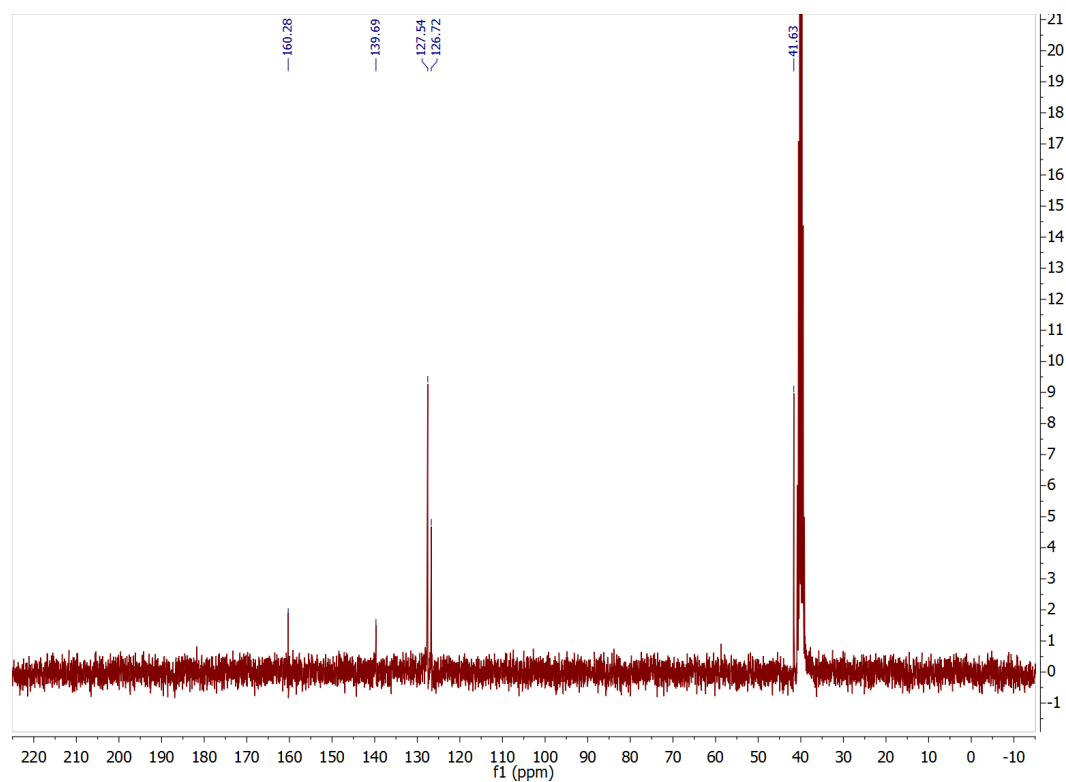


Figure A.7 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound **3**

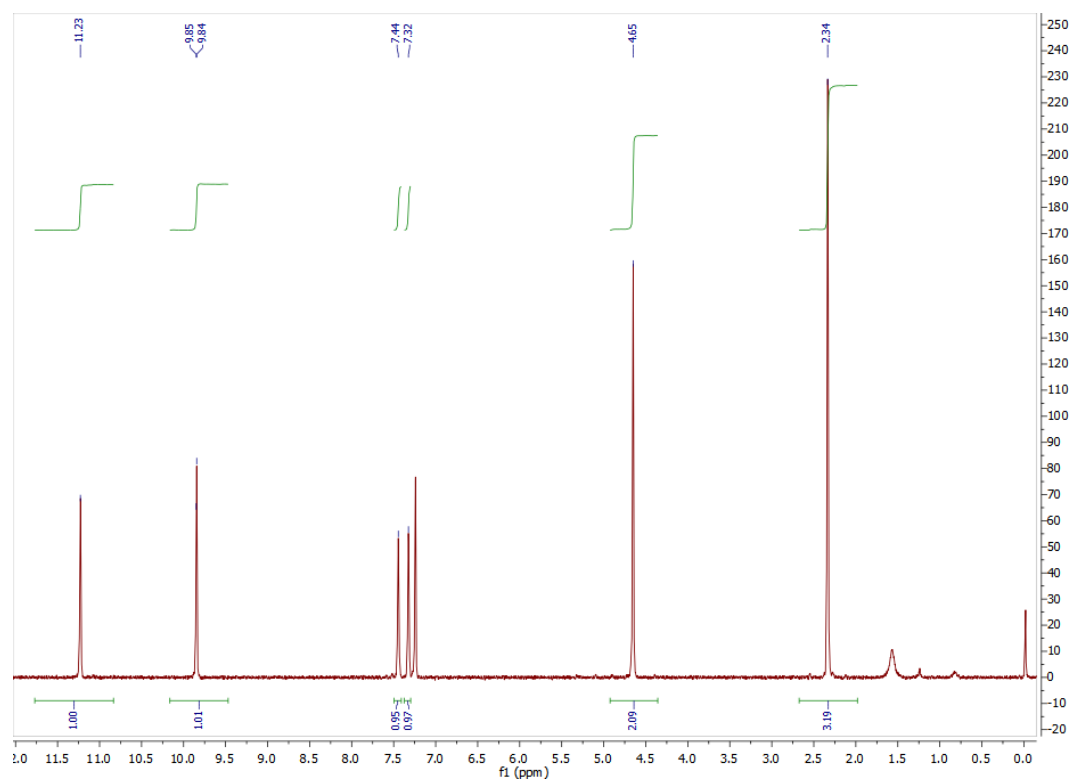


Figure A.8 ¹H NMR spectrum of compound 4

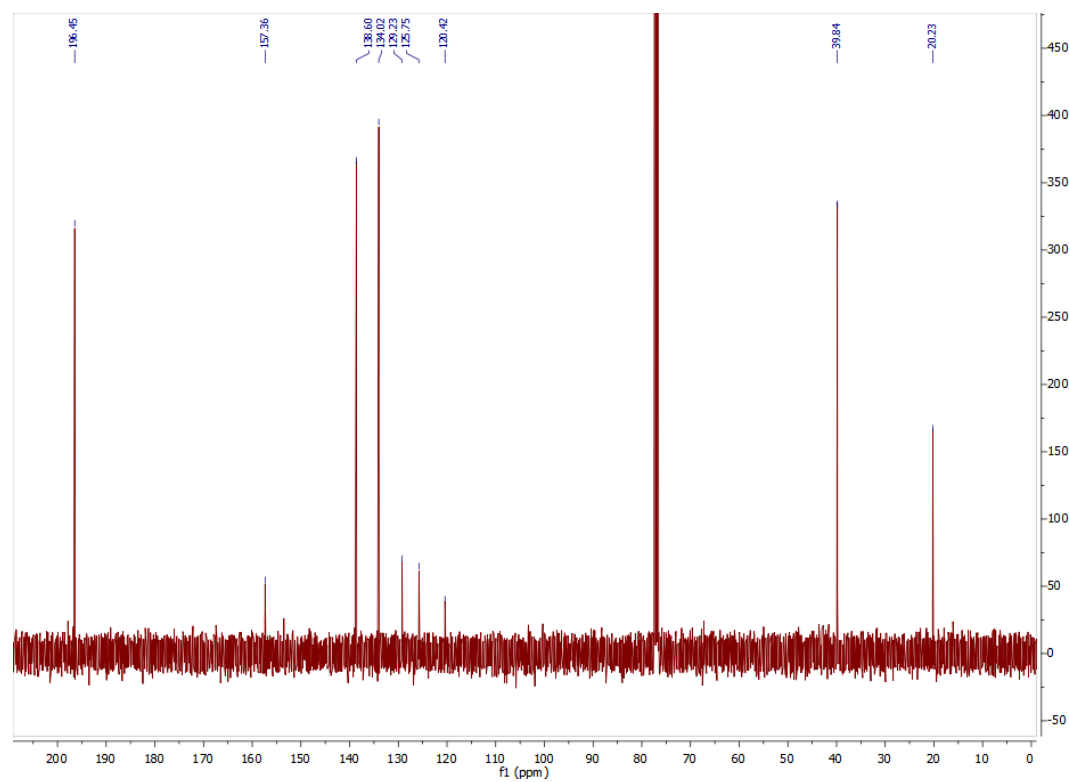


Figure A.9 ¹³C{¹H} NMR spectrum of compound 4

