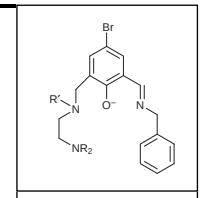
Metallobiosites and their synthetic analogues—a belief in synergism
1997–1998 Tilden Lecture



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Just as it is possible to use coordination compounds to try and gain insight into the nature of metallobiosites so it is also possible to use the knowledge acquired through crystallography concerning the structure of a metallobiosite to try to develop new chemistry. The aim of this lecture was to illustrate this philosophy by referring to the interplay that led to the confirmation of the dicopper site in haemocyanin and by speculating on the use of knowledge acquired concerning the dinuclear site in urease to facilitate simulation of the functioning of the site.

If one regards a *metalloprotein*, or a *metalloenzyme*, as a highly elaborated coordination complex the metal-containing site (*metallobiosite*) of which comprises one or more metal atoms and their ligands, then it is possible to contemplate simulating the immediate coordination environment of the metallobiosite by use of synthetic analogues derived from small molecule compounds. The purpose of this lecture is to promote the philosophy that there is a synergism between the knowledge acquired from model studies and that acquired from direct studies on metallobiosites which enables both a fuller understanding of the metallobiosite and the generation of new coordination chemistry. The first part will concern the application of models to understanding metallobiosites and the second the generation of new chemistry inspired by biology.

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'Life has evolved from inorganic materials . . . and in that evolution has incorporated every facet of inorganic chemistry that was profitable to it'

R. J. P. Williams

The discovery, in 1926, that enzymes could be crystallised led to James B. Sumner being awarded a share in the 1946 Nobel Prize for Chemistry.[1] He also proposed that 'enzymes could be proteins devoid of organic coenzymes and metal ions'. It has also been remarked that 'life has evolved from inorganic materials (generating organic chemistry as it went) and in that evolution has incorporated every facet of inorganic chemistry that was profitable to it'.[2] In the case of the enzyme that Sumner had crystallised, jack bean urease, this turned out to be true as in 1975 it was shown that the enzyme contained nickel. The discovery, by Blakely and his research group, of the presence of nickel came from a critical analysis of the uv-visible spectrum that showed a distinct long wavelength absorption characteristic of octahedral nickel(II) in an oxygen- and nitrogen-donor environment.[3] Further studies, by the same group, using site inhibitors indicated that there were two nickel ions per active site and this led to the postulation that the two nickel ions acted cooperatively in catalysing the hydrolysis of urea to ammonia and carbonic acid [reaction (1)].[3] Each nickel(II) was proposed

$$(NH_2)_2C{=}O + 2H_2O \rightarrow 2NH_3 + H_2CO_3 \qquad (1)$$

to undertake a different role in the mechanism of the reaction with one serving to polarise the urea and the second enhancing the nucleophilicity of water so that the hydroxide formed can attack the polarised carbonyl of the urea.

In 1995 Jabri and co-workers reported the crystal structure of microbial urease from *Klebsiella aerogenes* and this revealed that the active site did indeed consist of two nickel atoms.[4] These are sited 3.5 Å apart and were bridged by a carbamate group that had been formed by reaction of carbon dioxide with the ε-amino acid of a lysine residue. One of the nickel atoms is three coordinate and therefore coordinatively unsaturated, thus presenting a likely site for the binding of urea. The second nickel is pentacoordinated and the water molecule attached to the metal may be activated towards nucleophilic attack at the urea either by utilising the Lewis acidity of the metal or by base-catalysis promoted by a basic side chain proximal to the site (Fig. 1). It is quite evident that a close inspection of urease and its activity demonstrates the vitality of inorganic chemistry, an area which through time has been didactically linked to the inanimate.

From the above it is evident that the crystal structure of a metalloenzyme serves to act as a guide to the associated mechanistic pathway. In order to elucidate mechanisms further studies would require specific chemical modifications or site directed mutagenesis. The coordination chemist can provide the

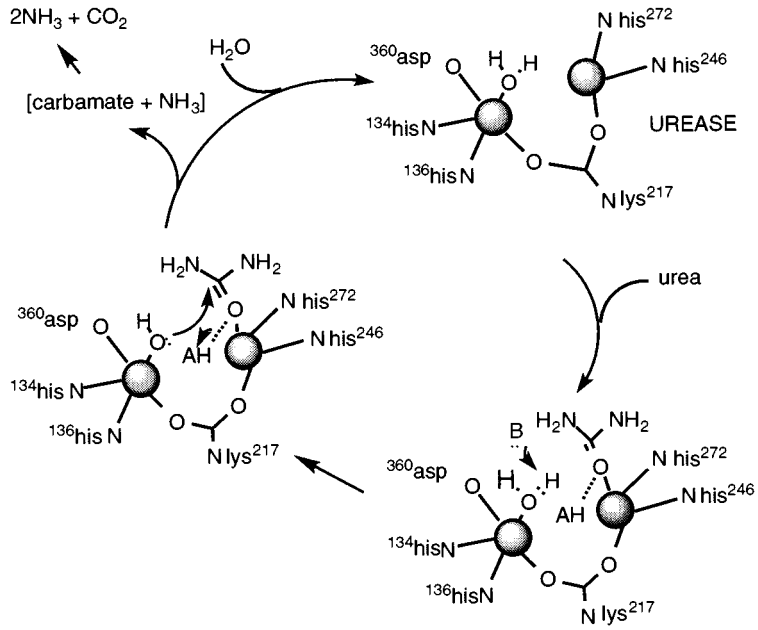


Fig. 1 A proposal for the mechanism of urease (based on S. J. Lippard, *Science*, 1995, **268**, 996).

opportunity to work with small molecule model compounds, or synthetic analogues. These can be designed to approach, or duplicate, the biological unit in terms of ligand and donor atom type and assembly, metal atom oxidation level and coordination geometry. They have been classified as *speculative* or *corroborative*.[5] A speculative model occurs when the structure of the microenvironment of the metal ion is unknown and the object is to reproduce some spectroscopic property of the metallobiomolecule using a simple complex. A corroborative model is used to try and directly imitate the coordination features of a structurally established site; the information recovered here can then be used to ascertain whether the properties of the site are dominated by interactions within the first coordination sphere of the metal. It is useful to recall that 'the extent to which a model resembles what it represents depends on its purpose' and so the Holy Grail for the bioinorganic modeller would be the acquisition of a *functional* model that simulated the natural site in terms of both structure and function. It is crucial to remember that the models may not be able to simulate the environmental effects of, and whatever structural constraints are imposed by, the natural environment—interactions beyond the first coordination sphere.

