Reversible Coordinative Bonds in Molecular Recognition

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1. Introduction

Metal complexes with open coordination sites have found wide use in molecular recognition. They serve as binding sites in the development of chemosensors,[1] to study metalloenzyme function in bioinorganic chemistry,[2] or to direct supramolecular self-assembly.[3] Lewis-acidic metal complexes

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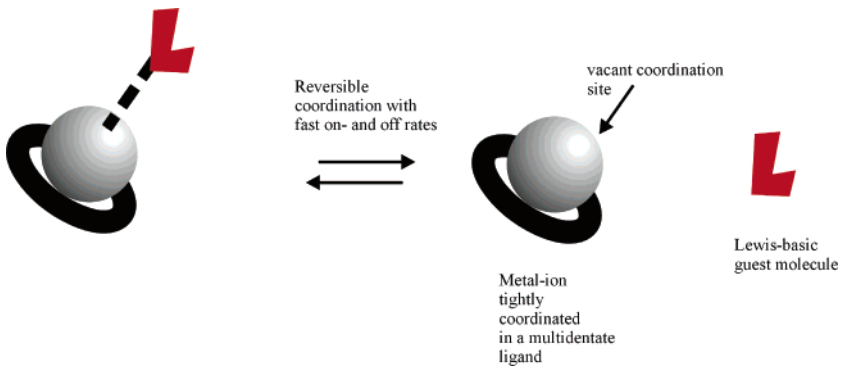
can target a large variety of Lewis basic functional groups, which makes them very suitable for the design of synthetic receptors. Coordination to metal ions occurs typically with large enthalpies compared to those for hydrogen bond formation, salt-bridges, or dipole−dipole interactions. This gives the opportunity to study molecular recognition and self-assembly in solvents competing for binding, such as water, using coordinatively unsaturated metal complexes as binding sites. A single coordinated bond from a guest to a metal-complex host may provide sufficient binding energy to result in stable and defined aggregates at micromolar concentrations in water. Typically, to achieve tight binding in such an environment by weaker intermolecular interactions, multiple interactions and large receptor structures are necessary to exclude competing solvents.[4] However, not all coordinatively unsaturated metal complexes are suitable binding sites for molecular recognition of guest molecules. The coordinative bond between the metal complex and the bound guest should be strong but not too tight (Scheme 1). Binding affinity and binding kinetics, the on- and off-rates of the ligand, should still allow reversibility to keep the important features of molecular recognition, such as self-assembly to the thermodynamically most favored structure, dynamics of supramolecular aggregates, and displacement of a bound guest by a better ligand. Metal complexes which bind ligands at their open coordination sites with milli- to micromolar affinity and rates in the millisecond time range or faster are most suitable for self-assembly and molecular recognition processes at the laboratory time scale.

The complementarity of shape and binding sites usually determines the binding selectivity of host molecules using hydrogen bonds or electrostatic interactions. This applies equally to the small number of oligonuclear metal complex receptors reported until now. However, even a single reversible bond between the metal complex and the ligand shows intrinsic selectivity toward the nature of the ligand exceeding its simple Lewis basicity. The Lewis basicity of a ligand correlates to the hydrogen bond donor or acceptor ability in hydrogen bond receptor binding. The strength of the coordinative bond depends additionally on the nature of the metal ion and the Lewis basic ligand atom as predicted by the HSAB principle. Such selectivity is well documented in metal ion complexation, e.g. by the preferential binding of imidodiacetato transition metal complexes to imidazole or pyridine nitrogen atoms. The selectivity is useful for receptor design in molecular recognition.

An obvious prerequisite for a metal complex to serve as a binding site is its stability. The metal ion should be coordinated in a thermodynamically and kinetically stable fashion. A reversibly bound Lewis basic guest should not displace the original ligand from the metal ion. This requires multidentate primary ligands for metal ion binding, and

Scheme 1. Principle of a Coordinatively Unsaturated Metal Complex as Binding Site for the Reversible Coordination of a Lewis Basic Guest Molecule



indeed all reported examples of reversible coordination of a guest to a metal ion binding site in molecular recognition use such ligands.

2. Scope and Limitations

This review will summarize the use of reversible coordination with selected classes of transition metal complexes of acyclic N,O ligands in molecular recognition. To be included, the stable metal complex of a primary ligand and a transition metal ion must possess unoccupied coordination sites and bind reversibly to a Lewis basic guest. The primary ligands of the unsaturated metal complex define the scope of this survey. We have selected the ligand classes for which the most applications as metal complex binding sites are reported. The general ligand types are given in Figure 1,

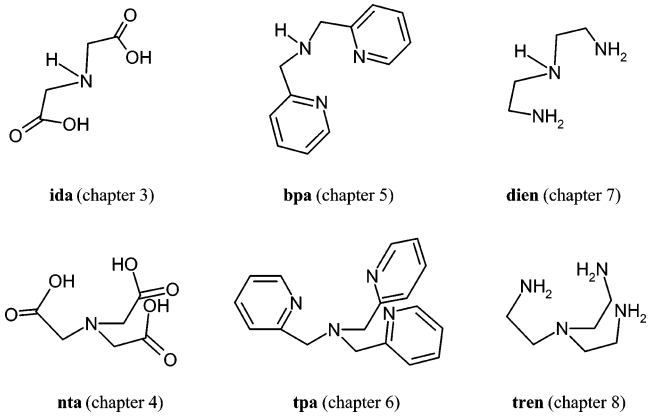


Figure 1. General ligand types of transition metal complex binding sites for molecular recognition covered in this review.

and we discuss for each ligand (i) the typical stability constants of the transition metal complexes, (ii) solid-state structures, in which the primary ligand−metal complex coordinates an additional complex ligand, (iii) reversible guest binding of the metal complex in homogeneous solution, and (iv) binding processes of metal complexes immobilized on a solid support, such as a polymer or gold. We exclude from the discussion the binding of simple inorganic ligands, such as halides or sulfate ions, in solid state or solution. The purpose of the review is to support the design of functionalized metal complexes as synthetic receptors for complex guests, such as peptides, amino acids, heterocycles, or nucleobases. The range of functional groups covered, which interact with metal complexes, is therefore limited to relevant ones for this purpose, such as carboxylate, phosphate, aromatic amines, diamines, imines, or thiols. The range of coordinatively unsaturated metal complexes covered in this survey is limited to the ligand types of Figure 1, which have found the most application in molecular recognition. A complete discussion of all coordination compounds which reversibly bind additional ligands is not possible within the scope of a review due to the very large number of possible examples.

We have excluded complexes with bipyridines or terpyridines as primary ligand from this review, because the few examples in which such complexes are solely used as metallo-binding sites are difficult to distinguish from the vast majority of applications of these complexes in photochemistry and photophysics. The key references for the use of bipyridine and terpyridine complexes as binding sites in molecular recognition by Anslyn[5,6] and others[7] are given.

Table 1. Binding Constants of Different Metal Ions to IDA[33]

ion	equilibrium	log *K*	ion	equilibrium	log *K*
Cr^{3+}	ML/M·L	10.9	Cu^{2+}	ML/M·L	10.56
	ML_2/M·L^2	21.4		ML_2/M·L^2	16.4
Co^{2+}	ML/M·L	6.54	Fe^{3+}	ML/M·L	10.72
	ML_2/M·L^2	11.95		ML_2/ML·L	9.42
Ni^{2+}	ML/M·L	8.3	Zn^{2+}	ML/M·L	7.15
	ML_2/M·L^2	14.5		ML_2/M·L^2	12.4

We do not cover porphyrin metal complexes as host binding sites in this review. Hamilton[8] very successfully used them for protein surface binding. Kral and Schmidtchen[9] reported sugar binding by porphyrin sandwich complexes. In these examples, the large hydrophobic porphyrin surface is significant for the binding events in water. The formation of a reversible coordinative bond to the central metal ion of the porphyrin ligand is ineffectual for this process. Functionalized porphyrin hosts were widely used for guest binding.[10] *Sanders* reported macrocyclic zinc(II) porphyrins and their use as synthetic enzymes directing and catalyzing Diels−Alder reactions.[11] Neutral zinc(II) porphyrin reversibly coordinates pyridine derivatives as additional ligands in dichloromethane solution. Synthetic porphyrin-based receptors for amino acids[12] or nucleobases[13] are known, but their binding ability is usually restricted to noncompeting solvents, such as dichloromethane or chloroform. Neutral porphyrin complexes of divalent metal ions, such as zinc(II), show only little Lewis-acidity and, therefore, weak coordination ability of additional ligands. Studies using trivalent porphyrin metal complexes as defined hosts reversibly coordinating guest molecules are limited.[14] The use of porphyrin complexes in composite materials or self-assembled aggregates in material science is well documented[15] but is outside the scope of this review.

Azamacrocyclic complexes of cyclen, cyclam, and related structures are widely used as binding sites in molecular recognition. Their application is well documented in many examples by Kimura,[16] Wieghard,[17,18] Fabbrizzi,[19−21] and others. Recent reviews[22−24] have covered the work, and we therefore exclude complexes of azamacrocyclic ligands from this review.

Overall, we provide a survey of reversible coordination to unsaturated acyclic metal complexes used in molecular recognition limited to the most important ligand classes used to date.[25] The selection of the ligands is defined by the ability of their corresponding transition metal complexes to reversibly and tightly bind Lewis basic guest molecules in competing solvents, such as water. This renders the selected complexes suitable as potential metal binding sites for synthetic receptors applicable in chemical biology or biotechnology, where their use would be most advantageous.

3. Iminodiacetato (IDA) Complexes

The iminodiacetato (IDA) ligand was first reported by Heintz[26] in 1862. Since then it has been widely used as a chelating ligand for various metal ions, e.g. Cu(II), Ni(II), Zn(II), Co(III), Fe(III), Al(III), and Cr(III).[27−32] Table 1 summarizes the metal ion binding constants of some typical complexes. Complexes with ligand to transition metal ion stoichiometries of 1:1 and 2:1 are typical. Metal ion binding constants and thermodynamic and kinetic stability are usually very high. Depending on the coordination number of the transition metal ion, complexation with the tridentate IDA ligand leaves coordination sites open for reversible ligand

binding. Therefore, unsaturated IDA complexes qualify perfectly as binding sites for molecular recognition of Lewis basic guests.

3.1. Structures of IDA Complexes in the Solid State

In the following, we discuss the solid-state structure of some typical IDA complexes, which have coordinated additional Lewis basic guests.

A large number of Cu(II)−IDA complexes have been reported and structurally characterized. Cu(II) complexes of IDA ([Cu(IDA)(H_2O)$_2$]) form polymeric structures in the solid state.[27,31] The Cu(II) ion coordination geometry is a distorted octahedron.[27]A large variety of complexes with IDA,[34−40] N-IDA,[37,41,42] or C-IDA[36,43] derivatives as primary chelating agent and imidazoles[34−37,41] as an additional ligand have been studied. The investigation of their structures in the solid state gave several interesting correlations: In compounds prepared from equimolar amounts of Cu(II), IDA, and N-heterocyclic donor, the Cu(II) exhibits a distorted pyramidal coordination (type 4+1) or, in some cases, an octahedral coordination geometry (type 4+2 or 4+1+1) is adopted.[37] The IDA or IDA derivative acts as a terdentate ligand with *mer*-chelation. In contrast, all known mixed-ligand complexes having a 1:1:2 Cu(II)/IDA/N-heterocyclic donor ratio show an elongated octahedral Cu(II) coordination with the IDA ligand as a *fac*-terdentate chelate.

The large number of solid-state structures known of transition metal complexes that bear an additional typically more weakly bound ligand prohibits their discussion in detail. Therefore, we have summarized in the Supporting Information all structures found in the Cambridge Structural Database where an IDA transition metal complex coordinates an additional complex ligand.[44] Noticeable is the ability of IDA complexes to bind additional N-heterocyclic ligands, in particular imidazole and pyridine. The summarized information may guide the design of functionalized IDA complexes as chemosensors, for self-assembly or other purposes.

3.2. Recognition Processes in Solution

3.2.1. Peptide and Protein Binding by IDA Transition Metal Complexes

The affinity of IDA complexes for histidine residues is well documented in the solid state and in solution. Its main application is protein purification using metal ion affinity chromatography (IMAC). However, some examples for peptide and protein binding in homogeneous solution are known.

To improve protein purification methods based on immobilized IMAC, a deeper understanding of the interactions between protein and metal complex is required. A polyhistidine tail (His$_{n=2-6}$) is introduced by standard methods of molecular biology into the protein. This creates affinity for an IMAC column.[45−47] By varying the amino acid sequence in the fused tail to SPHHGG, milder eluation conditions of tagged proteins were achieved.[48,49]

The coordination of proteins or their affinity tag region to immobilized Cu(II)-IMAC binding sites is difficult to study by direct physical methods. Therefore, Shepherd investigated which amino acids or side-chain donor atoms of SPHHGG and HHHHHH peptide sequences coordinate to the metal ion.[50] The diamagnetic, square planar [Pd^{II}(mida)] chelate (mida^{2-} = methyliminodiacetate) was used as a soluble model for a IMAC binding site to approximate the distorted tetragonal Cu(II)−IDA coordination geometry. The investigation of the binding motif by ^{1}H NMR measurements assumed equilibrium 1.

$$[Pd^{II}(mida)(D_2O)] + SPHHGG \rightleftharpoons [Pd^{II}(mida)(SPHHGG)] + D_2O \quad (1)$$

EPR spectra and the pH dependence of UV/vis spectra of [Cu(mida)(SPHHGG)] and [Cu(mida)(HHHHHH)] provided additional details. From the data, a binding model with a three-point contact of the protein-affinity tags to the Cu(II)−IMAC column at pH 7 was derived (Figure 2). Since

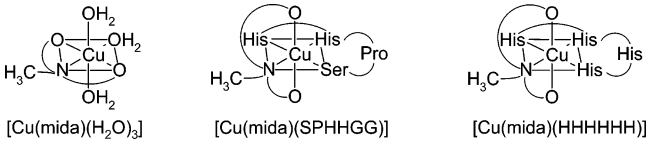


Figure 2. Geometry of Cu(II) complexes investigated by Shepherd.

2000, the group of Mallik reported IDA metal complexes capable of binding strongly to histidine patterns of flexible peptides in water.[51] To achieve strong and selective binding, the geometry of the IDA complex and the target histidine pattern need to be complementary. To prove this concept, Mallik designed and synthesized water-soluble peptides with three histidine moieties at particular distances (12−16 Å). This distance corresponds to the inter-histidine distances (His1, His7, and His12 or 14) on the surface of the protein carbonic anhydrase. Figure 3 shows the structures of the peptides.

From molecular modeling, the distances between the imidazole groups of the histidines were estimated to increase by approximately 4 Å when the peptide length increased by a glycine unit. The peptide **8** was designed to probe the role of flexibility in the recognition process (the inter-histidine distance for **8** was estimated to vary from ∼18 to ∼23 Å). Peptide **6** bears a hydrophobic methyl group, while **7** has a hydrophilic amide moiety in the side chain to study the effect of hydrophobicity on the recognition process. The monohistidine peptides **3** and **2** served as the controls for recognition studies.

They designed five Cu(II) complexes (Figure 4) as binding sites for these studies with distances between the Cu(II) ions estimated to be ∼12 Å for **10**, ∼16 Å for **11**, and ∼14−18 Å for **12** and **13**. Complex **9** with one Cu(II) ion serves as a control.

Using isothermal titration microcalorimetry (ITC), the binding constant (K_{st}), reaction stoichiometry (n), and enthalpy change (ΔH) of the recognition process were determined (Figure 5). A solution of metal complex (0.1−0.5 mM) in the ITC cell was titrated with a peptide (0.80−5.0 mM), both dissolved in 25 mM HEPES buffer at pH 7.0 and 25 °C. With the distance-matched peptide **5**, complex **12** showed a stronger binding affinity ($K_{st} = 1.19 \times 10^6$ M^{-1}) than those with a shorter peptide (**4**, $K_{st} = 104\,400 \pm 22\,800$ M^{-1}) or a longer peptide (**8**, $K_{st} = 243\,600 \pm 24\,500$ M^{-1}). A hydrophobic side chain on peptide **6** had a dramatic effect on the affinity for the metal complexes, due to unfavorable entropy changes. Introduction of a hydrophilic side chain on peptide **7** also led to large entropy losses and consequently decreased the affinity for the metal complexes. For all of

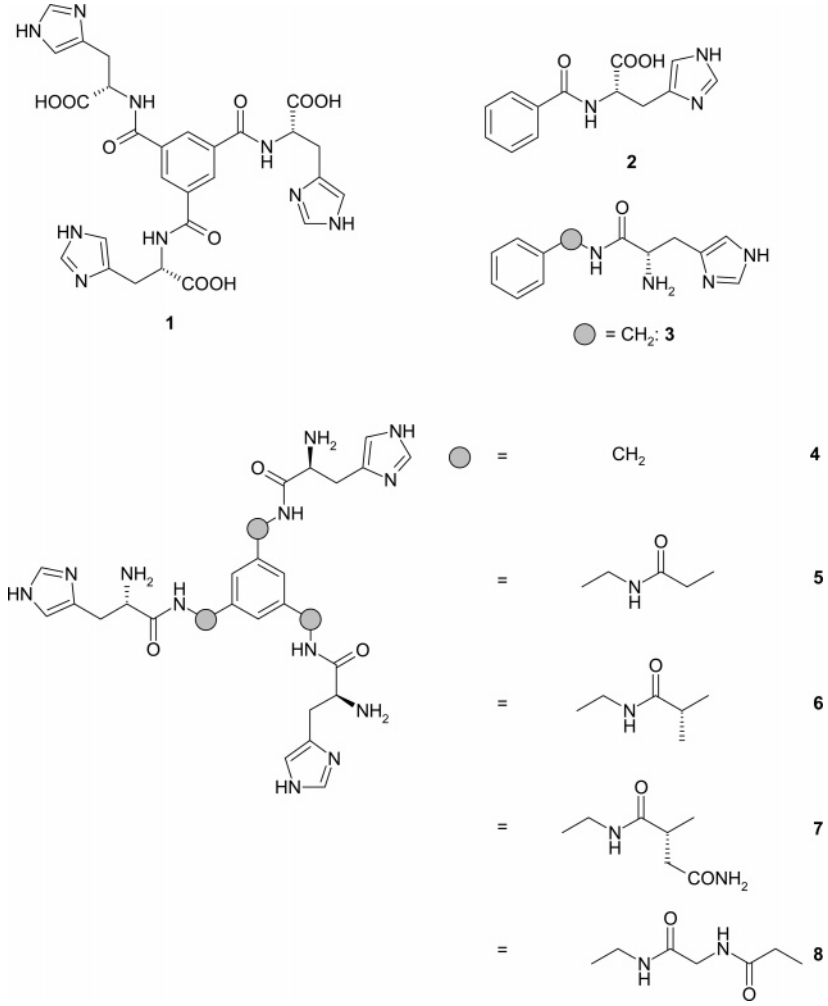


Figure 3. Structures of the histidine-containing peptides used in the binding studies.

the systems tested, the metal-ion-free ligands showed no measurable interaction by ITC under the same experimental conditions.

Extending the results from small peptide model compounds, Mallik et al. were able to selectively bind to peptides and proteins by recognizing unique patterns of surface-exposed histidines.[52] The target protein was carbonic anhydrase (CA, bovine erythrocyte). CA displays six histidines on the surface (the numbers of the histidines in the amino acid sequences of the peptides are 1, 7, 12, 14, 61, and 93). The distances separating histidines 1, 7, and 14 (or 12) are 13 ± 2, 16 ± 2, and 17 ± 2 Å, respectively. Three other proteins were used as controls (chicken egg albumin, horse skeletal muscle myoglobin, and chicken egg lysozyme). Chicken egg albumin (CEA) has the same number of solvent-exposed histidines as CA (amino acid numbers: 22, 23, 329, 332, 363, 371), but the pattern is different. Myoglobin (Mb) has seven histidines on the surface (amino acid numbers: 36, 48, 81, 97, 113, 116, 119), but with a different distribution. Lysozyme has only one histidine (15) exposed on the surface. All of these proteins (target and controls) are known to interact with transition metal ions and complexes through the surface-exposed histidines.[53] In addition to the already mentioned complexes **9**−**13**, interactions with cyclen **14** were also tested by ITC (Figure 6). Comparison of the binding constants indicated that complex **11** is very selective for CA compared to CEA (300:1); with myoglobin, the selectivity is moderate (20:1). For myoglobin, two of the cupric ions of **11** may simultaneously bind to two histidine residues on the protein surface (His 113 and 116 or 119). This leads to a lower selectivity of **11** in distinguishing CA from myoglobin. To demonstrate selective binding of **11** to the target protein, a mixture of CA and complex **9** ([CA] = 100 μM; [**9**] = 100 μM) was titrated with **11** ([**11**] = 0−500 μM). No change in the binding constant was observed for **11**. On the other hand, when a mixture of CA and **11** ([CA] = 100 μM; [**11**] = 100 μM) was titrated with **9** ([**9**] = 0−1 mM), no binding was detected. A mixture of the tested proteins (100 μM each) was titrated with complex **11** to obtain an unchanged affinity of **11** toward CA (280 000 M^{-1}). If CA was omitted from the protein mixture, only very weak binding (K < 1000 M^{-1}) was detected.

The experiments showed that a selective binding to a specific pattern of histidine residues on a protein surface becomes possible with suitable metal complexes of complementary geometry.

Another approach uses the combination of metal complex and enzyme inhibitor (Figure 7). To convert a poor inhibitor of carbonic anhydrase into a good inhibitor, surface-histidine coordination was used to increase its affinity.[54]

Benzene sulfonamide, a rather weak inhibitor for carbonic anhydrase (K_d = 120 μM), was converted to a very good inhibitor for the enzyme (K_d = 130 nM) as a result of this conjugation. For proof-of-concept, five Cu(II) complexes (Figure 8) were designed and synthesized.

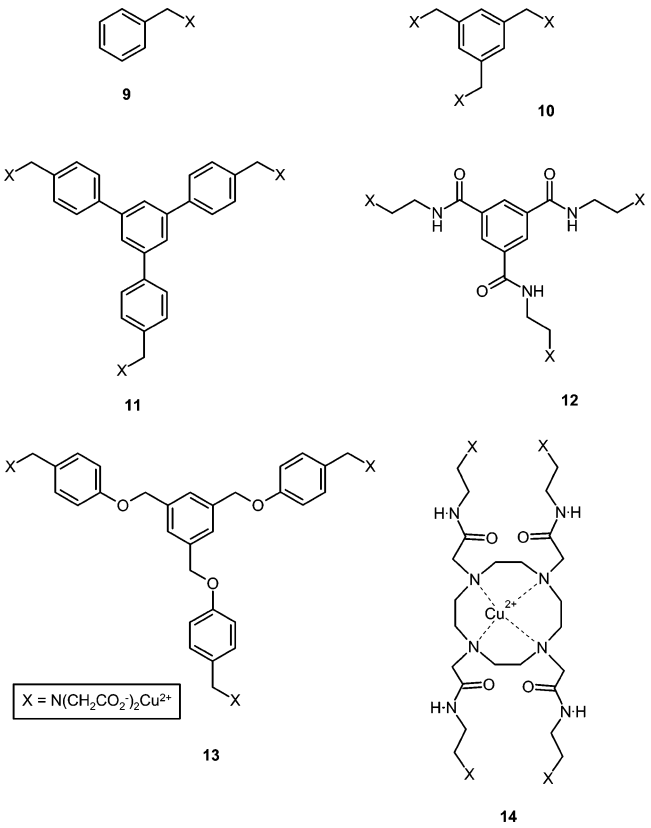


Figure 4. Structures of the metal complexes used in the recognition studies.

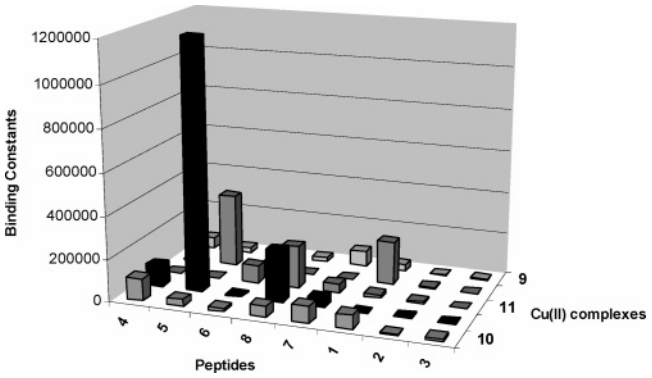


Figure 5. Binding constants of the model peptides with the metal complexes.

The length of the spacer separating the benzene sulfonamide group from IDA was varied in these complexes. Benzene sulfonamide **20** and the di-Cu(II) complex **21** (lacking the benzene sulfonamide moiety) were used as controls for these studies. The binding constants of the complexes with carbonic anhydrase (bovine erythrocyte) were determined with isothermal titration calorimetry (25 mM HEPES buffer, pH = 7.0).

The affinities of conjugates **15**−**19** were considerably higher compared to those of the controls (**20**, **21**; Table 2). Complex **17** showed the highest affinity for the enzyme, 3 orders of magnitude higher compared to the controls. The similar binding constants for complex **15** (one Cu(II) ion) and **17** (two Cu(II) ions) may indicate that one cupric ion binds to one histidine on the surface of the protein.

To amplify crown ether−ammonium ion binding in water, König et al. prepared a peptide binding luminescing crown ether, which contains a pendant Cu(II)−IDA complex (Figure 9).[55] No response of the emission properties is observed for **22** if it is treated with KSCN or *n*BuNH$_3$Cl in buffered aqueous solution (50 mM HEPES, pH 7.5, 1 c = 10−5 mol/L, up to 1000 equiv of ammonium ion), even with a large

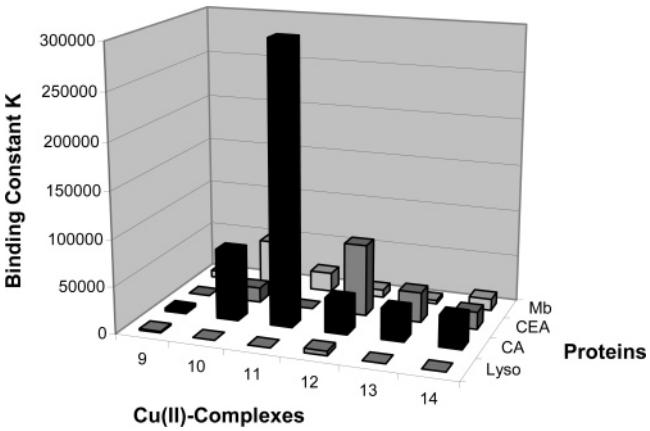


Figure 6. Binding constants of the metal complexes with the proteins in HEPES buffer, 25 mM, at pH = 7.0 and 25 °C.

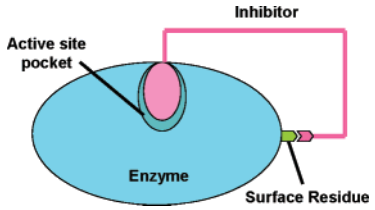


Figure 7. Surface-assisted enhancement in the binding of an inhibitor. The hybrid inhibitor binds at the active site pocket and to a surface-exposed His residue of the enzyme.[54] Reprinted with permission from ref 54b. Copyright 2004 American Chemical Society.

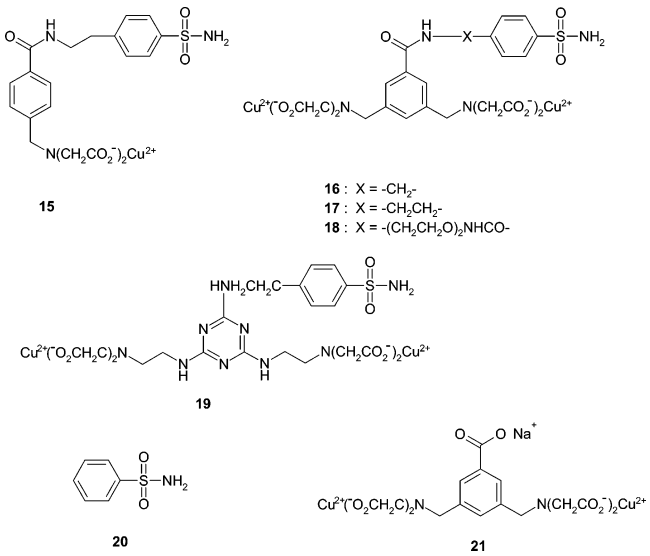


Figure 8. Structures of tested compounds.

Table 2. Binding Parameters of the Complexes with Carbonic Anhydrase

compound	binding constant (L/mol)	enthalpy (kcal/mol)
15	$(4.6 \pm 0.07) \times 10^6$	-26.4 ± 0.8
16	$(1.9 \pm 0.03) \times 10^5$	-51.7 ± 5.8
17	$(7.5 \pm 0.1) \times 10^6$	-36.9 ± 4.2
18	$(5.4 \pm 0.02) \times 10^5$	-30.3 ± 2.6
19	$(4.3 \pm 0.03) \times 10^5$	-45.5 ± 2.2
20	$(9.0 \pm 0.1) \times 10^3$	-31.2 ± 1.6
21	$(22.8 \pm 1.3) \times 10^3$	-129.0 ± 3.2

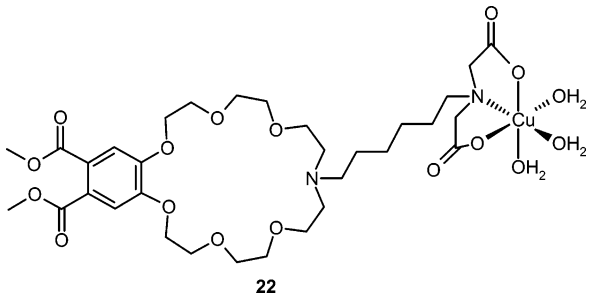


Figure 9. Luminescing benzo-crown ether with a pendant Cu(II)−IDA complex (**22**).

excess of the salts. The aqueous solvent competes with the crown ether for the cation binding, and the ammonium−crown ether interaction is intercepted. The situation changes if the dipeptide His-Lys-OMe is added to a solution of **22**. Coordination of the *N*-terminal His to the Cu(II)−IDA complex of **22** makes the crown ether−ammonium ion binding intramolecular and much more favorable (Figure 10).