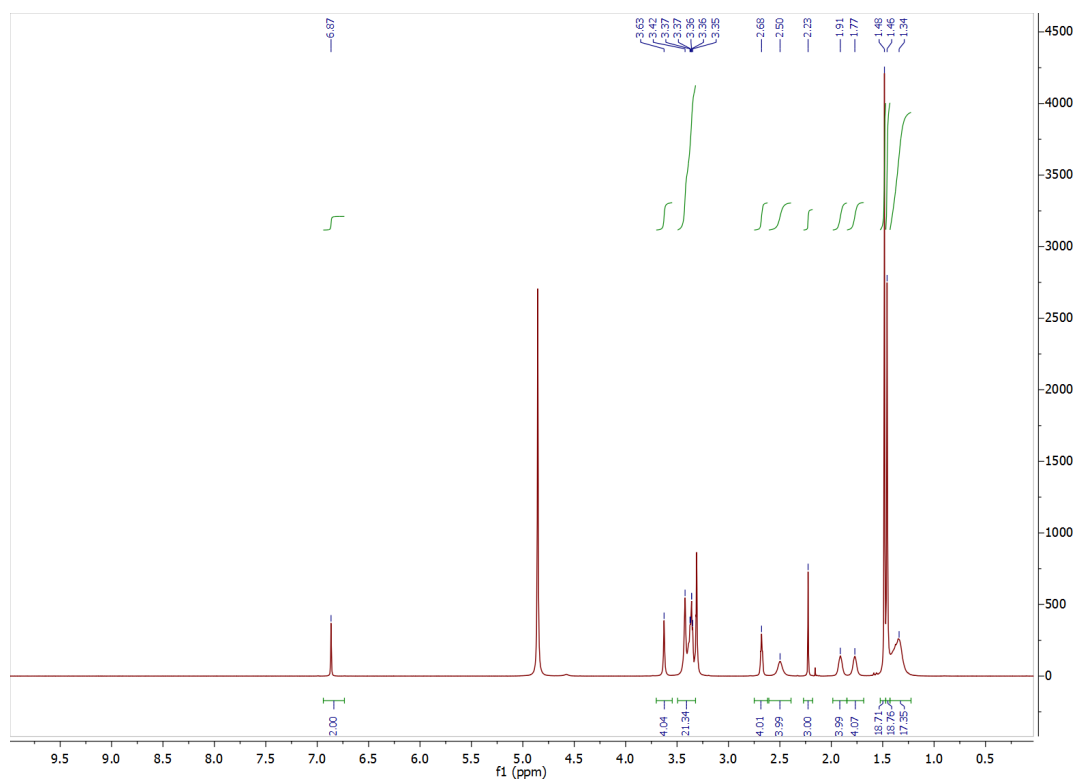


Figure A.10 ¹H NMR spectrum of compound **5**

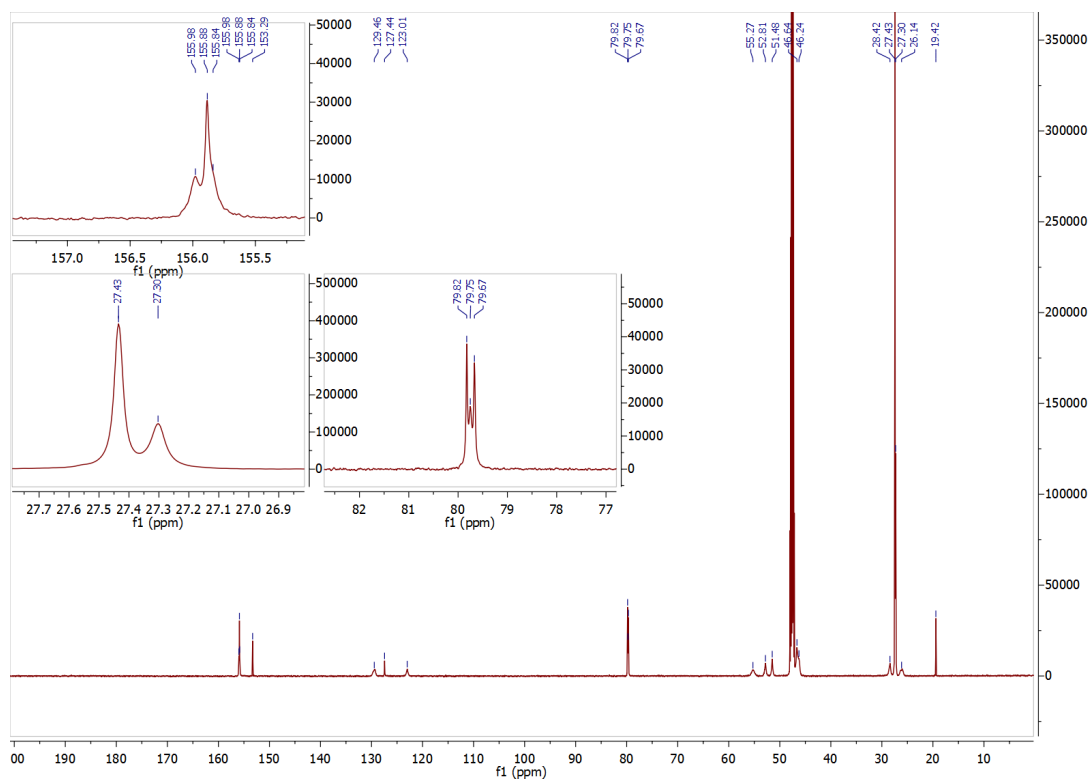


Figure A.11 ¹³C{¹H} NMR spectrum of compound **5**

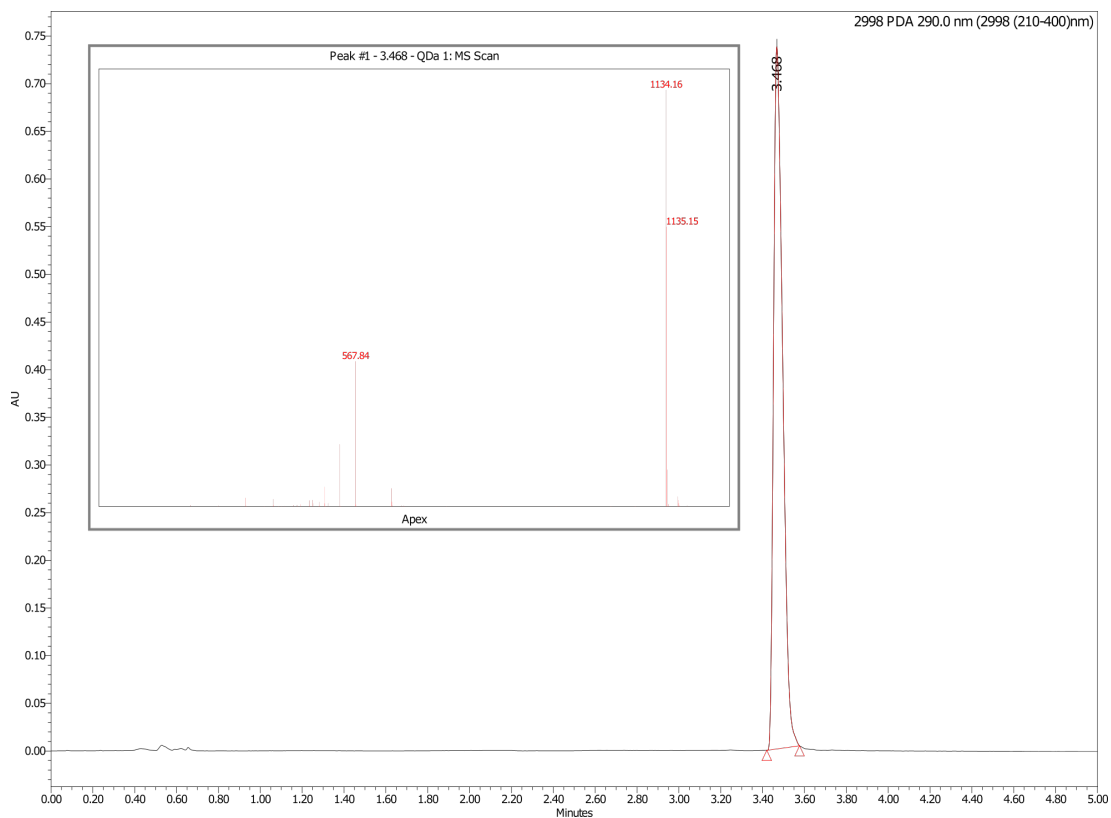


Figure A.12 HPLC-MS of compound **5**, method B.