Focused modelling therefore has a useful role to play in helping elucidate fundamental aspects of the structure, spectroscopic, magnetic and electronic properties, reactivity and mechanism pertaining at metallobiosites. The relationship between coordination chemistry and bioinorganic chemistry may be regarded as synergistic (Fig. 2). There is a learning process from Nature and an opportunity to adopt the knowledge retrieved to the generation of new chemistry and potential catalysts based on metalloenzymic processes; it is this aspect on which this lecture is focused. The philosophy is not new having first been expounded by Ken Karlin,[6] hence my creed is sequential.

My interest in the modelling of metallobiosites stems from a period of involvement with the Agricultural Research Council and the chemistry of macrocyclic polyethers and their selective coordination of alkali metal cations. Two structural motifs (Fig. 3)—the entrapment of two cations within the cavity of a single macrocycle[7] and the total encapsulation of a cation by a single macrocycle[8]—held my attention and stimulated interest in whether such motifs could be obtained for transition metals. The prospect of dinuclear encapsulation was further stimulated by the growing literature on the occurrence of dinuclear metallobiosites.[9]

What is a macrocycle?

A macrocycle has been defined as a cyclic molecule with three or more potential donor atoms in a heteroatom ring of at least

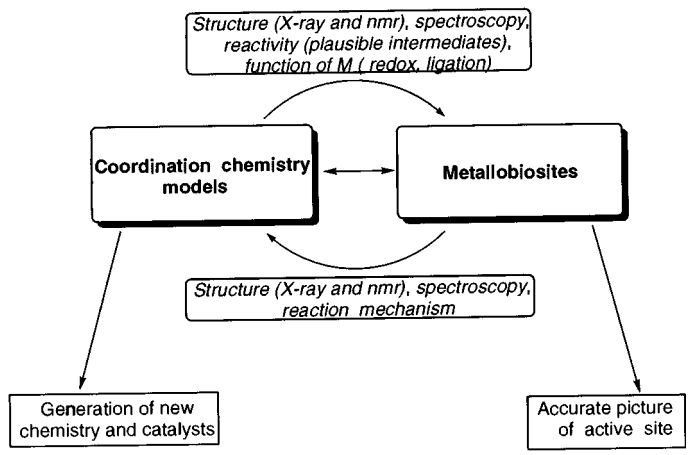


Fig. 2 The synergism between coordination chemistry and the chemistry of metallobiosites.

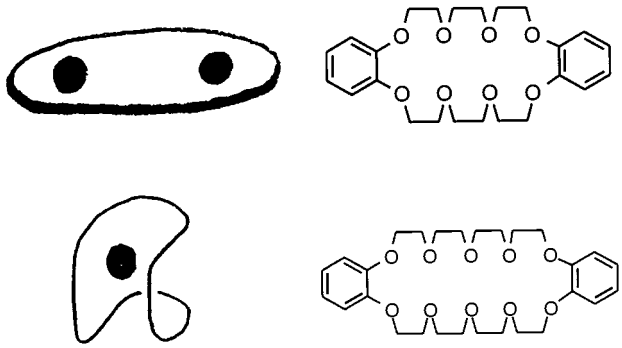


Fig. 3 Structural motifs from two crown ether complexes. Dibenzo-24-crown-8·2KNCS[7] (upper) and dibenzo-30-crown-10·KNCS[8] (lower).

nine atoms. Nature has exploited macrocycles—porphyrins and related systems—for an aeon but man has only used them in this century. Until the 1960's only the phthalocyanines and various isolated compounds such as van Alphen's cyclam (1,4,8,11-tetraazatetracyclodecane) and the polyethers of Luttringhaus were available. The early 1960's saw the advent of a range of polyazamacrocyclic ligands, formed by metal-template procedures and of Pedersen's crown polyethers. These discoveries led to more systematic studies of macrocycles and their metal complexes and as such provided the corner stones upon which supramolecular chemistry has been built (Fig. 4).

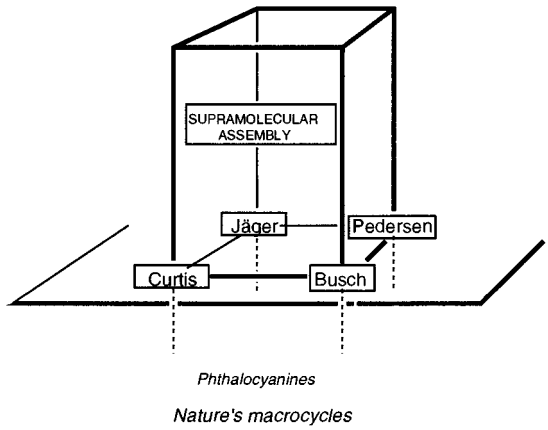


Fig. 4 The growth of supramolecular chemistry.

The synthesis of Schiff base macrocycles

Macrocycles containing Schiff base linkages have provided three of the corner stones mentioned above (Fig. 5a–c). The earliest example of a synthetic macrocyclic ligand containing an imine linkage stems from the work of Curtis and was derived from the mixed-aldol condensation of acetone with nickel(II) ethylenediamine complexes.[10] In 1964 Curry and Busch reported the iron(II)-templated condensation of 2,6-diacetylpyridine with triethylenetetramine to give iron(III) complexes of a pentaazadiimino macrocycle,[11] and Jäger showed that the reaction of β-ketoiminato complexes with 1,2-diaminoethane gave metal complexes of a tetraazadiimino macrocycle.[12] In all of these examples the product was recovered as a metal complex and no macrocycle was obtained in the absence of a metal ion. The requirement for a metal to be present in the reaction forming the macrocycle became known as the template effect.

The fourth cornerstone (Fig. 5d) was added by Pedersen with his discovery of the cyclic, or 'crown', polyethers, first synthesised from the reaction of catechol with α,ω-chloroethers in the presence of alkali metal cations.[13] In this case the metal

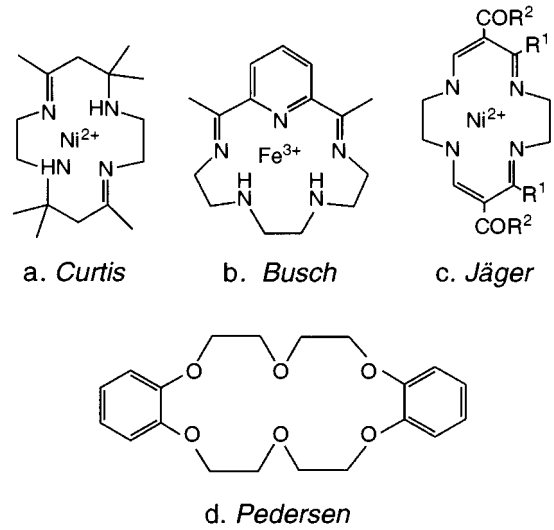


Fig. 5 The cornerstone macrocycles.

cation was not retained by the product and so it was proposed that the role of the metal was to organize the transition state which preceded formation of the macrocycle.