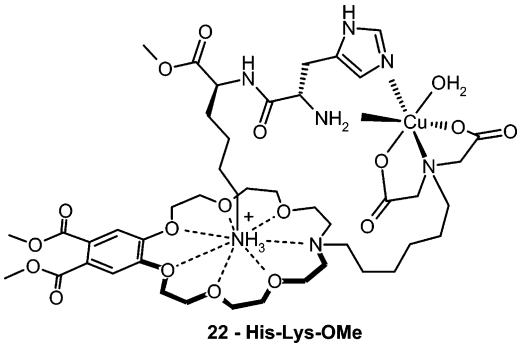


Figure 10. Proposed mode of binding of **22** and His-Lys-OMe.

Titration of **22** with His-Lys-OMe in HEPES buffer (50 mM, pH 7.5) resulted in a 1:1 complex as shown by a Job's plot analysis. With a log K of 4.22 ± 0.05, compound **22** binds the ammonium group of His-Lys-OMe with high affinity in buffered water. The emission intensity change of **22** in the presence of His-Lys-OMe can even be observed with the naked eye (see Figure 11). No emission response

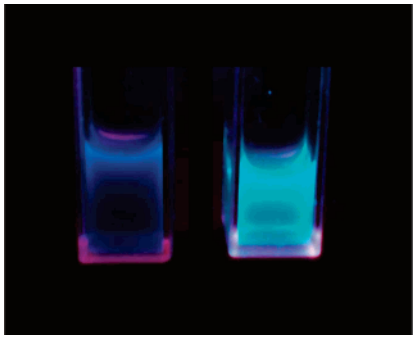


Figure 11. Emission intensity changes of solutions of **22** in buffered water in the absence (left) and presence (right) of His-Lys-OMe.

is detected under the same conditions with the *N*-terminal acylated dipeptide Ac-His-Lys-OMe, which proves the importance of an *N*-terminal His for the overall binding process.

The use of His-OMe resulted in a 2:1 stoichiometry for the His-OMe−**22** aggregate. After binding of one His-OMe to Cu(II)−IDA, one coordination site remains, which can accommodate the imidazole moiety of a second His-OMe while its ammonium group is bound by the crown ether, leading to an increased emission intensity. The overall binding constant of His-OMe to **22** was determined to be log $K = 3.8 \pm 0.1$. The binding motif allows the selective detection of N-terminal His groups, which is illustrated by the binding of tripeptide His-Gly-Gly. This peptide binds to **22** with the same 2:1 stoichiometry as observed for histidine and an overall affinity of log $K = 3.71 \pm 0.05$. In an additional binding experiment, it could be shown that the designed receptor **22** can selectively detect the amino acid His among 20 natural α-amino acids (Figure 12). The

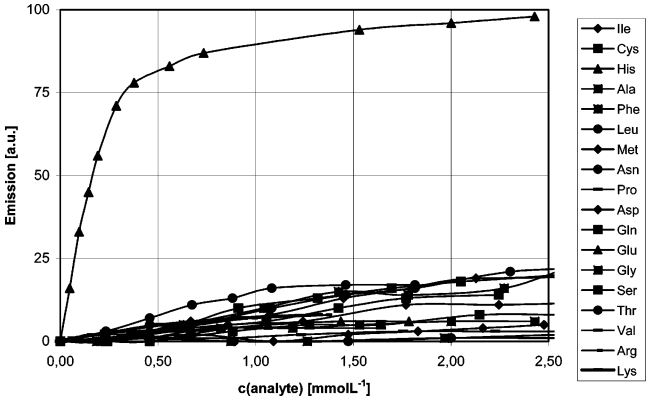


Figure 12. Response of the emission intensity of **22** to the presence of 20 natural α-amino acids in aqueous buffered solution (50 mM HEPES, pH 7.5). (Tyr is not soluble in HEPES buffer, and the indole emission of Trp interferes with the emission of **22**.)

examples illustrate that conjugation of enzyme inhibitors or crown ethers to unsaturated metal complex binding sites can enhance their substrate affinity and selectivity significantly.

3.2.2. Carbohydrate Recognition in Water

Several attempts to recognize carbohydrates selectively by means of synthetic receptors based on hydrogen bonding, charged interactions, or boronic acids have been made.[56,57] These suitable forces for sugar binding in apolar organic media become far less attractive in aqueous media.[58] Using metal coordination, Striegler et al.[59] built up ternary ligand Cu(II) complexes for sugar binding in aqueous solutions. To find a suitable metal complex for selective binding of various carbohydrates [glucose (**24**), galactose (**25**), mannose (**26**), or maltose (**27**)**],** they investigated several bi- and tridendate Cu(II) complexes (Figure 13). We discuss here the use of Cu(II)−IDA, Cu(II)−Dien (see section 7), and related complexes together, because they were used in the same work.

The Cu(II) complexes from the tridentate N- and N,O-ligands [(diethylenetriamine)copper(II)] dinitrate ([CuDien]-$(NO3)_2$, **23**), [(triazacyclononane)copper(II)] dichloride ([Cu-(TACN)]Cl_2, **28**), and *N*-[{(2-hydroxyethyl)ethylenediamine}-copper(II)] dichloride (CuHEN, **29**) coordinate hexoses **24**−**27** in identical stoichiometries. In contrast, [(ethylenediamine)copper(II)]sulfate (**30**), [(phenanthroline)-copper(II)] dinitrate (**31**), copper(II)iminodiacetic acid (**32**), and copper(II)-pyridinedicarboxylic acid (**33**) decompose in alkaline solution (pH > 10.4), which prevents complex formation with the investigated sugars. Since strong binding of carbohydrates requires highly alkaline pH values, only Cu(II) complexes which are stable under these conditions are useful. Complex [Cu(styDien)]$(HCOO)_2$ (**34**), a potential binding site for molecular imprinted devices, was also examined.

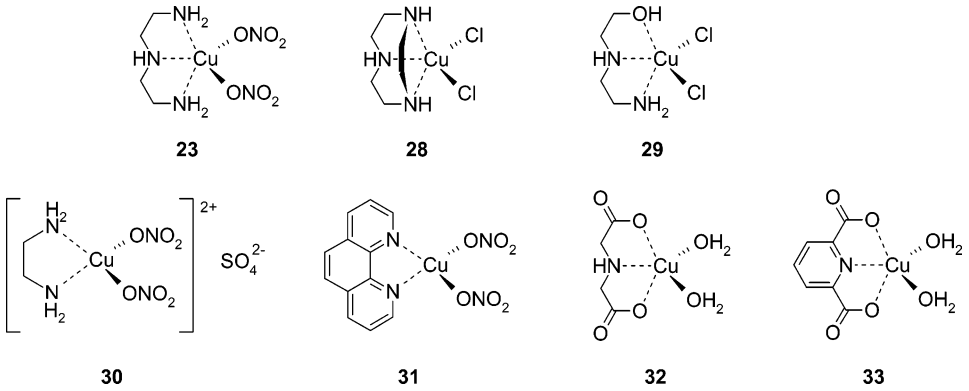


Figure 13. Structures of the Cu(II) complexes **23** and **28**−**33**.

Cis-diol fragments are expected to lead to higher binding affinities than *trans*-diol motifs.[60] The binding constants of the 1:1 complexes of **23**, **29**, and **34** with **24**−**27** (Scheme 2) were determined according to the method of Rose and Drago.[61] The ternary complexes formed from **24**, **25**, and **27** with **23**, **29**, or **34** show similar apparent binding constants (Table 3), while those formed with **26** are a little lower. Striegler et al. attributed this to a rearrangement of the ligand−Cu(II)−mannose complex when complex formation with a *cis*-1,2-diol occurs at C^2 and C^3 instead of the preferred chelation by the hydroxyl groups at C^1 and C^2.

Scheme 2. Complex Formation of [Cu(styDien)](HCOO)$_2$ 34 with 24−27 at pH = 12.40

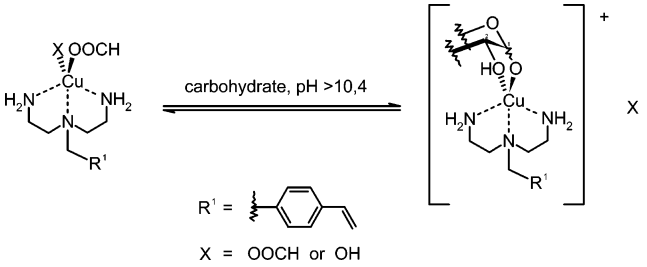


Table 3. Apparent Binding Constants (pK_{11}) for Ternary Complexes Formed from 23, 29, and 34 with 24−27

	pK_{11}		
compd	**23**	**29**	**34**
24	3.73 ± 0.12	3.37 ± 0.31	3.61 ± 0.17
25	3.70 ± 0.09	3.41 ± 0.43	3.64 ± 0.12
26	3.68 ± 0.12	3.05 ± 0.41	3.38 ± 0.19
27	3.75 ± 0.12	3.41 ± 0.26	3.62 ± 0.09

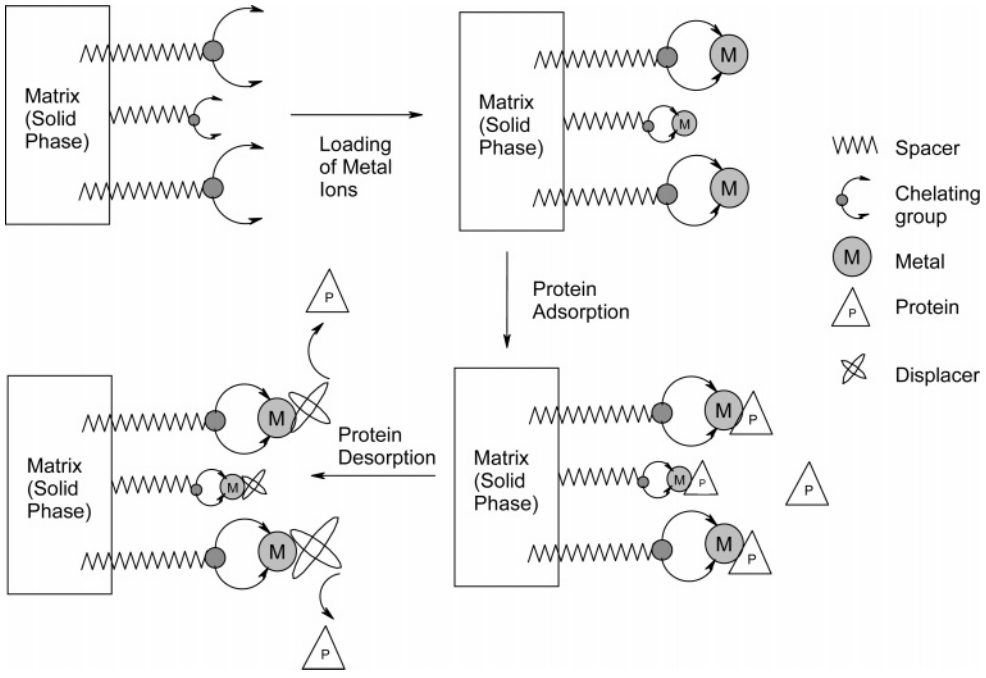
3.3. Recognition Processes on Solid Surfaces

3.3.1. Immobilized Metal Ion Affinity Chromatography (IMAC)

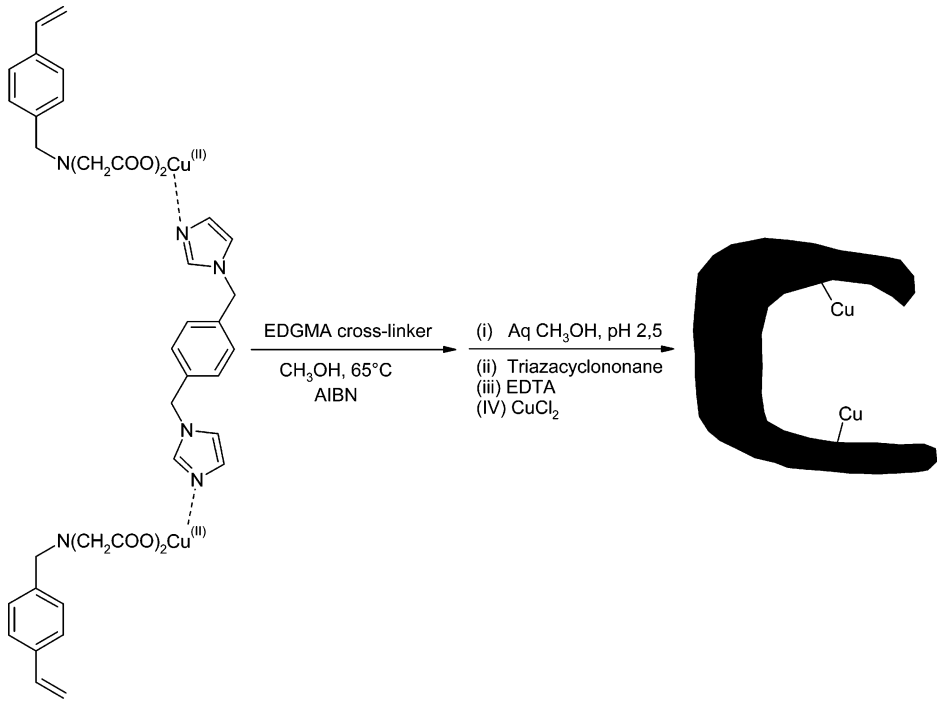
Immobilized metal ion affinity chromatography (IMAC) is a widely used technique. It is a separation and purification method based on interfacial interactions between biopolymers in solution and metal ions immobilized on a solid support (Scheme 3). The cross-linked polymer is hydrophilic. Introduced in 1975 by Porath and co-workers, this purification method is presently one of the most popular methods for purification of recombinant proteins.[62]

This interesting method of reversible coordination of His-tagged proteins to IDA metal complexes typically of Ni(II) or Cu(II) led to a huge number of review articles over the

Scheme 3. Schematic Principle of the IMAC Technique[63]



Scheme 4. Polymerization Recipe and Workup of Templated Chelating Polymers



past decades.[64] Due to this comprehensive coverage of the topic and its specific application, we will not discuss the IMAC technique in detail in this review and refer the interested reader to the cited literature.

3.3.2. Molecular Imprinting

The design of synthetic molecules capable of recognizing chemical entities in a specific and predictable manner is of great fundamental and practical importance. The principal paradigm of the molecular design of such materials involves the preorganization of binding sites of the host system (receptor) around complementary binding sites of the guest molecule (substrate).[65] Wulff and co-workers developed an approach to substrate-selective polymers.[66] This technique of template polymerization, also known as molecular imprinting (Scheme 4), has been used to prepare polymeric materials for applications in molecular recognition and chromatographic separations. Template polymerization produces cross-linked polymers containing functional groups strategically arranged in the polymer matrix. Arnold et al. report a variation of this template polymerization technique to synthesize rigid macroporous polymers containing Cu(II)−iminodiacetate (Cu(II)−IDA) complexes.[67,68]

For initial studies of template polymerization using metal ion complexes, a set of model templates was developed (Figure 14). Each of these templates **34**−**40** bears imidazole functionalities with slightly different arrangements. Compounds **41** and **42** are structural analogues of compounds **35** and **36** that contain no imidazole ligands for coordination of the metal ions.

To incorporate Cu(II) into the polymer at positions corresponding to the imidazole ligands of the templates, individual template−monomer assemblies were polymerized in the presence of a large excess of cross-linker. A control polymer with a random distribution of copper ions (P-1) was also prepared using the monofunctional ligand 1-benzylimidazole (**40**). Compound **35** was used as template to prepare polymer P-2, compound **36** was used as template for P-3, and **37** was used as template for P-4. In all cases, the templates and the Cu(II) used in the synthesis were recovered from the polymer nearly quantitatively.

To determine some of the factors that influence the ability of the templated polymers to discriminate template from nontemplate molecules, individual and competitive binding was studied. Experiments with the nontemplated polymer (P-1) were used to distinguish nonspecific metal ion binding from specific coordination. The random polymer P-1 binds nearly equal amounts of the four different bis(imidazole) substrates **35**−**38** (Table 4). P-1 also exhibits no selectivity in a competitive binding experiment (Table 5). In contrast, polymers prepared in the presence of a bis(imidazole) template show preferences for their own templates in both saturation and competitive rebinding experiments. The larger the structural differences between the substrates, the more pronounced are the selectivities. In the competitive rebinding

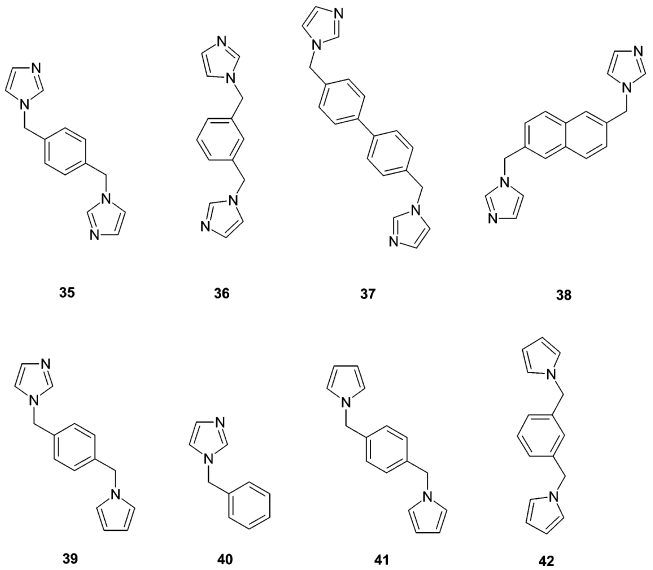


Figure 14. Set of imidazole templates.

Table 4. Substrate Binding by Templated and Nontemplated Polymers under Saturation Rebinding Conditions

polymer	substrate	substrate bound (mmol/g)
P-1	**35**	0.46
P-1	**36**	0.44
P-1	**37**	0.45
P-1	**38**	0.44
P-2	**35**	0.33
P-2	**36**	0.22
P-2	**37**	0.19
P-2	**38**	0.18
P-3	**35**	0.17
P-3	**36**	0.24
P-4	**37**	0.20
P-4	**38**	0.24

Table 5. Selectivity of Templated and Nontemplated Polymers during Competitive Rebinding of Bis(imidazole) Substrates

polymer	substrates	relative selectivity during rebinding
P-1	**35** + **36**	$\alpha_{35/36} = 1.02$
P-2	**35** + **36**	$\alpha_{35/36} = 1.17$
P-2	**35** + **37**	$\alpha_{35/37} = 1.35$
P-2	**35** + **38**	$\alpha_{35/38} = 1.32$
P-3	**35** + **36**	$\alpha_{36/35} = 1.15$
P-4	**35** + **37**	$\alpha_{37/35} = 1.22$

experiments, P-2 shows a small but measurable selectivity for its own template over the close structural analogue **36**, which differs only in the orientation of the imidazole groups. (**35** and **36** are in fact so similar that it is impossible to separate them by RP-HPLC.) The separation factor increases to above 1.3 when the polymer is used to distinguish template **35** from substrates **37** and **38**, which have imidazole groups with larger distances.

Molecular imprinted polymers can also be used as selective adsorbents. However, chromatographic separation with these polymers showed the need to optimize the imprinting process. The relatively high degree of cross-linking needed to capture a specific arrangement of functional monomers in the solid polymer prevents the diffusion of substrates in the particles, which leads to band spreading and poor peak resolution. Access of very large substrates to binding sites can thus be severely impeded. One approach to overcome these problems is to graft the monomer−template assemblies and a cross-linker onto a reactive support with the desired physicomechanical properties.[69,70] Arnold and his group have investigated poly(trimethylpropane trimethacrylate) (TRIM, **45**) as a reactive surface for template polymerization (Figure 15).[71]

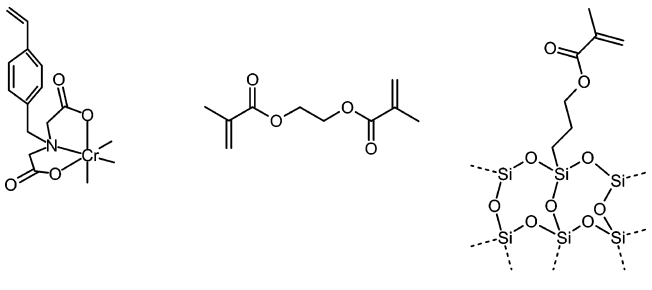


Figure 15. Monomers used for molecular imprinting.

During the polymerization it is necessary that the monomer−template assembly is held together by strong metal-to-ligand interactions. Unfortunately, very strong interactions with the template can interfere with the material's subsequent chromatographic performance. Tightly bound substrates experience very long retention times and excessive band spreading. Replacing the metal ion used during imprinting [Cu(II)] by others better suited for the chromatographic separation [i.e. Zn(II)] yields adsorbents capable of separating closely related bis-imidazole substrates. This "bait-and-switch" approach can significantly enhance the performance of molecular imprinted materials.

Table 6. Capacity Factors (k') and Chromatographic Separation Factors ($\alpha_{i,j}$) for Imidazole and Substrates 37−40 on Polymer-Coated Silica (LiChroshere 1000) Prepared Using 37−40 as Templates[a]

	material prepared using:							
	37		**38**		**39**		**40**	
substrate	k'_i	($\alpha_{37,i}$)	k'_i	($\alpha_{38,i}$)	k'_i	($\alpha_{39,i}$)	k'_i	($\alpha_{40,i}$)
imidazole	0.75	1.16	0.69	8.1	1.5	5.8	1.7	6.6
37	0.87		0.69	8.1	1.7	5.1	1.8	6.3
38	2.9	0.30	5.6		6.6	1.4	7.7	1.5
39	2.6	0.33	3.5	1.6	8.7		6.3	1.8
40	2.7	0.32	1.5	3.9	2.9	3.0	11.3	

[a] Elution volumes (V_e) were measured on 50 mm × 4.6 mm i.d. columns, at 0.5 mL/min 100% methanol and 65 °C, with a sample size of 10 μL of a 0.4 mM solution. The mobile phase contained zinc acetate in the following concentrations: experiments on **37**-templated material, 50 mM; **38**-templated material, 50 mM; **39**-templated material, 40 mM; **40**-templated material, 30 mM. $k' = (V_e - V_o)/V_o$; $\alpha_{i,j} = (V_e - V_o)_i/(V_e - V_o)_j$.

Chromatographic separation experiments with bis-imidazoles (**35**−**37**, **40**, **41**) showed the expected results (Table 6). Retention of the imidazole-containing compounds on materials loaded with Cu(II) was so strong that no peaks were observed during isocratic elution. Substrate retention times were greatly reduced when the Cu(II) ions were replaced with Zn(II).

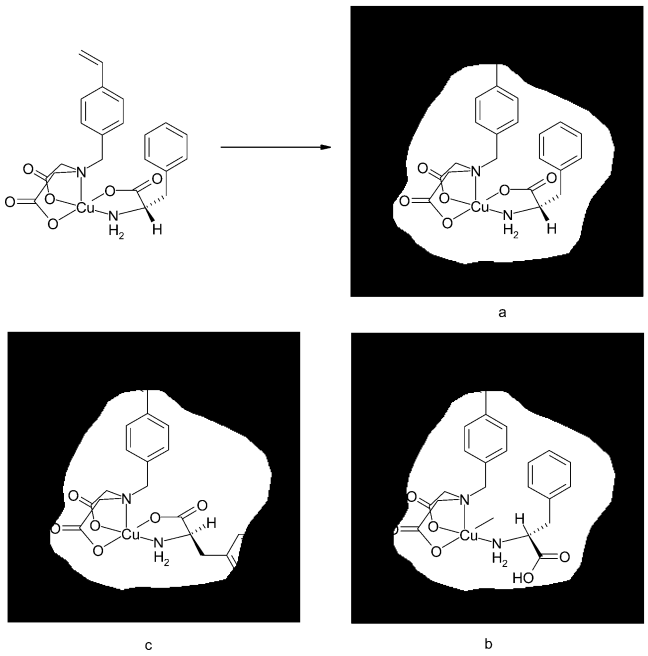
The material which was prepared with the monodentate coordinating template **37** retains the three bis-imidazole substrates **38**, **39**, and **40** to very similar extents. The binding sites in this "random" material have no basis to discriminate among the compounds. When the polymer-coated silica was prepared using bis-imidazole **38**, **39**, or **40**, as the template, the template was always the most strongly retained analyte.

In 1997 the same group reported an approach to preparing stereoselective ligand-exchange supports using molecular imprinting (Scheme 5).[72] Several aliphatic and aromatic amino acids were investigated in order to evaluate the role of the side group in stereodifferentiation. Comparison with an achiral monomer based on iminodiacetate showed that the enantioselectivity of the adsorbent arises from the chirality of recognition sites created during polymerization.

3.3.3. Metal Chelating Lipids

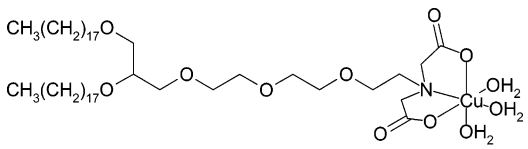
Arnold et al. prepared chelating amphiphiles, which then can be incorporated in biological membranes.[73] This is of interest to specific target proteins on surfaces for applications in biomedicine and to study protein interactions in biological membranes. For the purpose of targeting proteins to monolayer and bilayer assemblies, they synthesized a lipid with an IDA moiety in the head group (**46**, Figure 16). When loaded with Cu(II), small quantities of this IDA-lipid in monolayers and liposomes of distearoyl phosphatidylcholine (DSPC) effectively bind a small histidine-rich protein, myoglobin. Horse heart myoglobin contains 11 histidines, of which at least four can coordinate to Cu(II)−IDA.[74]

Scheme 5. Source of Enantioselectivity in Imprinted Ligand-Exchange Materials[a]



[a] Molecular imprinting with L-Phe gives a cavity that is selective for L-Phe. (a) The L-isomer can simultaneously chelate to a metal ion and fit into the shape-selective cavity. (c) Rebinding of the D-isomer is hindered because chelation of the metal ion by the D-isomer is sterically unfavorable. (b) Alternately, if the molecule fits into the cavity, it cannot chelate Cu(II). This idealized picture of the origin of enantioselectivity is probably only true for a small fraction of the binding sites.

The binding studies focus on myoglobin interacting with monolayers and vesicles of DSPC and IDA-lipid 1 containing Cu(II) and Ca(II). Because imidazole is not a good ligand for Ca(II) ($K_a = 1.2\ M^{-1}$),[75] this divalent ion provides a noncoordinating surface for comparison. Monolayer surface pressure−area ($\pi-A$) isotherms, measurements of protein binding to liposomes, and ESR analyses of Cu(II)-containing liposomes in the presence of unmodified and diethyl pyrocarbonate (DEPC)-modified protein were used to examine the lipid−protein interactions. The results indicated that myoglobin is binding to these artificial membrane assemblies. The binding is significantly enhanced by coordination of surface histidines to Cu(II) ions immobilized at the membrane surface ($K_a > 10^6\ M^{-1}$).



46

Figure 16. Cu(II)−IDA−lipid **46**.

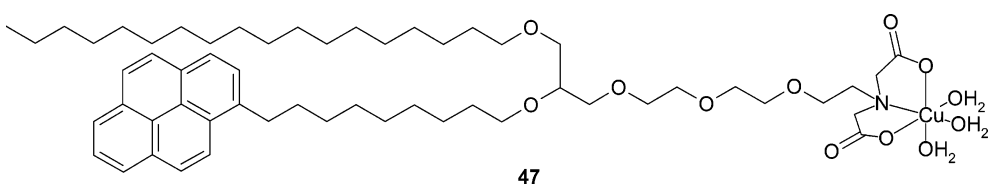
One year later, in 1995, the same group synthesized a new IDA-lipid unit.[76] In addition to the targeting function, it is also desirable to have the capability to directly probe molecular redistribution or assembly that accompanies protein binding. Thus, the new IDA-lipid **47** (PSIDA) incorporated a luminescent pyrene label in the lipid tail (Figure 17).

These emitting lipid membrane probes are sensitive to protein binding and recognition of lipid components. In the course of studying ligand-induced reorganization of the luminescent lipid **47** in membrane assemblies, Arnold observed that metal ion binding can strongly affect the ratio of the excimer to monomer emission intensities. The pyrene-labeled IDA lipid also serves as a membrane "receptor mimic" in that binding of external ligands can induce its reorganization in the membrane and change its emission properties.[77,78] Using these properties of the modified lipid, they show that a small globular protein, the soluble domain of cytochrome b_5, can be targeted with high affinity to PSIDA-containing lipid monolayers via metal coordination to a six-histidine peptide appended to the protein's C-terminus (6-His cyt b_5) (Figure 18). The poly(histidine) fusion peptide and metal-chelating IDA lipid comprise an effective and versatile system for targeting proteins to lipid assemblies.

In 1997 Arnold[79] reported the use of such metal-chelating lipids in the application of 2D protein crystallization. A 2D protein crystallization requires the immobilization and orientation of a protein at the monolayer surface in high concentration, without loss of mobility.[80] This mobility is supplied largely by the lipid monolayer. Crystallization is generally performed using fluid-phase monolayers. For targeting proteins to the interface, various classes of interactions have been employed. In most cases, specific ligands have been used to promote crystallization. In each instance, a unique lipid containing the appropriate affinity ligand was required. Using the concept of targeting proteins through coordination of amino acid side chains to lipid-bound metal ions, one and the same metal-containing lipid can be used to bind to a large variety of peptides. For this purpose, lipid **48** (Figure 19) was specifically designed for 2D crystallization. Unsaturated oleyl tails were utilized to provide the required monolayer fluidity. The utility of this lipid for 2D protein crystallization was demonstrated using the tetrameric protein streptavidin. Fluorescence microscopy was used to confirm and further characterize the streptavidin crystallization process.

4. Nitrilotriacetato Complexes

Nitrilotriacetic acid (NTA), first published in the same year as the IDA ligand, has been widely used as a chelate for more than 60 metal ions.[81] Most common are NTA complexes of Al(III),[82] Cu(II),[83] Cd(II),[84] Ni(II),[85] Zn(II),[86] Cr(II and III),[87] Fe(II and III),[88] and Co(II and III).[89] Table 7 summarizes the metal ion binding constants for some typical NTA complexes. The higher stability of the NTA complexes in comparison to the corresponding IDA analogues is the result of an additional coordination site. Carboxylate donor groups containing a central nitrogen atom allow tetradentate chelation. The chelation of a Cu(II) ion



47

Figure 17. Luminescent IDA−lipid **47**.