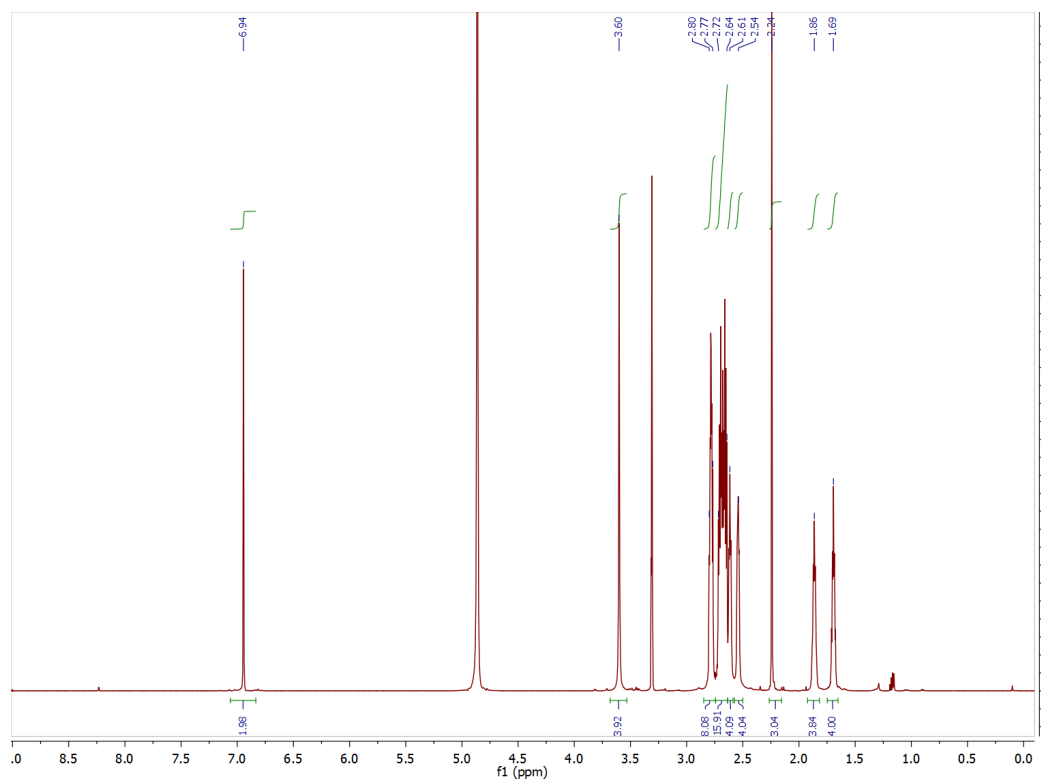


Figure A.13 ^1H NMR spectrum of compound **HL¹**

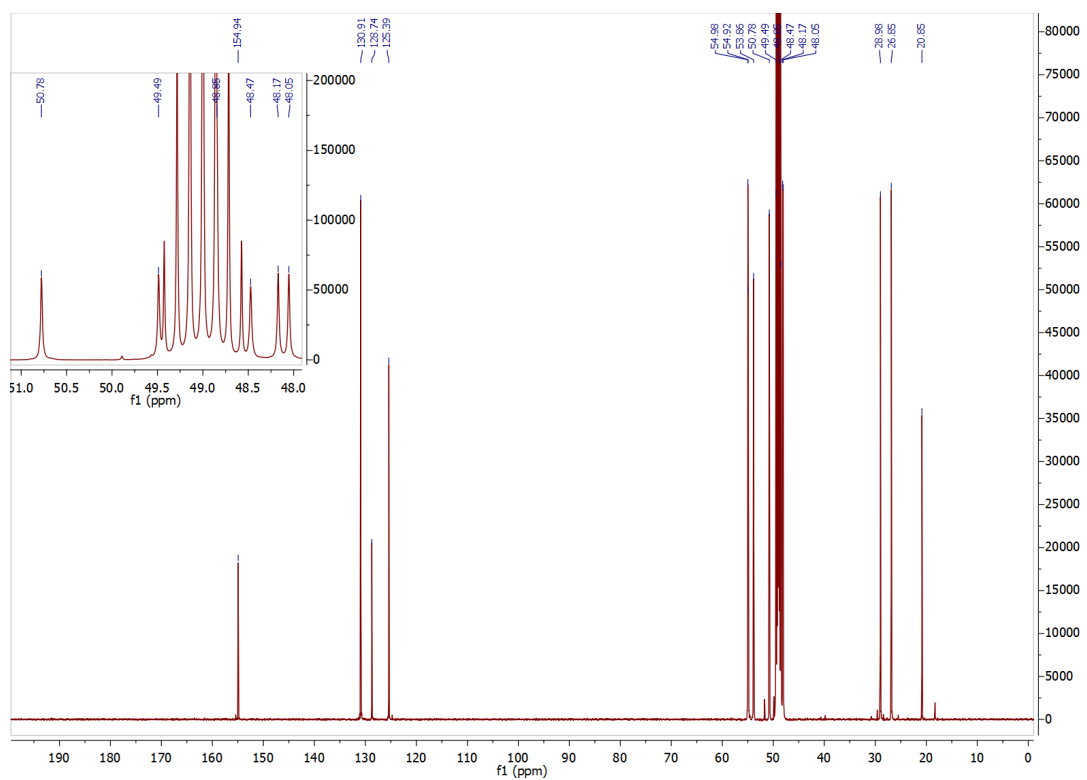


Figure A.14 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound **HL**¹

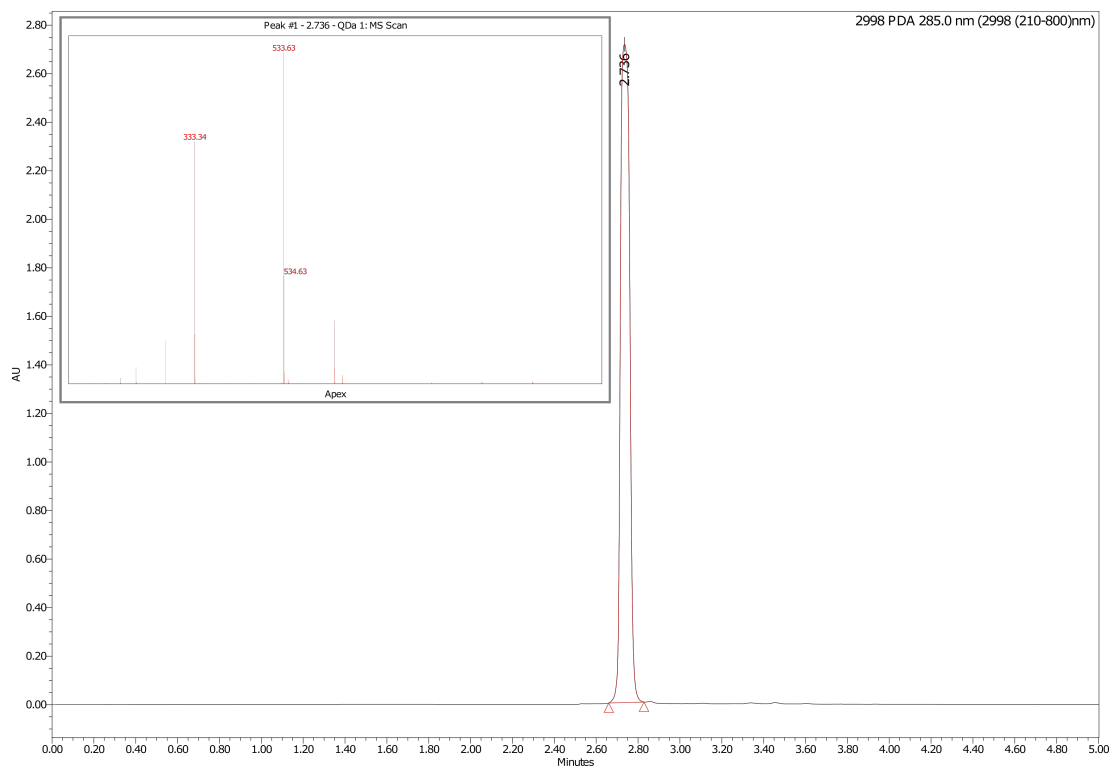


Figure A.15 HPLC-MS of compound **HL**¹, method A.

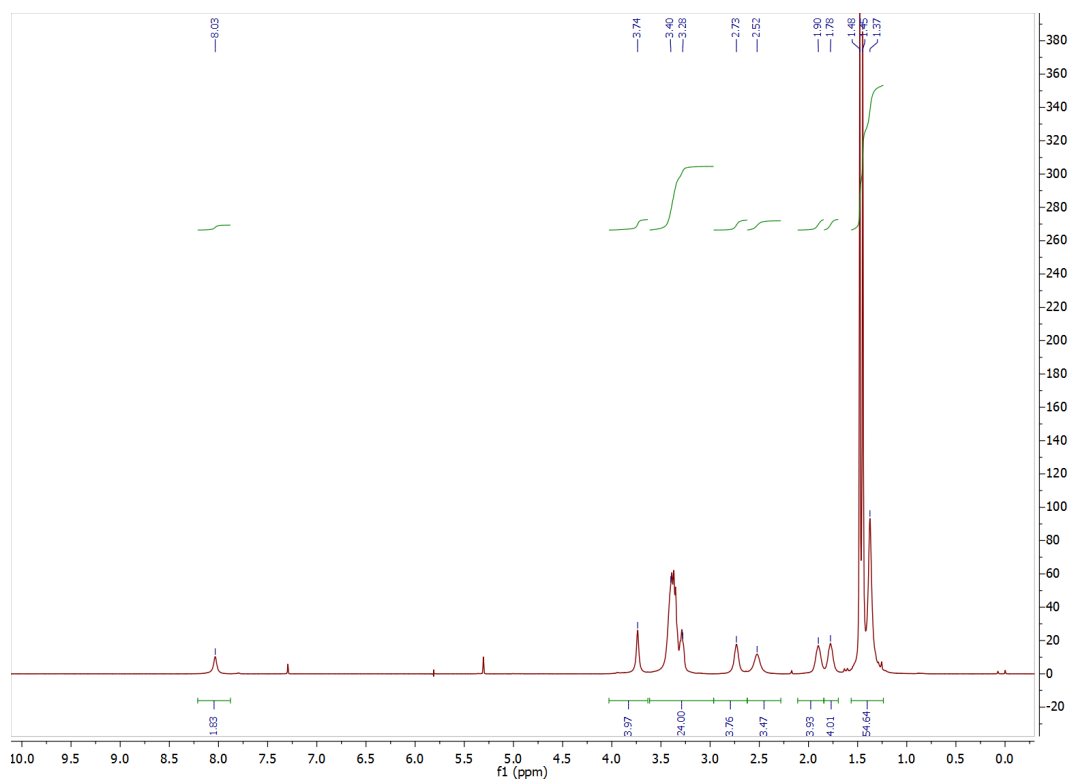


Figure A.16 ^1H NMR spectrum of compound **6**

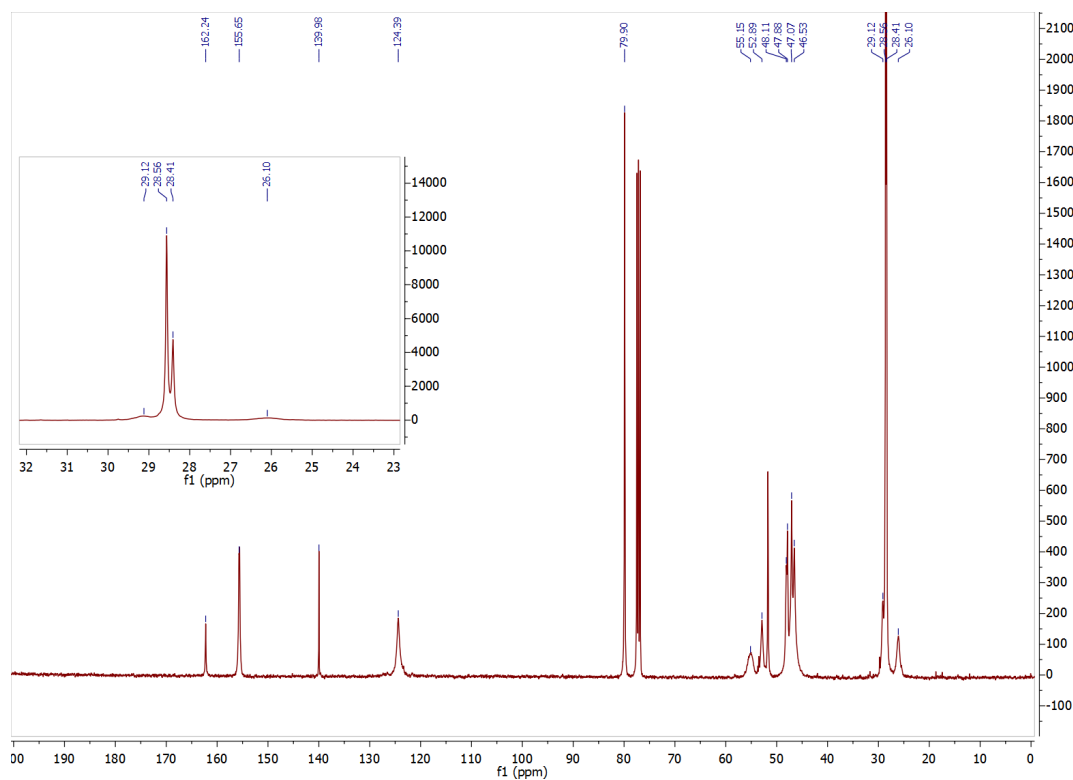


Figure A.17 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound **6**

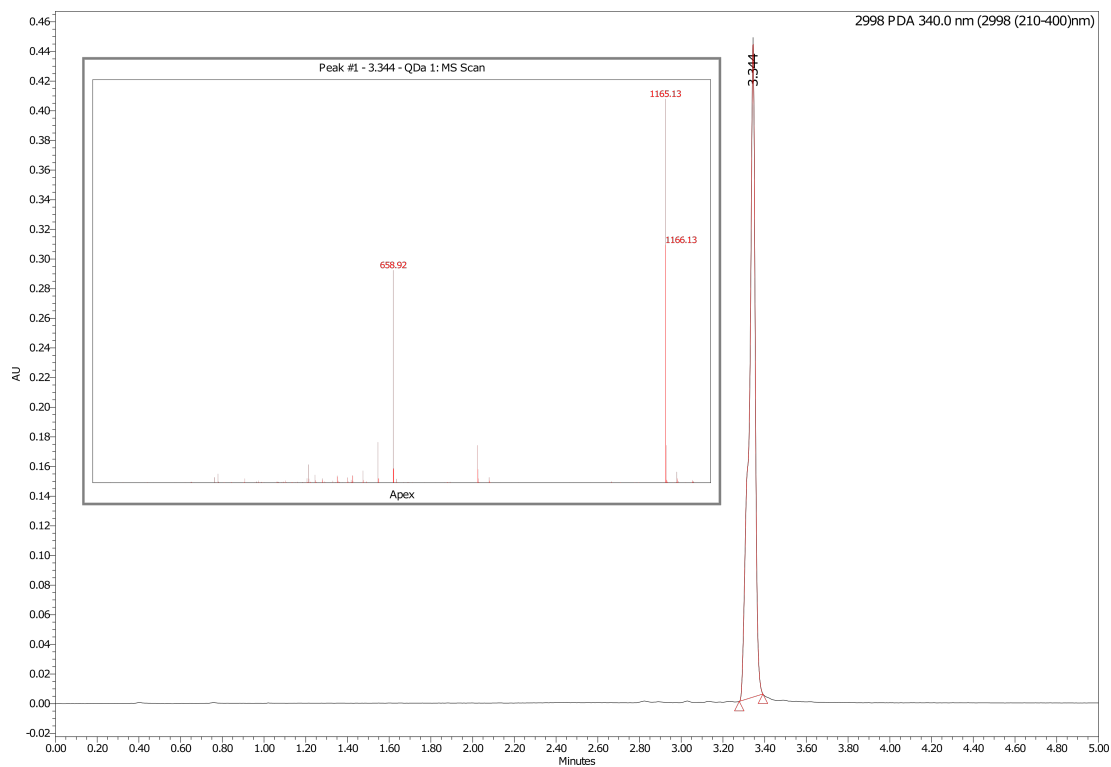


Figure A.18 HPLC-MS of compound **6**, method B.