'I don't believe that any scientist should be deprived of the joy of getting a research idea from the chance juxtaposition of two titles in a journal'
Ephraim Banks

A wide range of Schiff base macrocycles has now evolved from the early studies. Many involve the use of 2,6-diacetylpyridine (PDA) or 2,6-diformylpyridine (PDF) as building blocks and it is possible to find an oligomeric series of macrocycles based on the condensation of these pyridine dicarbonyls with 1,*n*-diaminoalkanes.[14] Routes to the formation of [1 + 1] and [2 + 2] Schiff base macrocycles, that is macrocycles based on the condensation of one dicarbonyl with one diamine, and two dicarbonyls with two diamines respectively, are shown in Fig. 6. The reaction of PDA with α,ω-diaminoethers is used as an example as these were the first that we attempted. The principle of using units from both the transition metal orientated template procedures of Busch and the ether donors exploited by Pedersen was based on the Banksian philosophy shown in the above heading. The role of the metal ion in these metal-ion templated cyclisations is to control the supramolecular assembly of pre-cyclisation fragments, most likely through metal complexes derived from the precursors. The desired cyclisation product then results from an intramolecular interaction in the transition state. In the syntheses using α,ω-diaminoethers alkali metal cations and transition metal ions are ineffective as templates but alkaline earth cations and lead(II) promote cyclisation. The size and ionic potential of the template were shown to be important factors in these reactions. In the formation of [1 + 1] macrocycles the larger cations Ca^{2+}, Sr^{2+}, and Ba^{2+} give metal complexes of the hexadentate 18-membered macrocycle derived from 1,11-diamino-3,6,9-trioxaundecane whereas the smaller Mg^{2+} cation gives only a complex of the pentadentate 15-membered macrocycle derived from 1,8-diamino-3,6-dioxaoctane. The reaction of Ba^{2+} under conditions which could have given the [1 + 1] product derived from 1,8-diamino-3,6-dioxaoctane promotes formation of a mononuclear complex of a 30-membered [2 + 2] macrocycle and, interestingly, when Pb^{2+} is used as the template a homodinuclear complex of the [2 + 2] macrocycle is found. The influence of the donor atoms is noted here as in the former the Ba^{2+} is located centrally within

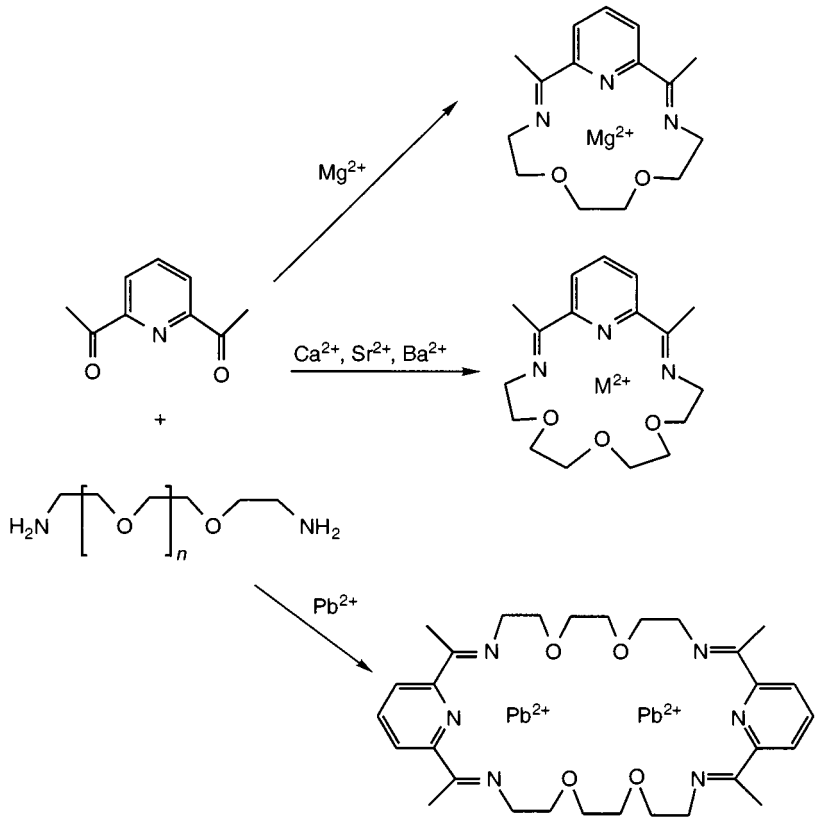


Fig. 6 Routes to macrocyclic Schiff bases.

the macrocycle cavity and interacts with all of the ligand donors whereas in the Pb^{2+} complex the metal is coordinated by the softer donors from the head units.

By varying the nature of the heterocyclic dicarbonyl ('head unit') and the 1,*n*-diamine ('lateral unit') a wide range of dinucleating tetraimine Schiff base macrocycles can be synthesised (Fig. 7).

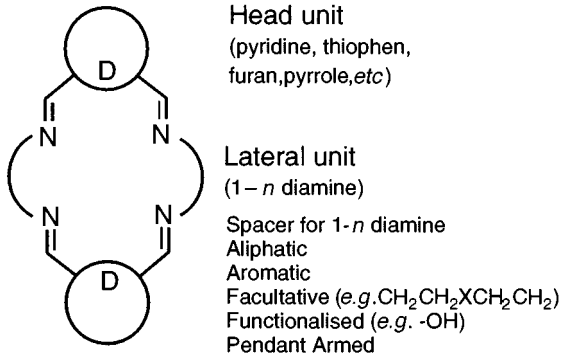


Fig. 7 The range of Schiff base macrocycles.

That cation size is not the ultimate factor involved in delineating the success of a templated cyclocondensation has recently been demonstrated by Busch and his co-workers.[15] If in the above synthesis a 20-membered [1 + 1] macrocycle is synthesised rather than the 18-membered [1 + 1] macrocycle by using a linker modified by addition of two methylene units—$NH_2(CH_2)_3O(CH_2)_2O(CH_2)_3NH_2$—to increase the flexibility of the ligand then Cu^{2+} may be used as a template. The copper is coordinated by the head unit and a chloride ion, the ether oxygens are not involved, and this shows that as long as the resultant macrocycle can accommodate the preferred coordination geometry of the metal it is likely that cyclocondensation will occur.

'The extent to which a model resembles what it represents depends on its purpose'

Jonathan Miller

Haemocyanin is the dioxygen-carrying metalloprotein in arthropods and molluscs. Cumulative spectroscopic studies on oxyhaemocyanin led to the presentation of a proposal that the active site was a dinuclear copper centre in which the two copper atoms were each coordinated by three histidine units together with an endogenously bridging donor atom, proposed as an oxygen from a tyrosine residue, and an exogenously bridging *cis*-1,2-peroxide.[16] This speculation led to the derivation of many modelling studies, the thrust of which were to produce an endogenous bridge capable of holding the copper atoms *ca.* 3.6 Å apart and mediating strong antiferromagnetic coupling.