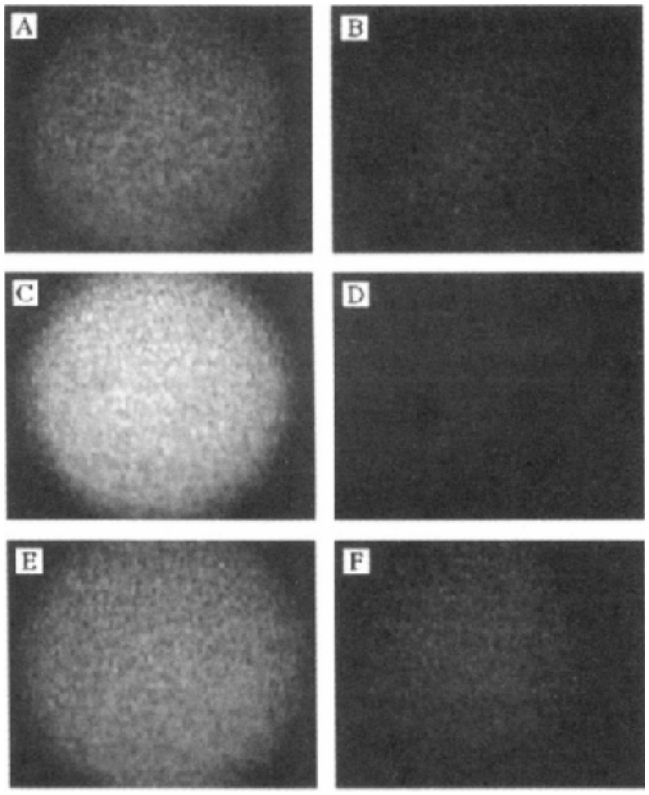


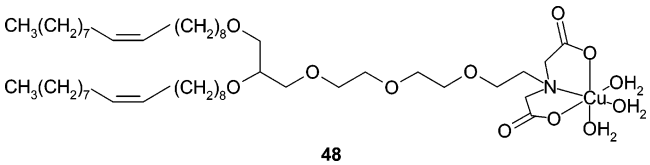
Figure 18. Fluorescence micrographs of fluorescein labeled 6-His cyt b_5 in the presence of metal-chelating lipid monolayers spread on a buffered subphase (20 mM MOPS, 100 mM NaCl, pH 7.5) at $\pi = 25$ mN/m. (A) 10% PSIDA−Ni(II); (B) 10% PSIDA, no metal; (C) 30% PSIDA−Ni(II); (D) 30% PSIDA, no metal; (E) 100% PSIDA−Ni(II); (F) 100% PSIDA, no metal.

Table 7. Overview of Binding Constants of Different Metal Ions toward NTA[33] Measured at 20 °C

ion	equilibrium	log *K* (25 °C)	ion	equilibrium	log *K* (25 °C)
Fe^{2+}	ML/M·L	8.33[a]	Fe^{3+}	ML/M·L	15.9
	ML_2/M·L^2	12.8[a]		ML_2/M·L^2	24.3[a]
Co^{2+}	ML/M·L	10.38	Cd^{2+}	ML/M·L	9.78
	ML_2/M·L^2	14.33		ML_2/M·L^2	14.39
Ni^{2+}	ML/M·L	11.5	Zn^{2+}	ML/M·L	10.66
	ML_2/M·L^2	16.32		ML_2/M·L^2	14.24
Cu^{2+}	ML/M·L	12.94	Al^{3+}	ML/M·L	11.4
	ML_2/M·L^2	17.42			

[a] Measured at 25 °C.

leads to the formation of three five-membered rings.[90] The two remaining coordination sites on the copper(II) can bind to Lewis basic ligands.[91]



48

Figure 19. Metal chelating lipid **48** for applications in 2D crystallization.

4.1. Structures of NTA Complexes in the Solid State

Ni(II)−NTA complexes were used to coordinate nucleobases as additional Lewis basic ligands.[92] The crystal structures of cocrystals of Ni(II)−NTA and adenine or cytosine demonstrate the potential of NTA complexes to form aggregates with such biologically interesting ligands. However, the X-ray structure reveals that hydrogen bonds, but not reversible coordination, stabilize the aggregate.[93]

The structure of $Na[Zn(NTA)(H_2O)]$ is isomorphous with $Na[Cu(NTA)(H_2O)]$ discussed above.[94] The structure consists of an intricate network of ligand-bridged coordination complexes. Two coordination sites, which remain vacant after NTA coordination of the Zn(II) ion, are occupied by nonchelating oxygen atoms from other $[Zn(NTA)]^-$ ions.

Chelating Cr(III) by NTA leads to various species in solution, which are pH dependent.[95] Solutions of pH = 5.5 result in the formation of $Cs_2[Cr(nta)_2(\mu\text{-}OH)_2]\cdot 4H_2O$, while solutions at pH = 1 give $[Cr(nta)(H_2O)_2]$, and $[Cr(nta)(H_2O)(OH)]^-$ is obtained at pH = 5−6. A similar complex is formed when acetate is added (Figure 20).[96]

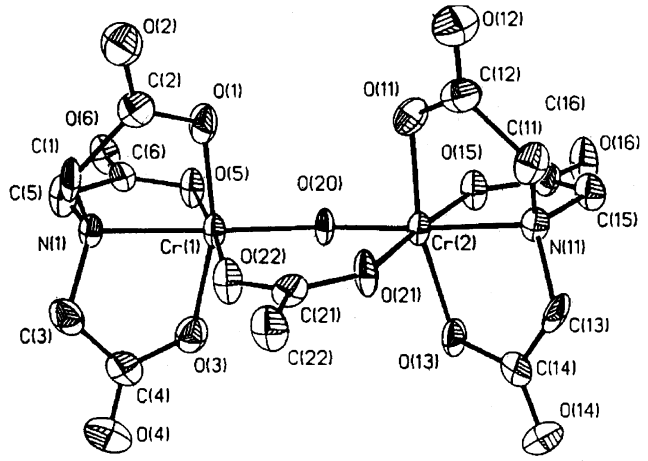


Figure 20. Crystal structure of the dimeric unit in $K_2[Cr(nta)(OH)(acetato\text{-}O,O)Cr(nta)]\cdot 4H_2O$.[96] Reprinted with permission from ref 96. Copyright 1990 Elsevier Science B.V.

The best investigated ligand for Cr(III)−NTA is imidazole.[97] The crystal structure of Figure 21 shows two imidazole residues coordinating to the Cr(III) ion.

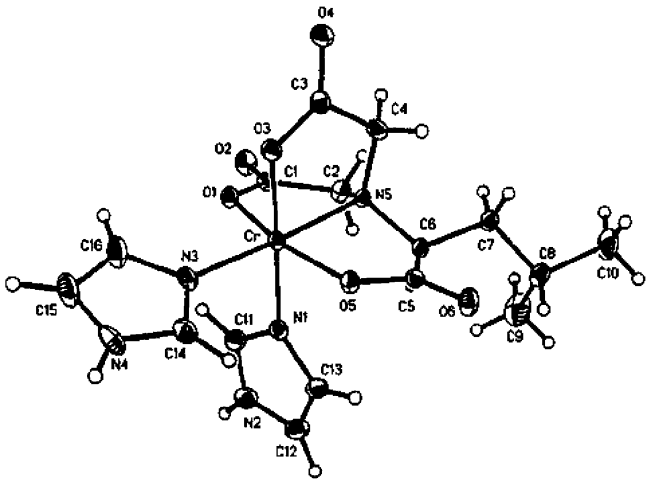


Figure 21. Crystal structure of Cr(III)−NTA coordinating additional imidazole ligands.[97] Reprinted with permission from ref 97b. Copyright 1990 American Chemical Society.

A more oxophilic NTA metal complex can be obtained with Fe(III). The octahedral coordination geometry of the complexes, such as $[Fe(nta)Cl_2][pyH]_2$,[98] is similar to structures obtained with other metals.[99] The oxophilic character

of these Fe(III)−NTA complexes is illustrated by their ability to bind to β-keto-carboxylates. The antibiotic ciprofloxacin coordinates to the iron center by its carboxy and keto oxygen atoms, giving [Fe(cip)(nta)] (cip = ciprofloxacin).[100]

NTA metal complexes containing Co(III) have been investigated in detail. Crystal structures utilizing NTA as a tridentate ligand,[101] a tetradentate NTA ligand,[102] oxo-bridged Co(III)−NTA complexes,[103] complexed carbonate (see Figure 22),[104] and complexed amino acids[105] have been reported.

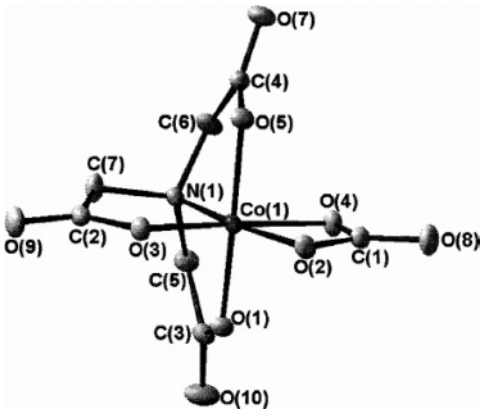


Figure 22. Structure of the $[Co(nta)(CO_3)]^{2-}$ anion in the solid state.[104] Reprinted with permission from ref 104a. Copyright 2001 Elsevier Science B.V.

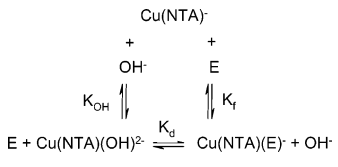
Overall, the reported crystal structure analyses of NTA complexes containing Cu(II), Cr(III), Zn(II), Co(III), or Fe(II), which coordinate additional ligands, show similar coordination geometries for the metal ion and the NTA ligand.

4.2. Recognition Processes in Solution

4.2.1. Interactions of Metal NTA Complexes with Carboxylic Acids, Amino Acids, and Their Esters

Angelici et al. studied the interactions of complexes of divalent metals and quadridentate ligands with amino acids and their esters.[106] Divalent ions used for these experiments were manganese, cobalt, nickel, copper, zinc, and lead. Binding constants of amino acids to these metal complexes were determined using potentiometric measurements. Titration data of stoichiometric mixtures of $[Cu(NTA)]^-$ and Gly or EtVal were fitted to the theoretical equilibrium equation shown in Scheme 6, which involves the formation of a Cu(II)

Scheme 6. Binding Equilibrium between an Ester (E), a Hydroxy Anion, and [Cu(NTA)]−



hydroxy species. The titration data illustrate that hydroxy complex formation occurs only by the displacement of the acid or ester (E).

$K_d = [MZOH^{2-}][E]/[MZE^-][OH^-] = K_{OH}/K_f$, where K_f is the formation constant of the acid or ester with $[Cu(NTA)]^-$.[107] Similar experiments were carried out with the other metals; Table 8 summarizes the results. The coordination of His and MeHis to $[Cu(NTA)]^-$ (Table 9) leads to a more complex equilibrium because these ligands

Table 8. Aggregate Formation Constants of $[M(NTA)]^-$ with Glycine and Ethyl Valinate at 25.0 °C

M^{2+}	log K_{fEtVal}	log K_{fGly}
Mn	2.39 ± 0.02	2.24 ± 0.005
Co	1.88 ± 0.014	3.65 ± 0.014
Ni	2.03 ± 0.011	4.95 ± 0.011
Cu	2.88 ± 0.002	5.46 ± 0.008
Zn	1.58 ± 0.08	3.64 ± 0.007
Pb	1.55 ± 0.10	1.93 ± 0.009

Table 9. Aggregate Formation Constants of $[Cu(NTA)]^-$ with Amino Acids and Their Esters at 25.0 °C (log Standard Deviation < 0.01)

acid	pK_a	log K_f	ester	pK_a	log K_f
Gly	2.35		Me	7.62	3.06
	9.78	5.44	Et	7.68	3.15
			n-Bu	7.78	3.33
Ala	2.34				
	9.87	5.42	Et	7.91	3.10
Phe	1.83				
	9.13	4.99	Et	7.12	2.77
Leu	2.36				
	9.60	5.35	Et	7.64	2.79
Val	2.32				
	9.62	5.10	Et	7.75	2.88
β-Ala	3.55				
	10.35	4.56	Et	9.13	3.65
His	1.85	4.16 (monodentate)			3.98
	6.04		Me	7.23	
	9.12	5.73 (bidentate)			4.90

are potentially tridentate. Cu(II)−His systems contain mixtures of species in which His is coordinated either as an anion or in its zwitterionic form.[108]

Blakney measured the binding constants of Gly to Cd(II)− and Zn(II)−NTA using NMR techniques.[109] The results confirm previously reported data from potentiometric titrations. In 2003 Crumbliss reported ternary complexes of Fe(NTA) complexes with phosphate and acetohydroxamic acid.[110] The investigated overall reaction is shown in eq 2,

$$Fe(NTA)_{(aq)}^{m-} + H_xPO_4^{n-} \rightleftharpoons Fe(NTA)H_xPO_{4(aq)}^{p-} \quad (2)$$

where $Fe(NTA)_{(aq)}^{m-}$ and $H_xPO_4^{n-}$ represent the various hydrolyzed and protonated forms of the reactants, depending on pH. The kinetics of complex formation was monitored by UV spectroscopy. In a second series of reactions, phosphate was substituted by acetohydroxamic acid.

4.2.2. Hexahistidine-Tag-Mediated Fluorescent Labeling with [Ni(II): Nitrilotriacetic Acid]$_n$−Fluorochrome Conjugates[111]

Structural and mechanistic characterization of proteins by fluorescence resonance energy transfer (FRET) requires the ability to incorporate fluorescent probes at specific, defined sites. Proteins lacking these sites must be modified. One strategy involves use of the "hexahistidine tag"[112] (i.e., the amino acid sequence His_6) to target site-specific fluorescent labeling.[113] The hexahistidine tag is known to interact tightly with transition-metal complexes, including nickel(II) nitrilotriacetic acid (Ni(II)−NTA). The hexahistidine tag can be introduced to protein termini or internal sites by using standard molecular-biology procedures. It is hypothesized that these tags interact tightly with $(Ni(II):NTA)_n$−fluorochrome conjugates (Figure 23).

To test these hypotheses, Kapanidis et al. synthesized derivatives of the widely used cyanine fluorochromes Cy3

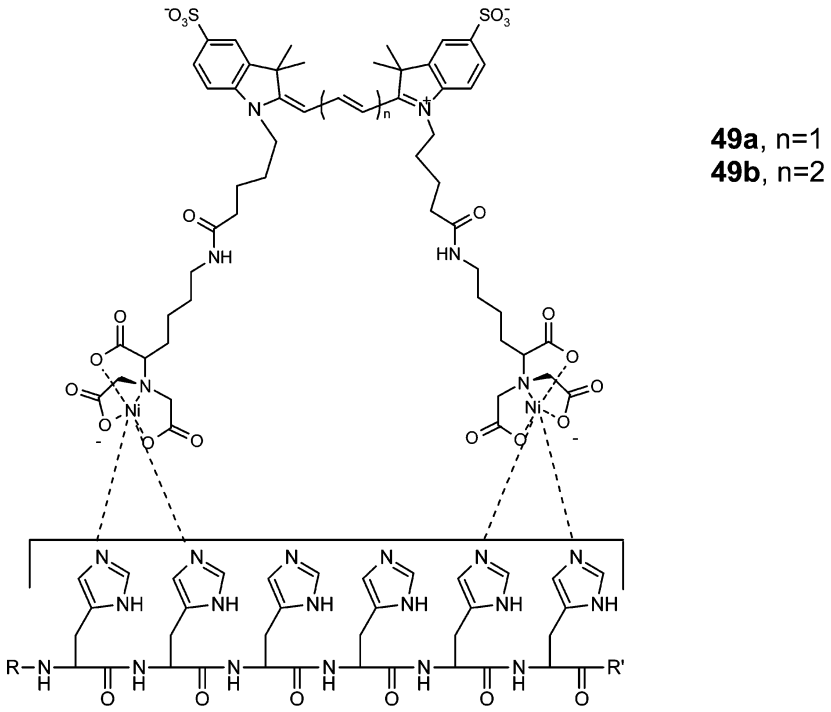
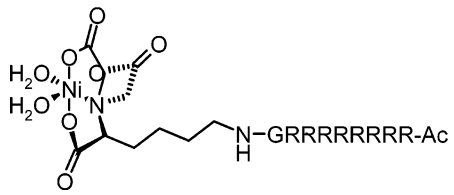


Figure 23. Schematic representation of the proposed interation of **49a** or **49b** with a hexahistidine tag.

and Cy5.[111] Fluorescence anisotropy and FRET experiments established that **49a** and **49b** exhibit high affinity and specificity for the hexahistidine tag ($K_D = 1.0\ \mu$M for **49a**; $K_D = 0.4\ \mu$M for **49b**; specificity > 95%). Thus, titration of **49a** and **49b** with an otherwise identical protein lacking a hexahistidine tag results in little or no increase in the emission signal.[114]

4.2.3. Arginine Carrier Peptide Bearing Ni(II)–NTA

The ability of NTA to bind very tightly to poly-His-tag proteins stimulated Futaki et al. to combine this property with a carrier peptide which is able to invade living cells.[115] Octaarginine was used as such a peptide and was linked to a NTA chelator ligand (Figure 24). Covalent cross-linking



50

Figure 24. Arginine carrier peptide (R8-NTA−Ni(II), **50**) with a binding site for His-tagged proteins.

between the cargo proteins and the carrier peptide is necessary for cellular introduction. The histidine-tagged, enhanced green fluorescent protein (H6-EGFP) forms a noncovalent complex with the carrier peptide. The interactions ($K_D = 4.2 \times 10^{-7}$ M) are sufficiently strong, which allows the protein to pass through the cell membrane. Internalization of H6-EGFP was monitored by laser confocal microscopy after 3 h of incubation of the protein with HeLa cells at 37 °C. Significant internalization of H6-EGFP was observed when the cells were treated with H6-EGFP (10 μM) in the presence of R8-NTA−Ni(II) (50 μM).

4.2.4. Metal-Dependent Cell-Penetrating Peptides (CPPs)

To enable oligohistidine peptides to function as CPPs, Peterson et al. synthesized an artificial cell surface receptor (**51**, Figure 25) comprising the plasma membrane anchor *N*-alkyl-3β-cholesterylamine linked to the metal chelator NTA.[116] *N*-Alkyl-3β-cholesterylamine derivatives can function as prosthetic molecules active on the surface of living mammalian cells because this steroid can insert into cellular plasma membranes.

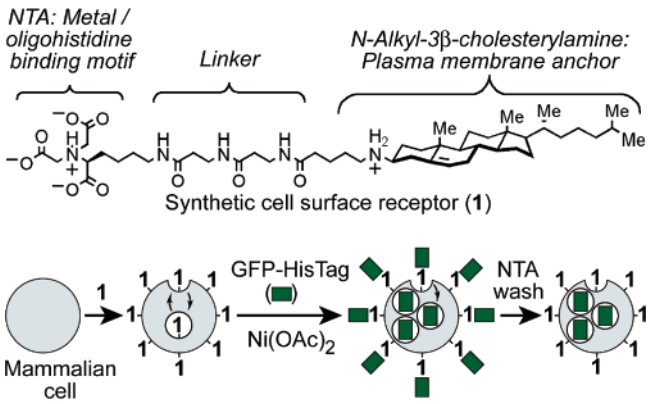


Figure 25. (top) Structure of the synthetic cell surface receptor **51** that binds metal and His tags. (bottom) Strategy for delivery of His-tagged proteins into mammalian cells by synthetic receptor-mediated endocytosis. Reprinted with permission from ref 116. Copyright 2006 American Chemical Society.

To qualitatively examine the ability of compound **51** to mediate the cellular uptake of His-tagged proteins, human Jurkat T-lymphocytes were treated with **51** (10 μM) for 1 h to load this compound into the outer leaflet of the cellular membrane. These cells were washed and subsequently treated with a solution of AcGFP$(His)_{10}$ (3.2 μM) and $Ni(OAc)_2$ (100 μM) for an additional 4 h. As shown in Figure 26 (panel A), examination of these cells by confocal laser scanning

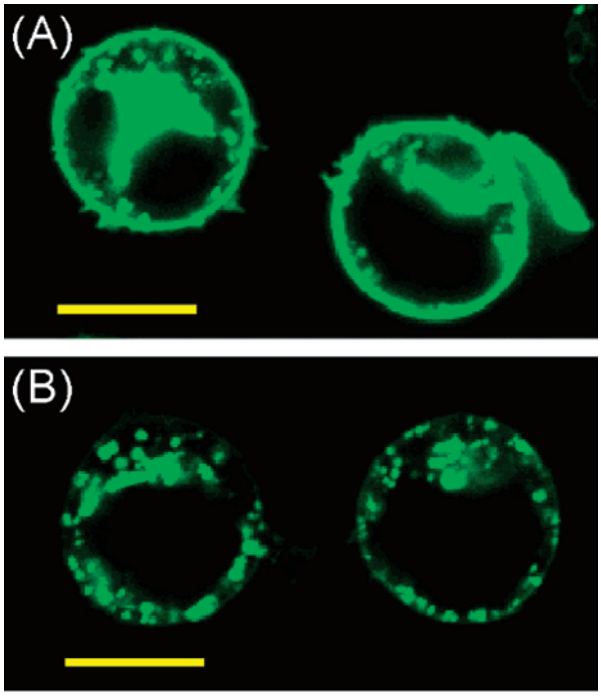


Figure 26. Confocal laser scanning micrographs of living Jurkat lymphocytes. Cellular plasma membranes were preloaded with receptor **51** (10 μM) for 1 h at 37 °C, cells were washed with fresh media and media containing AcGFP$(His)_{10}$ (3.2 μM) and $Ni(OAc)_2$ (100 μM) was added for an additional 4 h at 37 °C. The cells shown in panel A were imaged immediately after this treatment. The cells shown in panel B were washed with NTA prior to microscopy to remove noninternalized protein from the cell surface. Reprinted with permission from ref 116. Copyright 2006 American Chemical Society.

microscopy revealed fluorescent protein both on the cell surface and in defined intracellular compartments. Washing the cells with disodium NTA (400 μM) as a chelator removed the bound protein from the cell surface, facilitating examination of the intracellular distribution (Figure 26, panel B).

4.2.5. Enhanced Peptide β-Sheet Affinity by Metal to Ligand Coordination

The combination of metal complexes and hydrogen binding sites creates interesting polytopic receptors with binding affinities in aqueous and DMSO solutions. The concept was illustrated by using a histidine-coordinating NTA complex and methoxy pyrrole amino acids (MOPAS), as hydrogen sites for peptides (Figure 27).[117] The peptide binding properties of a Zn(II)−NTA complex with two MOPAS were investigated by NMR. The NMR spectra of a 1:1 mixture of receptor and H-His-Leu-Leu-Val-Phe-OMe in DMSO-$[D_6]$ shows a single set of resonances, which were structurally assigned. The temperature dependence of the chemical shifts confirmed the expected hydrogen bond interaction of the MOPAS amide NH with methoxy groups, which should keep the structure planar. Two-dimensional NOESY spectra revealed four intrastrand contacts (Figure 28). The interactions of the conformationally restricted MOPAS units with the bound pentapeptide induce a conformational preference of the peptide, which has a β-sheet like structure in the bound state. The peptide conformation was calculated using experimentally determined distances as constraints.

4.3. Recognition Process on Solid Surfaces

4.3.1. Immobilized Metal Ion Affinity Chromatography (IMAC)

Immobilized metal ion affinity chromatography (IMAC) on resins coupled with chelating agents is widely used to purify proteins containing exposed histidine and cysteine side chains. Recent reviews thoroughly discuss applications of this technique and previous research in the field.[64] Thus, we will not elaborate on IMAC in this overview.

4.3.2. Immobilization of Ni(II)–NTA on Microtiter Plates

Paborsky et al. describe the application of a derivative of NTA, *N*,*N*-bis[carboxymethyl]lysine (BCML), which can be coupled by simple amide chemistry to carboxyl groups on commercially available microtiter plates coated with maleic anhydride (Figure 29).[118,119] The NTA modified plate was charged with Ni(II) and was used to capture either recombinant IL8 or sTF, both engineered to contain 6His tags. The amount of each protein captured by the Ni(II)−NTA plate was measured by sandwich ELISA. To determine the specificity of the Ni(II)−NTA plate for proteins containing 6His tags, the capture of IL8-6His was compared with native IL8 (lacking a 6His tag) while 6His-sTF was compared with FLAG-sTF. The IL8-6His was bound by the Ni(II)−NTA plate in a concentration-dependent manner, reaching saturation at approximately 5 nM. In contrast, the native IL8 did not bind the plate, indicating that the binding was specifically through the six histidine residues. Results comparing 6His-sTF and sTF lacking the 6His tag were comparable.

4.3.3. Molecular Imprinting of Ni(II)–NTA Complexes

The preparation of molecular imprinted polymers (MIPs) selective for amino acids and small peptides has been limited to traditional imprinting formulations, such as polyacrylates,

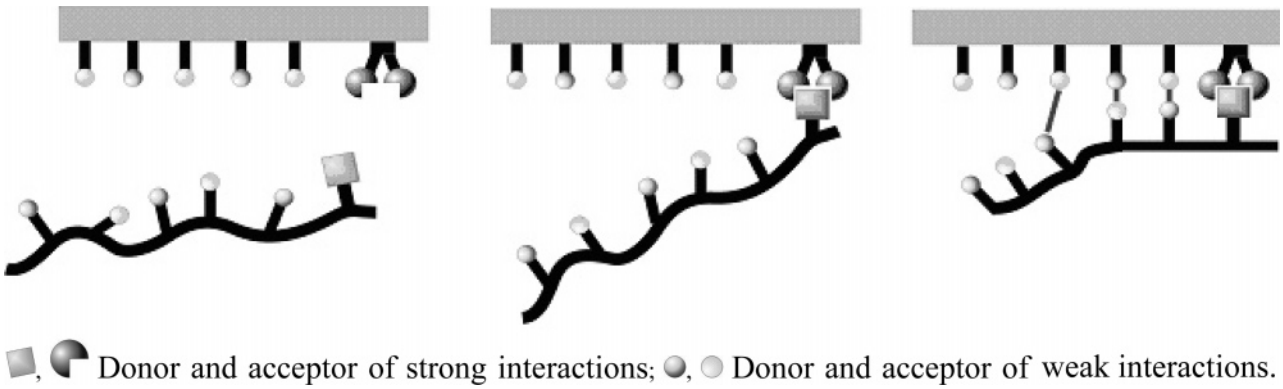


Figure 27. Schematic illustration of a binding process combining strong metal to ligand coordination and weaker interactions.

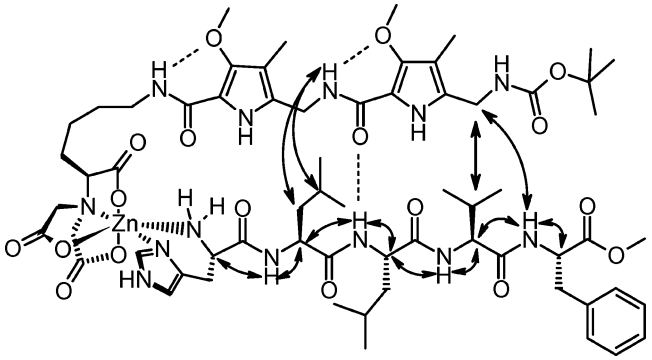


Figure 28. Spectroscopically detected interactions of a Zn(II)−NTA complex and H_2N-His-Leu-Leu-Val-Phe-OMe in DMSO solution: dotted lines, hydrogen bonding suggested from the temperature dependence of the chemical shift; arrows, intrastrand NOE contacts; green arrows, intramolecular distances within the pentapeptide, which for the determination of its conformation.

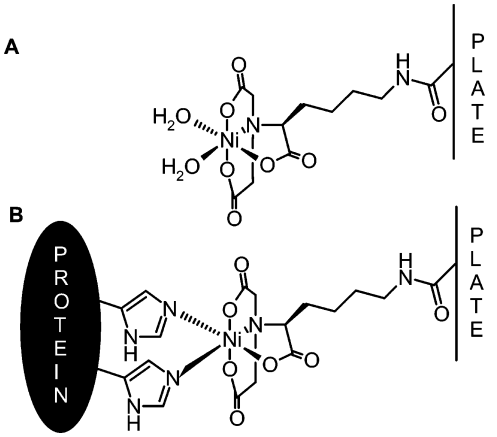


Figure 29. Immobilization of proteins on microtiter plates derivatized with *N*,*N*-bis[carboxymethyl]lysine (BCML). Two adjacent histidine residues in a protein containing a 6His tag bind in bidentate fashion (B) to a BCML-derived plate (A).

where methacrylic acid is the functional monomer in organic solvents (Scheme 7). Hart et al. developed a polymerizable acrylamide-functionalized NTA ligand.[120] By incorporating a Ni(II)−NTA complex into the polymer, they provided a "handle" to bind peptides containing N-terminal His residues in water. The ligand occupies four positions in the octahedral coordination sphere of Ni(II), leaving the remaining two sites for selective binding of His. The polymerizable methacrylamide−NTA−Ni(II) mixed complex was prepared by combining aqueous solutions of the NTA monomer with $NiSO_4$. The prepolymerization template complex was then formed by addition of the *N*-terminal histidine peptide His-Ala. Copolymerization of this complex (5 mol %) with *N*,*N*′-ethylenebisacrylamide as cross-linking monomer (82 mol %) and acrylamide (13 mol %) provided a pale blue monolith.