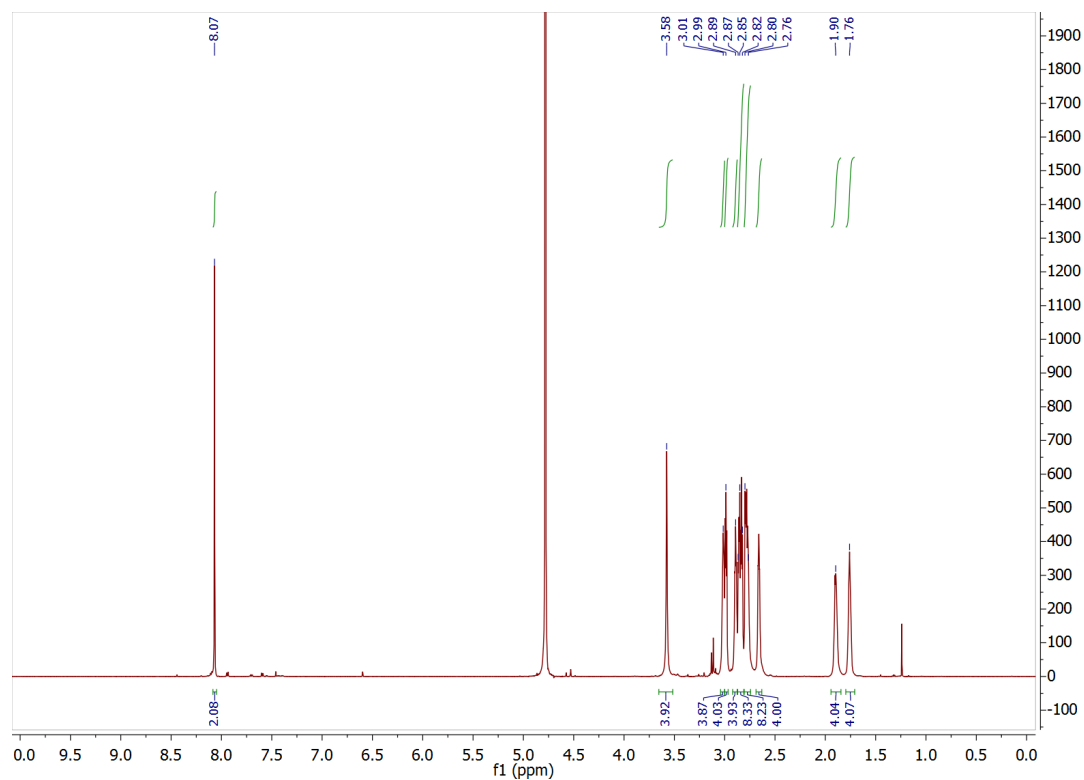


Figure A.19 ^1H NMR spectrum of compound **HL²**

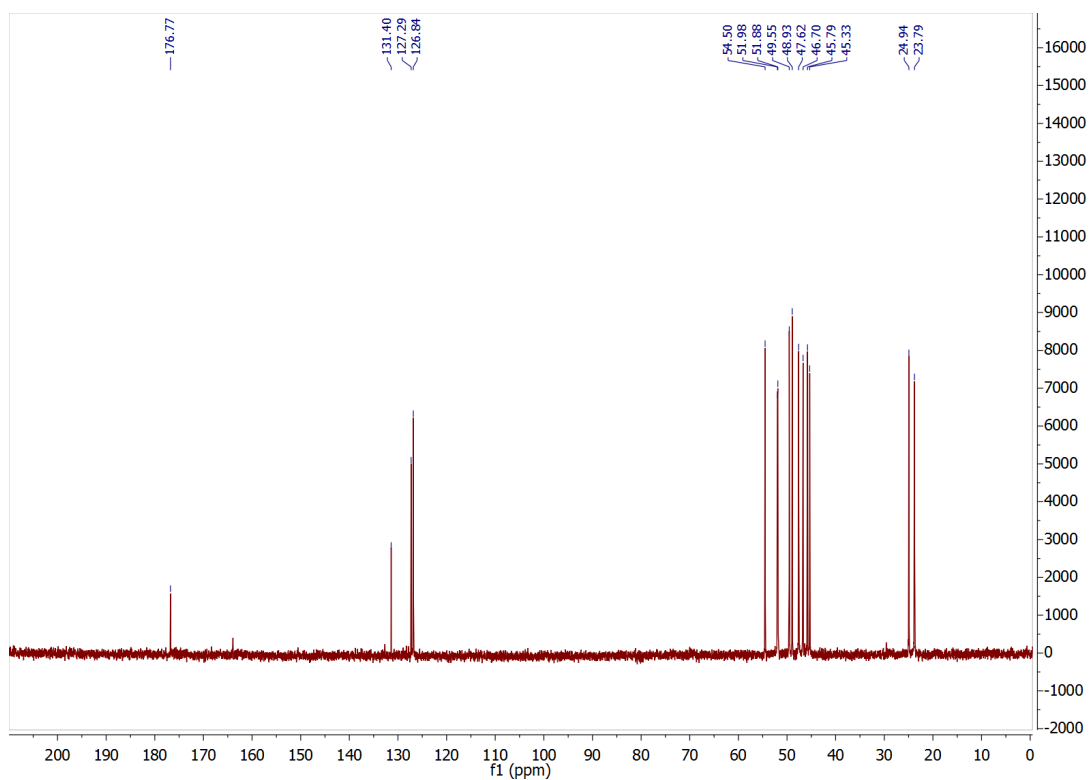


Figure A.20 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound HL^2

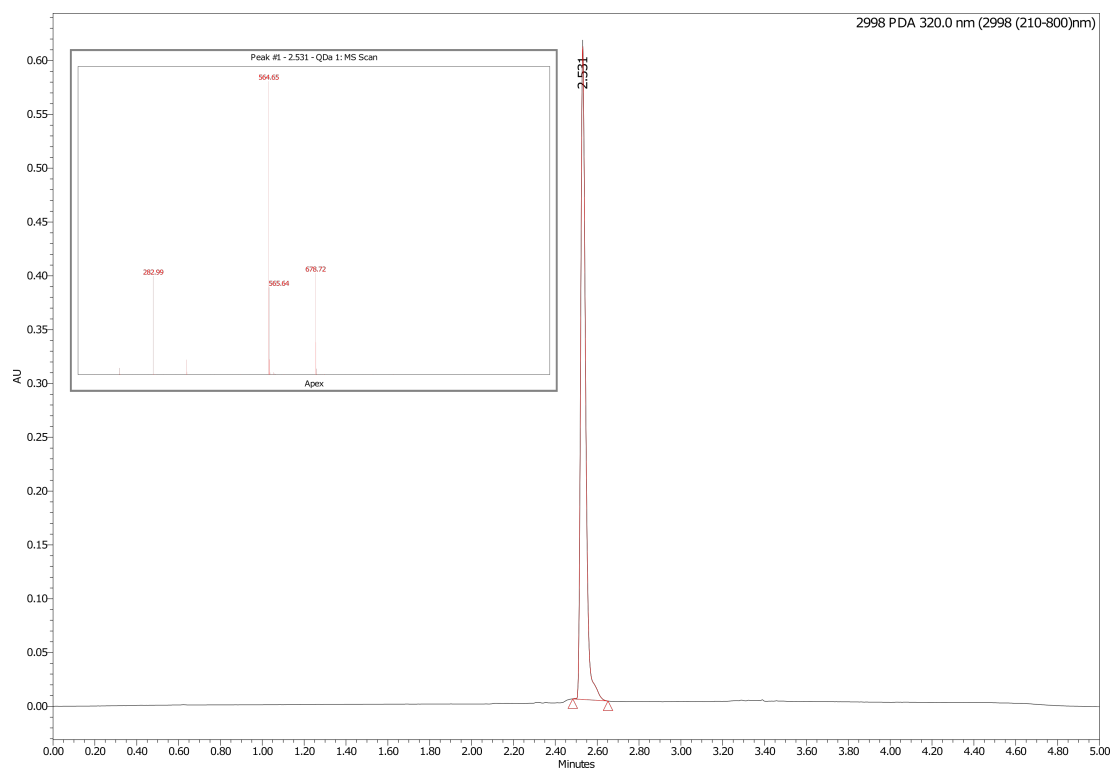


Figure A.21 HPLC-MS of compound HL^2 , method A.