Our system was derived from the barium-templated cyclocondensation of 2,6-diacetylpyridine and 1,3-diaminopropan-2-ol followed by transmetallation with copper to give the dinuclear complex **1** shown in Fig. 8, the crystal structure of which was obtained.[17] This revealed a Cu···Cu separation of 3.64 Å, and provided the first example of a structurally-characterised copper dimer with a single alkoxo-bridge. The complex showed a charge-transfer band at 330 nm and a small antiferromagnetic coupling ($2J = -84$ cm^{-1}) and so complex **1** was viewed as a speculative model for the spectroscopically-derived haemocyanin site. This however was a false dawn, and indeed part of a cautionary tale, as when the structure of colourless deoxy-haemocyanin from *Panulirus interruptus* (spiny lobster) was solved it showed no endogenous or exogenous bridging group to be present at the active site. Each Cu^{I} ion was co-ordinated by three histidine residues with an inter-copper distance of *ca.* 3.7 Å.[18] The nature of the spectroscopically-derived site was questioned and it fell to the coordination chemist to present a route forward with the publication of 'an accurate synthetic model of oxyhaemocyanin' by Kitajima and his co-workers.[19]

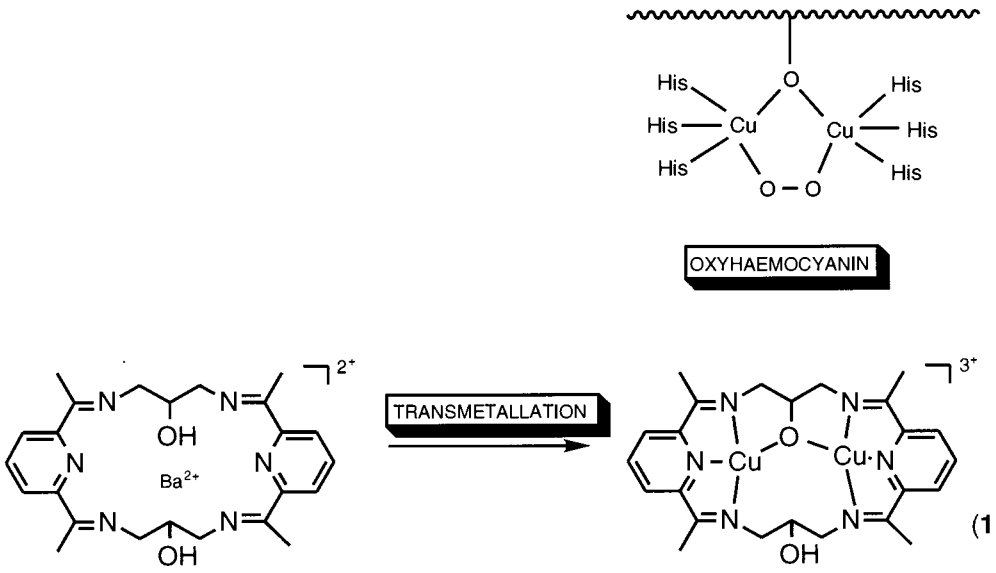


Fig. 8 Proposed replication of features of the spectroscopically-derived dinuclear site in oxyhaemocyanin [tetragonal Cu, λ_{max}, 345 nm (ε 20 000) (spectroscopy); Cu···Cu, 3.64 Å (EXAFS); ν(O–O), 750 cm^{-1} (resonance Raman); diamagnetic].

Using sterically hindered tripodal ligands Kitajima showed that it was possible to prepare dinuclear complexes bearing the 'CuO_2Cu' group in the absence of an endogenous ligand bridge and that these complexes closely replicated the spectroscopic properties of the natural site.[19] The crystal structure of the complex derived from hydrotris(3,5-diisopropylpyrazolyl)borate revealed the presence of an unexpected, and unusual, μ-η^2:η^2-peroxide bridge and when the X-ray crystal structure of oxyhaemocyanin from *limulus polyphemus* was solved by Magnus *et al.*,[20] it confirmed that there is no endogenous bridge present at the dinuclear site in oxyhaemocyanin and that the structure contains a μ-η^2:η^2-peroxide bridge as shown schematically in Fig. 9. In this instance the validity of using small molecule models to help in the understanding of the coordination environment of a metallobiosite is clear.[19]

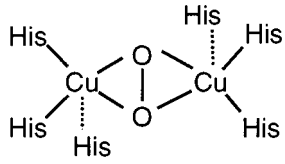


Fig. 9 Schematic representation of the dinuclear site in oxyhaemocyanin.

A change in direction—the post-endogenous bridge era

During the course of studies on ligands capable of providing endogenous bridges it became apparent from the structures of several mononuclear barium complexes of functionalised tetraimine Schiff base macrocycles that the macrocyclic ligands had folded to present molecular clefts into which the metal ions coordinated—particularly if the 'lateral unit' of the macrocycle contained an odd number of carbon atoms the central one of which was functionalised.[21] This mode of metal incorporation is not dissimilar to that of metalloproteins in which the requisite metal is bound in a pocket or cleft produced by the conformational arrangement of the protein. The objective then became the synthesis of flexible macrocycles capable of generating clefts for metal coordination without the presence of a ligand-based endogenous bridge. In order to do this a series of bibracchial macrocycles containing two pendant arms strategically attached to the heteroatom ring were synthesised from 2,6-diacetylpyridine and a range of *N*,*N*-bis(3-aminopropyl)- and *N*,*N*-bis(2-aminoethyl)-alkylamines using barium or silver(I) templates (Fig. 10).[22] The resulting mononuclear barium

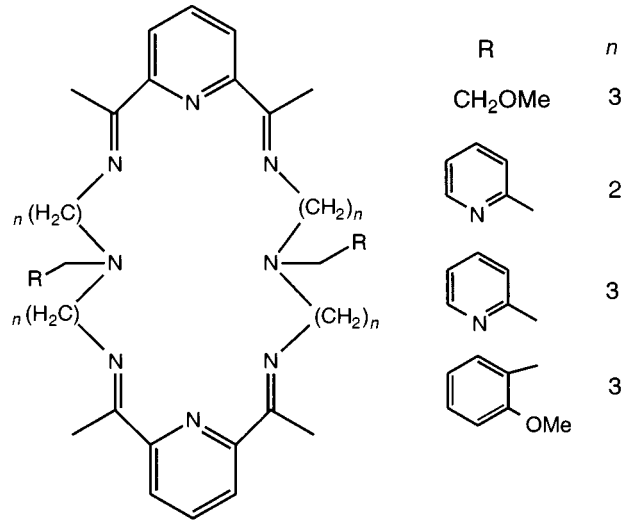


Fig. 10 Bibracchial tetraimine Schiff base macrocycles.