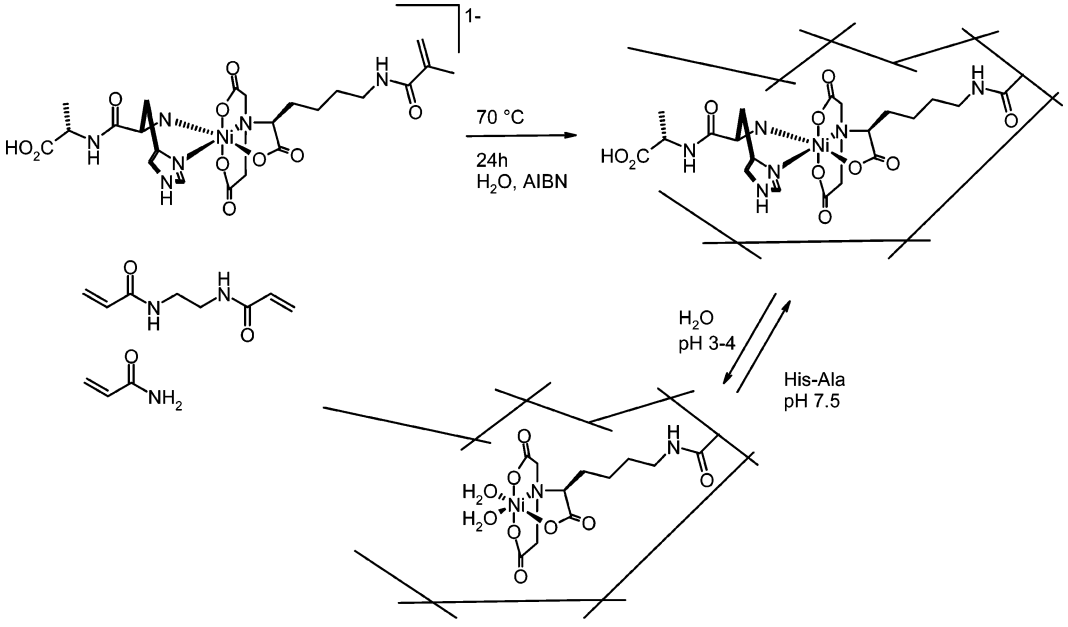
The polymer-bound NTA−Ni(II) complex is pale green in the absence of peptide. Upon binding of an N-terminal histidine peptide, the color changes to pale blue. This color change allows colorimetric detection of peptide binding to the complex. Di- and tripeptides His-Ala, His-Ala-Phe, His-Phe, and Ala-Phe were tested for binding. The (His-Ala)-imprinted polymer has the highest binding capacity and affinity for the template peptide, while His-Ala-Phe and His-Phe show weaker binding. Non-histidine containing peptides, such as Ala-Phe, have almost no affinity for the polymer.[121]

4.3.4. Immobilization on Gold Surfaces[122]

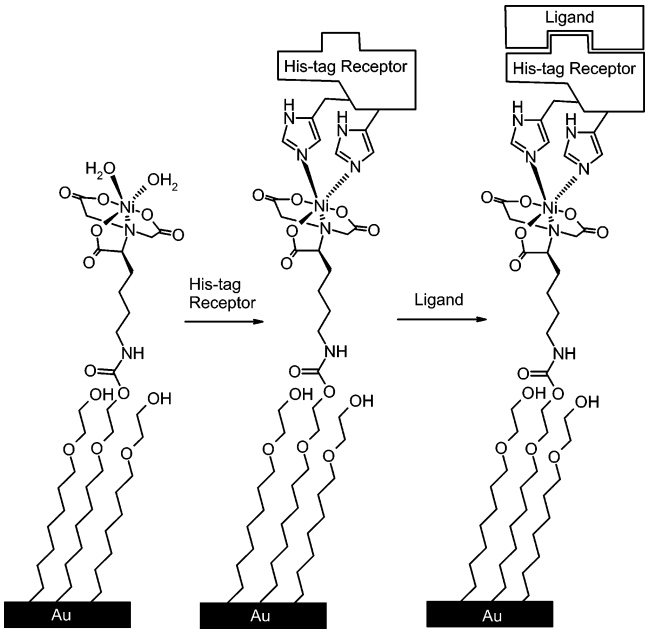
Surface plasmon resonance (SPR) is a useful technique for measuring the kinetics of association and dissociation of ligands from proteins in aqueous solution.[123] A variety of methods have been used to immobilize proteins on silver or gold films for studies using SPR (Scheme 8).[123,124] Whitesides et al. developed procedures to modify the surface of gold films by formation of self-assembled monolayers (SAMs) of alkanethiolates.[125] They describe the generation of a SAM functionalized with the Ni(II)−NTA group. Using this method to immobilize proteins bound to a sequence of His tags, a surface was created that would specifically immobilize a protein of interest while resisting nonspecific binding of other proteins. Therefore, the requirement for nonspecific, covalent modification of the protein was evaded.

After the His-labeled receptor is bound to the modified gold surface, interactions between the receptor and a ligand in solution could be studied by SPR. A similar approach was

Scheme 7. Schematic Representation of the Peptide Imprinting Process



Scheme 8. Protocol for Immobilization of Proteins onto Surfaces To Probe for the Ability of Immobilized Proteins To Interact with Ligands in Solution



used by Hoggett et al. to measure the interaction between and the core of bacterial (*E. Coli*) RNA and the immobilized His-tagged form of the σ^{70} factor.[126] Vogel demonstrated the reversible binding of an anti-lysozyme F_{ab} fragment modified with a C-terminal hexahistidine extension on similar gold surfaces.[127] The dissociation constants, determined by SPR and IR spectroscopy, showed that immobilization did not affect the secondary structure of the protein.

Powell et al. report the use of Ni(II)−NTA immobilized on gold particles to label a His tag attached to a protein.[128] The label is detected by electron microscopy or, with metal enhancement (e.g., silver), even by light microscopy or by the naked eye.

The Ni(II)−NTA His tag approach has been used in several examples to create strong intermolecular interactions. However, the strength of the coordination bond between a histidine tag and Ni(II)−NTA was not well understood until 2000, when Samorì et al. published their results.[129] Their experimental design simulated the Ni(II)-NTA protein binding process. Approach, binding, stretching, and unbinding of individual His tags and individual Ni(II)−NTA chelating groups were studied under external forces by scanning force microscopy (SFM) in a single-molecule experiment. An n-His-tagged peptide ($n = 2$ or 6), HCys-$(Gly)_6$-$(His)_n$-OH, was covalently attached on its Cys end to a gold-coated SFM tip. The tip was placed in proximity to a substrate having exposed Ni(II)−NTA groups covalently bound through carboxymethylated dextran linkers, which were attached to a gold surface bearing an alkane C_{18} chain. When the $n \times$ His tag of the peptide formed a chelate with a Ni(II)−NTA group, a molecular bridge was established between the tip and the substrate. The tip was subsequently retracted at a constant pulling velocity until the bridge ruptured. The probability of binding events increased from 28% for the 2 $\times$ His tag to 42% for a 6 $\times$ His tag. Most of the unbinding traces exhibited a single rupture: the distribution of the forces was very broad and peaked at about 500 pN for the 6 $\times$ His tag and about 300 pN for the 2 $\times$ His tag.

4.3.5. Electropolymerization of NTA Complexes

The electrosynthesis of polymers allows the reproducible and precise functionalization of conductive microsurfaces of complex geometry with organic molecules.[130] Gondran et al. successfully synthesized and electropolymerized an NTA derivative bound to a poly(pyrrole) film (Scheme 9).[131] The ability of this polymer to bind Cu(II) was investigated using cyclic voltammetry.

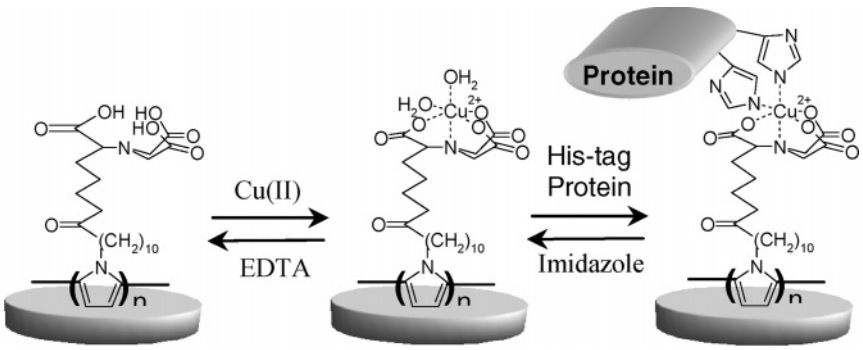
To examine the potential affinity of the metal−polymer film for histidine-tagged proteins, two platinum electrodes containing the chelating polymer with coordinated Cu(II) were soaked in a 0.1 M phosphate buffer, where one was in the presence of His-tagged glucose oxidase (his-GOX, 0.1 mg/mL) and the other was in the presence of regular glucose oxidase (GOX, 0.1 mg/mL). In the presence of oxygen, this enzyme catalyzes the oxidation of glucose with the concomitant production of H_2O_2. Its activity can be monitored via the electrochemical oxidation by H_2O_2. Using this technique, the electrode coated with his-GOX resulted in markedly higher maximum current density (j_{max}, determined in glucose saturated conditions) than the electrode incubated with GOX.

4.3.6. Metal Chelating Lipids

To link lipid technology to the concept of fusion proteins, Tampé et al. synthesized lipid molecules, which contain NTA as a metal chelator head group and bind electron donating groups such as imidazole or histidine.[132] The synthesis was carried out with two different amphipatic molecules, a phospholipid (DPPE, **52**) and a dioctadecylamine (DODA, **53**) (Figure 30).

The lipids were characterized chemically and physically by FTIR spectroscopy, mass spectroscopy, and film balance

Scheme 9. Schematic Representation of the Reversible Immobilization of His-Tagged Biomolecules on an Electrogenerated Poly(pyrrole)−NTA Film[a]



[a] Reprinted with permission from ref 131. Copyright 2005 American Chemical Society.

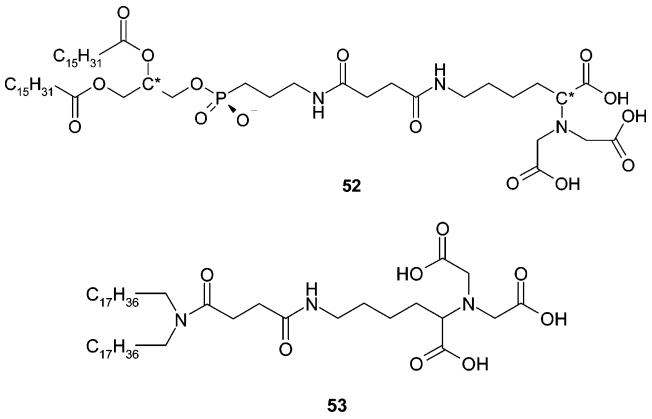
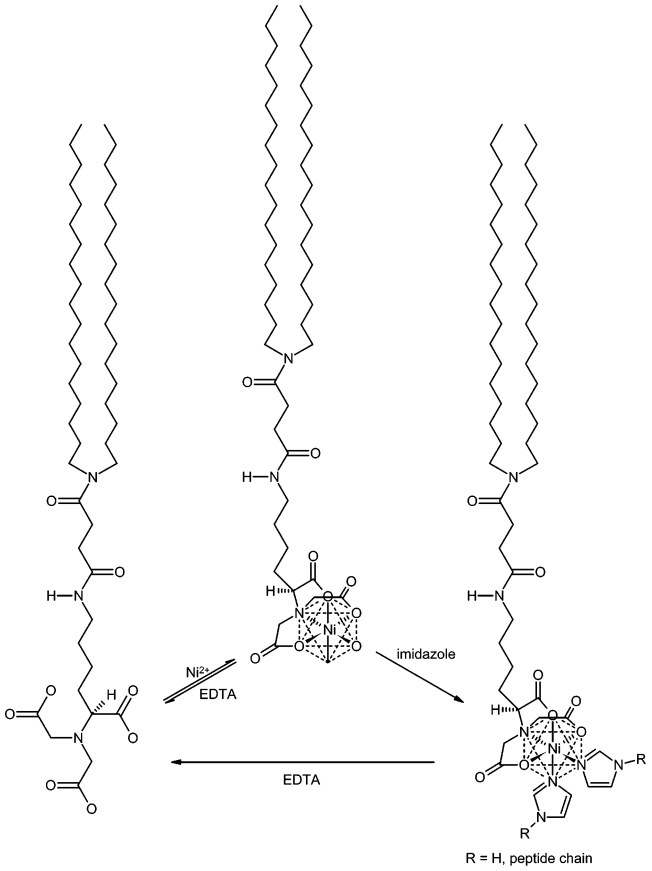


Figure 30. Structures of two lipid NTA conjugates.

experiments. Both NTA lipids spread at the air/water interface. The addition of Ni(II) resulted in complexed Ni(II)−NTA−DODA, which coalesces at the air/water interface. Imidazole interacts specifically with the metal complexes on the lipid surface (Scheme 10). Addition of

Scheme 10. Schematic Illustration of Complex Formation and Imidazole Binding to Ni(II)−NTA−DODA at the Lipid Surface



EDTA, which has a higher binding affinity for Ni(II) than NTA, reverses the binding event. Therefore, the immobilization of any bound ligand can be disrupted under mild conditions. In 1995, the same group was able to immobilize fluorescence-labeled, histidine-tagged biomolecules.[133] The two-dimensional organization is visualized by epifluorescence microscopy and film balance studies.

Similar lipid surfaces have been used to grow two-dimensional crystals and to determine structures of various

Table 10. Selected Binding Constants of BPA Complexes[33]

ion	equilibrium	log *K*
Co^{2+}	$ML/M{\cdot}L$	7.74
	$ML_2/M{\cdot}L^2$	13.05
Ni^{2+}	$ML/M{\cdot}L$	8.7
	$ML_2/M{\cdot}L^2$	16.60
Cu^{2+}	$ML/M{\cdot}L$	14.4
	$ML_2/M{\cdot}L^2$	19.0
Zn^{2+}	$ML/M{\cdot}L$	7.57
	$ML_2/M{\cdot}L^2$	11.93
Cd^{2+}	$ML/M{\cdot}L$	6.44
	$ML_2/M{\cdot}L^2$	11.74

His-tagged proteins.[134] This method of surface modifications using lipid bound metal complexes inspired Nolan et al.[135] to modify microspheres by the formation of self-assembled bilayer membranes where the metal chelators have lateral mobility. Interactions on this surface were studied by flow cytometry.

4.3.7. NTA-Modified Magnetic Nanoparticles

In an effort to develop a simpler and more versatile platform for protein purification than IMAC, Xu et al. invented surface-modified magnetic nanoparticles (Scheme 11) as the binder, carrier, and anchor for histidine-tagged proteins.[136] Using NTA to attach to FePt magnetic nanoparticles covalently, they developed a simple procedure to obtain pure proteins directly from the mixture of lysed cells within 10 min (Scheme 12). The procedure to use particles, such as **59**, for protein separation consists of three simple steps: (1) adding **59** into the suspension of the lysed cells and shaking for 5 min, (2) using a small magnet to attract the nanoparticles to the wall of the vial and washing them with deionized water to remove the residual protein solution, and (3) using a concentrated imidazole solution to wash the nanoparticles to yield pure proteins. After releasing the proteins, washing with EDTA, buffer, and $NiCl_2{\cdot}6H_2O$ solution, **59** can be recovered and reused.

4.3.8. Immobilization on Quartz Surfaces

Vogel et al. reversibly immobilized His-tagged proteins on quartz, preserving the protein function.[137] The chelator, NTA, is covalently bound to the hydrophilic quartz surface and is subsequently loaded with the divalent metal cation [e.g. Ni(II)]. Binding of the protein to NTA is highly specific with reasonable affinities. In addition, it is fully reversible upon addition of a competitive ligand, reprotonation of the histidine residue, or removal of the metal ion via EDTA complexation. Binding and removal from the surface of the modified quartz is visualized using green fluorescent protein using real-time measurements.

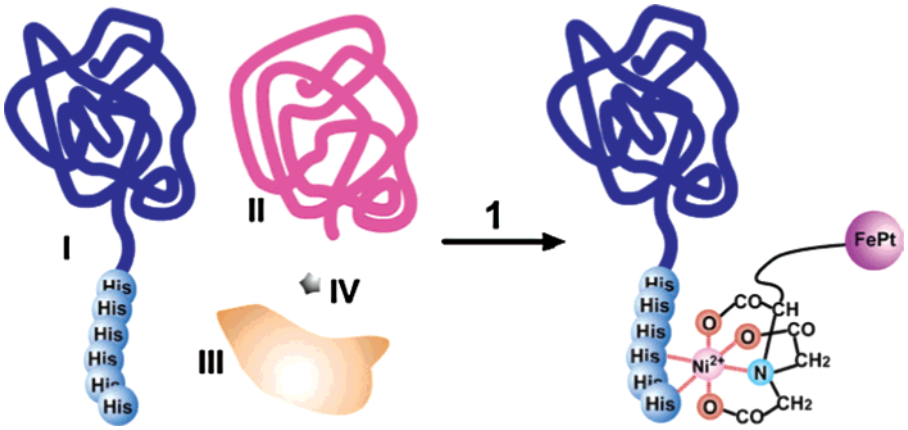
5. Bis(2-pyridylmethyl)-amine (BPA, DPA, BISPMA, DIPICA, BISPICAM)[138] Complexes

In bis(2-pyridylmethyl)-amine (BPA) ligands the carboxylic acid functionalities of IDA are replaced by pyridines. First reported in 1964 by Kabzinska, this ligand and derivatives of it are used for complexation of over 50 different metal ions.[139] Emphasis was put on the investigation of coordination compounds with Cu(II),[140] Zn(II),[141] and Fe(III) ions (Table 10).[142]

5.1. Structures of BPA Complexes in the Solid State

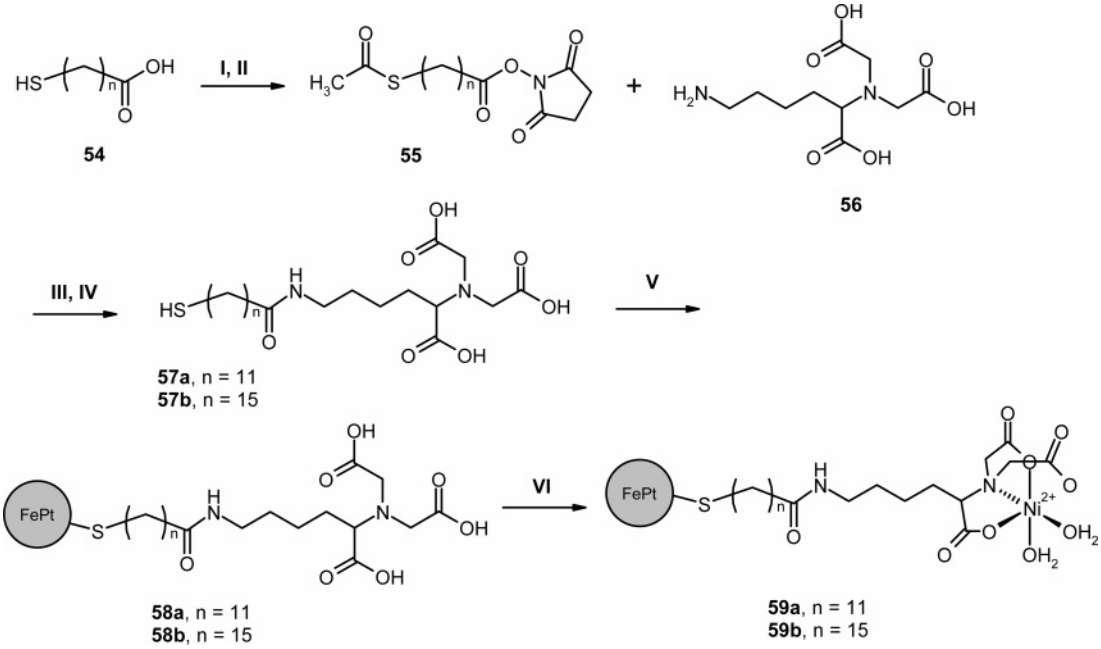
The Cu(II)−BPA complex $[Cu(bpa)Cl_2]$ was structurally examined by Choi et al.[143] In this compound the Cu(II) is

Scheme 11. Surface-Modified Magnetic Nanoparticles Selectively Bind to Histidine-Tagged Proteins in a Cell Lysate: I, 6His-Tagged Protein; II, Other Proteins; III, Cell Debris; IV, Colloid Contaminants[a]



[a] Reprinted with permission from ref 136. Copyright 2004 American Chemical Society.

Scheme 12. Synthesis of NTA-Modified Magnetic Nanoparticles[a]



[a] (I) AcOCl, Zn, 85−90%; (II) NHS, DCC; (III) $NaHCO_3$, 40−46%; (IV) H_2NNH_2, AcOH, 88−90%; (V) FePt (3−4 nm); and (VI) $NiCl_2 \cdot 6H_2O$ and buffer.

coordinated by three nitrogen atoms of the BPA ligand and two chlorine anions in a distorted square pyramidal environment.

Beside complexes of 1:1 stoichiometry, BPA−Cu(II) coordination compounds with 2:1 stoichiometry are known, which have no vacant coordination sites and are therefore not discussed here.[144] The 1:1 complex coordinates additional electron donating groups. Murakami et al. reported X-ray structures which show picolinate (Figure 31) and α-amino acids such as L-Phe coordinated to the Cu(II)−BPA complex.[145]

A better understanding of the redox reactions between the metal center and the pterin cofactor in phenylalanine hydroxylase led Yamauchi and co-workers to examine the binding of pterin-6-carboxylate (ptc, as a cofactor model) to $[Cu(bpa)(ptc)]\cdot H_2O\cdot CH_3OH$.[146] The study revealed different Cu(II) binding modes of oxidized and reduced pterins.

The structure of $[Zn(bpa)(H_2O)_2](ClO_4)_2$ shows a distorted octahedral coordinated Zn(II).[147] This geometry results from the three nitrogen donor groups of the chelating ligand BPA

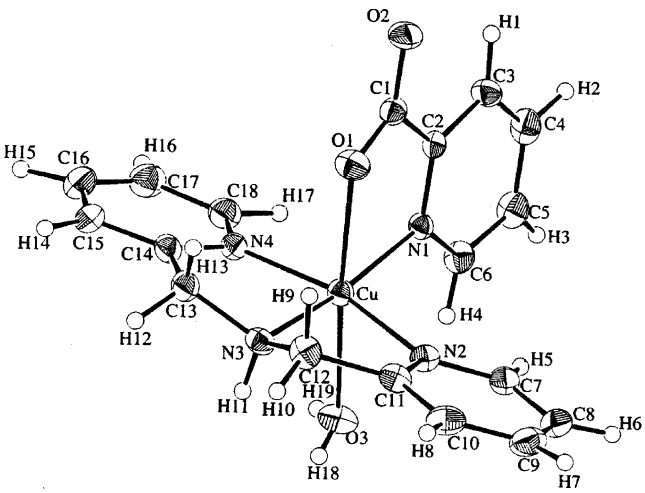


Figure 31. ORTEP drawing of $[Cu(bpa)(pic)]PF_6\cdot H_2O$. Reprinted with permission from ref 145a. Copyright 2000 Elsevier Science B.V.

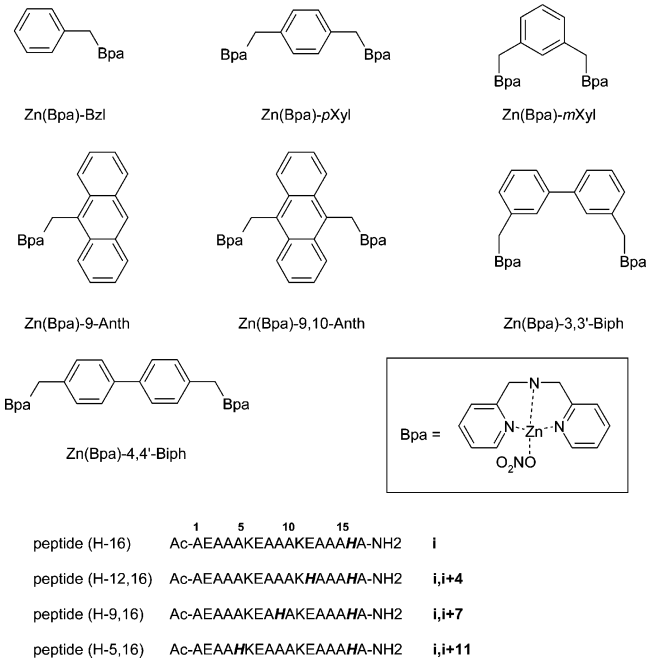


Figure 32. Molecular structures of $[Zn(bpa)]^{2+}$-based receptors and their target peptide sequences.

and the additional two water molecules. In contrast to this structure, Wirbser reported a pentacoordinated Zn(II) complex: $[Zn(bpa)Cl_2]$ shows a trigonal bipyramidal coordination geometry in the solid state.[148]

As the BPA ligand uses only three coordination sites of an octahedral coordinating Zn(II), $[Zn(bpa)_2]^{2+}$ complexes with a 2:1 ligand to metal ion stoichiometry are known. [149] One of the ions which can additionally bind to $[Zn(bpa)]^{2+}$ complexes are phosphates. Vahrenkamp et al. reported the crystal structure of a phosphate−Zn(II)−BPA complex of the composition $[(BPA{\cdot}Zn)_3(tolyl\text{-}OPO_3)_2]HPO_4$.[150] The ability of $[Zn(bpa)]^{2+}$ to bind phosphate is discussed in detail in the section on recognition processes in solution.

Crystal structures of $[Fe(bpa)Cl_3]$[151] and $[Fe(bpa)_2(BF_4)_2]$[152] show similar structures with the metal ion in an octahedral coordination.

5.2. Recognition Processes in Solution

5.2.1. Surface Recognition of α-Helical Peptides in Aqueous Solution

Hamachi et al. used $[Zn(bpa)]^{2+}$-based coordination chemistry for the design of artificial peptide receptors (Figure 32).[153,154] A Zn(II) bispicolylamine complex is employed as a binding site for histidine residues because of its good affinity in aqueous solution. Five dinuclear Zn(II)−BPA complexes having *m*- or *p*-xylene, 9,10-dimethylanthracene, and 3,3′- or 4,4′-dimethylbiphenyl as a linker were prepared. Two mononuclear Zn(II) complexes based on benzyl-BPA or 9-anthrylmethyl-BPA were used for comparison. The target molecules for binding are four helical model peptides with one or two His residues located on one side of the α-helix.

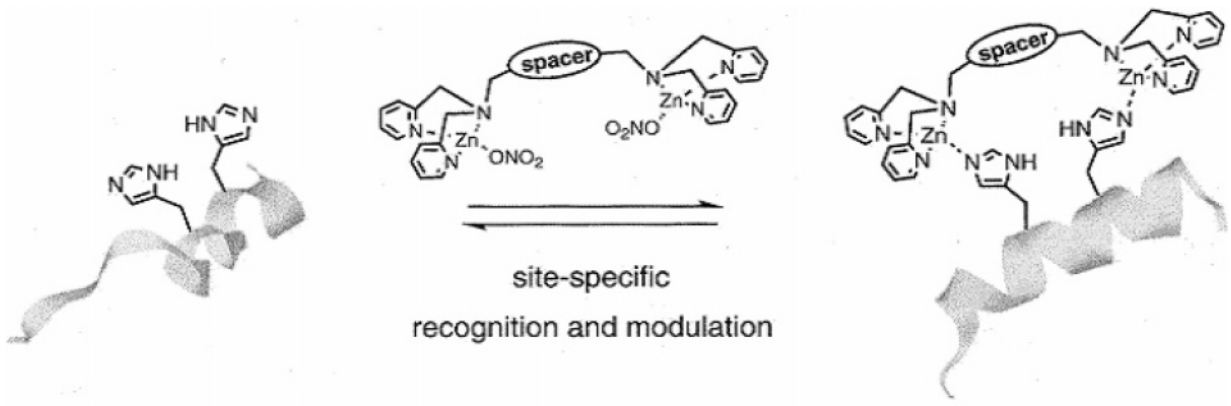
For the rapid screening of the affinity of these Zn(II)−BPA based receptors for the four peptides, the receptor-induced conformational change of the peptides was examined by circular dichroism spectroscopy. The helicity significantly increased upon receptor addition for peptides having two His residues but not for the mono-His peptide. The helical conformation of a H-12,16 peptide was selectively stabilized by Zn(BPA)-9,10-anthracene (Scheme 13). For a H-9,16 peptide, Zn(BPA)-*p*-xylylene, Zn(BPA)-9,10-anthracene, and Zn(BPA)-4,4′-biphenylene showed similar effects. In the case of a H-5,16 peptide, Zn(BPA)-4,4′-biphenylene selectively induced a helical conformation. Neither Zn(II)-cations nor a Zn(BPA)-Bzl mononuclear complex effectively induced a conformational change of the peptides. The Job's plot analysis based on CD measurements showed its maximum at 0.5 of the molar fraction of the receptor. Consistently, ESI-TOF mass spectroscopy, under identical conditions, displayed a 1:1 complex between the receptor and the peptide as a major species. All receptor−peptide combinations selected by screening had an affinity in the range of 10^4 to 10^5 L/mol.

5.2.2. Artificial Receptors for Phosphorylated Peptides

Hamachi and his group have used $[Zn(bpa)]^{2+}$ based receptors to selectively bind phosphorylated peptides and ATP.[154−156] Anthracene derivatives having two Zn(II)-BPA moieties selectively coordinate phosphorylated amino acids and signal the binding by a change in emission. When phosphorylated tyrosine (p-Tyr) was added to the aqueous solution of the dinuclear complex **60** (Figure 33), Hamachi observed a significant emission increase from the anthracene chromophore (Figure 34).

These receptors strongly bind and fluorometrically sense phosphorylated species such as phosphate and p-Tyr in a concentration of 10^{-6} to 10^{-5} M in neutral aqueous solution. No fluorescent response is induced by other anions, such as sulfate, nitrate, acetate, and chloride. Job's plot analyses of

Scheme 13. Schematic Representation of Side Chain Selective Peptide Recognition by a $[Zn(bpa)]^{2+}$-Based Receptor[a]



[a] Reprinted with permission from ref 153. Copyright 2001 Elsevier Science B.V.

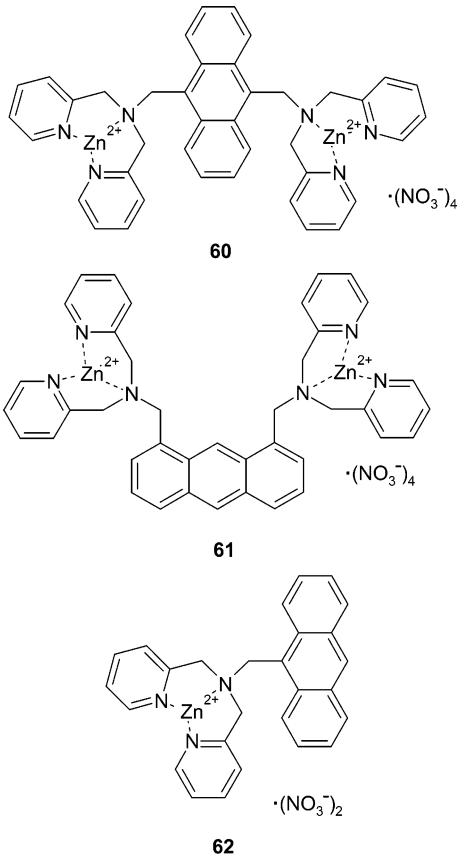


Figure 33. Emitting mono- and dinuclear Zn(II) complexes.

the binding of these receptors and p-Tyr reveal a 1:1 stoichiometry (inset of Figure 35).