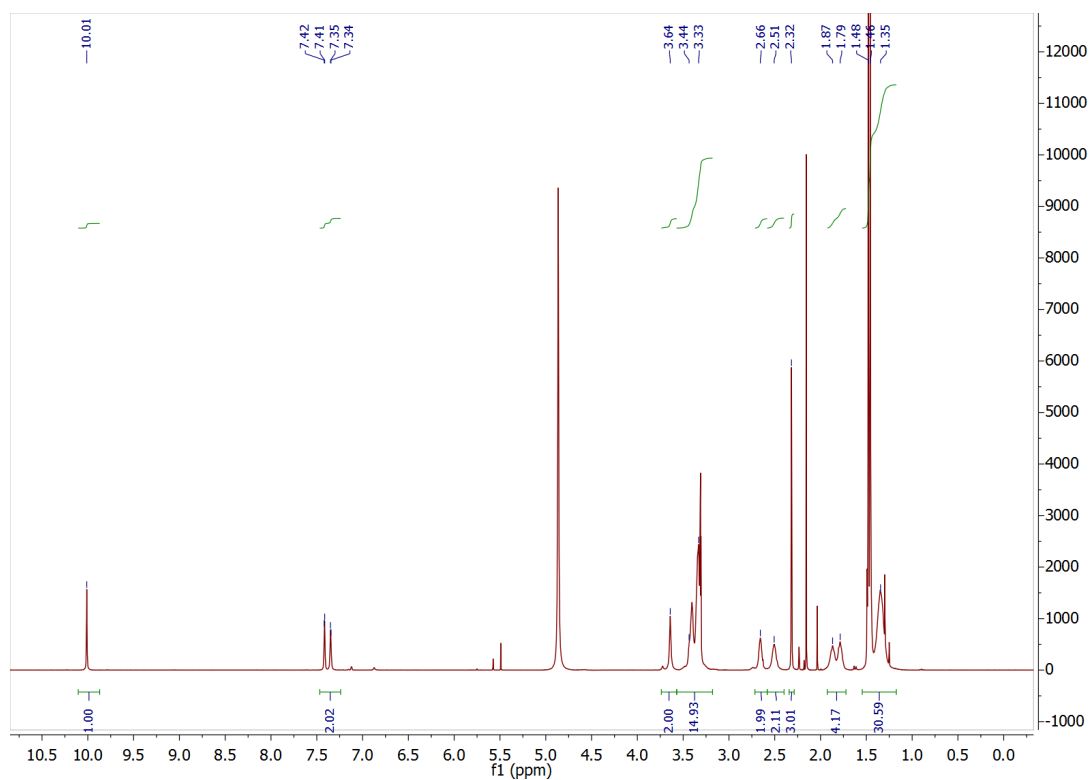


Figure A.22 ¹H NMR spectrum of compound **7**

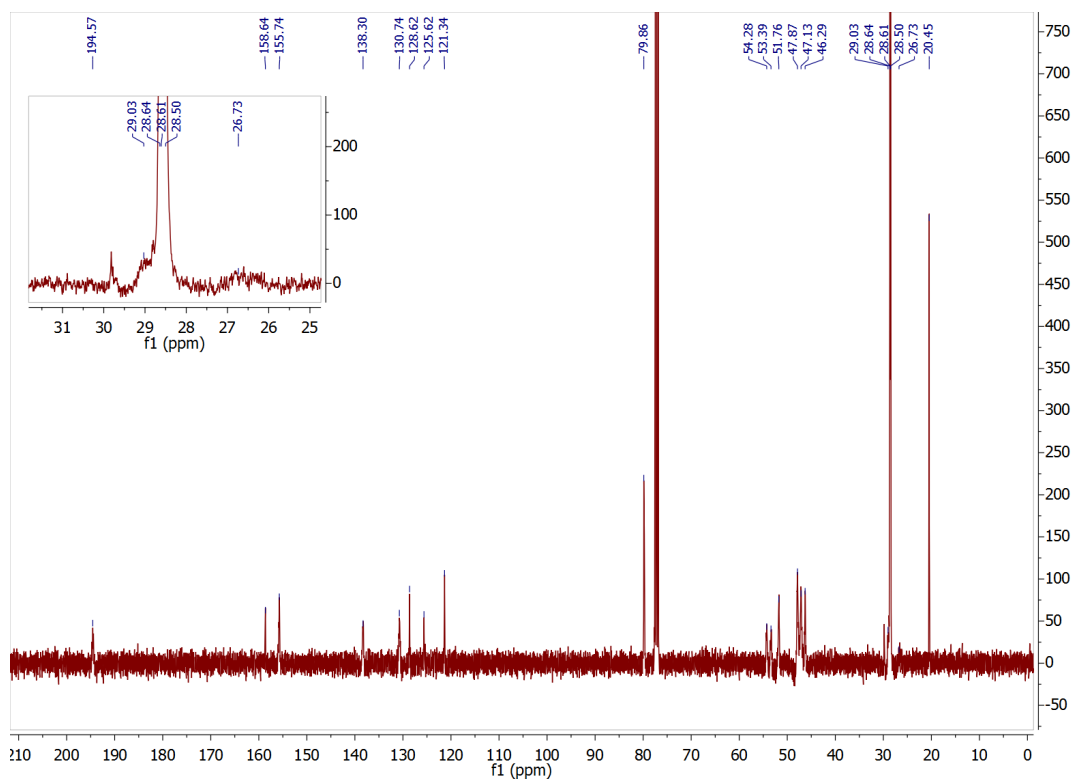


Figure A.23 ¹³C{¹H} NMR spectrum of compound **7**

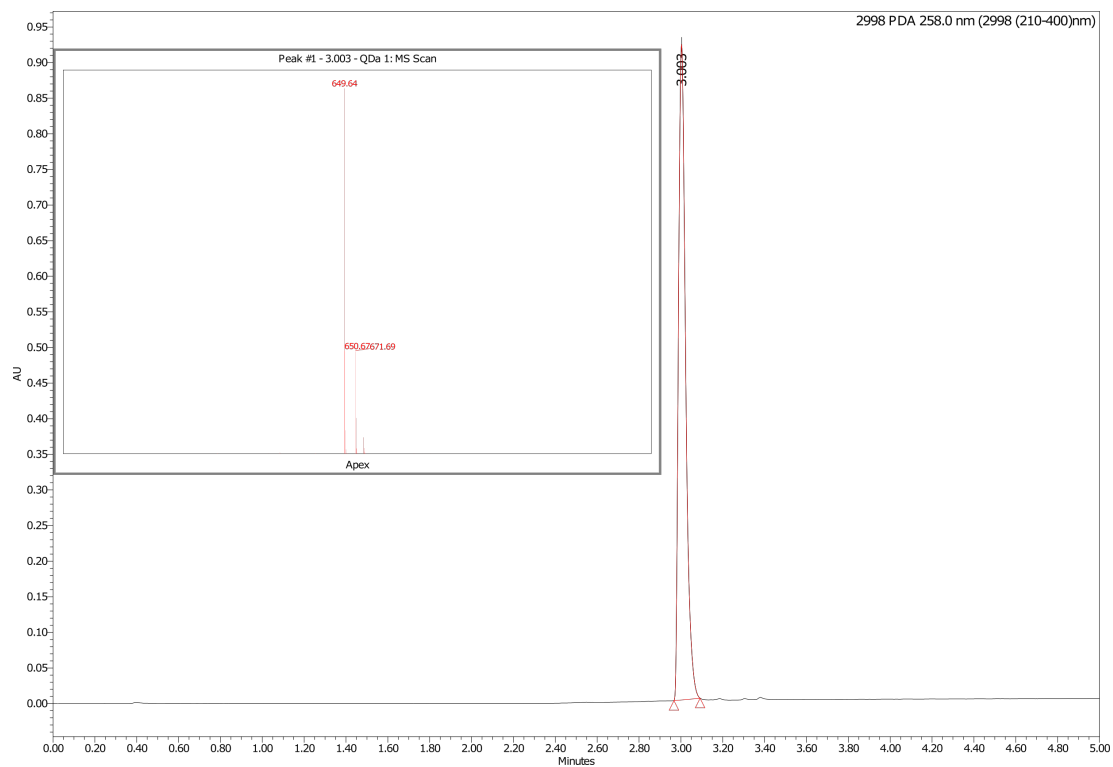


Figure A.24 HPLC-MS of compound **7**, method B.

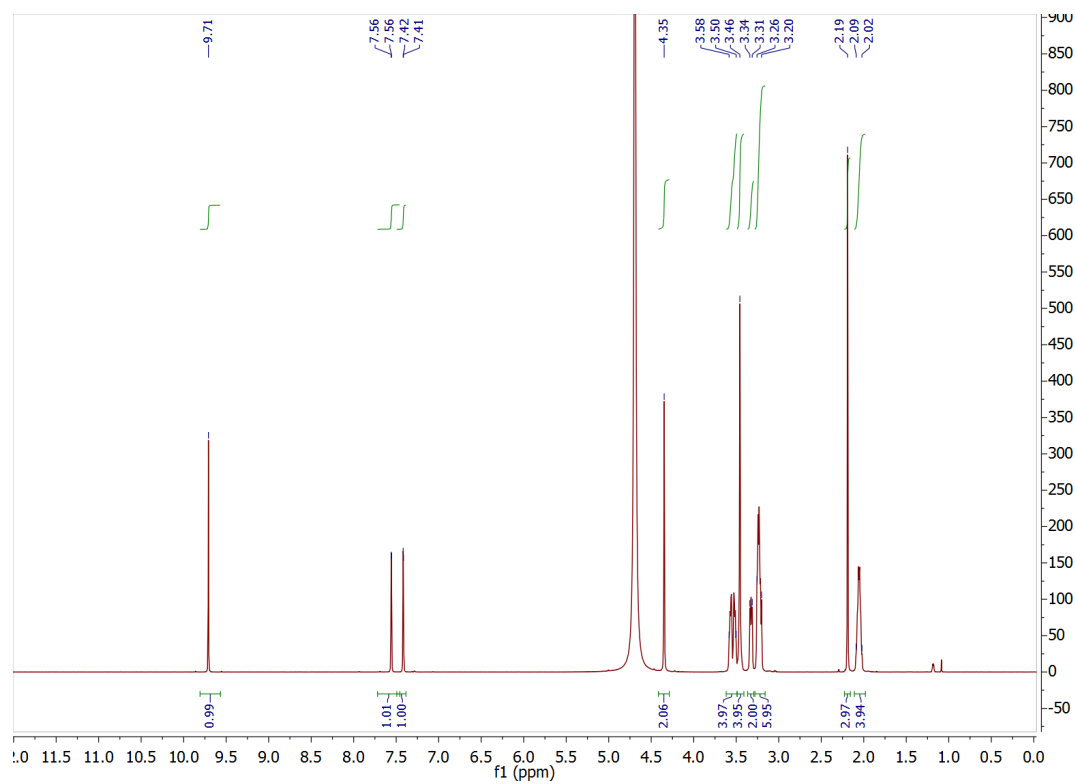


Figure A.25 ^1H NMR spectrum of compound **HL³**

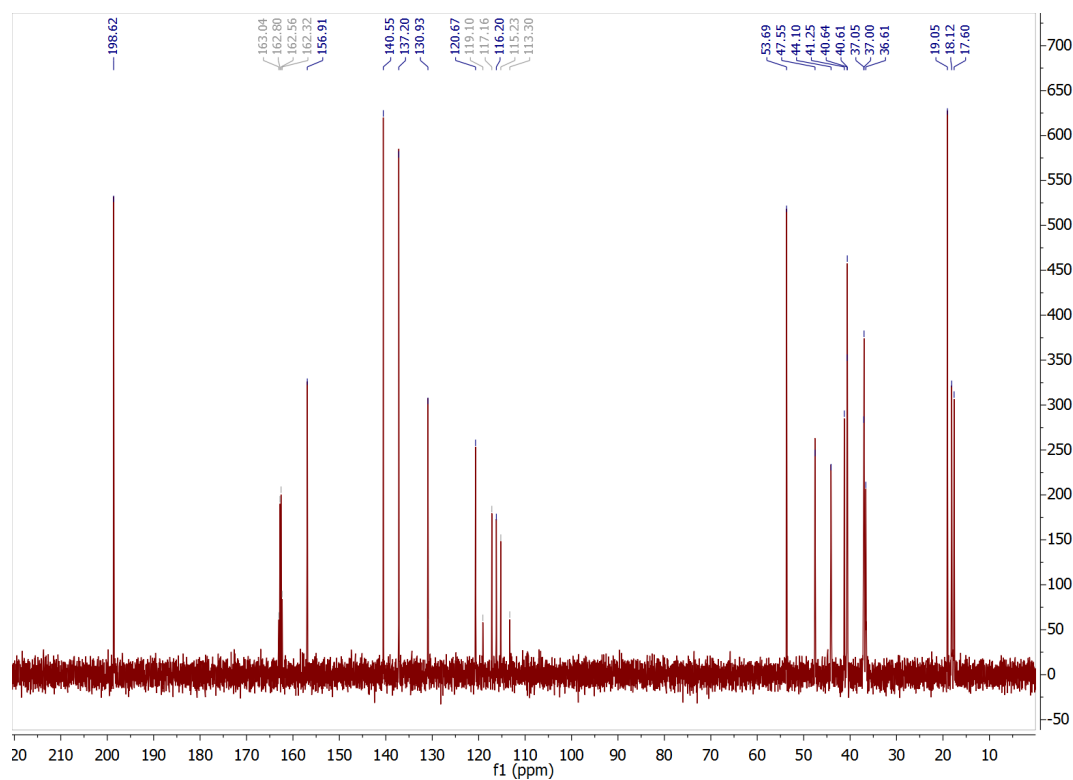


Figure A.26 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound **HL³**

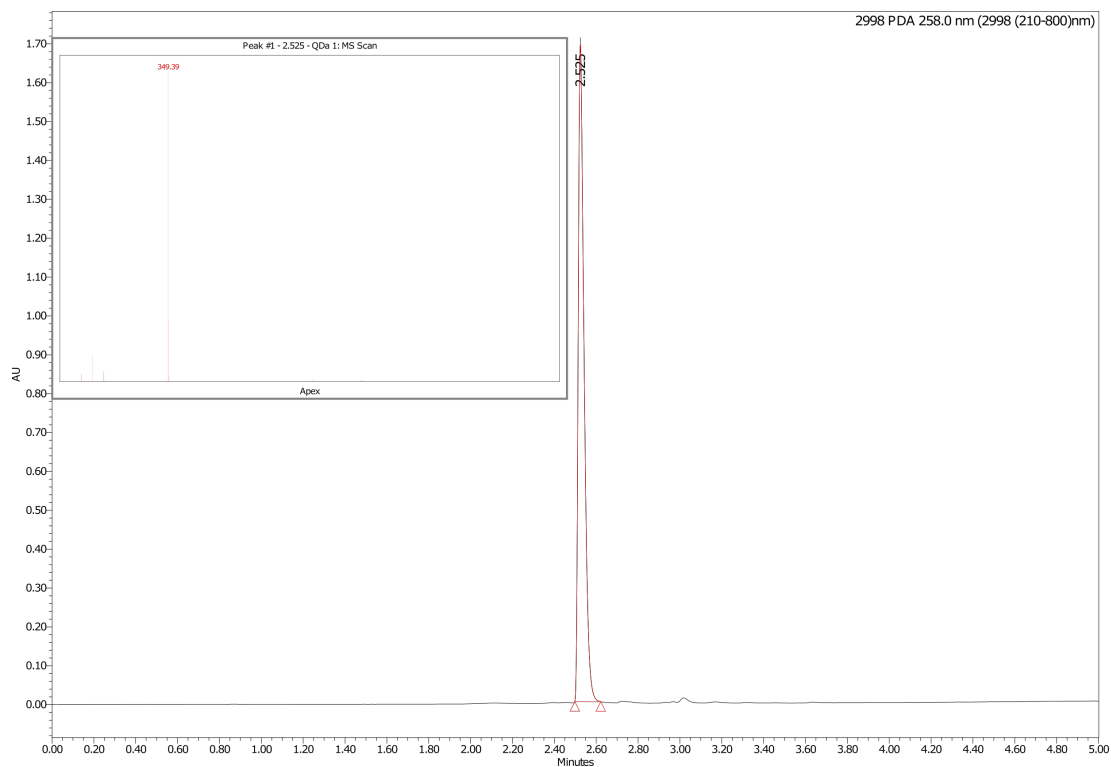


Figure A.27 HPLC-MS of compound **HL³**, method A.