complexes and dinuclear silver(I) complexes were found to have the required conformation and in the latter cases the metals were separated by distances ranging from 2.9–6.0 Å depending on the nature of the donor groups in the pendant arms and on the length of the carbon atom chains present in the lateral diamine derived spacers. The transmetallation of the barium complex of the corresponding macrocycle derived from *N*,*N*-bis(2-aminoethyl)-2-methoxyethylamine readily gave a dinuclear copper(II) complex. However the X-ray crystal structure of this copper complex showed that the cleft conformation had been destroyed to give a much more planar macrocyclic conformation.[23]

Back to the drawing board

All was not entirely lost as it was possible to use the bibracchial macrocycle derived from tris(2-aminoethyl)amine and 2,6-diacetylpyridine as a precursor for the derivation of a trinuclear copper complex which served as a first generation model for the trinuclear site in ascorbate oxidase—this has been recounted

elsewhere.[21] A fresh route was required to try to place the dinuclear copper fragment in a molecular cleft and here serendipity played its hand.

The reaction of a diprotonated hexaiminocryptand (**2**), prepared by [2 + 3] cyclocondensation of 2,6-diacetylpyridine and tris(2-aminoethyl)amine in the presence of hydrochloric acid, with $Cu(BF_4)_2 \cdot nH_2O$ in methanol (Fig. 11) gave the

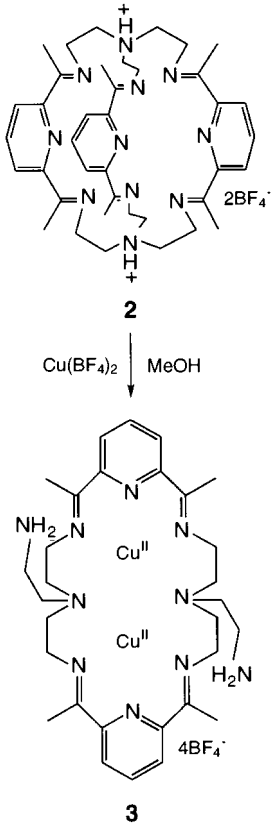


Fig. 11 Synthesis of the dicopper(II) complex.

dinuclear complex $[LCu_2](BF_4)_4 \cdot H_2O$, **3**.[21] The crystal structure confirms that a single ring-opening of the Schiff base cryptand, caused by scission of one pyridinyl-diimine unit, has occurred such that the dicopper(II) moiety is held inside a cleft with a Cu(II)···Cu(II) separation of 4.53 Å. Although this separation is comparable with the dicopper(I) separation of 4.6 Å found in deoxygenated haemocyanin from *Limulus polyphemus*,[20] we have not yet been able to prepare a dicopper(I) complex of L. The N-donor atoms of the pendant arms in this complex approach the metal atoms from the same side of the macrocyclic ring ('*cis*') consistent with the clipping out of one bridge from the cryptate precursor. This may be contrasted with the approach of the pendant arms from opposing sides ('*trans*') found when the dicopper(II) complex of the directly related tetraimine macrocycle bearing methoxyethyl pendant arms is prepared by transmetallation of the mononuclear barium precursor complex.[23]

Although we have not yet been able to prepare a dicopper(I) complex of L it has been possible to prepare related complexes of bibracchial tetraimine Schiff base macrocycles in which the head units are derived from furan-2,5-dicarbaldehyde or thiophene-2,5-dicarbaldehyde and the arms are simply aliphatic chains. In these complexes the heterocyclic head group serves as an inert spacer unit and the copper(I) atoms are four coordinate with ligation from three donors from the lateral units and a tightly bound acetonitrile of solvation. The inter copper separations are *ca.* 5.2 Å and the molecules are quite air-stable, presumably due to the coordinative saturation at each copper(I). This area of our work has recently been reviewed.[21]

Learning from biology—a new chemistry

At this point in time there is no precise model for the reversible uptake and release of dioxygen by haemocyanin. However it is clear that the use of small molecule models has provided useful information relating to the mode of binding of dioxygen to the dinuclear copper centre therein. At the same time it is apparent that the biological problem stimulated the interest of coordination chemists and that this in turn has provided seminal studies on the nature of interaction of dioxygen with copper, particularly by the groups of Karlin, Kitajima, Martell, Sorrell and Tollman. This area therefore serves as an excellent example of the interplay alluded to in the title with biologists learning from chemistry and chemists applying knowledge from biology to generate new chemistry (Fig. 12). To further illustrate this latter approach the development of unsymmetric dinucleating ligands will be discussed.

Site asymmetry at metallobiosites

Homodinuclear metallobiosites may be exemplified by those structurally characterised in non-haeme manganese catalase [Mn,Mn],[24] urease [Ni,Ni],[4] alkaline phosphatase[25] and phospholipase C [Zn,Zn],[26] the last two sites are actually trinuclear constellations, $[Zn_2Mg]$ and $[Zn_3]$ respectively, and it is the homodinuclear fragment that has relevance here. Representative heterodinuclear metallobiosites are those structurally characterised in purple acid phosphatase [Fe,Zn][27] and human protein phosphatase 1 [Mn,Fe].[28]

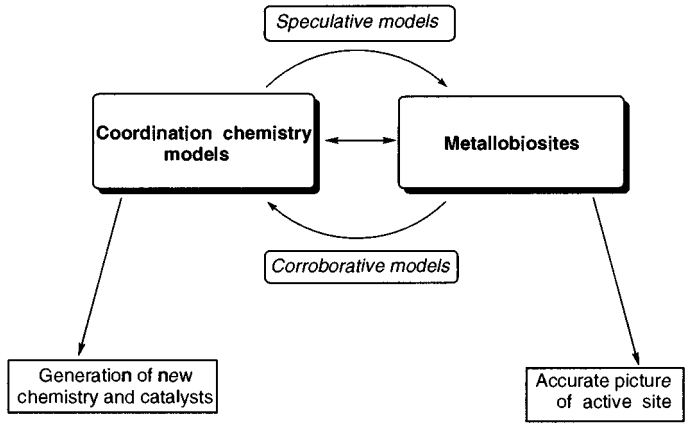


Fig. 12 Models and the synergism between coordination chemistry and the chemistry of metallobiosites.