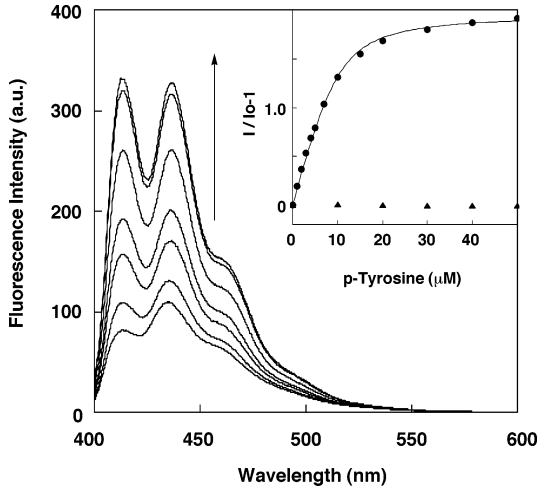


Figure 34. Emission changes of compound **60** (10 μM) upon addition of p-Tyr: [p-Tyr] = 0, 1.0, 3.0, 5.0, 10, 30, 50 μM from the lowest to the top trace. The spectra were measured in 10 mM HEPES buffer (pH 7.2) at 20 °C, λ_{ex} = 380 nm. Reprinted with permission from ref 155a. Copyright 2002 American Chemical Society.

NMR studies suggested that two Zn (BPA) sites equally contribute to the phosphate ion binding. Cocrystal structures of the complex with phenyl phosphate support the expected coordination geometry.[157] Hamachi subsequently examined the sensing ability of these receptors toward phosphorylated peptides in aqueous solution. Three kinds of phosphorylated peptides (peptide-a, -b, -c) and a nonphosphorylated peptide

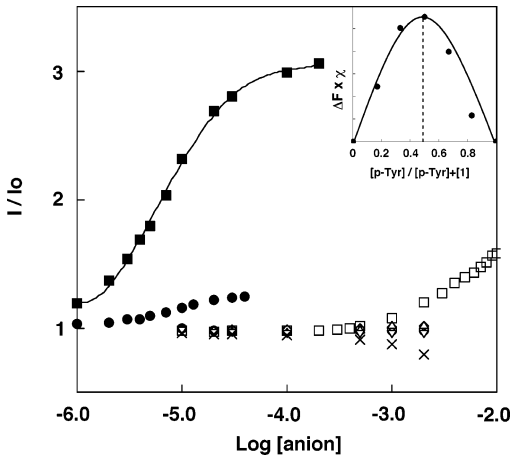


Figure 35. Relative emission response of compound **60** to the anion concentration (log[anion]): phosphate (●), p-Tyr (■), chloride (○), acetate (◇), sulfate (△), nitrate (▽), carbonate (□), azide (×). λ_{ex} = 380 nm. Inset: Job's plot of compound **60** (=1) and p-Tyr. Reprinted with permission from ref 155a. Copyright 2002 American Chemical Society.

(peptide-d, Scheme 14) for comparison were used to investigate receptor binding.

The fluorescence intensity of **60** increases significantly by addition of less than 1 μM of peptide-a (Figure 36). The

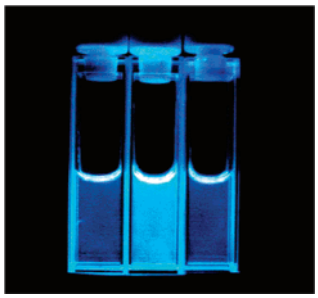


Figure 36. Increased emission from receptor **60** in the presence of phosphorylated peptide-a (middle): solution of **60** only (left); **60** in the presence of nonphosphorylated peptide-*d* (right). Reprinted with permission from ref 155a. Copyright 2002 American Chemical Society.

binding is remarkably strong with an affinity of 10^7 M^{-1}.

Apparently, the blue fluorescence is enhanced by the phosphorylated peptide-a, whereas the nonphosphorylated peptide-d does not induce a change in luminescence. Such visualizations stimulated the same group to immobilize the artificial receptors **60** (2 in Scheme 16) and **62** (3 in Scheme 16) in a gel matrix without loss of their binding capability displayed in solution.[158] The binding constant in the gel is reduced compared to the case of homogeneous solution. However, easy discrimination between phosphorylated and nonphosphorylated peptides is still possible. For rapid sensing, the hydrogel was miniaturized and arranged into an array on a glass support.

This chip was used for receptor screening as shown in Figure 37. Variations of receptors, pH, metal salts, and presence of phosphorylated peptide are realized on a single chip. The detection system for phosphorylated peptides based on compound **60** was also used to spot the presence of phosphorylated peptides in SDS−PAGE gel electrophoresis.[159]

Recent research in the area of phosphate recognition focused on a real-time assay to monitor glycosyltransferase

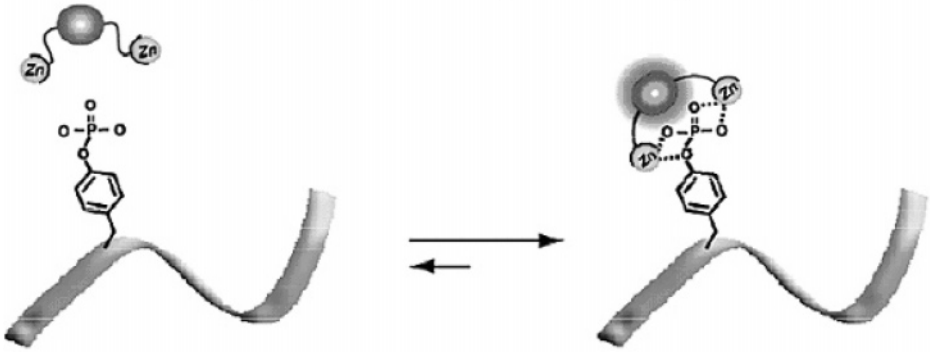
Scheme 14. (top) Amino Acid Sequences of the Phosphorylated Peptides and (bottom) Schematic Depiction of the Interaction of Receptor 60 or 61 with a Phosphorylated Peptide[a]

Peptide a: Glu-Glu-Glu-Ile-pTyr-Glu-Glu-Phe-Asp

Peptide b: Arg-Arg-Phe-Gly-pSer-Ile-Arg-Arg-Phe

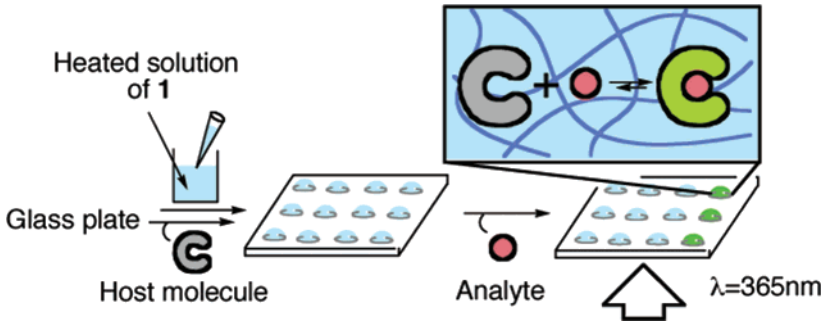
Peptide c: Lys-Ser-Gly-pTyr-Leu-Ser-Ser-Glu

Peptide d: Glu-Glu-Glu-Ile-Tyr-Glu-Glu-Phe-Asp



[a] Reprinted with permission from ref 155a. Copyright 2002 American Chemical Society.

Scheme 15. Preparation Scheme of a Semiwet Sensor Array (1 Is the Monomer of the Hydrogel)[a]



[a] Reprinted with permission from ref 158. Copyright 2004 American Chemical Society.

activity.[160] In biological glycosyl transfer processes, a glycolsylated nucleotide, such as uridine 5′-diphosphate (UDP)-glcoside, is used to form new glycoside bonds with a glycosyl acceptor. As the glycosylated nucleotide is converted into the corresponding nucleotide during the reaction, it is conceivable that monitoring of nucleotide formation correlates with the glycosyl transfer reaction. The key issue in Hamachi's assay is the selectivity of **60**, which can distinguish a nucleotide from a glycosylated nucleotide in a multisubstrate enzymatic reaction medium (see Scheme 16).

The increase in fluorescence upon coordination to the pyrophosphate monoester is a consequence of the suppression of photoinduced electron-transfer (PET) quenching that

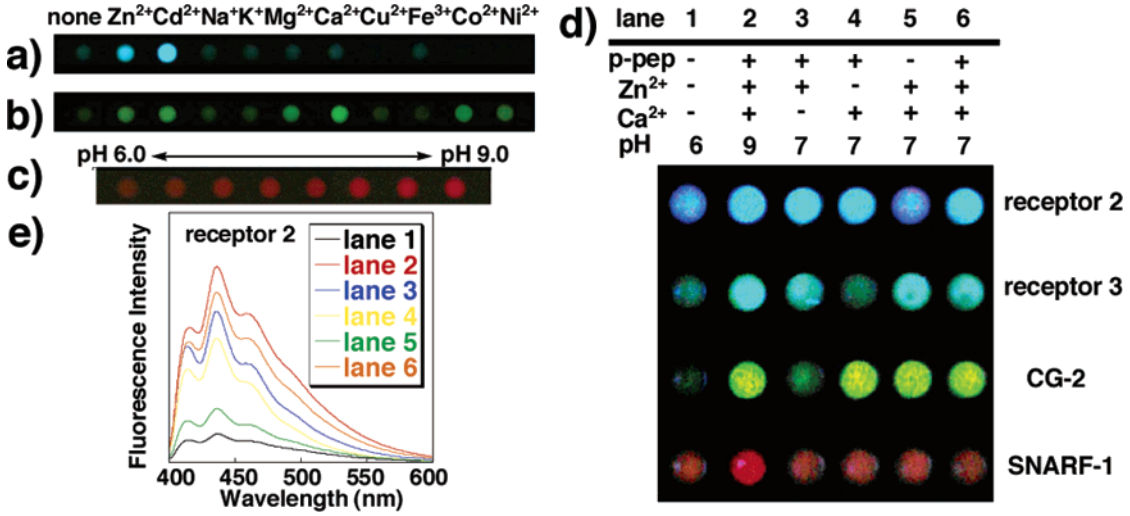
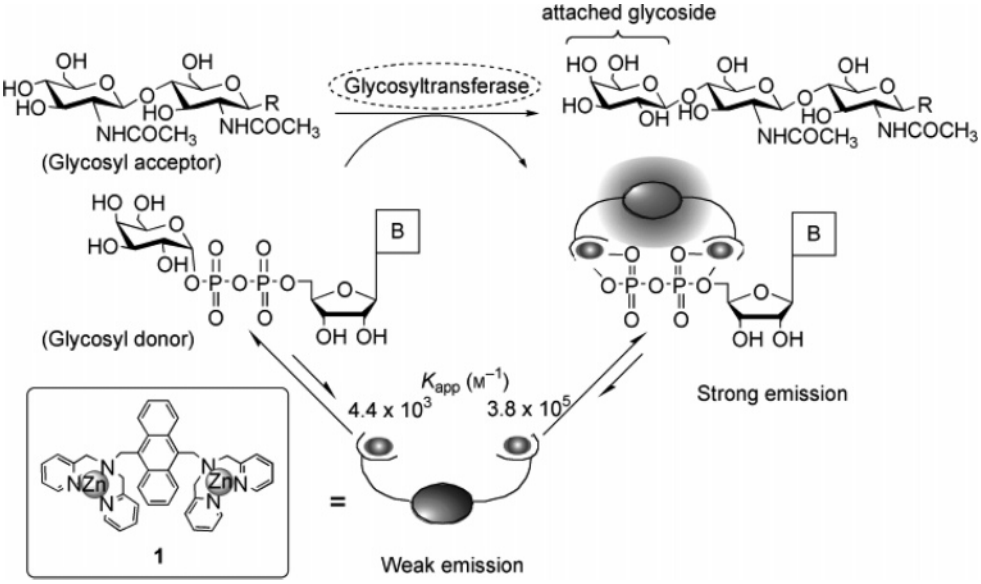


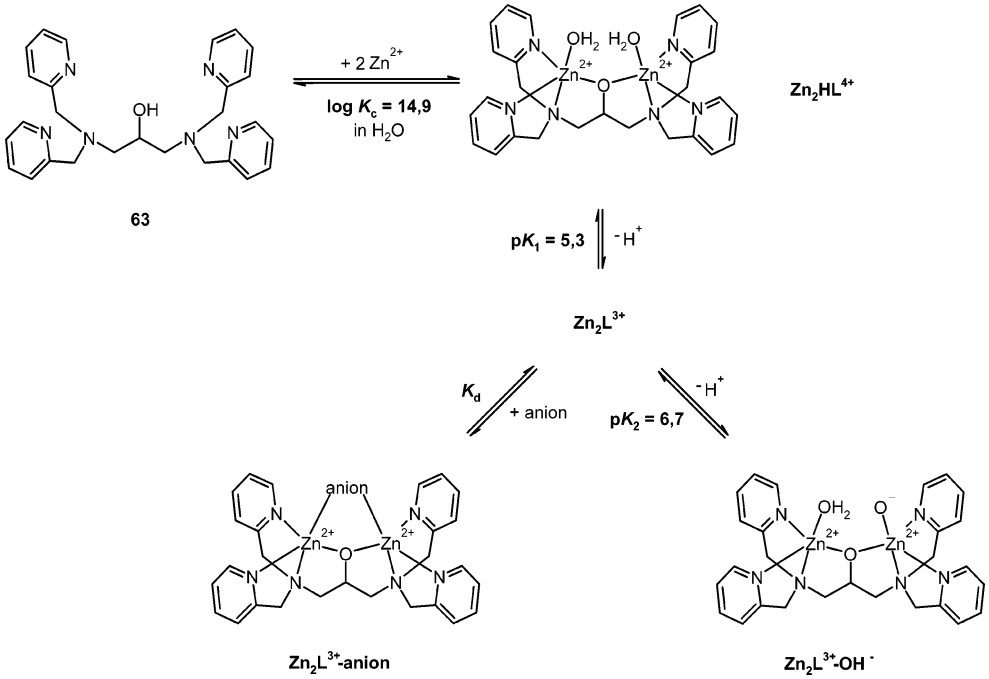
Figure 37. Images of sensing patterns of a semiwet chemosensor chip containing (a) **60** (80 μM), (b) CG-2 (Ca (II) probe) (50 μM) in the presence of various metal cations, or (c) SNARF-1 (pH probe) (100 μM) at various pHs. (d) An image of an integrated molecular recognition hydrogel chip for mixed solution assay. Reprinted with permission from ref 158. Copyright 2004 American Chemical Society.

Scheme 16. Chemosensor-Based Glycosyltransferase Assay (B = Base Content of the Nucleotide)[a]



[a] Reprinted with permission from ref 160. Copyright 2006 Wiley-VCH.

Scheme 17. Structure of BPA Ligand Used for Complexation Experiments



results from the phosphate-assisted coordination of the second Zn(II) ion. Other groups synthesized modified BPA ligands to bind to phosphates (Scheme 17).[161−163] Here, two $[Zn(bpa)]^{2+}$ binding units and additional hydroxy coordination were used for anion binding.[161]

The solution binding processes were investigated by potentiometric titration. The species distribution plot shows the Zn_2L^{3+} phenyl phosphate aggregate as the only species between pH 5 and 8. This compound is present in the solid state, as found by X-ray structure analysis.

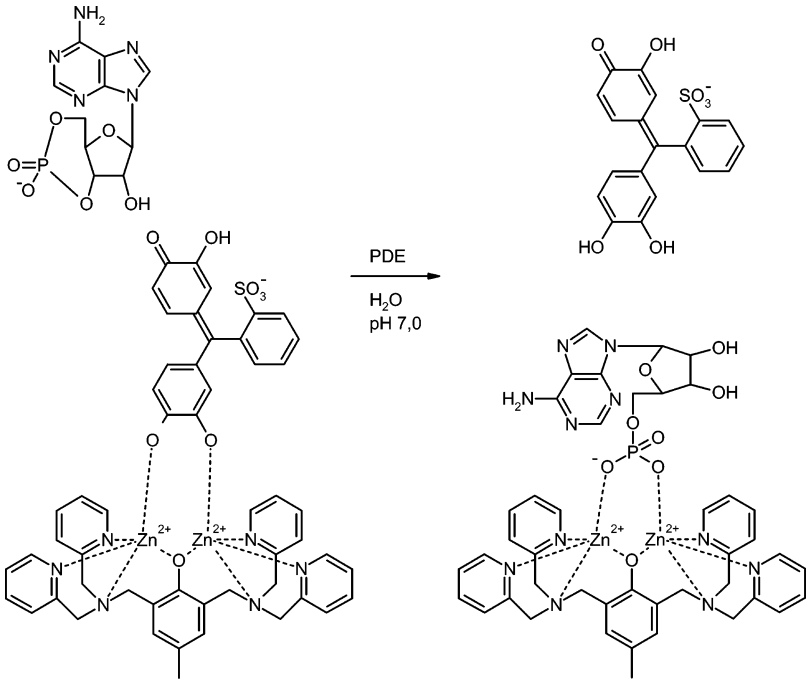
Kim et al. designed a receptor for AMP binding based on BPA ligands.[162] Using a 4-methyl-phenyl group as linker to bridge the BPA ligands, they created a binuclear complex with similar structure as the compound from Koike.[164] The visual detection of di- and triphosphates in aqueous solution of neutral pH using a bis-BPA Zn(II) complex and indicator displacement was reported by Han et al.[165]

Sensing AMP is achieved by displacement of pyrocatechol violet (Scheme 18). The AMP binding is monitored by the amount of free pyrocatechol violet in solution. The binding was investigated spectrophotometrically and by isothermal titration calorimetry.

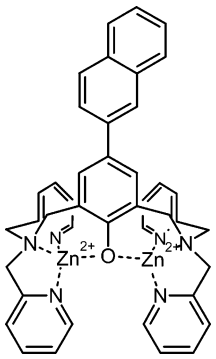
Using a similar approach, Smith et al. reported an indicator displacement system for fluorescent detection of phosphate oxyanions under physiological conditions.[166] Hong and coworkers reported a fluorescent pyrophosphate ($P_2O_7^{4-}$, PPi) sensor based on a naphthalene−BPA system, which shows high sensitivity and selectivity over a wide pH range for pyrophosphate ions relative to other anions, including ATP and ADP (Figure 38).[163] The presence of pyrophosphate is signaled by changes in the emission intensity of the luminescent receptor.

Upon addition of pyrophosphate, a 6-fold increase of the emission intensity is noted. Added ATP leads to a 2-fold

Scheme 18. Schematic Representation of the AMP Chemosensor



increase of emission intensity. The binding mode for pyrophosphate to **64** is illustrated in Scheme 19, which is based on previous work involving a structurally similar sensor and its X-ray crystal structure.



64

Figure 38. Structure of *Hongs* pyrophosphate sensor **64**.

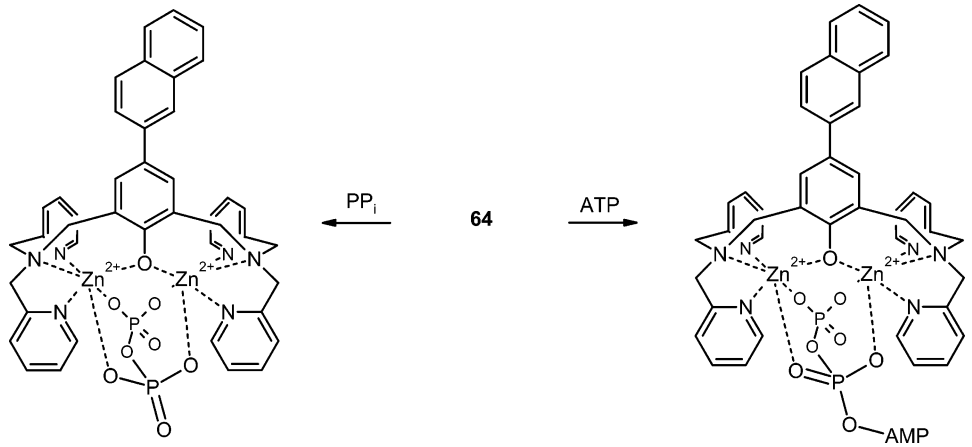
The observed selectivity for pyrophosphate over ATP can be understood from the structure of the guest and differences in the charge density of guest oxygen atoms involved in the complexation. The total anionic charge density of the four phosphate oxygen atoms involved in the complexation of ATP with **64** is lower if compared with pyrophosphate. Therefore, the binding affinity of ATP is drastically reduced, and observed luminescence changes are smaller relative to pyrophosphate binding.

5.3. Recognition Processes on Solid Surfaces

5.3.1. Sensors for Phosphatidylserine-Containing Membranes

Using the sensor metal complex **60** from Hamachi, the Smith group detected the presence of anionic phospholipids, particularly phosphatidylserine (PS), on the surface of vesicles and cells.[167] However, the excitation wavelength is in the UV range and therefore not compatible with the lasers in most flow cytometers. This prompted Smith and coworkers to design a second generation of sensors (Figure 39).[168] The titration experiments involved addition of unilamellar vesicles composed of 1:1 POPC:POPS, 1:1 POPC:POPA, 1:1 POPC:POPG, or 100% POPC to a 1 μM solution

Scheme 19. Proposed Mechanism for the Complexation of Sensor 64 with Pyrophosphate and ATP



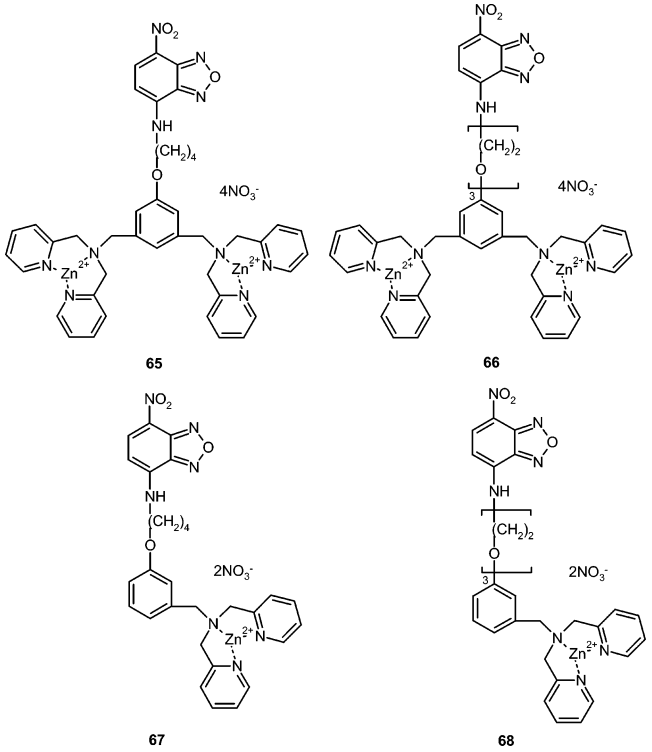


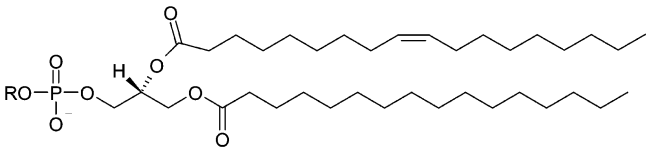
Figure 39. Complexes **65**−**68** as potential PS sensors.

Table 11. Sensor/Phospholipid Binding Constants (K_a)

	K_a[a] ($\times 10^4$ M^{-1})			
sensor	100% POPC	1:1 POPC:POPS	1:1 POPC:POPG	1:1 POPC:POPA
65	<1	23.3 ± 1.7	14.2 ± 3.8	11 ± 6.0
66	<1	5.3 ± 2.0	2.0 ± 0.5	2.0 ± 0.3
67	<1[b]	11.5 ± 5.5		
68	<1[b]	7.7 ± 3.3		

[a] Values are the average of at least three independent measurements. [b]Values are the average of two independent measurements.

of **65** or **66** (Figure 40). The resulting isotherms fit to a 1:1 binding model, which allowed calculation of apparent phospholipid binding constants (Table 11). As the sensors were not expected to bind the zwitterionic POPC head group, this titration was performed as a control to determine the specificity of the sensors for anionic PS.



Formula for R	Phospholipid
-H	POPA
$-CH_2CH_2N(CH_3)_3^+$	POPC
$-CH_2CH(NH_3^+)COO^-$	POPS
$-CH_2CH(OH)CH_2OH$	POPG

Figure 40. Head group structure of common phospholipids.

The effect of only one Zn(II)−BPA unit in the sensor structure was evaluated using control compounds **67** and **68**. Vesicles composed of 100% POPC or 1:1 POPC:POPS were added to a 1 μM solution of **67** or **68**. The emission of butyl-linked sensor **67** with 1:1 POPC:POPS is about two times that observed with 100% POPC vesicles, whereas the intensity ratio with TEO-linked sensor **68** is about five. Thus, it is clear that the hydrophilic TEO-linker is more useful for detecting PS-containing membranes over PC-only membranes. The emission of sensor **66** with two $[Zn(bpa)]^{2+}$ units is significantly higher than that obtained with **68**, indicating that the second $[Zn(bpa)]^{2+}$ unit increases the interactions that produce a stronger response to PS-containing membranes. The PS sensitivity of sensor **66** was investigated in additional titration experiments with POPC vesicles enriched with various amounts of POPS (Table 12). A solution of **66** (1 μM) was titrated with vesicles composed of POPC and 0−50% POPS. Sensor **66** can readily detect the presence of bilayer membranes containing 5% PS.

Until now, no surface-immobilized BPA complexes and their use in molecular recognition have been reported.

6. Tris(2-pyridylmethyl)-amine (TPA, TMPA) Complexes

Substitution of the carboxylic acid functionalities of NTA by pyridine leads to the TPA ligand. The ligand was first reported in 1969 by da Mota, and a large number of derivatives have been used for complexation of metal ions.[169] Besides Ni(II)[170] and Co(III)[171] complexes, most reports cover investigations of Cu(II),[172] Zn(II),[173] and Fe(III) ion complexation (Table 13).[174]

6.1. Structures of TPA Complexes in the Solid State

The structure of the copper(II) complex of TPA was reported by Karlin et al.[175] As indicated by the similar Cu(II)−N bond lengths and bond angles, the copper ion is found in a trigonal bipyramidal coordination geometry (Figure 41).

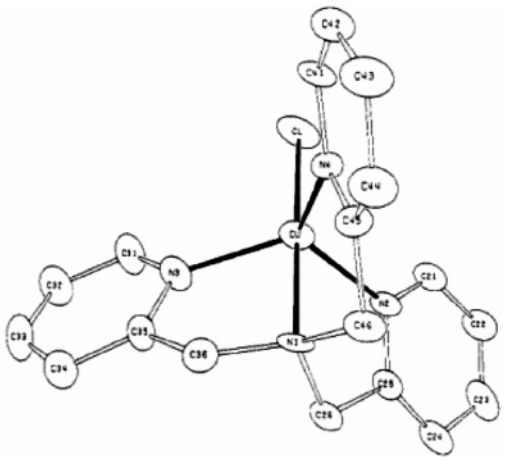


Figure 41. Structure of $[Cu(tpa)(Cl)]^+$ in the solid state. Reprinted with permission from ref 175. Copyright 1982 American Chemical Society.

These copper complexes have the ability to bind additional phosphate ions, which is illustrated by the structure of the 1:1 complex of $[Cu(tpa)]^{2+}$ and BNPP (=bis(*p*-nitrophenyl) phosphate) (Figure 42).[176]

The crystal structure of $[Zn(tpa)Cl]ClO_4$ shows the ligand maintaining a helical twist in which the dihedral angle between the plane of each pyridine ring and the axis of the

Table 12. Sensor 66/Phospholipid Binding Constants (K_a) for Vesicles with Varying PS Content

	95:5 POPC:POPS	90:10 POPC:POPS	80:20 POPC:POPS	50:50 POPC:POPS
K_a[a] ($\times 10^4$ M^{-1})	1.0 ± 0.3	2.2 ± 0.5	5.7 ± 0.5	5.3 ± 2.0

[a] Values are the average of at least three independent measurements.

Table 13. Typical Binding Constants of Metal Ions to the TPA Ligand[33]

ion	equilibrium	log K
Co^{2+}	M/M·L	11.4
Ni^{2+}	M/M·L	14.5
Cu^{2+}	M/M·L	16.2
Zn^{2+}	M/M·L	11.0
Fe^{2+}	M/M·L	8.7

M−N(11) bond is between 12 and 22°.[177] Thus, the overall shape of the cationic portion of the molecule resembles a propeller. In the crystal, the compounds are found as racemates with their mirror images. Similar to the Cu(II) complex, the analogous Zn(II) complex binds to BNPP.[178]

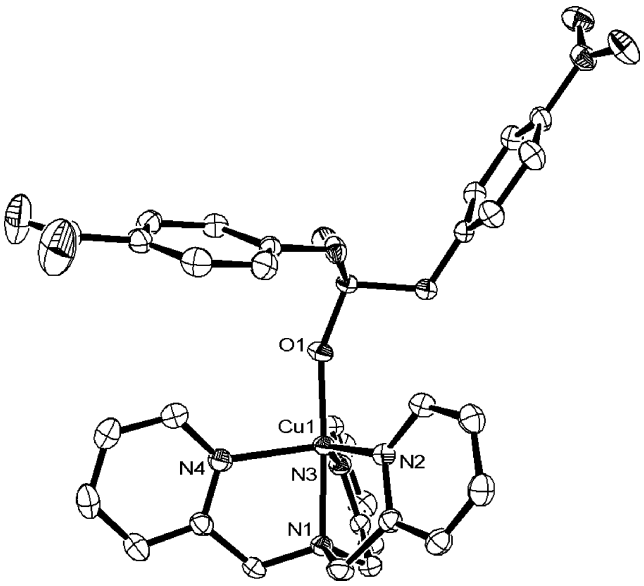


Figure 42. Structure (50% probability ellipsoids) of the cationic portion of [Cu(tpa)(BNPP)]ClO_4 in the solid state. Hydrogen atoms are omitted for clarity. Reprinted with permission from ref 176a. Copyright 2003 American Chemical Society.