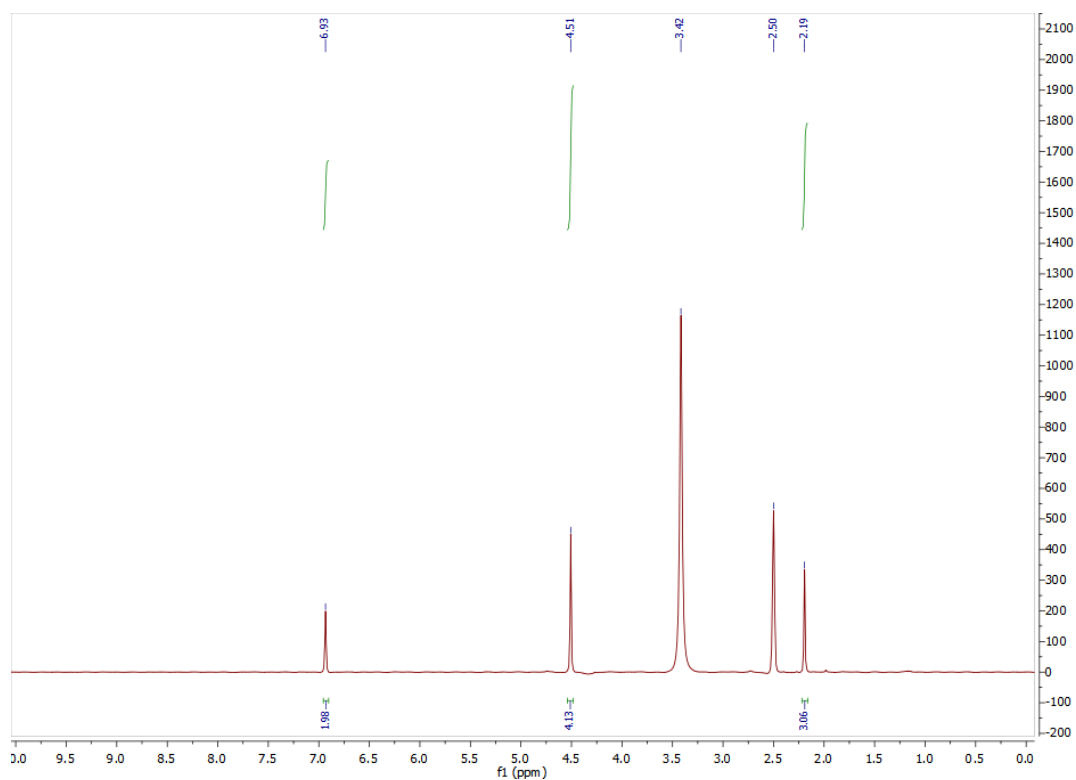


Figure A.28 ^1H NMR spectrum of compound **8**

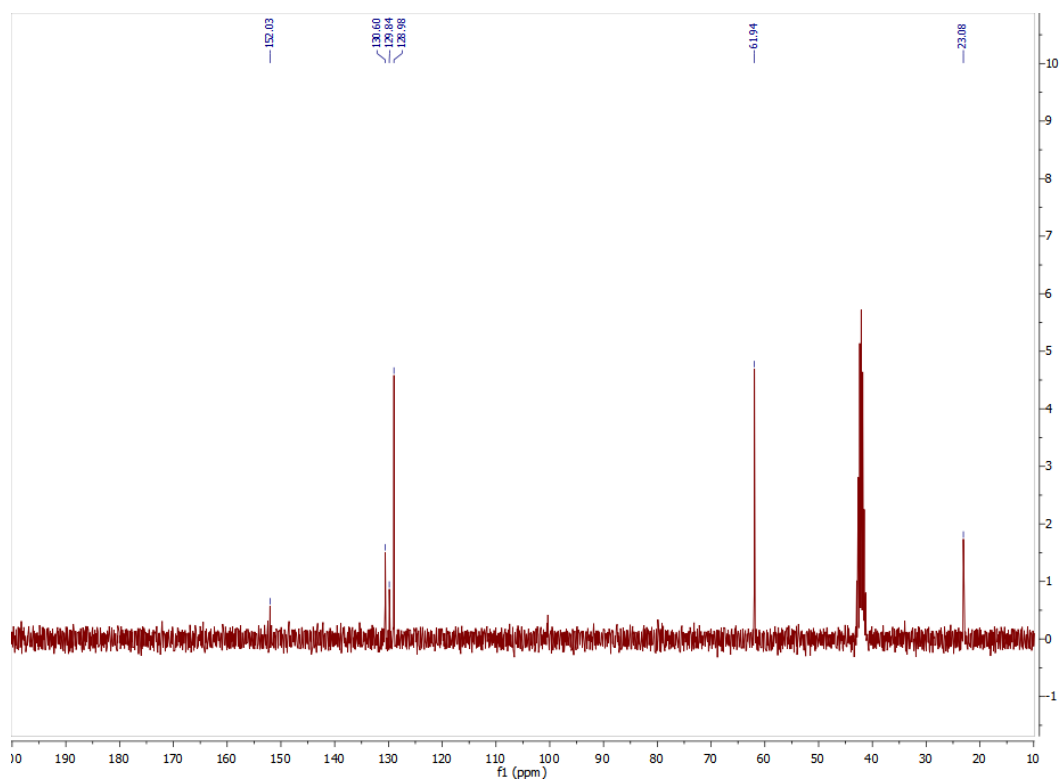


Figure A.29 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound **8**

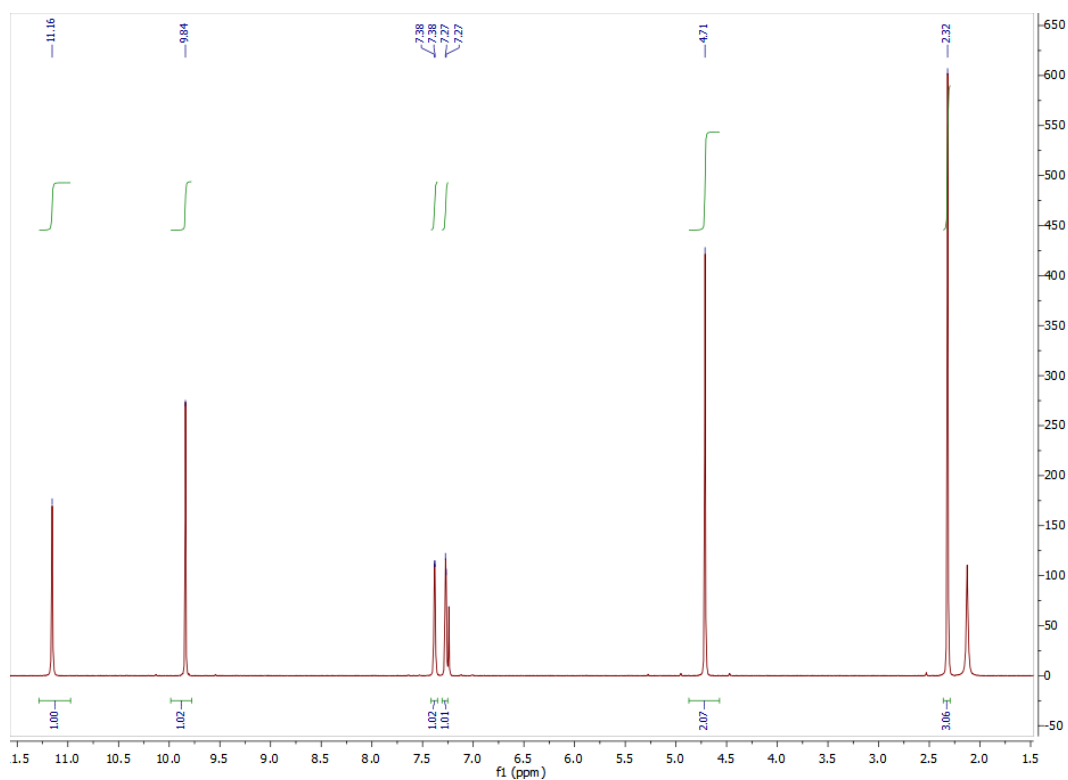


Figure A.30 ¹H NMR spectrum of compound **9**

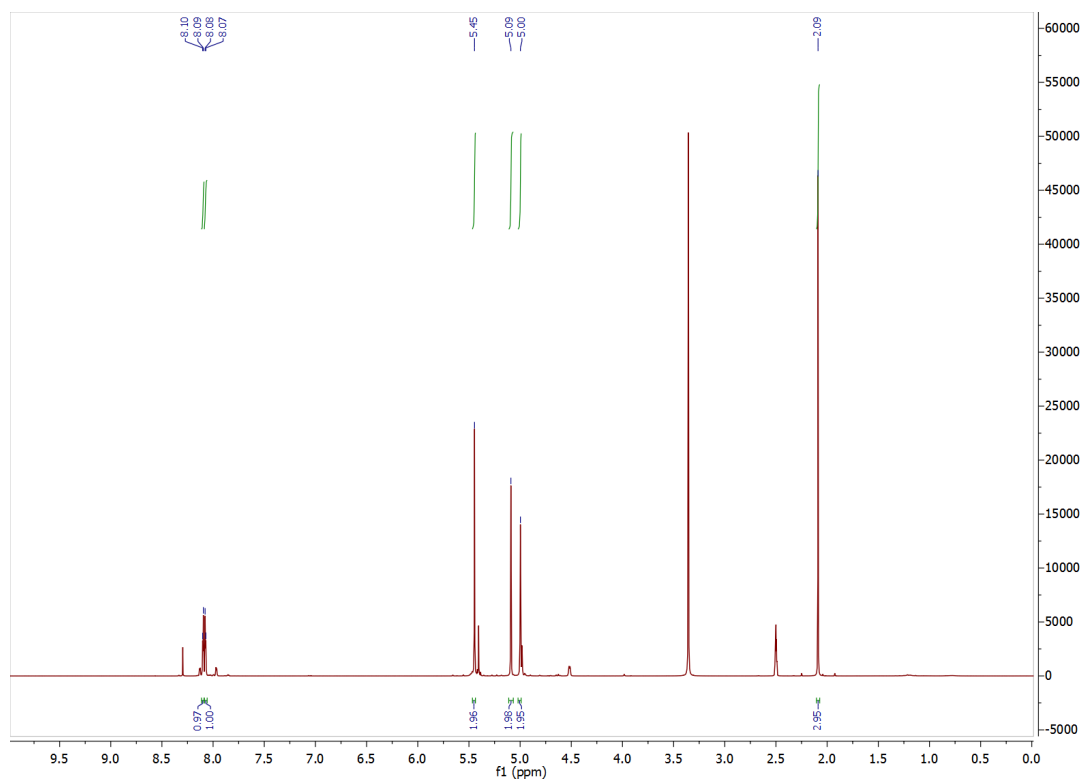


Figure A.31 ¹H NMR spectrum of compound **10**

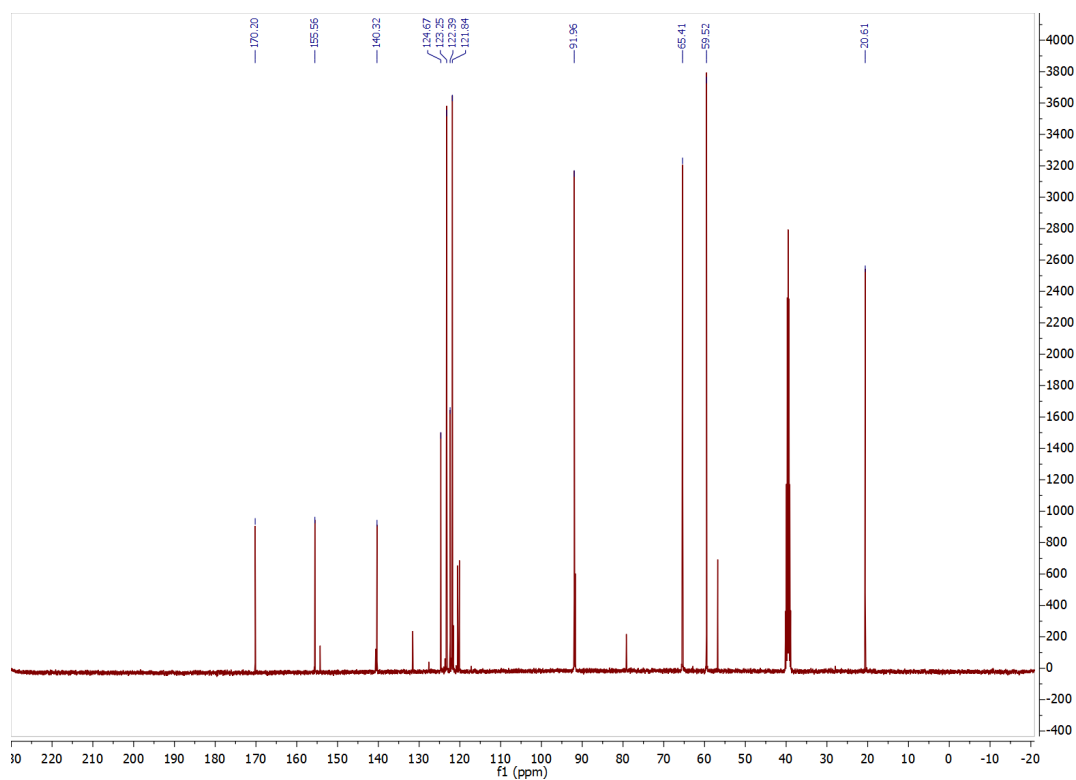