The roles of the individual metal ions present at a biosite may be quite distinct in character. For example one metal may play a *structural role* by helping to maintain the structural integrity of the protein whilst the second metal has a *functional role* through binding to a substrate.[5] The role of the metals may also be defined as *co-catalytic* whereby the two metal atoms in close proximity operate together as a catalytic site.[29] In order to facilitate these modes of behaviour the metal ions can be found in chemically distinct environments. These have been classified in four distinct groupings (Fig. 13). (a) *Symmetric*—in which an

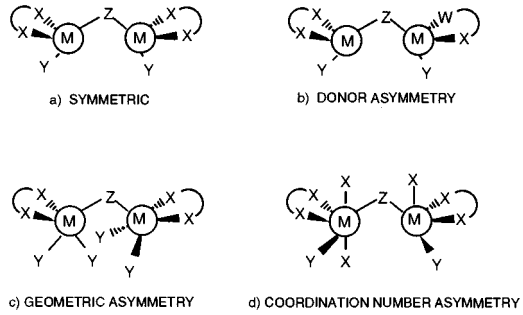


Fig. 13 A classification of metal coordination environments found at transition metal-derived dinuclear centres present in metallobiosites (M is a transition metal and W, X, Y and Z are ligand donor atoms such as N, O, S *etc.*).

identical number of donor atoms of the same type are bound to each metal atom in similar geometries. (b) *Donor asymmetry*—different types of donor atom coordinate to each metal atom. (c) *Geometric asymmetry*—there are inequivalent geometric arrangements of the donor atoms about each metal atom. (d) *Coordination number asymmetry*—an unequal number of donor atoms are coordinated to each metal atom.[30] To a first approximation the nature of the donor atom may be restricted to simply O, N, S *etc.* but a more accurate definition would specify the functional grouping associated with the donor atom and so differentiate between O in water and carboxylate or S in a thiolate or a thioether. The combination of different types of asymmetry may also occur at a dinuclear centre.

'For modelling···unsymmetrical, binuclear complexes are desirable synthetic targets'

T. N. Sorrell

Although many examples of coordination complexes derived from symmetric acyclic dinucleating ligands have been prepared and investigated as potential model complexes for metallobiosites, polydentate ligand systems that would give necessarily asymmetric dinuclear complexes remain rare; site asymmetry was often only accessed through good fortune. This, and the awareness of the asymmetric nature of many metallobiosites, led to the suggestion that for modelling studies unsymmetrical dinucleating ligands should be viewed as desirable targets.[31]

Complexes of dinucleating ligands have been divided into two general classes (Fig. 14).[32] The first group consists of those complexes in which the metals share at least one donor atom in species containing adjacent sites in which the central donor atom(s) provide a bridge; the ligands giving these 'bridging donor sets' have been collectively termed *compartmental ligands*. The second group consists of those complexes in which donor atoms are not shared and so *isolated donor sets* exist. If the arms are constituted of different donor atoms then an unsymmetric ligand results.

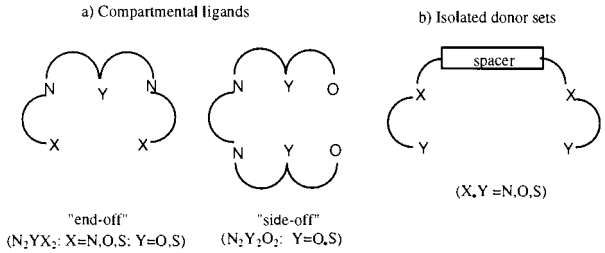
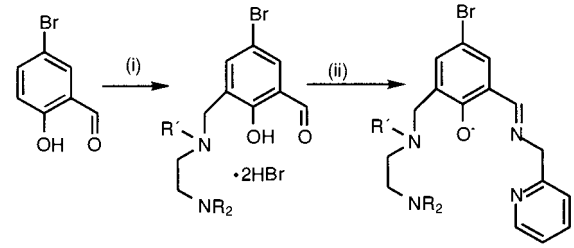


Fig. 14 Schematic representations of dinucleating ligands. Mono- or bibracchial pendant arms may be attached to the N atoms in the 'end-off' compartmental ligands and to the X atoms in the isolated donor sets. The spacers in the isolated donor sets do not provide bridging atoms.

Dinuclear access through use of unsymmetric endogenous phenoxo-bridge ligands

Our inaugural work on this topic utilised 'end-off' compartmental ligands and involved the introduction of a single pendant arm into 5-bromosalicylaldehyde by using the Mannich reaction followed by condensation of the resulting aminomethylsalicylaldehyde with a primary amine (Scheme 1) to give



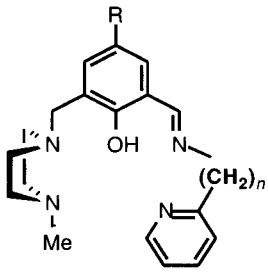
Scheme 1 *Reagents and conditions*: (i) $R_2NCH_2CH_2NHR'$, CH_2O_{aq}; (ii) 2-aminomethylpyridine, $HC(OEt)_3$.

a range of unsymmetric dinucleating Schiff base proligands exemplified by HL^1, HL^2, HL^5, HL^6, H_2L^7, and H_2L^8.[33] The first four of these proligands have N_5O donor sets and are donor atom unsymmetric as one donor set incorporates two sp^3 N atoms and the second set has present two sp^2 N atoms, the last two proligands are built up to adjacent N_2O and NO_2 donor sets.

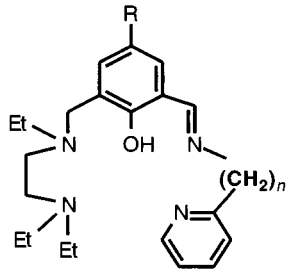
The reaction of the asymmetric proligands with copper(II) salts gave homodinuclear copper(II) complexes. The crystal structures of $[Cu_2Br(HCO_2)L^1]ClO_4{\cdot}H_2O$ (**4**)[33] showed that both copper atoms present in **4** are five-coordinate and in distorted square pyramidal environments with one copper atom (Cu1) more distorted towards a trigonal bipyramid than the second copper atom (Cu2). The indices of trigonality (τ) are 0.4 and 0.29 respectively where τ is the index of the degree of trigonality within the structural continuum between square pyramidal and trigonal bipyramidal geometries and has values of 0 for a square pyramid and 1 for a trigonal bipyramid.[34] Each copper is coordinated by the oxygen atom of the non-symmetrically bridging phenoxide, and by atoms from two exogenous bridging groups—a bromide and a formate anion. Coordination at each metal is completed by interaction with two nitrogen atoms from the appropriate pendant arm.There is also a spatial asymmetry present in that the square pyramidal environment at Cu1 has a formate oxygen atom at its apex whilst that at Cu2 has the bromide anion at its apex; the Cu···Cu separation is 3.24 Å.