Adams et al. reported a 2:1 complex of $[Zn(tpa)]^{2+}$ and BNPP.[179] The ability of this metal complex to bind imidazole is demonstrated by the structure of the 2:1 complexation of $[Zn(tpa)]^{2+}$ and deprotonated imidazole.[180] Each of the two zinc atoms is pentacoordinated by four nitrogen atoms from TPA and one nitrogen atom from bridging imidazolate ligands in an approximately trigonal bipyramidal structure. The distance between the two zinc atoms in the Zn−Im−Zn moiety is 6.078 Å.

Fe(III) complexes of TPA were found to crystallize as an oxo-bridged dimer (Figure 43).[181] An interesting substrate which can bind to Fe(III)−TPA complexes is dithiolate 1,2-benzenethiol (=H_2dtc) (Figure 44).[182] Next to complexes with diphenyl phosphates[183] and acetates,[184] structures with natural amino acids and dipeptides were investigated.[185]

6.2. Recognition Processes in Solution[186]

6.2.1. Cysteine Binding

Holwerda and co-workers examined the complexation of $[Cu(tpa)]^{2+}$ with cysteine.[187] An intense, symmetric absorption band with λ_{max} at 396 nm was observed upon mixing cysteine with $[Cu(tpa)]^{2+}$ throughout the pH range 4.0−6.0. Stopped-flow studies at 396 nm indicate that complexation was complete within the mixing time (3 ms), even at low cysteine concentrations. Excellent linear correlations of absorption changes and cysteine concentration were found, indicating the exclusive formation of a 1:1 cysteine−$[Cu(tpa)]^{2+}$ adduct. The formation constant (K_f) was calculated to be $K_f = (1.21 \pm 0.06) \times 10^2$ M^{-1}. The pH dependence of K_f is consistent with the formation of a thiolate sulfur−Cu(II) bond:

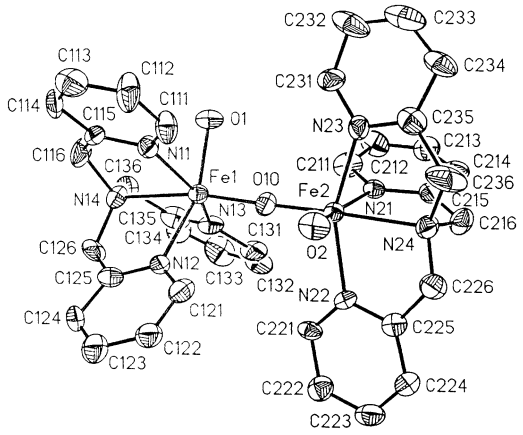


Figure 43. Structure of the dinuclear cation in $[\{Fe(tpa)(H_2O)\}_2O](ClO_4)_4 \cdot 3H_2O \cdot 0.25CH_3CH_2OH$ in the solid state. Reprinted with permission from ref 181. Copyright 1999 Elsevier Science B.V.

$$\text{Cys}-\text{SH} + \text{Cu(tpa)}^{2+} \rightleftharpoons [(\text{tpa})\text{Cu}-\text{S}-\text{Cys}]^{+} + \text{H}^{+} \quad (3)$$

6.2.2. Phosphate Binding

As known from X-ray structure analyses, $[Zn(tpa)]^{2+}$ has the ability to coordinate phosphates.[178,179] In 2003, Mareque Rivas et al.[188] reported $^{31}P\{^1H\}$ NMR titration experiments of dibenzyl phosphate (=dbp) with $[Zn(tpa)]^{2+}$ in D_2O at pD 7.4. The addition of increasing amounts of the Zn(II) complex to solutions of DBP caused a progressive upfield shift of the ^{31}P signal relative to free DBP. During these studies, other ligands with additional amino groups were used.

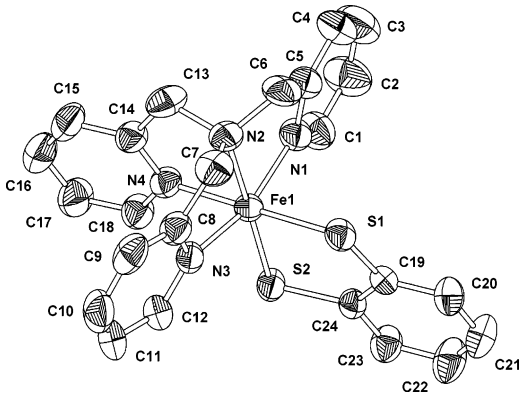


Figure 44. Structure of the cation in $[Fe(tpa)(dtc)]ClO_4 \cdot 0.25$ MeOH. Reprinted with permission from ref 182a. Copyright 2003 Wiley-VCH.

The additional electron donating amino groups do not participate in metal but form hydrogen bonds to the coordinated phosphate ion, as confirmed by the X-ray structure analysis of $[Zn(bpapa)]^{2+}$ (bpapa is a tpa with one additional amino groups; see Figure 45). Supported by this

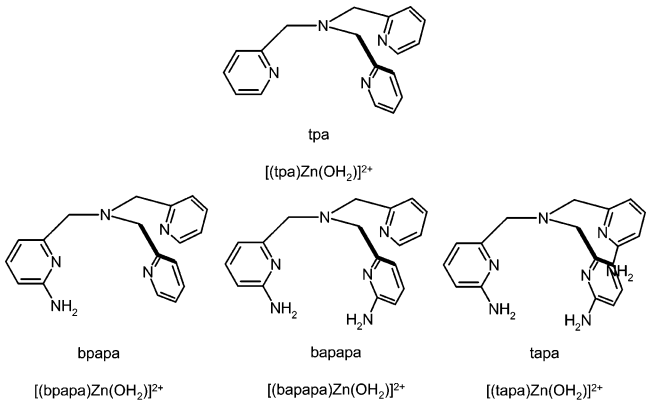
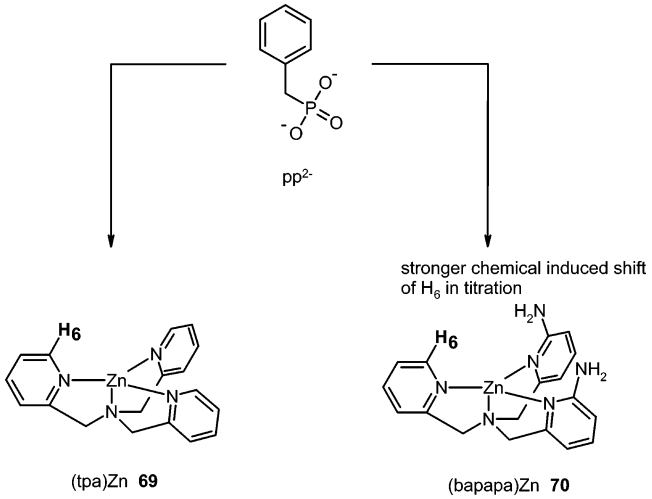


Figure 45. TPA ligands with additional amino groups.[188]

additional interaction, the binding of the phosphate ion to the BPAPA complex is stronger than the binding to the TPA complex. This effect was also observed in binding studies of phenyl phosphate (PP^{2-}) to the metal complexes **69** and **70** (Scheme 20).[189]

Scheme 20. A Larger Chemical Induced Shift of Proton H_6 of Complex 70 Compared to Complex 69 (1 mM in D_2O) Is Observed upon Addition of Increasing Amounts of Phenyl Phosphate at pH 7 ± 0.1 (50 mM HEPES)



A related system binds phosphate ions supported by additional interactions to guanidinium moieties.[190] The chemosensor developed in this study takes advantage of an indicator-displacement assay comprised of metallohost **71** and 5(6)-carboxyfluorescein (Scheme 21).

Upon addition of aliquots of **71** to a solution of 5(6)-carboxyfluorescein, binding proceeds in a 1:1 stoichiometry with a color change from yellow to light orange. Added aliquots of a phosphate solution change the light orange color of the host−dye complex into the yellow color of free dye in solution. This indicates that the phosphate ion displaces the dye from the host cavity.

Table 14. Typical Binding Constants of Dien Metal Complexes at 25 °C

ion	equilibrium	log *K*
Co^{2+}	$ML/M{\cdot}L$	8.0
	$ML_2/M{\cdot}L^2$	13.9
Ni^{2+}	$ML/M{\cdot}L$	10.5
	$ML_2/M{\cdot}L^2$	18.6
Cu^{2+}	$ML/M{\cdot}L$	15.9
	$ML_2/M{\cdot}L^2$	20.9
Zn^{2+}	$ML/M{\cdot}L$	8.8
	$ML_2/M{\cdot}L^2$	14.3
Cd^{2+}	$ML/M{\cdot}L$	8.05
	$ML_2/M{\cdot}L^2$	13.84

6.3. Recognition Processes on Solid Surfaces

Until now, no applications are reported using immobilized TPA complexes for binding of guest molecules on solid surfaces.

7. Diethylenetriamine (Dien) Complexes

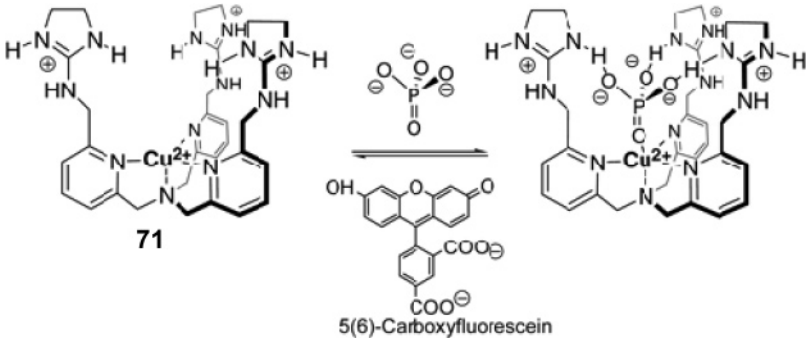
Known since 1959, this ligand was intensively used by many research groups to examine its complexation properties.[191] Most studied are complexes with Cu(II),[192] Ni(II),[193] Zn(II),[194] Co(III),[195] Pd(II),[196] Fe(III),[197] and Pt(II) (Table 14).[198]

7.1. Structures of Dien Complexes in the Solid State

In the following, we discuss the solid-state structure of some typical Dien complexes, which have coordinated additional Lewis basic guests.

Reactions of Dien based ligands bearing a tethered amine ligand (e.g. abba = *N*-(4-aminobutyl)-*N*,*N*-bis(2-aminoethyl)-amine) with Cu(II) ions gives complexes in which the tripodal ligand coordinates to the Cu(II) ion in a hypodentate fashion, with the longest arm remaining protonated and unbound.[199] The crystal structure of $[Cu(abbaH)Cl_2]Cl{\cdot}H_2O{\cdot}CH_3OH$ reveals a five-coordinate Cu(II) ion in a slightly distorted square pyramidal geometry (Figure 46).[200]

Scheme 21. Assembly for Phosphate Ion Detection Using an Indicator-Displacement Assay



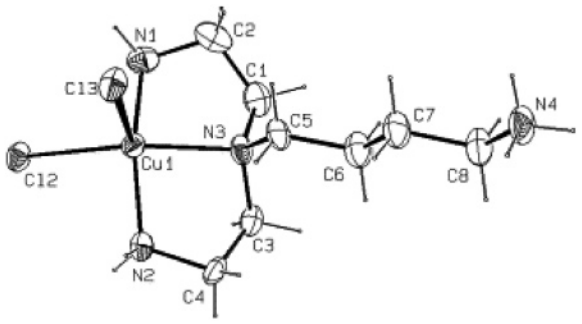


Figure 46. Structure of $[Cu(abbaH)Cl_2]^+$ in the solid state. Reprinted with permission from ref 200a. Copyright 2004 Blackwell Publishing.

The formation of a 2:1 Dien:Cu(II) complex was studied by Garcia-Granda et al.[201] In this complex Cu(II) adopts a 4+2 distorted octahedral environment. The affinity to oxygen atoms is show by the formation of a blue 2:1 $[Cu(Dien)]^{2+}$: oxalato complex (Figure 47).[202]

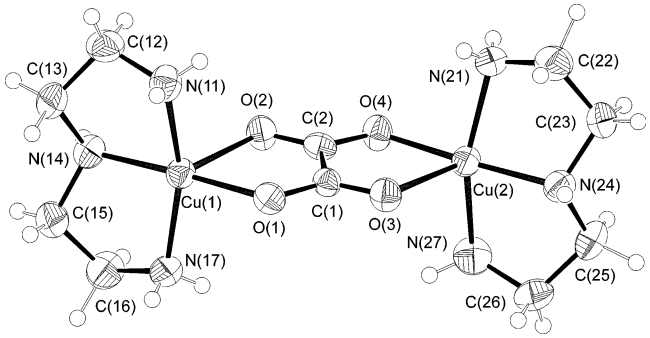


Figure 47. Structure of $[\{Cu(Dien)\}_2(ox)]^{2+}$ in the solid state. Reprinted with permission from ref 202a. Copyright 1999 Elsevier Science B.V.

The metal complexes $[Cu(Dien)(2\text{-}PhIm)(ClO_4)]^+$ and $[Cu(Dien)(2\text{-}MeBzIm)(ClO_4)]^+$ (Figure 48) are interesting, because they resemble the Cu(II) active site in many metalloproteins containing binuclear metallosites with a square pyramidal geometry and a weakly bound additional ligand.[203] Structural data for both complexes support geometries between square planar and square pyramidal and a weak axial contact of a perchlorate anion.

Interactions between $[(Dien)Cu(ONO_2)]NO_3$ and guanosine and adenine were observed and examined by Bolos.[204] The blue crystals formed by $[(Dien)Cu(Guo\text{-}N^7)(ONO_2)]NO_3$ show a distorted square pyramidal coordination sphere around the Cu(II) ion (Figure 49). The incorporation of two Dien units in a macrocyclic multidentate ligand results in a complex which is able to bind 4,4′-bipyridine in its cavity (Figure 50).[205] Several macrocyclic ligands bearing only one Dien unit were investigated over the last few years.[206]

Surrounded by two Dien ligands, the $[Ni(Dien)_2]^{2+}$ cation adopts a coordination geometry which is best described as octahedral (Figure 51).[207] The conformation of the complex is s-facial.

The oxophilic character of the Ni(II)−Dien complex is demonstrated by complex formation with an oxalato ion.[208] In the 2:1 complex of $[Ni(Dien)]^{2+}$ and the oxalate ligand, each Ni(II) cation is surrounded by a distorted octahedral complexation sphere.

In the presence of $[Fe(CN)_6]^{4-}$ anions and water molecules, a $[Zn(Dien)_2]^{2+}$ cation is obtained.[209] The coordination geometry of the Zn(II) ion is distorted octahedral. Dinuclear complexes consisting of two $[Zn(Dien)]^{2+}$ units are capable of binding butanol and form a complex with azides.[210] Each Zn(II) ion is coordinated by a Dien ligand and the phenylic oxygen, reaching pentacoordination. The coordination polyhedron around the Zn1 atom can be described as a trigonal bipyramid, while a square pyramid arrangement is found around Zn2 (Figure 52). The binding properties of bis-zinc and bis-copper complexes of this type to small anions and di-aza hetereocycles were investigated by potentiometric titration. Only imidazole is coordinated by the zinc and the copper complex, while pyrazoles and pyridazine show affinity to the copper complex only.

The reaction of $[Pd(Dien)]^{2+}$ with isocytosine results in the formation of yellow crystals.[211] Pd(II) is coordinated through the N3 position of the isocytosine in a distorted square planar geometry (Figure 53).

Seven isomers of a Co(III)−Dien−2-(aminomethyl)-pyridine (ampy) complex are possible. Five (three *mer*- and two *fac*-) of the seven possible isomeric forms of $[Co(Dien)(ampy)Cl]^{2+}$ were isolated, and their structures were determined by single-crystal X-ray diffraction.[212] Similar results were obtained from complexation studies of $[Co(Dien)Cl]^{2+}$ with 2-amino-2,2-dimethylpropylamine.[213]

Ru(III)−Dien diolato complexes coordinate sugar molecules.[214] From aqueous solutions of *trans*-dichloro-*mer*-diethylentriamine-nitrosyl ruthenium(III) salts, Klüfers obtained diolato complexes of ethylendiol, (*R,R*)-cyclohexane-1,2-diol, anhydroerythritol, and methyl-β-D-ribofuranoside.[214a]

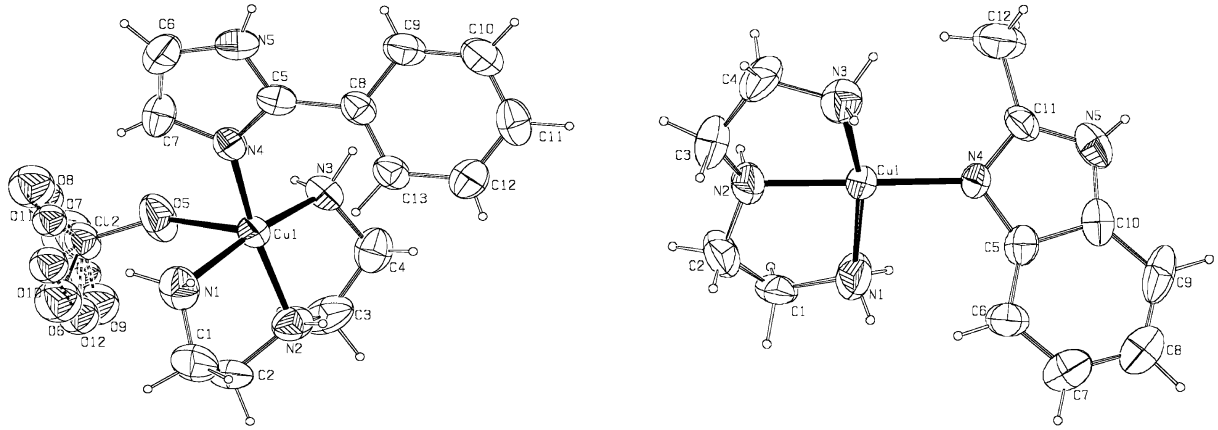


Figure 48. Structure of $[Cu(Dien)(2\text{-}PhIm)(ClO_4)]^+$ and $[Cu(Dien)(2\text{-}MeBzIm)(ClO_4)]^+$ in the solid state. Reprinted with permission from ref 203a. Copyright 2005 Elsevier Science B.V.

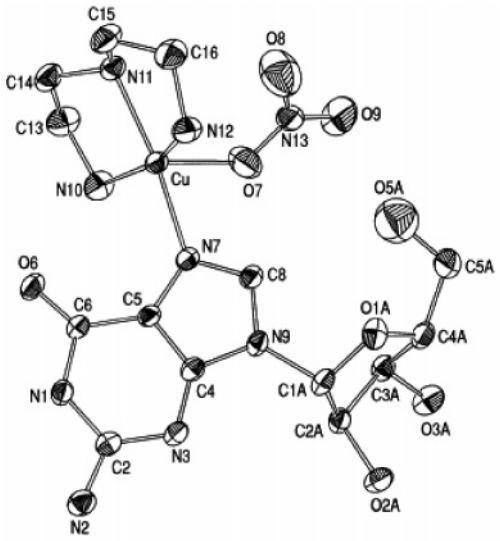


Figure 49. X-ray structure of [(Dien)Cu(Guo-N^7)(ONO_2)]NO_3. Reprinted with permission from ref 204. Copyright 1998 Elsevier Science B.V.

The red crystals of [*mer*-(Dien)(NO)Ru(Me-β-D-Rib*f*2,3H_{-2})]-BPh_4·5.5H_2O (Figure 54) reveal an octahedral coordinated Ru(III) center. ^{13}C NMR studies of this complex showed a

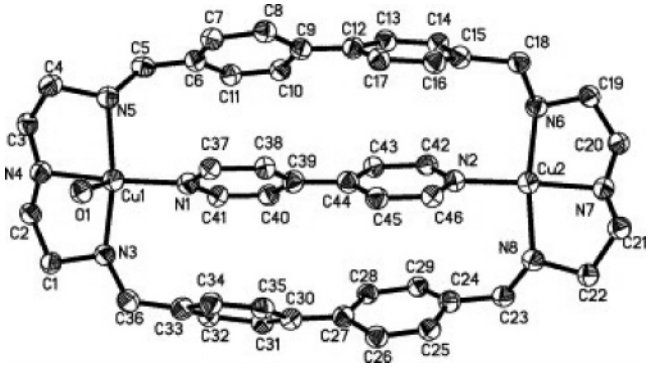


Figure 50. Structure of [Cu_2L(4,4′-bpy)(H_2O)](ClO_4)$_4$·3H_2O in the solid state. Reprinted with permission from ref 205a. Copyright 2005 Elsevier Science B.V.

coordination-induced downfield shift of the resonance signals of the diol carbon atoms which bear metal-binding oxygens.

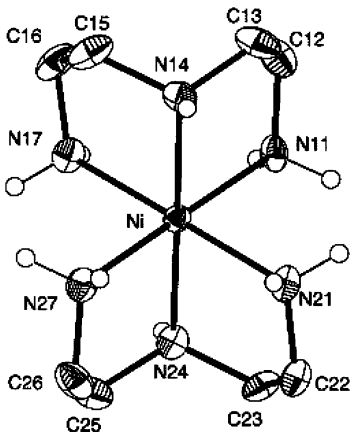


Figure 51. Structure of $[Ni(Dien)_2]^{2+}$ in the solid state. Reprinted with permission from ref 207a. Copyright 2005 Elsevier Science B.V.

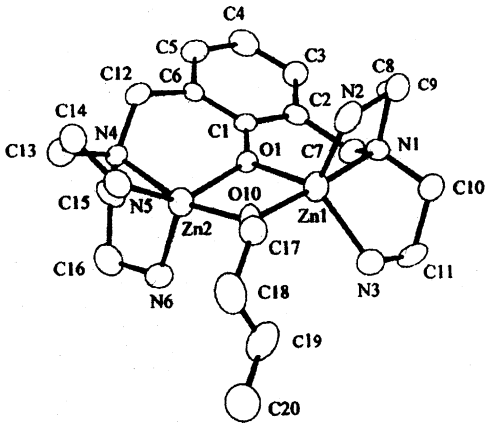


Figure 52. X-ray structure of [Zn_2(H_{-1}L)(butanolat)(ClO_4)$_2$]. Reprinted with permission from ref 210. Copyright 2001 American Chemical Society.

7.2. Recognition Processes in Solution

7.2.1. Imidazole Binding in Solution

Combinations of Dien and Tren metal complexes with a bridging imidazole ligand were used to mimic the active center of superoxide dismutase.[215] To understand the important role of the imidazolate ligand in copper(II)−

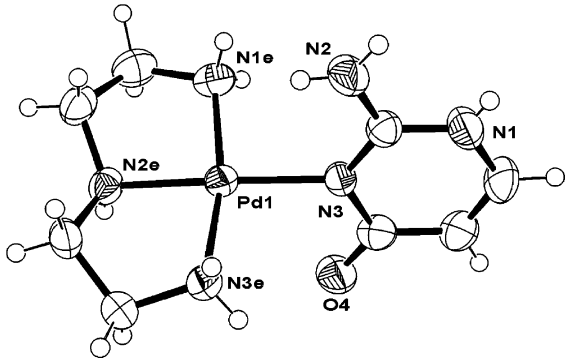


Figure 53. Structure of [(Dien)Pd(ICH-*N*3)](NO_3)$_2$ in the solid state. Reprinted with permission from ref 211. Copyright 2004 American Chemical Society.

diethylenetriamine(**A**)−μ-imidazolate(**B**)−zinc(II)−tris(2-aminoethyl)amine(**C**) (**ACuBH**$_{-1}$**ZnC**) complexes, mixtures

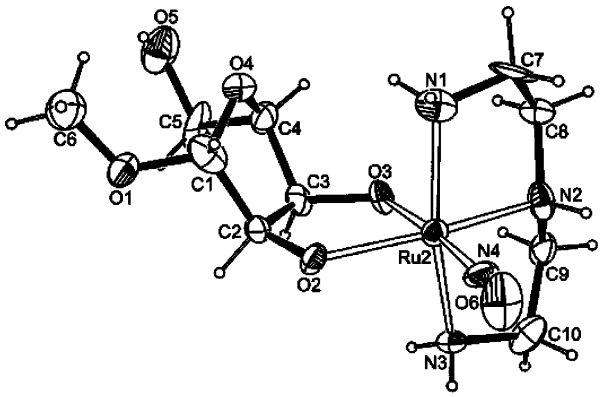


Figure 54. Structure of [*mer*-(Dien)(NO)Ru(Me-β-D-Rib*f*2,3H_{-2})]-BPh_4·5.5H_2O in the solid state. Reprinted with permission from ref 214a. Copyright 2005 Wiley-VCH.

of the metal ions, ligands, and imidazole were examined using potentiometric measurements.[216] To determine the

stability constants of this five-component system, all possible two- and three-component complexes were investigated separately (e.g. Cu(II)−**A**, Cu(II)−**B**, Cu(II)−**A**−**B**, etc.). With these data in hand, the five-compound system can be described. The metal to ligand concentration ratios were always 1:1. Above pH 7, the **ACuBH$_{-1}$ZnC** is the major species. EPR, UV/vis spectroscopy, and MS analysis supported these results. Measuring the superoxide dismutase activity of the system showed that the complex **ACuBH$_{-1}$ZnC** had higher activity than the copper(II) complexes from which it was prepared. The activity of all complexes was significantly smaller compared to that of the native enzyme. Furthermore, the interactions of Dien metal complexes and histidine containing peptides were investigated.[217] NMR studies of Pt(II)− and Pd(II)−Dien complexes with His-Ala and His-Gly-Ala showed binding of these complexes to N-terminal His.[218]

7.2.2. Nucleotide Binding

To understand the parameters leading to stable adducts of platinum amine complexes and DNA,[219] ^{15}N-labeled Dien complexes of Pt(II) were synthesized.[220] The NH groups on platinum amine ligands are expected to be important to form hydrogen bonds to DNA. [^{1}H,^{15}N] HSQC NMR was used to monitor the binding between 5′-GMP, as an initial model for DNA, and [Pt([$^{15}N_3$]Dien)Cl]$^{+}$ (Figure 55). The NH protons are found to be magnetically nonequivalent.

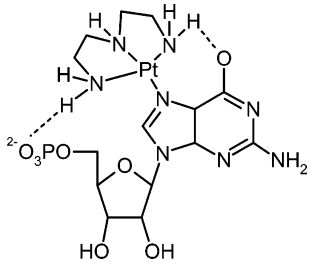


Figure 55. Complex formation between 5′-GMP and [Pt([$^{15}N_3$]-Dien)Cl]$^{+}$ in solution.

Additionally, NMR techniques were used to analyze the binding of [(Dien)Pd]$^{2+}$ to uracil and azauracils.[221] The role of the phosphate group for specific metal-fragment-nucleotide recognition by chelation was investigated in 2002 by Sheldrick.[222] The pH-dependences of interactions of Pd(II)−Dien and Pt(II)−Dien complexes with adenosine and guanosine 2′- and 3′-monophosphates at different molar ratios were determined by potentiometry and NMR. The results confirmed that intramolecular N−H O(phosphate) hydrogen bonding plays a decisive role in enhancing specific interactions of (amine)M(II) complexes with both purine nucleoside 2′- and 5′-monophosphates. In related work, the ability of Cu(II) triamine complexes to bind to adenosine 5′-monophosphate or cytidine 5′-monophosphate was studied.[223]

For the use of Cu(II)Dien complexes for carbohydrate binding, see section 3.2.2. The compounds are discussed with Cu(II)−IDA and related complexes, because they were all used in the same work.

7.2.3. Binding Processes in Oligo(ethylenediamino)-Bridged Bis-β-cyclodextrins

Natural cyclodextrins (CD) bind hydrophobic guests in water usually with affinities in the range of 10^2 to 10^4 M^{-1}. To enhance their binding ability, CD units can be tethered by an oligo(ethylenediamino) chain. Liu et al. investigated the binding of oligo(ethylendiamine) tethered β-CD to fluorescent dyes and found that Cu(II) ions coordinated to the oligo(ethylendiamine) tether enhance the binding ability and selectivity (Figure 56).[224,225] The affinity of several dyes

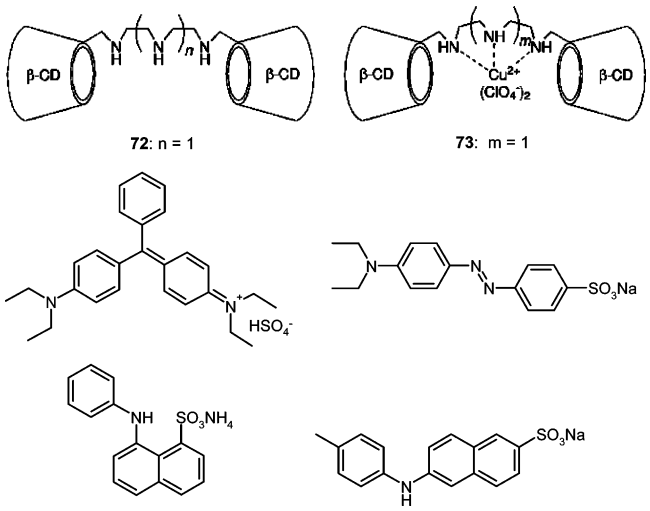
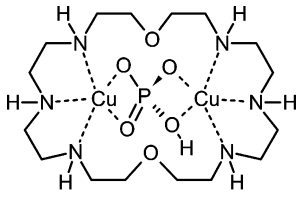


Figure 56. Cyclodextrins linked by a Cu(II)−Dien complex and fluorescent dyes, which bind to the complex. Parts reprinted with permission from ref 224. Copyright 2001 Wiley-VCH.

was determined by UV, CD, and emission titration. A good size/shape match of the bis-β-CD complex and the dye is crucial for the complex stability.