Figure A.32 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound 10

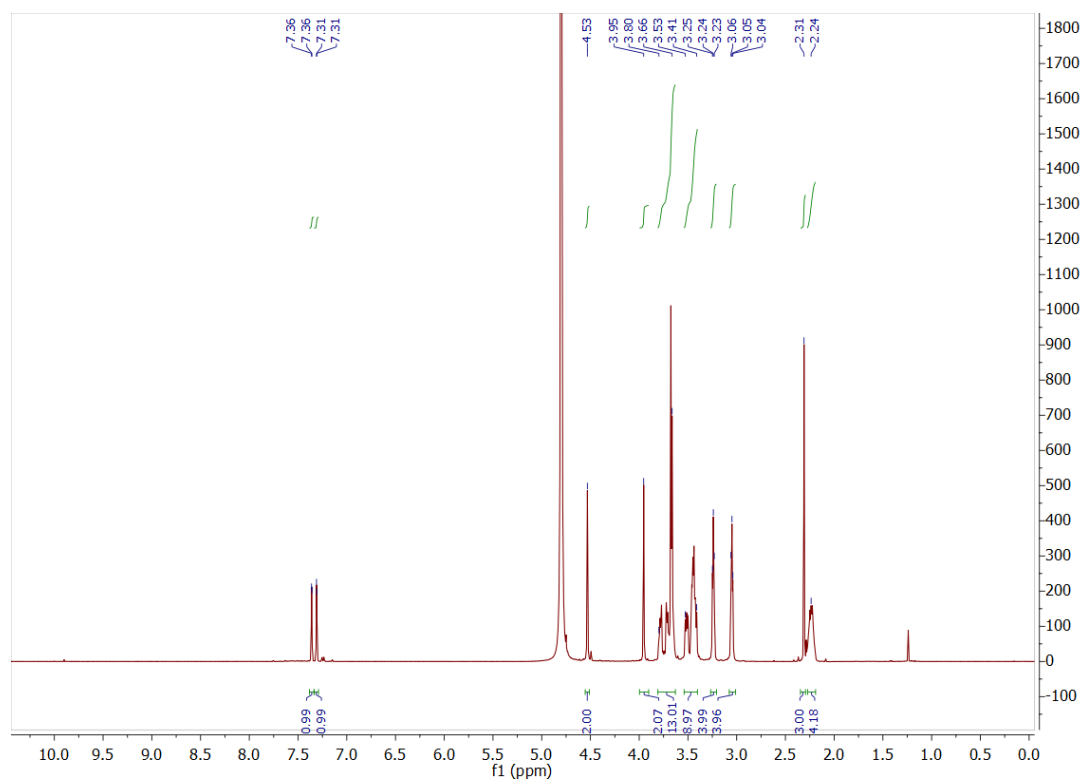


Figure A.33 ^1H NMR spectrum of compound 12

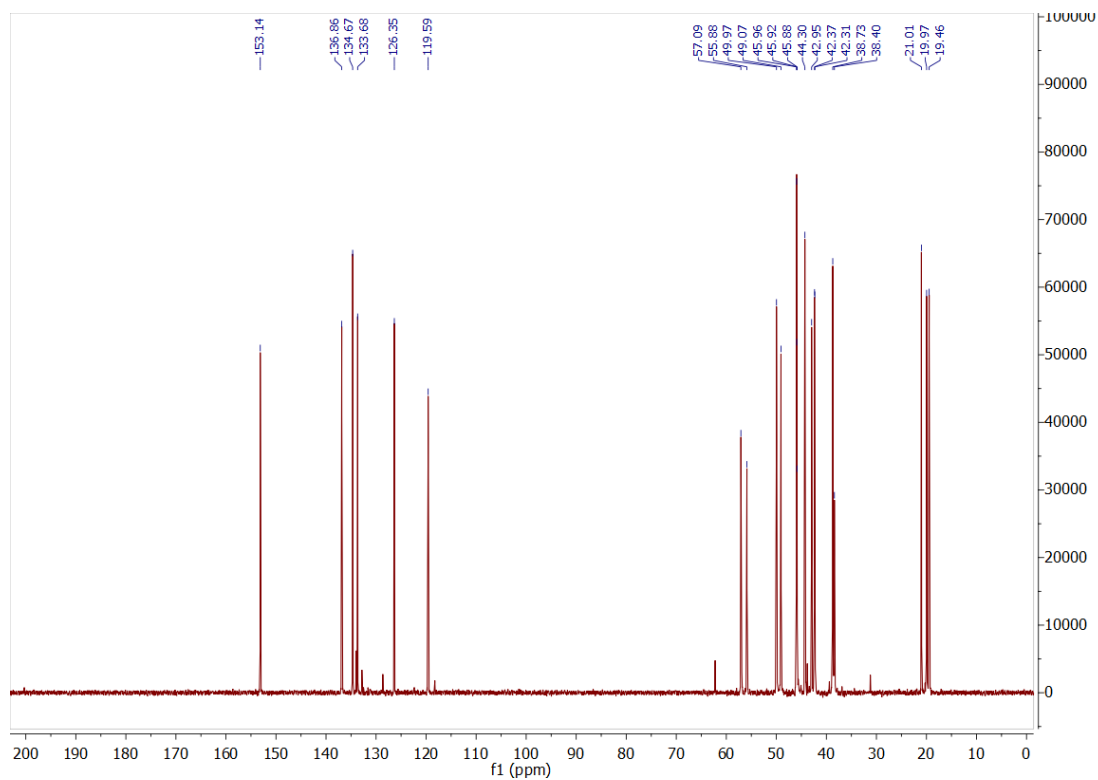


Figure A.34 $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound **12**

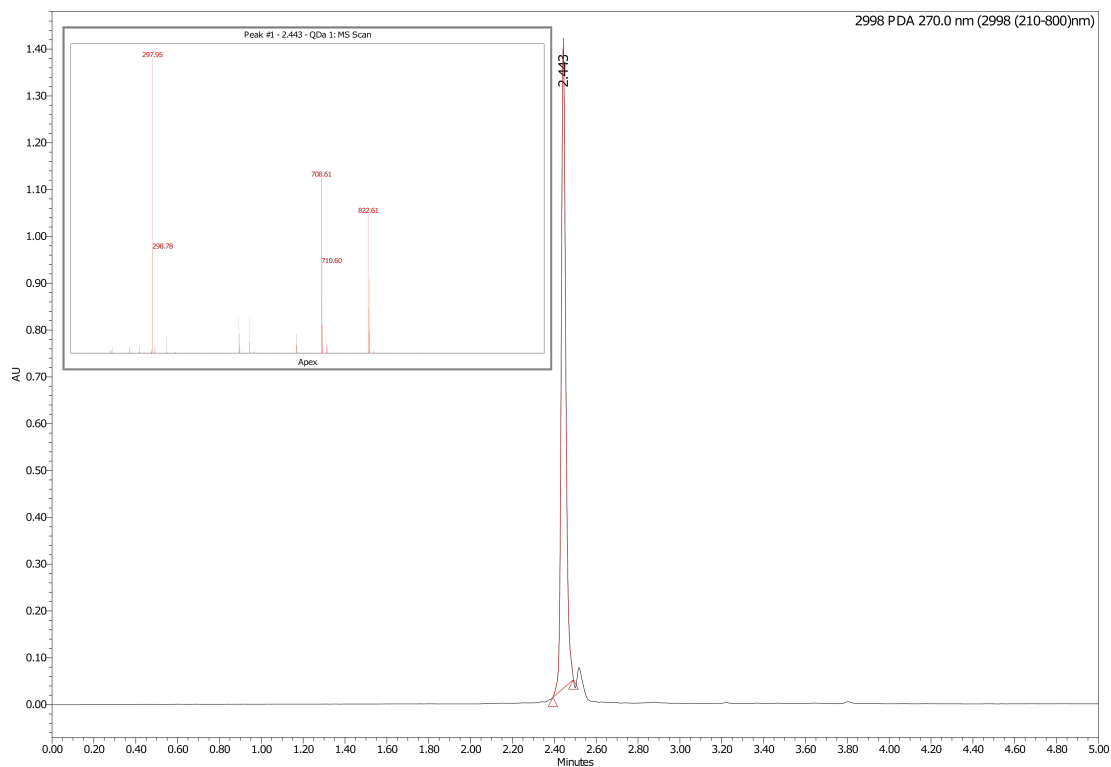


Figure A.35 HPLC-MS of complex $[\text{Cu}(\text{HL}^1)]\text{SO}_4$, method A.