In order to introduce a wider range of O and N donor atoms into the proligands and so extend the range of this type of 'end-off' ligand the necessary Mannich bases have been prepared under non-aqueous aprotic conditions prior to Schiff base condensation.[33] Here it was found that the preponderance of O donors led mainly to the synthesis of mononuclear complexes.[33]

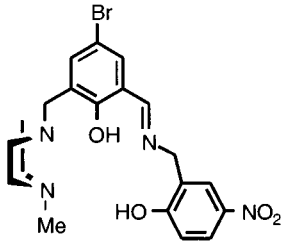
Feringa[35] later applied the Mannich-derived synthetic procedure to the generation of unsymmetric proligands such as HL^3



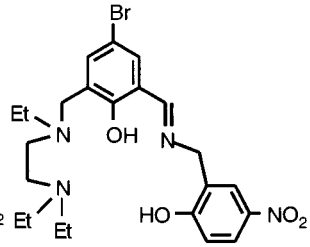
$HL^{1–4}$



$HL^{5,6}$



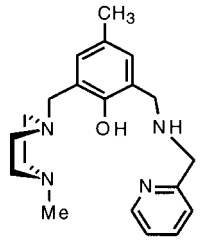
H_2L^7



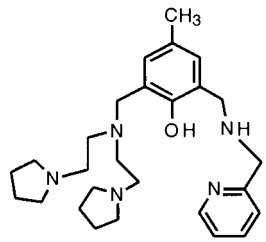
H_2L^8

R	*n*	
Br	1	HL^1
Br	2	HL^2
H	2	HL^3
CH_3	2	HL^4
Br	1	HL^5
Br	2	HL^6

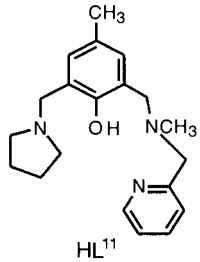
and HL^4 and showed that reduction of the Schiff base ligand gave the corresponding diamino compound *e.g.* HL^9. Such



HL^9



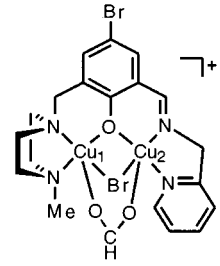
HL^{10}



HL^{11}

compounds were also prepared by reductive amination of the monoaminomethylated salicylaldehyde with a secondary amine (HL^{10}) or by starting from *p*-cresol and employing sequential Mannich reactions using two different amines (HL^{11}).

The dinuclear copper(II) complex $[Cu_2(CH_3CO_2)L^4]ClO_4$] (**5**)[35] was studied and the structure revealed that each copper is five coordinate. One copper (Cu1) is less distorted towards a trigonal bipyramid than is the second copper atom (Cu2) with indices of trigonality of 0.07 and 0.25 respectively and a Cu···Cu separation of 3.029 Å. In addition to the geometric asymmetry, spatial asymmetry is again noted; copper(1) has an oxygen atom from a bidentate acetate at an apex whilst copper(2) has the oxygen atom of a monodentate acetate at its apex. Both of the copper(II) complexes referred to here exhibit small antiferromagnetic couplings [$J = -42$ cm^{-1} (**4**) and -15



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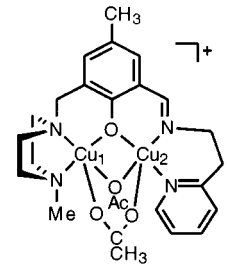

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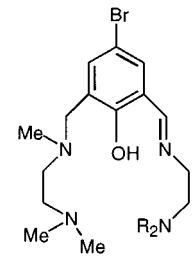
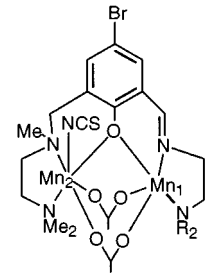
cm^{-1} (**5**)]. Although these values do not model accurately the strongly antiferromagnetically coupled Type 3 copper centres found in haemocyanin and tyrosinase it has been remarked that the presence of different environments for the two copper atoms may be related to the different modes of bonding proposed for the two copper atoms in tyrosinase.[35] A recent study by Reim and Krebs on the catecholase activity of dinuclear copper(II) complexes has included complexes derived from unsymmetric dinucleating Schiff base proligands. In this study it was shown that the unsymmetric complexes did show high catecholase activity compared with that of related symmetric complexes.[36]

Unsymmetric ligands have also been used to prepare manganese(II) complexes which have been exploited in the functional modelling of manganese catalase, a dimetalloenzyme which catalyses the disproportionation of hydrogen peroxide into dioxygen and water.[37] The proligands HL^{12} and HL^{13} were



R = Me HL^{12}
R = Et HL^{13}



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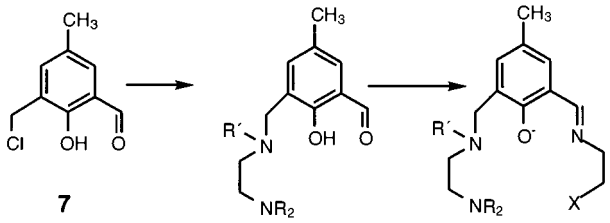
synthesised by the Mannich route and provide donor asymmetric ligands in which one compartment includes one sp^2 and one sp^3 nitrogen and the second compartment includes two sp^3 nitrogen atoms. The structure of the dimanganese(II) complex $[Mn_2L^{13}(MeCOO)_2NCS]$ (**9**) shows that three types of asymmetry are involved—donor atom, coordination number and geometrical. One manganese atom, Mn^1, is in a distorted square pyramidal geometry ($\tau = 0.34$) provided by the imino-N, two acetato-O, the phenoxo-O and an amino-N atom whereas the second manganese atom, Mn^2, is in a distorted octahedral environment provided by the thiocyanate anion, two acetato-O, the phenoxo-O and two amino-N atoms; the manganese atoms are separated by 3.376 Å. There is a significant difference between the behaviour of the unsymmetric complexes and the corresponding symmetric complexes with respect to their disproportionation. For the symmetric complexes the theoret-

ical yield of dioxygen is evolved whereas for the unsymmetric complexes only 60–70% of the expected dioxygen is evolved and a side reaction is found to occur to consume H_2O_2 when the manganese atoms are not in electronically equivalent environments. These results support the view that a symmetric environment is required for the active dimetallobiosite in the metalloenzyme to operate efficiently.