7.2.4. Binding with Bis-Dien Complexes

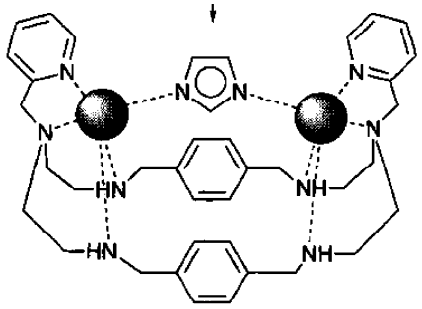
Macrocycles consisting of two Dien binding sites are able to coordinate two transition metal ions. The close proximity of two metal centers allows the binding of small anions, such as hydrogen phosphate, phosphates,[226] pyrophosphate,[227] glycinate, or malonate.[228] The binding of hydrogen phosphate to macrocylic Cu bis-Dien and bis-Cu bis-Dien complexes was investigated by potentiometric titrations in detail (Figure 57). Similar studies examined the binding of malonate and glycinate to Co(III) complexes of the bis-Dien ligand.



74

Figure 57. Macrocyclic bis-Dien bis-copper(II) complex **74** binding hydrogen phosphate.

Related bis-Dien bis-copper complexes bind imidazole as bridging ligand between the two Cu(II) ions with the simultaneous extrusion of a proton.[229,230] Fabbrizzi modified the macrocycle by *p*-xylyl spacers and two 2-picolyl arms (Figure 58).[231] Each pendant arm saturates the coordination sphere of a Cu(II) center, preventing the coordination of additional imidazole fragments. The coordinating property of the octadentate ligand in aqueous solution was investigated by pH titration. The 1:1 binding between Cu(II)−bis-Dien was additionally verified by spectrophotometrical titrations, and a binding constant of log $K = 4.7 \pm 0.1$ was derived. Similar results were obtained for histamine (log $K = 4.3 \pm 0.1$) and L-His (log $K = 5.5 \pm 0.1$).



75

Figure 58. Fabbrizzi's macrocyclic bis-Cu(II) bis-Dien complex **75** binding imidazolate. Reprinted with permission from ref 231. Copyright 1995 Royal Society of Chemistry.

The bis-Cu(II) complex of a similar bis-Dien ligand **76** was use by the same group for luminescent sensing of amino acids (Figure 59).[232] Indicator displacement allowed the selective sensing of His in the presence of other amino acids, including Ala, Phe, Leu, Pro, and Gly.

The titration of each indicator with $[Cu_2(\mathbf{76})]^{4+}$ at pH = 7 resulted in a complete quenching of its emission. Aggregates were found to have a 1:1 stoichiometry and milli- to micromolar binding constants (cumarine, log $K = 4.5$; fluorescein, log $K = 5.9$; eosine Y, log $K = 7.2$). After these measurements, each receptor/indicator pair was titrated with His, Ala, Phe, Leu, Pro, plus Gly.

The $[Cu_2(\mathbf{76})]^{4+}$/cumarine aggregate does not discriminate between His and Gly. Both amino acids displace the indicator equally and restore its full emission. The fluorescein indicator complex shows a different response to His and Gly, which is even more pronounced with the $[Cu_2(\mathbf{76})]^{4+}$/eosine Y system. This indicator complex interacts with His only and selectively signals the presence of the amino acid.

Bis-Dien complexes bridged by pyrazole units were used to bind dopamine (Figure 60).[233] The formation of mixed complexes Cu(II)−L-dopamine was monitored by potentiometric titration experiments. The model fitting best the experimental data suggests the formation of ternary complexes with Cu(II):ligand:L-dopamine in 2:1:1 stoichiometries. Depending on the pH of the solution, L-dopamine has a binding constant of log $K = 3.6$ (at pH = 2.5−4.5, log $K = 3.9$ (at pH = 5−7) or log $K = 4.0$ (at pH > 8.5).

Table 15. Typical Binding Constants of Some Tren Transition Metal Complexes at 25 °C

ion	equilibrium	log K
Fe^{2+}	ML/M·L	8.7
Co^{2+}	ML/M·L	12.7
Ni^{2+}	ML/M·L	14.6
Cu^{2+}	ML/M·L	18.5
Zn^{2+}	ML/M·L	14.5
Cd^{2+}	ML/M·L	12.3[a]

[a] $T = 20$ °C.

7.3. Recognition Processes on Solid Surfaces

Using a molecular imprint approach (MIP), Striegler et al. immobilized [Cu(styDien)]$(HCOO)_2$ (**34**) (see section 3.2.2 for binding properties) by polymerization to achive selective binding of carbohydrates between 5.5 < pH < 7.5.[234,235] In homogeneous aqueous solution, the complex binds carbohydrates only at alkaline pH of 12.4. Rebinding studies under physiological conditions revealed a selective affinity for glucose over other hexoses after complex immobilization by MIP.

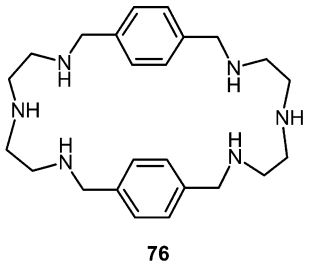
Expanding the research, disaccharide imprinting and binding was investigated by the same group.[236] The epimeric disaccharides D-lactulose and D-lactose were chosen to prove the concept. After complexation studies in solution, the imprinted polymers of both disaccharides were prepared and examined for their ability to rebind the imprinted disaccharide. The rebinding competition experiments were conducted at neutral pH with an excess of equimolar mixtures of the two epimers D-lactulose and D-lactose. The concentration of unbound carbohydrate in solution was determined by HPLC, showing high selectivity and moderate capacity of both templated polymers for rebinding of the original templates.

8. Tri(2-aminoethyl)-amine (Tren) Complexes

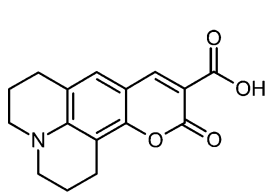
Tren complexes of various metals have been known since 1959.[191] Among all reported complexes, Cu(II),[237] Cu(I),[238] Ni(II),[239] Zn(II),[240] Co(III),[241] Rh(III),[242] Fe(III),[243] and Cr(III)[244] complexes have received the most attention (Table 15).

8.1. Structures of Tren Complexes in the Solid State

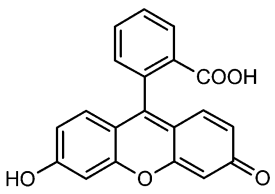
The complexation properties of Cu(II)−Tren complexes to heteroaromatic amine bases were studied in detail.[245−247]



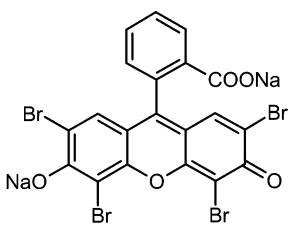
76



Coumarine 343



Fluorescein



Eosine Y

Figure 59. Ligand **76** and fluorescent indicator dyes used for amino acid sensing.

The structure of a [Cu(II)(Tren)(imidazole)]$^{2+}$ complex (Figure 61), a typical example, shows a distorted trigonal bipyrimidal geometry.[245]

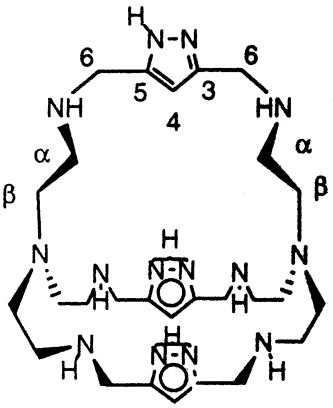


Figure 60. A capped bis-Dien ligand for dopamine binding. Reprinted with permission from ref 233a. Copyright 2001 American Chemical Society.

The same coordination geometry was reported for the complexation of acetylhistamine and methylimidazole.[248] Using imidazole as a bridging ligand, Lu and co-workers prepared [Cu(Tren)(im)Zn(Tren)](ClO$_4$)$_3$·MeOH (Figure 62).[249]

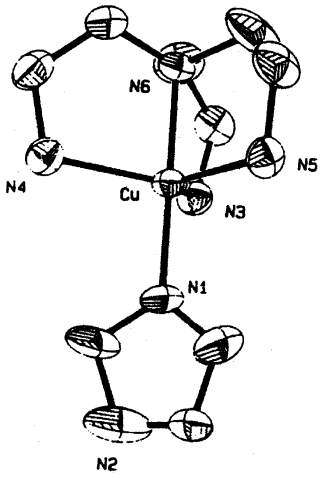


Figure 61. Molecular structure of [(C$_6$H$_{18}$N$_4$)Cu(C$_3$H$_4$N$_2$)]$^{2+}$ in the solid state. Reprinted with permission from ref 247. Copyright 1996 American Chemical Society.

Cu(II)−Tren complexes with coordinated nucleobases have been reported.[250] By coordinating the Lewis basic nitrogen of adenine, the 1:1 complex [Cu(Tren)(adeninato)]$^+$ is formed (Figure 63).[251] The trigonal bipyramidal Cu(II) ion

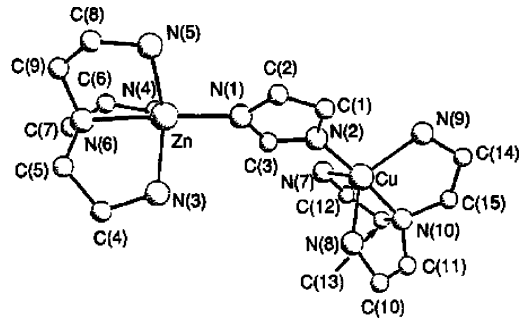


Figure 62. X-ray structure of [Cu(Tren)(im)Zn(Tren)](ClO$_4$)$_3$·MeOH. Reprinted with permission from ref 249. Copyright 1990 Royal Society of Chemistry.

is capped by a tripodal tetradentate Tren ligand, with the equatorial sites occupied by the three primary amino nitrogen atoms and the axial position occupied by the tertiary amino nitrogen. The adenine anion is bound through the axial position, supported by the formation of a hydrogen bond to the ligand. Each adenine moiety forms several hydrogen bonds, which creates a sheet structure with an infinite adenine−adenine zigzag molecular array.

The deep blue Ni(II) complex of Tren shows an octahedral coordination sphere.[252] Different coordination modes of a carbonate ligand are found in the 3:1 complex of Ni(II)−Tren and a μ_3-CO$_3^{2-}$ ligand, [Ni$_3$(Tren)$_3$(H$_2$O)$_2$(μ_3-CO$_3$)](ClO$_4$)$_4$·2H$_2$O.[253] Similar metal complexes were reported for Cu(II) and Zn(II).[254]

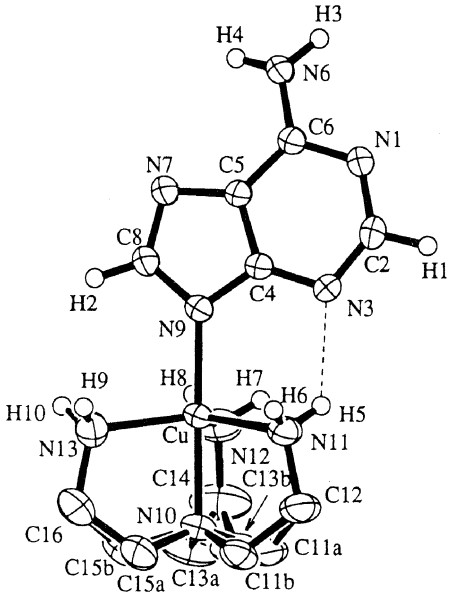


Figure 63. Molecular structure of [Cu(Tren)(adeninato)]$^+$. Reprinted with permission from ref 251a. Copyright 2001 Elsevier Science B.V.

The 1:1 complexation of amino acids was reported by Cai et al.[255] They examined the homochiral−heterochiral assembly processes of racemic mixtures of DL-valine, DL-phenylalanine, and DL-leucine and isolated the trans(N, *t*-N) isomer of [Ni(Tren)(DL-valinato)](Cl)·2H$_2$O, [Ni(Tren)(DL-phenylalaninato)](Cl)·3H$_2$O, and [Ni(Tren)(DL-leucinato)](Cl)·2H$_2$O. In all crystals the tertiary N-atom of the Tren ligand is *trans* to the N-atom of the amino acidato ligand in the coordination sphere.

Another substrate binding to this Ni(II) complex is dulcit.[256] The isolated metal μ-dulcitolato-O$^{2,3;4,5}$−Ni(II)−Tren complex [(Tren)$_2$Ni$_2$(Dulc2,3,4,5H$_{-4}$)]·20 H$_2$O is surrounded by a three-dimensional network of water molecules. The use of methylated Me$_3$Tren ligands instead of Tren leads to nearly the same structure. Amine substitution of the ligand is possible without significantly changing its coordination properties, which discloses opportunities to incorporate the ligand in more extended molecules.

The formation of [Ni(Tren)(9-ethylguanine-0.5H)-(H$_2$O)]$_2$·(ClO$_4$)$_{2.5}$·(ClO$_3$)$_{0.5}$[251] demonstrates the ability of the complex to coordinate to Lewis basic nitrogen atoms of nucleobases.

Zn(II) complexes of Tren are reported as {[Tren]Zn-(HOMe)}·ClO$_4$·BPh$_4$[257] and as a perchlorate-bridged tetranuclear Zn(II) structure {[Zn(Tren)]$_4$(μ_4-ClO$_4$)}$^{7+}$.[258] A simple Zn(II)−Tren complex with slightly distorted trigonal bipyr-

amidal configuration coordinating one water molecule is shown in Figure 64.[259] The water molecule can be displaced

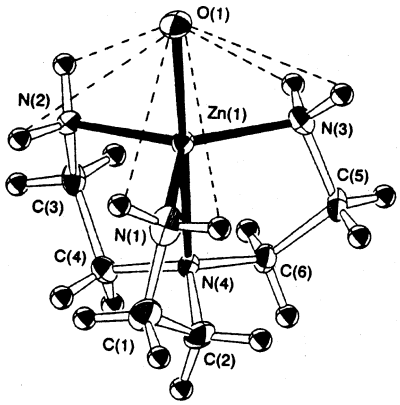


Figure 64. Molecular structure of $[Zn(Tren)(H_2O)]^{2+}$. Reprinted with permission from ref 259. Copyright 2001 Elsevier Science B.V.

by a 4-nitrophenyl phosphate anion acting as a monodentate ligand. The X-ray structure of the trigonal bipyramidal Zn(II)−Tren complex $[Cu(1\text{-benzylimidazole})(Tren)](ClO_4)_2$ coordinating 1-benzylimidazole in the axial position has been reported.[260]

The structure of p-$[Co(Tren)(NH_3)Cl]$-$S_2O_6 \cdot H_2O$ with an ammonia and a chloride ligand completing the distorted octahedral coordination sphere of the cobalt ion was reported by Chun.[261] Similar to the Ni(II) complexes of Tren, the Co(III) analogues coordinate amino acids as bidentate ligands.[262] X-ray structures are reported for *trans*-(N,t-N)-$[Co(Tren)(DL\text{-leucinato})]^{2+}$ and *trans*-(N,t-N)-$[Co(Tren)(DL\text{-methioninato})]^{2+}$. *trans*-(N,t-N) describes the geometric isomer in which the tertiary nitrogen atom of Tren is located *trans* in the coordination sphere to the amide nitrogen of the amino acid ligand.

Thioacetate as ligand is found in a Co(III)−Tren complex of the composition $[Co(CH_3\text{-}COS)_2(Tren)]^+$.[263]

8.2. Recognition Processes in Solution

8.2.1. Binding of Nucleotides

Torres et al. examined the different complexation properties of 5′-, 3′-, or 2′-nucleotides toward Rh(III)−Tren complexes.[264] An aqueous solution of $[Rh(Tren)(H_2O)_2]^{3+}$ (0.056 M) and adenosine in a 1:1 ratio was brought to 60 °C. After 48 h, essentially no evidence of reaction was observed by ^1H NMR. On the other hand, guanosine and inosine both react rapidly with $[Rh(Tren)(H_2O)_2]^{3+}$ ($t_{1/2} < 2$ h) under these conditions to form complexes. In contrast to adenosine, 5′-AMP does react with the Rh(III)−Tren complex. Further investigation revealed the important role of the 5′-phosphate group for the complexation process. In complexes of 3′- and 2′-AMP, participation of phosphate in the complexation process is weak. With these results, Torres concluded that the reported metal complex $[Rh(Tren)(H_2O)_2]^{3+}$ selectively binds adenine nucleobase only when it is a constituent of a 5′-nucleotide. In related studies, GMP and ApG binding to Ni(II)−Tren complexes was investigated.[265,266]

8.2.2. Binding of Carboxylates

The development of receptors for different organic carboxylates based on Tren ligands turned out to be challenging.[267] The Tren subunit typically shows a trigonal bipyramidal structure of the complexed metal center. The axial position opposite to the tertiary amine group is vacant and available for the coordination of an anion. Fabbrizzi et al. extended the core ligand by an anthracenyl substituent (**77**; Figure 65) or *N*,*N*-dimethyl-4-amino benzyl groups to obtain

Table 16. Binding Constants and Emission Quenching of Carboxylates to [Zn(78)(anion)]$^+$

$$[Zn(\mathbf{78})]^{2+} + X^- \rightleftharpoons [Zn(\mathbf{78})(X)]^+$$

anion	log *K*	quenching[a]
benzoate	4.69 ± 0.04	yes
4-nitrobenzoate	4.43 ± 0.06	yes
9-anthracenoate	4.47 ± 0.04	yes
acetate	4.35 ± 0.08	no
cyclohexylcarboxylate	4.08 ± 0.08	no

[a] Anion binding induces partial quenching of the emission of the DMA fragment of **78**.

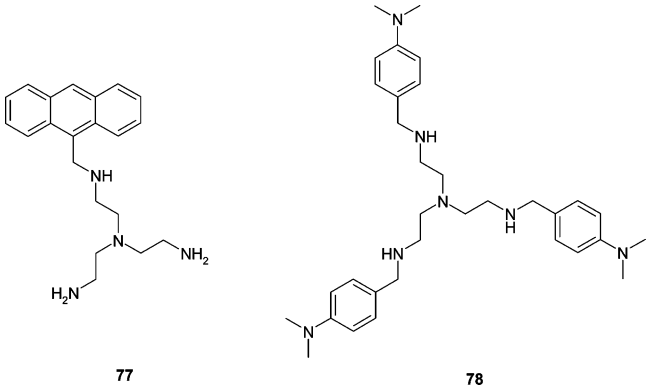


Figure 65. Structures of the Tren ligands **77** and **78**.

ligands for fluorescent Zn(II) carboxylate receptors.[268,269]

The Zn(II) complex of **77** binds aromatic carboxylates, but only those benzoates bearing either a donor or an acceptor substituent quench the anthracene emission.[270] Spectrofluorometric titration experiments of the related complex Zn(II)−**78** in MeOH indicated the formation of 1:1 adducts. The determined binding constants are summarized in Table 16.

Anslyn analyzed the cooperative effect of metal ion coordination and ionic interactions on the carboxylate binding of Cu(II)−Tren complex **79**.[271,272] The host complex was designed to provide a total of four binding sites (three ammonium groups and a metal center) to complement guests having negatively charged functional groups (carboxylates). Host−guest binding was expected to occur through the action of multiple complementary electrostatic interactions between functional groups of each of the binding partners. The binding of the different carboxylates (see Figure 66) to **79** was quantified by monitoring the absorbance changes of **79** upon carboxylate addition (Table 17). To uncover the enthalpic/entropic origin of the cooperativity, isothermal titration calorimetry (ITC) was used.

The affinity constants, and therefore the Gibbs free energies of binding, are comparable from both the UV/vis and ITC data. The trend is identical: acetate and glutarate binding affinities are similar and the tricarballate and 1,2,3,4-butanetetracarboxylate binding affinities are of the same order of magnitude. Additionally, there is a large increase in affinity when going from acetate or glutarate to tricarballate

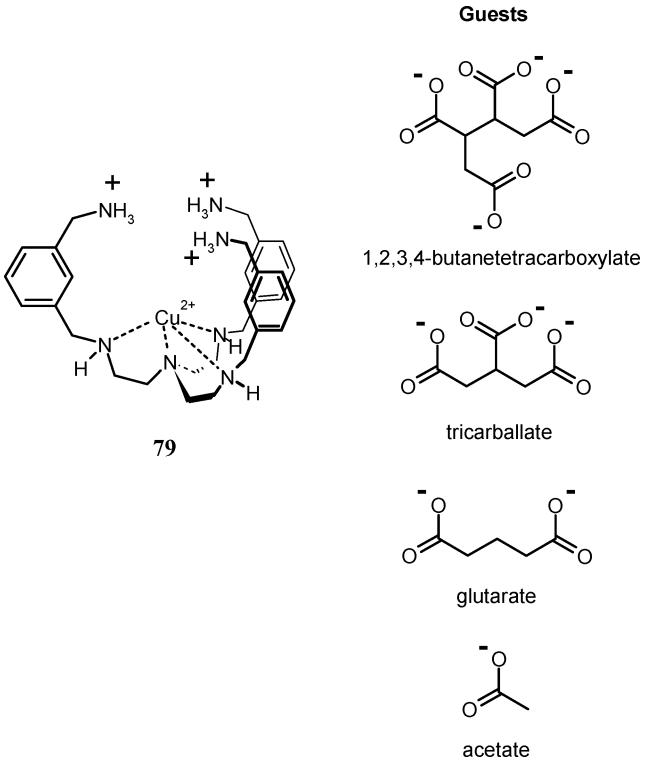


Figure 66. Metal complex **79** used for cooperative binding studies with carboxylates. Reprinted with permission from ref 271. Copyright 2003 American Chemical Society.

or 1,2,3,4-butanetetracarboxylate. The ammonium groups play an important role, as they add 2.5 to 3.2 kcal/mol in binding energy for potentially three ammonium−carboxylate interactions, but this total is less than the single coordinative interaction with Cu(II).

8.2.3. Binding of Imidazole and Amino Acids[273]

Moreton et al. examined simple Zn(II)−Tren complexes coordinating 1,2-diaminoethane, 1,3-diaminopropane, glycine, and L-alanine by ^{13}C NMR.[274] In 1997 Fabbrizzi and co-workers reported a luminescent sensor[275] for histidine based on a Zn(II)−Tren complex.[276] The octamine **80** (Figure 67) provides two quadridentate binding sites for Cu(II) or Zn(II) ions, leaving a free coordination site on each metal ion for an additional ligand. The anthracene fragment linking the tetraamine subunits may signal binding processes. Although the bis-Cu(II) complex is an excellent receptor for imidazole, it cannot function as a fluorosensor, since the Cu(II) ions fully quench the antrachene emission both in $[Cu(II)_2(\mathbf{80})]^{4+}$ and in $[Cu(II)_2(\text{imidazole})(\mathbf{80})]^{3+}$.

In the corresponding Zn(II) complex $[Zn(II)_2(\mathbf{80})]^{4+}$, the anthracene fluorescence revives. The formation of the imidazolate bridged complex $[Zn(II)_2(\text{imidazole})(\mathbf{80})]^{3+}$ induces a substantial fluorescence quenching. In particular, the

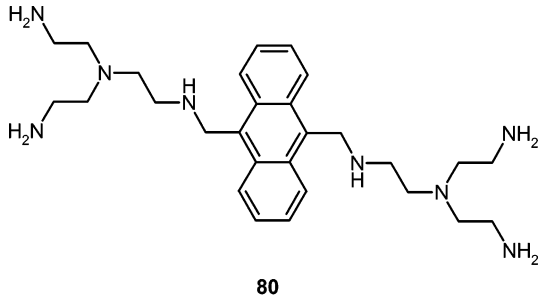
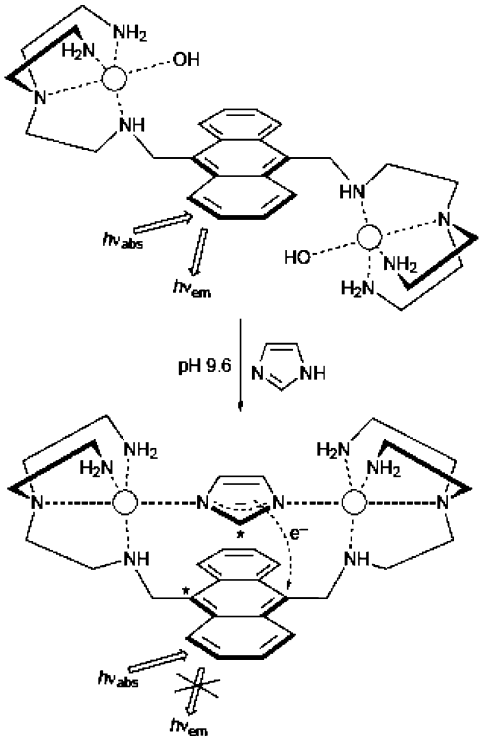


Figure 67. Octamine ligand **80**.

minimum of the anthracene emission intensity corresponds to the maximum concentration of $[Zn(II)_2(\text{imidazole})(\mathbf{80})]^{3+}$ at around pH 10. As emission quenching mechanism, the authors suggest an electron-transfer process from a π-orbital of the electron rich imidazolate anion to a π-orbital of the photoexcited anthracene fragment. The emission quenching that follows imidazolate binding opens the way to sense imidazole and molecules bearing an imidazole residue (Scheme 22). This was demonstrated by L-histidine, which shows a binding constant of $\log K = 2.9$.[277]

Scheme 22. Luminescent Bis-Zn(II) Sensor for Imidazole and Suggested Emission Quenching Mechanism[a]



[a] Reprinted with permission from ref 276. Copyright 1997 Royal Society of Chemistry.

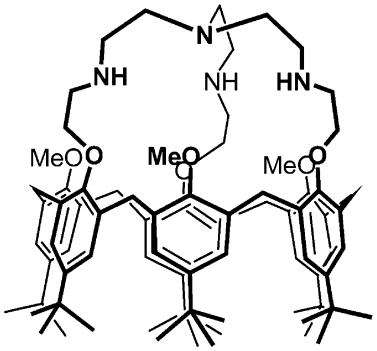
Table 17. Binding Constants and Thermodynamic Parameters of Carboxylate Binding to 79

guest	K_a, M^{-1} UV/vis	K_a, M^{-1} ITC	$\Delta G°$, kcal/mol UV/vis	$\Delta G°$, kcal/mol ITC	$\Delta H°$, kcal/mol	$T\Delta S°$, kcal/mol
1,2,3,4-butane tetracarboxylate	2.2×10^5 ($\pm 2 \times 10^4$)	1.8×10^4 ($\pm 1 \times 10^3$)	−7.3	−5.8	−0.29 (±0.01)	+5.4
tricarballate	9.0×10^5 ($\pm 4 \times 10^3$)	1.9×10^4 ($\pm 3 \times 10^3$)	−6.8	−5.8	−0.47 (±0.01)	+5.4
glutarate	2×10^3 ($\pm 2 \times 10^2$)	4×10^2 ($\pm 1 \times 10^2$)	−4.5	−3.6	+3.3 (±0.5)	+6.8
acetate	9×10^2 ($\pm 2 \times 10^2$)	3×10^2 ($\pm 1 \times 10^2$)	−4.1	−3.4	+0.7 (±0.5)	+4.1

The scope of Tren based receptors for binding and sensing of amino acids was extended by the same group to amino acids bearing aromatic residues.[278] Using a similar design as in ligand **78**, they prepared a substituted Tren ligand. The Zn(II) complex of the ligand combines metal−ligand interactions with π-stacking interactions of aromatic moieties of the ligand and on the coordinated amino acid. By this approach, tryptophane is bound exclusively, forming a 1:1 adduct, which is signaled by a strong fluorescence quenching.

8.2.4. Binding of Organic Guests by Calix[6]tren

A combination of metal complex coordination with additional hydrophobic binding sites was used by Reinaud et al. to design a calix[6]tren ligand (**81**; Figure 68) for synthetic



81

Figure 68. Structure of a calix[6]tren ligand **81**. Reprinted with permission from ref 280. Copyright 2005 American Chemical Society.

receptors.[279,280] This macrocyclic system has a size well-adapted for the selective inclusion of small organic molecules.

The metal free calix[6]tren ligand **81** already shows interesting affinities toward small organic ammonium ions, which were investigated by NMR. Complexation of the calix[6]tren with Zn(II) led to a funnel-shaped complex with the metal ion coordinated by the Tren cap (Scheme 23). Neutral molecules bind to the metal ion and are located in the hydrophobic calixarene pocket. Larger basic guests, such as imidazole or benzylamine, show affinity but lead to decoordination of Zn(II). No coordination of bulkier molecules such as phenylethylamine or 4-(dimethylamino)pyridine could be detected. This shows that the calixarene *funnel* controls the access to the metal center and selects suitable guest molecules by size.

Table 18. Relative Affinities of Ligands L toward $[Zn(calix[6]tren)]^{2+}$

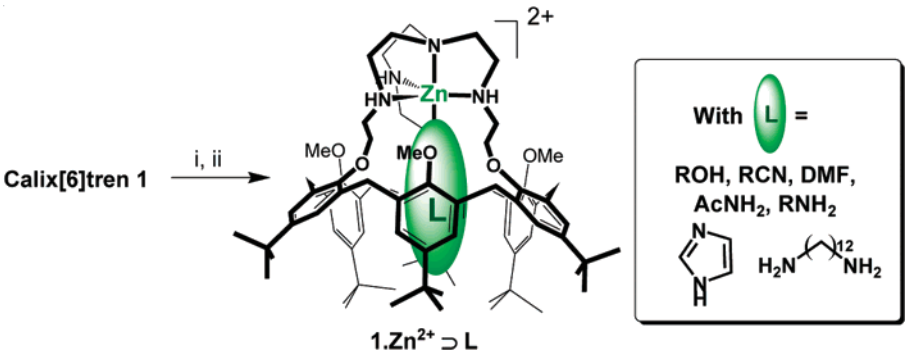
L	relative affinity
DMF	1
$AcNH_2$	0.39
EtOH	0.41
$PrNH_2$	1610
octylamine	0.95

The affinities of different ligands L to this receptor were determined by NMR and are summarized in Table 18. The relative affinity is defined as $[L_{in}]/[DMF_{in}] \times [DMF_T]/[L_T]$ and is measured at 293 K (the indexes "in" and "T" stand for "included" and "total amount", respectively).

8.2.5. Binding to Dimetal Bis-Tren Cages

Caged, bis-Tren cryptands (i.e. octamine cages consisting of two tripodal tetramine subunits covalently linked by given spacers) are able to incorporate first two metal ions and then an ambidentate anion, according to a cascade mechanism. This topic has been recently reviewed, and we refer interested readers to this overview by Fabbrizzi and cited references.[281,282] Recent developments in this area use an indicator displacement approach with receptor **82** (Figure 69), which is capable of distinguishing between dicarboxylates of the

Scheme 23. Preparation and Host−Guest Behavior of $[Zn(calix[6]tren)]^{2+}$ [a]



[a] (i) $Zn(ClO_4)_2(H_2O)_6$, DCM, MeOH; (ii) $CDCl_3$, L; calix[6]tren 1 = compound **81**. Reprinted with permission from ref 280. Copyright 2005 American Chemical Society.