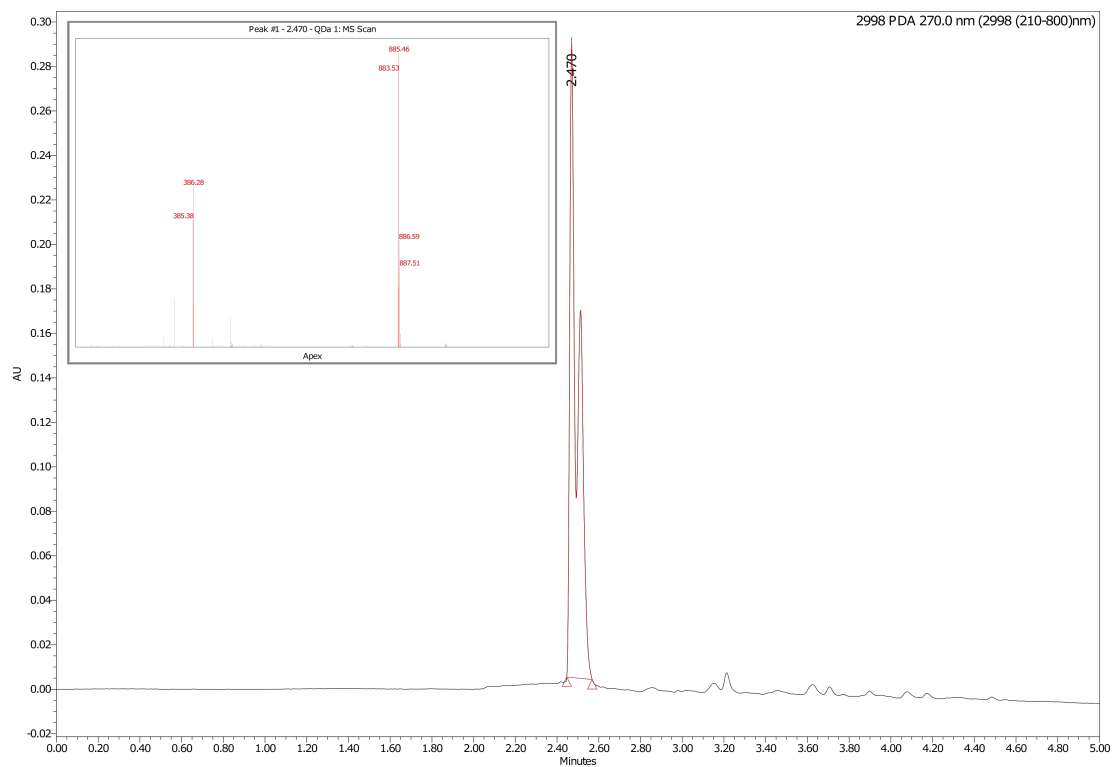


Figure A.36 HPLC-MS of complex $[\text{Cu}_2(\text{HL}^1)(\text{SO}_4)_2]$, method A, both peaks show the same pattern in MS.

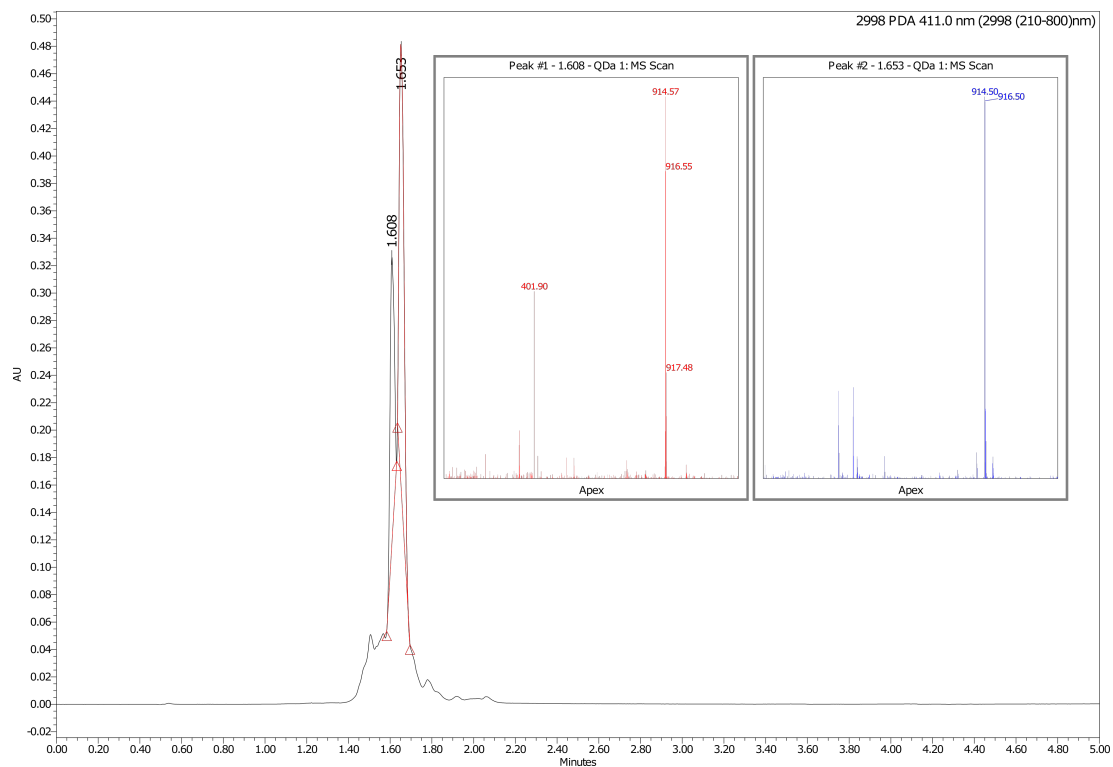


Figure A.37 HPLC-MS of complex $[\text{Cu}_2(\text{HL}^2)\text{Cl}_4]$, method A.

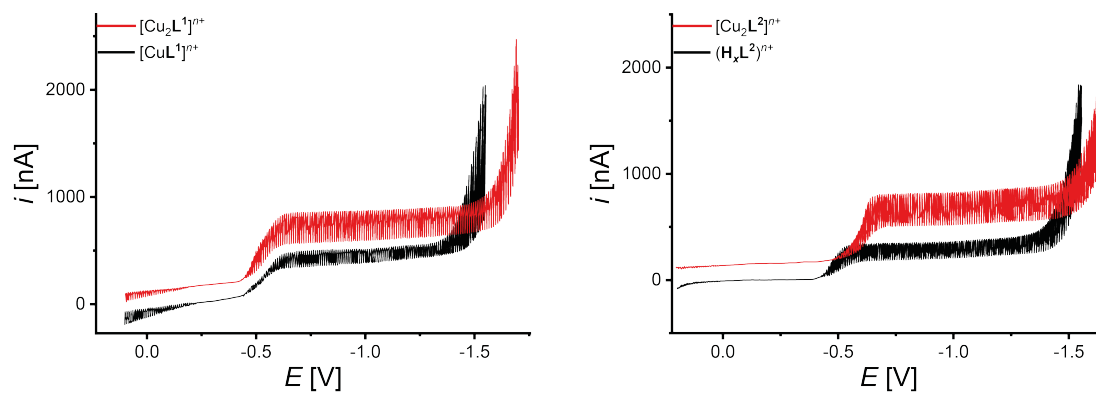


Figure A.38 Example polarograms of mono and dinuclear Cu^{II} complexes with L^1 (left) and ligand L^2 and its dinuclear Cu^{II} complex (right), the red lines are manually shifted by 150 nA up for clarity

Table A.1 X-ray Crystallography Data

Compound	$[\text{Cu}_2(\text{HL}^1)(\text{SO}_4)(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 8\text{H}_2\text{O}$	$[\text{Cu}_2(\text{HL}^1)\text{Cl}_2]\text{Cl}_2 \cdot 5\text{H}_2\text{O}$	$[\text{Cu}_2(\text{HL}^1)\text{Cl}_2(\text{H}_2\text{O})_2] [\text{Cu}_2(\text{HL}^1)\text{Cl}_4]\text{Cl}_2 \cdot 18\text{H}_2\text{O}$	$[\text{Cu}_2(\text{HL}^2)(\text{ClO}_4)_4] \cdot 4.5\text{H}_2\text{O}$	Compound 2b
Formula	$\text{C}_{29}\text{H}_{76}\text{Cl}_2\text{Cu}_2\text{N}_8\text{O}_{15}\text{S}$	$\text{C}_{29}\text{H}_{66}\text{Cl}_4\text{Cu}_2\text{N}_8\text{O}_6$	$\text{C}_{58}\text{H}_{152}\text{Cl}_8\text{Cu}_4\text{N}_{16}\text{O}_{22}$	$\text{C}_{28}\text{H}_{56}\text{Cl}_4\text{Cu}_2\text{N}_9\text{O}_{20} \cdot 5$	$\text{C}_{27}\text{H}_{27}\text{Cl}_3\text{O}_3$
M_r	1007.01	891.77	1963.71	1115.69	505.83
Crystal system	Monoclinic	Triclinic	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1$	$P-1$	$P2_1/m$	Cm	$P-1$
a [Å]	14.1858(6)	9.4063(5)	8.5959(3)	9.5228(3)	9.7147(3)
b [Å]	8.7463(3)	10.0450(5)	39.2336(11)	33.0240(13)	11.4594(3)
c [Å]	18.0578(8)	22.2333(13)	13.2328(4)	8.2639(3)	11.5865(4)
α [°]	90	79.116(2)	90	90	91.765(1)
β [°]	100.057(2)	80.041(2)	98.477(1)	111.279(1)	111.585(1)
γ [°]	90	77.443(2)	90	90	101.994(1)
U [Å ³]	2206.06(16)	1994.45(19)	4414.0(2)	2421.66(15)	1165.04(6)
Z	2	2	2	2	2
d [g·cm ⁻³]	1.516	1.485	1.477	1.604	1.442
F_{000}	1068	940	2080	1214	528
Unique refl.	10036	13089	10247	5585	5305
Obsd. refl.	9961	12436	9364	5545	5109
Parameters	504	445	515	334	301
Restraints	25	0	0	2	0
$R(I > 2\sigma(I)); R'$	0.0326; 0.0329	0.0366; 0.0412	0.0924; 0.0979	0.0257; 0.0262	0.0334; 0.0343
$wR(I > 2\sigma(I)); wR'$	0.0893; 0.0896	0.0857; 0.0925	0.02524; 0.2570	0.0673; 0.0678	0.0916; 0.0923
CDCC ref.	2354226	2354224	2354228	2354225	2354227