The ongoing problem

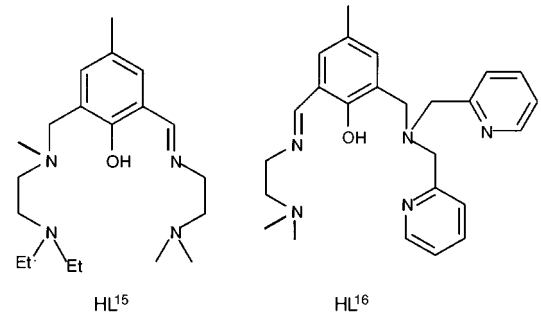
In our present work we have adopted a synthetic procedure based on that reported by Latour and co-workers.[38] This derives from 2-(chloromethyl)-6-formyl-4-methylphenol (**7**) which is available in 2 steps from commercial 2,6-bishydroxymethyl-4-methylphenol. Reaction of **7** with functionalised secondary amines followed by Schiff base condensation with functionalised primary amines provides a further range of unsymmetric ligands (Scheme 2).[39]



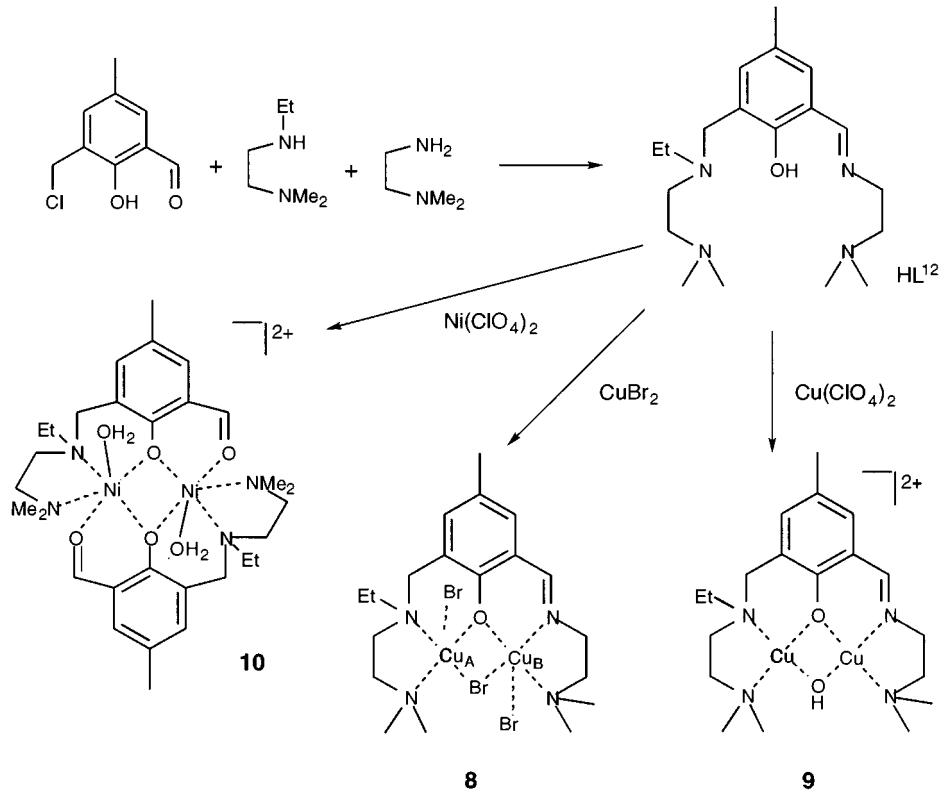
Scheme 2 X = range of functionalites.

The reaction of HL^{14} with copper(II) and nickel(II) salts is given in Scheme 3 and serves as a representative example.[39] The crystal structure of the dicopper(II) complex **8** showed that whilst the two copper(II) atoms were both square pyramidal the two environments were spatially asymmetric. Cu_A has the bridging bromide in an axial position and the non-bridging bromide in an equatorial position whereas Cu_B has the bridging bromide equatorial and the non-bridging bromide axial. This results in the square planes being tilted at 180° to each other. As a consequence of this, and despite the copper–copper separation of 3.23 Å, the magnetic properties of the complex show no coupling between the two copper atoms.[39] This is presumably the result of an orbital mismatch and corresponding loss of exchange pathway.

Changing the anion to perchlorate gives a different result and a hydroxo-bridged dicopper(II) complex $[Cu_2L^{14}](ClO_4)_2$ (**9**) is obtained. Good crystals of this compound proved elusive but the crystal structure of the directly related complex $[Cu_2L^{15}](ClO_4)_2$ prepared from proligand HL^{15}, in which the positions of the *N*-alkyl groups on the saturated arm have been reversed, was solved and showed that each copper(II) was in a square coplanar environment composed of the articular nitrogen donors, the bridging phenolate oxygen atom and the bridging hydroxide oxygen atom. The copper(II)–copper(II) separation was 2.97 Å and there was antiferromagnetic coupling with $J = -215$ cm^{-1}.[39]



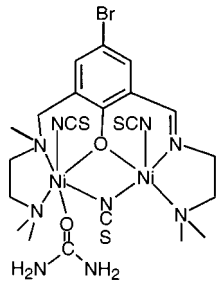
The nickel(II) complex $[Ni_2(L)_2](ClO_4)_2 \cdot 2H_2O$ (**10**) resulted from slow evaporation of a methanolic solution and is the consequence of hydrolysis of the Schiff base arm. The crystal structure showed that each nickel(II) atom is in an octahedral environment with the planar environment at each nickel(II)



Scheme 3

being provided by a N_{Et} atom, an aldehydic O atom, and two bridging phenolato-O atoms. The apical sites are occupied by the terminal N_{Me2} atom and a water molecule; the two water molecules are *trans* to each other. It is possible to speculate that the initial product in this reaction might be a μ-hydroxo bridged species, as in the copper complexes above. The hydroxide would then be available to initiate hydrolytic cleavage of the side-arm.

In effect we have almost come through a circle starting with urease and in reaching dinickel(II) complexes that circle is beginning to close. Recently it has been shown that urea will bind to a nickel(II) atom in dinuclear complexes of phenol-based 'end-off' compartmental ligand.[40] One proligand used was HL^{12} and from this the complex $[Ni_2(L^{12})(NCS)_3(urea)]$



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(**11**) was obtained. The crystal structure showed that the nickel–nickel separation was 3.16 Å and that the nickel atoms were coordination number asymmetric. The nickel held by the iminic arm was 5-coordinated and that held by the aminic arm was 6-coordinated. The urea was bound through its oxygen atom to the nickel in the aminic compartment (Ni–O, 2.13 Å). If the proligand is changed to HL^{16} then the dinickel(II) complex $[Ni_2(L^{16})(OAc)(NCS)_2]$ can be prepared. The structure of this complex reveals that there is an asymmetric dinuclear core with a mixed 5/6 coordination number set—the pair of nickel atoms are bridged by a phenolate and acetate group, and an isothiocyanate group coordinates to each nickel atom together with the nitrogen atoms of the appropriate arm. Reaction with urea then generates $[Ni_2(L^{16})(OAc)(NCO)(NCS)]$.[41] As a very slow dissociation of urea into ammonium and isocyanate ions is known under acidic conditions, it is of interest to note that the dinickel core appears to assist in the conversion of urea to isocyanate at moderate pH with absolute alcohol or acetonitrile as the solvent. To close the circle completely it is necessary to demonstrate that a synthetic dinuclear core can hydrolyse urea. This serves as a goal and may be possible, perhaps not with the efficiency of nature, but even a fraction of that efficiency would constitute a significant advance.

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