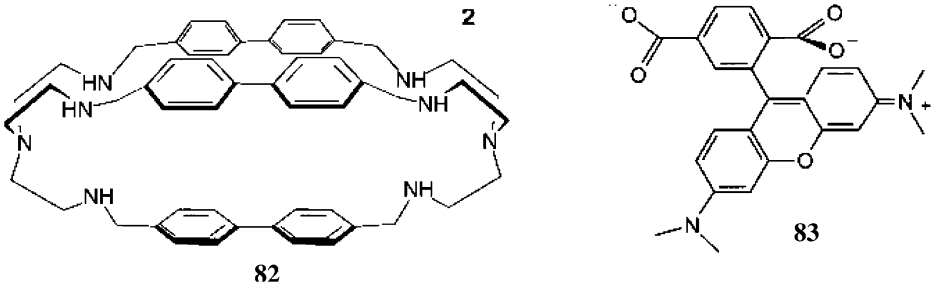


Figure 69. Bis-Tren cryptand **82** and fluorescence indicator **83**. Reprinted with permission from ref 283b. Copyright 2004 Wiley-VCH.

general formual $^{-}O_2C-(CH_2)_n-CO_2^{-}$ having different spacer lengths.[283]

8.3. Recognition Processes on Solid Surfaces

Only a few applications for the use of Tren ligands on solid surfaces are reported.[284] These applications can all be allocated to IMAC protein purification techniques, and interested readers are referred to recent reviews of this field.[64]

9. Conclusion

We have discussed in this review the use of coordinatively unsaturated transition metal complexes of six important acyclic N,O ligand classes as receptor binding sites in molecular recognition. From this selection of ligands, a number of metal complex receptor binding sites for functional groups of biological interest originated over the past decade. Transition metal complexes based on IDA, NTA, or BPA ligands are widely used for the recognition of N-terminal histidine and the imidazole group in the histidine side chain. Some NTA complexes show affinity to other amino acids or phosphate. Cu(II)−IDA complexes bind to hydroxyl groups of carbohydrates at basic pH. Bis-BPA and TPA complexes are good binding sites for phosphate anions. Depending on the structure, a selective binding of AMP, ATP, or pyrophosphate can be achieved. Transition metal complexes of the Dien and Trien ligands reversibly coordinate heterocyclic N-donor groups, e.g. of nucleobases or imidazole. Trien complexes serve as carboxylate binding sites. Although several impressive examples of luminescent chemosensors, peptide binders, or immobilization devices based on these ligands have been reported, the number of functional groups which can be selectively bound is still rather limted. Imidazole moieties and phosphate and carboxylate anions are selectively addressed with ease, but general metal complex binding sites for other important functional groups of biomolecules, such as thiol, hydroxyl, indol, or phenol moieties, are lacking.

The selectivity that can be achieved with metal complexes as binding sites is based on the specific binding properties of the complex, additional steric bias by substituents of the ligand, and, for di- and oligonuclear complexes, the geometrical arrangement of the metal centers. In some examples, impressive affinities and selectivities, even under physiological conditions, have been demonstrated. The recently introduced strategy of differential synthetic receptor arrays[285] can improve detection selectivites further. The receptor array shows a highly analyte specific pattern response, although the selectivity of each receptor site is limited.[286,287] The strategy mimics biological receptor response, and even similar proteins can be distinguished.

Overall, coordinatively unsaturated transition metal complexes are well established as binding sites for synthetic receptors. High binding constants to Lewis basic donor groups, even in water, make applications as probes for biomolecules particularly favorable. More examples of sophisticated luminescent chemosensors for peptides, proteins, or nucleotides based on metal complexes are foreseen for the next few years, while immobilization and differential receptor array strategies pave the way to sensor applications with very high detection selectivities.

10. Acknowledgments

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11. Supporting Information

Summary of all structures found in the Cambridge Structural Database where an IDA transition metal complex coordinates an additional complex ligand.[44] This material is available free of charge via the Internet at http://pubs.acs.org.

12. References

(1) (a) Rogers, C. W.; Wolf, M. O. *Coord. Chem. Rev.* **2002**, 341. (b) Pin, F.; Bernardo, M. A.; Garcia-Espana, E. *Eur. J. Inorg. Chem.* **2000**, *10*, 2143.
(2) (a) Armstrong, R. N. *Biochemistry* **2000**, *39*, 13625. (b) Costamagna, J.; Ferraudi, G.; Matsuhiro, B.; Campos-Vallette, M.; Canales, J.; Villagram, M.; Vargas, J.; Aguirre, M. J. *Coord. Chem. Rev.* **2000**, *196*, 125. (c) Holm, R. H.; Kennepohl, P.; Solomon, E. *Chem. Rev.* **1996**, *96*, 2239.
(3) (a) Ward, M. D. *Annu. Rep. Prog. Chem., Sect. A: Inorg. Chem.* **2002**, *90*, 55. (b) Borovik, A. S. *Comments Inorg. Chem.* **2002**, *23*, 45. (c) Amendola, V.; Fabrizzi, L.; Pallavicini, P. *Coord. Chem. Rev.* **2001**, *216*, 435.
(4) (a) Schmuck, C.; Geiger, L. *Chem. Commun.* **2004**, *15*, 1698. (b) Schmuck, C.; Geiger, L. *J. Am. Chem. Soc.* **2004**, *126*, 8898. (c) Nowick, J. S.; Chung, D. M.; Maitra, K.; Maitra, S.; Stigers, K. D.; Sun, Y. *J. Am. Chem. Soc.* **2000**, *122*, 7654. (d) Nowick, J. S.; Smith, E. M.; Ziller, J. W.; Shaka, A. J. *Tetrahedron* **2002**, *58*, 727. (e) Tsai, J. H.; Waldman, A. S.; Nowick, J. S. *Bioorg. Med. Chem.* **1999**, *7*, 29.
(5) Ait-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296.
(6) (a) Wright, A. T.; Anslyn, E. V. *Org. Lett.* **2004**, *6*, 1341. (b) Wright, A. T.; Anslyn, E. V.; McDevitt, J. T. *J. Am. Chem. Soc.* **2005**, *127*, 17405.
(7) (a) Smith, J. A.; Collins, J. G.; Patterson, B. T.; Keene, F. R. *Dalton Trans.* **2004**, *9*, 1277. (b) Rice, C. R.; Baylies, C. J.; Harding, L. P.; Jeffery, J. C.; Paul, R. L.; Ward, M. D. *J. Chem. Soc., Dalton Trans.* **2001**, *20*, 3039. (c) Goshe, A. J.; Crowley, J. D.; Bosnich, B. *Helv. Chim. Acta* **2001**, *84*, 2971. (d) Jurek, P. E.; Martell, A. E. *Inorg. Chem.* **1999**, *38*, 6003. (e) Wu, L.; Li, Y. *Anal. Chim. Acta* **2004**, *517*, 145.
(8) Baldini, L.; Wilson, A. J.; Hong, J.; Hamilton, A. D. *J. Am. Chem. Soc.* **2004**, *126*, 5656.
(9) Kral, V.; Rusin, O.; Schmidtchen, F. P. *Org. Lett.* **2001**, *3*, 873.
(10) Zhang, C.; Suslick, K. S. *J. Am. Chem. Soc.* **2005**, *127*, 11548.
(11) (a) Bonar-Law, R. P.; Mackay, L. G.; Walter, C. J.; Marvaud, V.; Sanders, J. K. M. *Pure Appl. Chem.* **1994**, *66*, 803. (b) Nakash, M.; Sanders, J. K. M. *J. Org. Chem.* **2000**, *65*, 7266.
(12) (a) Hayashi, T.; Aya, T.; Nonoguchi, M.; Mizutani, T.; Hisaeda, Y.; Kitagawa, S.; Ogoshi, H. *Tetrahedron* **2002**, *58*, 2803. (b) Wang, C. Z.; Zhu, Z. A.; Li, Y.; Yun, T. C.; Wen, X.; Fang, M. M.; Chan, W. L.; Chan, A. S. C. *New J. Chem.* **2001**, *276*, 9093. Binding in water: (c) Imai, H.; Munakata, H.; Uemori, Y.; Sakura, N. *Inorg. Chem.* **2004**, *43*, 1211. (d) Mizutani, T.; Wada, K.; Kitagawa, S. *J. Am. Chem. Soc.* **1999**, *121*, 11425.
(13) (a) Malinovski, V.; Tumir, L.; Piantanida, I.; Zinic, M.; Schneider, H. J. *Eur. J. Org. Chem.* **2002**, *22*, 3785. (b) Munakata, H.; Kanzaki, T.; Nakagawa, S.; Imai, H.; Uemori, Y. *Chem. Pharm. Bull.* **2001**, *49*, 1573.
(14) Wada, K.; Mizutani, T.; Kitagawa, S. *J. Org. Chem.* **2003**, *68*, 5123.
(15) (a) Prodi, A.; Chiorboli, C.; Scandola, F.; Iengo, E.; Alessio, E.; Dobrawa, R.; Wuerthner, F. *J. Am. Chem. Soc.* **2005**, *127*, 1454. (b) You, C.; Wuerthner, F. *Org. Lett.* **2004**, *6*, 2401. (c) Wuerthner, F.; Vollmer, M. S.; Effenberger, F.; Emele, P.; Meyer, D. U.; Port, H.; Wolf, H. C. *J. Am. Chem. Soc.* **1995**, *117*, 8090.
(16) (a) Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805. (b) Kimura, E.; Shionoya, M.; Hoshino, A.; Ikeda, T.; Yamada, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10134.
(17) (a) Wieghardt, K.; Schmid, W.; Herrmann, W.; Kppers, H.-J. *Inorg. Chem.* **1983**, *22*, 2953. (b) Pavlishchuk, V.; Birkelbach, F.; Weyhermller, T.; Wieghardt, K.; Chaudhuri, P. *Inorg. Chem.* **2002**, *41*, 4405. (c) Weyhermller, T.; Wieghardt, K.; Chaudhuri, P. *J. Chem. Soc., Dalton Trans* **1998**, 3805.
(18) Pavlishchuk, V.; Birkelbach, F.; Weyhermuller, T.; Wieghardt, K.; Chaudhuri, *P. Inorg. Chem.* **2002**, *41*, 4405.
(19) Amendola, V.; Fabbrizzi, L.; Mangano, C.; Pallavicini, P. *Acc. Chem. Res.* **2001**, *34*, 488.

(20) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P. *Acc. Chem. Res.* **1999**, *32*, 846.

(21) (a) Fabbrizzi, L.; Licchelli, M.; Mancin, F.; Pizzeghello, M.; Rabaioli, G.; Taglietti, A.; Tecilla, P.; Tonellato', U. *Chem.—Eur. J.* **2002**, 94. (b) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Perotti, A.; Taglietti, A.; Sacchi, D. *Chem.—Eur. J.* **1996**, *2*, 75.

(22) Kimura, E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 207.

(23) Kimura, E. *Acc. Chem. Res.* **2001**, 34, 171.

(24) (a) Aoki, S.; Kimura, E. *Chem. Rev.* **2004**, *104*, 769. (b) Aoki, S.; Kimura, E. *Rev. Mol. Biotechnol.* **2002**, *90*, 129.

(25) Further examples of tri- and tetradentate *O,N*-ligands as receptors for amino acids, phosphates, and small molecules which do not fit into the chosen ligand pattern illustrated in Figure 1 can be found in: (a) Chin, J.; Lee, S. S.; Lee, K. J.; Seogsoon, K.; Dong, H. *Nature* **1999**, *401*, 254. (b) Chin, J.; Shung, S.; Kim, D. H. *J. Am. Chem. Soc.* **2002**, *124*, 10948. (c) Mancin, F.; Chin, J. *J. Am. Chem. Soc.* **2002**, *124*, 10946. (d) Bandyopadhyay, I.; Lee, H. M.; Tarakeshwar, P.; Cui, C.; Oh, K. S.; Chin, J.; Kim, K. S. *J. Org. Chem.* **2003**, *68*, 6571.

(26) Heintz, W. *Liebigs Ann. Chem*. **1862**, *122*, 257.

(27) Podder, A.; Dattagupta, J. K.; Saha, N. N.; Saenger, N. *Acta Crystallogr.* **1979**, *B35*, 53.

(28) Kramanenco, F. G.; Polynova, T. N.; Porsai-Koshits, M. A.; Chalvi, V. P.; Kumivanova, G. N.; Martynenko, L. I. *Zh. Strukt. Khim.* **1973**, *14*, 744.

(29) (a) Wu, Y. R.; Long, L. S.; Huang, R. B.; Zheng, L. S.; Ng, S. W. *Acta Crystallogr.* **2003**, *E59*, m390. (b) Mammano, N. J.; Templeton, D. H.; Zalkin, A. *Acta Crystallogr.* **1977**, *B33*, 1251. (c) Burshtein, I. F.; Poznyak, A. L. *Kristallografiya* **2000**, *45*, 465. (d) Valach, F.; Hoang, N. N.; Lukes, I. *Chem. Pap.* **1996**, *50*, 115. (e) Morel, A. C.; Choquesillo-Lazarte, D.; Alarcon-Payer, C.; González-Pérez, J. M.; Castiñeiras, A.; Niclós-Gutiérrez, J. *Inorg. Chem. Commun.* **2003**, *6*, 1354.

(30) (a) Walters, M. A.; Vapnyar, V.; Bolour, A.; Incarvito, C.; Rheingold, A. L. *Polyhedron* **2003**, *22*, 941. (b) Napoli, A. *J. Inorg. Nucl. Chem.* **1972**, *34*, 1347.

(31) (a) Román-Alpiste, M. J.; Martín-Ramos, J. D.; Castiñeiras-Campos, A.; Bugella-Altamirano, E.; Sicilia-Zafra, A. G.; González-Pérez, J. M.; Niclós-Gutiérrez, J. *Polyhedron* **1999**, *18*, 3341. (b) For deposited structures, see CCDC numbers 105855 and 103136.

(32) (a) Schmitt, W.; Jordan, P. A.; Henderson, R. K.; Moore, G. R.; Anson, C. E.; Powell, A. K. *Coord. Chem. Rev.* **2002**, *228*, 115. (b) Klyén, M.; Lakatos, A.; Latajka, R.; Labádi, I.; Salifoglou, A.; Raptopoulou, C. P.; Kozlowaki, H.; Kiss, T. *J. Chem. Soc., Dalton Trans.* **2002**, *18*, 3578. (c) Hong, L.; Duan-Jun, X.; Kai-Liang, Y. *Acta Crystallogr.* **2003**, *E59*, m671. (d) Mootz, D.; Wunderlich, H. *Acta Crystallogr.* **1980**, *B36*, 445.

(33) Martell, A. E.; Smith, P. M. *Critical Stability Constants*; Plenum Press: New York, 1975; Vol. 2.

(34) Dung, N. H.; Viossat, B.; Busnot, A.; Pérez, J. M.; Niclós-Gutiérrez, J.; Gardette, F. *Inorg. Chim. Acta* **1990**, *174*, 145.

(35) Castiñeiras-Campos, A.; Busnot, A.; Abarca, M. E.; Sicilia-Zafra, A. G.; González-Pérez, J. M.; Niclós-Gutiérrez, J. *Inorg. Chim. Acta*, **1994**, *215*, 73.

(36) Castiñeiras-Campos, A.; Tercero, J. M.; Matilla, A.; González, J. M.; Sicilia, A. G.; Niclós, J. *J. Coord. Chem.* **1995**, *35*, 61.

(37) Castiñeiras-Campos, A.; Sicilia-Zafra, A. G.; González-Pérez, J. M.; Niclós-Gutiérrez, J.; Chinea, E.; Mederos, A. *Inorg. Chim. Acta* **1996**, *241*, 39.

(38) Dung, N. H.; Viossat, B.; Busnot, A.; Sicilia-Zafra, A. G.; González-Pérez, J. M.; Niclós-Gutiérrez, J. *Inorg. Chim. Acta* **1990**, *169*, 9.

(39) Nardin, G.; Randaccio, L.; Bonomo, R. P.; Rizzareli, E. *J. Chem. Soc., Dalton Trans.* **1980**, *3*, 369.

(40) Castiñeiras, A.; Abarca, M. E.; De la Cueva, I.; González, J. M.; Niclós, J. *J. Coord. Chem.* **1993**, *30*, 273.

(41) González Pérez, J. M.; Dung, N. H.; Niclós-Gutiérrez, J.; Viossat, B.; Busnot, A.; Vincente-Gelabert, M. L. *Inorg. Chim. Acta* **1989**, *166*, 155.

(42) Niclós-Gutiérrez, J.; Abarca-García, E.; Viossat, B.; Dung, N. H.; Busnot, A.; Hemidy, J. F. *Acta Crystallogr.* **1993**, *C49*, 19.

(43) Rojas-González, P. X.; Choquesillo-Lazarte, D.; González-Pérez, J. M.; Ruíz-García, S. A.; Carballo, R.; Castiñeiras, A.; Niclós-Gutiérrez, J. *Polyhedron* **2003**, *22*, 1027.

(44) See section 2, Scope and Limitations, for selection criteria of additional ligands.

(45) Hochuli, E.; Bannwarth, W.; Dobeli, H.; Gentz, R.; Suder, D. *Bio/Technology* **1988**, *6*, 1321.

(46) Genz, R.; Certa, U.; Takacs, B.; Matile, H.; Dobelji, H.; Pink, P.; Mackay, M.; Bone, N.; Scaife, J. G. *EMBO J.* **1988**, *7*, 225.

(47) (a) Skerra, A. I.; Pfitzinger, I.; Plunkthun, A. *Bio/Technology* **1991**, *9*, 273. (b) Lilius, G.; Persson, M.; Bulow, L.; Mosbach, K. *Eur. J. Biochem.* **1991**, *198*, 499.

(48) Goud, G. N.; Patwardhan, A. V.; Beckmann, E. J.; Ataai, M. M.; Koepsel, R. R. *Int. J. Biol. Chromatogr.* **1997**, *3*, 123.

(49) Patwardhan, A. V.; Goud, G. N.; Koepsel, R. R.; Ataai, M. M. *J. Chromatogr.* **1997**, *787*, 91.

(50) Chen, Y.; Pasquinelli, R.; Ataai, M.; Koepsel, R. R.; Kortes, R. A.; Shepherd, R. E. *Inorg. Chem.* **2000**, *39*, 1180.

(51) (a) Sun, S.; Fazal, M. A.; Roy, B. C.; Mallik, S. *Org. Lett.* **2000**, *2*, 911. (b) Sun, S.; Fazal, M. A.; Roy, B. C.; Chandra, B.; Mallik, S. *Inorg. Chem.* **2002**, *41*, 1584.

(52) (a) Roy, B. C.; Fazal, M. A.; Sun, S.; Mallik, S. *J. Chem. Soc., Chem. Commun.* **2000**, 547. (b) Fazal, M. A.; Roy, B. C.; Sun, S.; Mallik, S.; Rodgers, K. R. *J. Am. Chem. Soc.* **2001**, *123*, 6283.

(53) Sadler, P. J.; Viles, J. H. *Inorg. Chem.* **1996**, *35*, 4490.

(54) (a) Bildhan, C. R.; Hegge, R.; Rosendahl, T.; Jia, X.; Lareau, R.; Mallik, S.; Srivastava, D. K. *Chem. Commun.* **2003**, *18*, 2328. (b) Banerjee, A. L.; Swanson, M.; Roy, B. C.; Jia, X.; Haldar, M. K.; Mallik, S.; Srivastava, D. K. *J. Am. Chem. Soc.* **2004**, *126*, 10875. (c) Roy, B. C.; Banerjee, A. L.; Swanson, M.; Jia, X.; Haldar, M. K.; Mallik, S.; Srivastava, D. K. *J. Am. Chem. Soc.* **2004**, *126*, 13206.

(55) Kruppa, M.; Mandl, C.; Miltschitzky, S.; König, B. *J. Am. Chem. Soc.* **2005**, *127*, 3362.

(56) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2979.

(57) James, T. D.; Samankumara Sandanayake, K. R. A.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910.

(58) Nagai, Y.; Kobayashi, K.; Toi, H.; Aoyama, Y. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2965.

(59) Striegler, S.; Tewes, E. *Eur. J. Inorg. Chem.* **2002**, *2*, 487.

(60) Angyal, S. J. *Adv. Carbohydr. Chem. Biochem.* **1991**, *49*, 19.

(61) Connors, K. A. *Binding constants—The Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987.

(62) Porath, J.; Carlsson, J.; Belfrage, G. *Nature* **1975**, *258*, 598.

(63) Ueda, E. K. M.; Gout, P. W.; Morganti, L. *J. Chromatogr., A* **2003**, *988*, 1.

(64) (a) Suen, S.-Y.; Liu, Y. C.; Chang, C.-S. *J. Chromatgr., B* **2003**, *797*, 305. (b) Shepherd, R. E. *Coord. Chem. Rev.* **2003**, *247*, 147. (c) Gaberc-Porekar, V.; Menart, V. *J. Biochem. Biophys. Methods* **2001**, *49*, 335. (d) Chaga, G. S. *J. Biochem. Biophys. Methods* **2001**, *49*, 313. (e) Arnold, F. H. *Bio/Technology* **1991**, *9*, 151. (f) Porath, J. *TrAC, Trends Anal. Chem.* **1988**, *7*, 254.

(65) Rebek, J., Jr. *Acc. Chem. Res.* **1990**, *23*, 399.

(66) For a review, see: Wulff, G. In *Biomimetic Polymers*; Gebelein, C. G., Ed.; Plenum Press: New York, 1990; p 1.

(67) Dhal, P. K.; Arnold, F. H. *J. Am. Chem. Soc.* **1991**, *113*, 7417.

(68) Dhal, P. K.; Arnold, F. H. *Macromolecules* **1992**, *25*, 7051.

(69) Arnold, F. H.; Plunkett, S.; Dhal, P. K.; Vidyasankar, S. *Polym. Prepr.* **1995**, *36*, 97.

(70) Plunkett, S. D.; Arnold, F. H. *J. Chromatogr., A* **1995**, *708*, 19.

(71) Dhal, P. K.; Vidyasankar, S.; Arnold, F. H. *Chem. Mater.* **1995**, *7*, 154.

(72) Vidyasankar, S.; Ru, M.; Arnold, F. H. *J. Chromatogr., A* **1997**, *775*, 51.

(73) Shnek, D. R.; Pack, D. W.; Sasaki, D. Y.; Arnold, F. H. *Langmuir* **1994**, *10*, 2382.

(74) Wuenschell, G. E.; Wen, E.; Todd, R.; Schnek, D. R.; Arnold, F. H. *J. Chromatogr.* **1991**, *543*, 345.

(75) Schubert, J. *J. Am. Chem. Soc.* **1954**, *76*, 3442.

(76) Ng, K.; Pack, D. W.; Sasaki, D. Y.; Arnold, F. H. *Langmuir* **1995**, *11*, 4048.

(77) Sasaki, D. Y.; Shnek, D. R.; Pack, D. W.; Arnold, F. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 905.

(78) Pack, D. W.; Arnold, F. H. *Chem. Phys. Lipids* **1997**, *86*, 135.

(79) Pack, D. W.; Chen, G.; Maloney, K. M.; Chen, C.; Arnold, F. H. *J. Am. Chem. Soc.* **1997**, *119*, 2479.

(80) Kornberg, R. D.; Darst, S. A. *Curr. Opin. Struct. Biol.* **1991**, *1*, 642.

(81) Rajabalee, F. J. M. *J. Inorg. Nucl. Chem.* **1974**, *36*, 557.

(82) (a) Valle, G.; Bombi, G. G.; Corain, B.; Favarato, M.; Zatta, P. *J. Chem. Soc., Dalton Trans.* **1989**, *8*, 1513. (b) Tewari, B. B. *J. Chromatogr., A* **1995**, *718*, 454.

(83) (a) Shepherd, R. E.; Hodgson, G. M.; Margerium, D. W. *Inorg. Chem.* **1971**, *10*, 989. (b) Dung, N. H.; Viossat, B.; Busnot, A.; González Pérez, J. M.; González García, S.; Niclós Gutiérrez, J. *Inorg. Chem.* **1988**, *27*, 1227. (c) Fábián, I. *Inorg. Chem.* **1993**, *32*, 1184. (d) Saxena, V. K.; Srivastava, M. N. *J. Inorg. Biochem.* **1990**, *38*, 37.

(84) (a) Laham, S.; Rajabalee, F. J. M. *Bioinorg. Chem.* **1973**, *2*, 243. (b) Almeida Paz, F. A.; Klinowski, J. *J. Phys. Org. Chem.* **2003**, *16*, 772. (c) Rabenstein, D. L.; Blakney, G. *Inorg. Chem.* **1973**, *12*, 128.

(85) (a) Jackons, N. E.; Margerum, D. W. *Inorg. Chem.* **1967**, *6*, 2038. (b) Erickson, L. E.; Ho, F. F.-L.; Reilley, C. N. *Inorg. Chem.* **1970**, *9*, 1148.

(86) (a) Budkuley, J. S.; Naik, G. K. *Thermochim. Acta* **1998**, *320*, 115. (b) Sharma, G.; Tandon, J. P. *J. Inorg. Nucl. Chem.* **1970**, *32*, 1273.

(87) (a) Sharma, C. L.; Jain, P. K.; De, T. K. *J. Inorg. Nucl. Chem.* **1980**, *42*, 1681. (b) Visser, H. G.; Leipoldt, J. G.; Purcell, W.; Mostert, D.

Polyhedron **1994**, *13*, 1051. (c) Bhattacharyya, S. K.; Banerjee, R. *Polyhedron* **1997**, *16*, 3371.
(88) (a) Gustafson, R. L.; Martell, A. E. *J. Phys. Chem.* **1963**, *67*, 576. (b) Cox, D. D.; Que, L. *J. Am. Chem. Soc.* **1988**, *110*, 8085.
(89) (a) Mentasti, E. *J. Chem. Soc., Dalton Trans.* **1982**, *4*, 721. (b) Chattopadhyay, S. P.; Banerjea, D. *Polyhedron* **1994**, *13*, 1981. (c) Visser, H. G.; Purcell, W.; Basson, S. S. *Trans. Met. Chem.* **2002**, *27*, 461. (d) Visser, H. G.; Purcell, W.; Basson, S. S. *Trans. Met. Chem.* **2001**, *26*, 175. (e) Smith, B. B.; Sawyer, D. T. *Inorg. Chem.* **1968**, *7*, 922. (f) Meloon, D. R.; Harris, G. M. *Inorg. Chem.* **1977**, *16*, 434.
(90) Whitlow, S. H. *Inorg. Chim.* **1973**, *12*, 2286.
(91) (a) Baolong, L.; Bazong, L.; Jianping, L.; Yong, Z. *Inorg. Chem. Commun.* **2003**, *6*, 725. (b) Baolong, L.; Bazong, L.; Xia, Z.; Yong, Z. *Inorg. Chem. Commun.* **2003**, *6*, 1304. (c) Huawa, Y.; Juen S.; Liaorong, C.; Baosheng, L. *Polyhedron* **1996**, *15*, 3891.
(92) Abdus Salam, Md.; Aoki, K. *Inorg. Chim. Acta* **2000**, *311*, 15.
(93) Structures are deposited, see: CCDC 150197, 150198, and 150199.
(94) Oliver, J. D.; Barnett, B. L.; Strickland, L. C. *Acta Crystallogr.* **1984**, *B40*, 377.
(95) (a) Visser, H. G.; Purcell, W.; Basson, S. S. *Polyhedron* **1999**, *18*, 2795. (b) Koine, N.; Bianchini, R. J.; Legg, J. I. *Inorg. Chem.* **1986**, *25*, 2835.
(96) Green, C. A.; Koine, N.; Legg, J. I.; Willett, R. D. *Inorg. Chim. Acta* **1990**, *176*, 87−93.
(97) (a) Bocarsly J. R.; Barton, J. K. *Inorg. Chem.* **1989**, *28*, 4189. (b) Bocarsly, J. R.; Chiang, M. Y.; Bryant, L.; Barton, J. K. *Inorg. Chem.* **1990**, *29*, 4898. (c) Bocarsly, J. R.; Barton, J. K. *Inorg. Chem.* **1992**, *31*, 2827.
(98) (a) Walters, M. A.; Vapnyar, V.; Bolour, A.; Incarvito, C.; Rheingold, A. L. *Polyhedron* **2003**, *22*, 941. (b) Structure coordinates are deposited at CCDC 196921 and 196922.
(99) (a) Malfant, I.; Morgenstern-Badarau, I.; Philoche-Levisalles, M.; Lloret, F. *J. Chem. Soc., Chem. Commun.* **1990**, *19*, 1338. (b) Heath, S. L.; Powell, A. K.; Utting, H. L.; Helliwell, M. *J. Chem. Soc., Dalton Trans.* **1992**, *2*, 305.
(100) Wallis, S. C.; Gahan, L. R.; Charles, B. G.; Hambley, T. W. *Polyhedron* **1995**, *14*, 2835.
(101) Polyakova, I. N.; Poznyak, A. L.; Egorova, O. A. *Russ. J. Coord. Chem.* **2001**, *27*, 852.
(102) Battaglia, L. P.; Corradi Bonamartini, A.; Vidoni Tani, M. E. *Acta Crystallogr.*, **1975**, *B31*, 1160.
(103) Visser, H. G.; Purcell, W.; Basson, S. S.; Claassen, Q. *Polyhedron* **1997**, *16*, 2851.
(104) (a) Visser, H. G.; Purcell, W.; Basson, S. S. *Polyhedron* **2001**, *20*, 185. (b) Structure coordinates are deposited at CCDC 136408.
(105) (a) Kumita, H.; Jitsukawa, K.; Masuda, H.; Einaga, H. *Inorg. Chim. Acta* **1998**, *283*, 160. (b) Kumita, H.; Kato, T.; Jitsukawa, K.; Einaga, H.; Masuda, H. *Inorg. Chem.* **2001**, *40*, 3936. (c) Jitsukawa, K.; Morioka, T.; Masuda, H.; Ogoshi, H.; Einaga, H. *Inorg. Chim. Acta* **1994**, *216*, 249. (d) Kumita, H.; Morioka, T.; Ozawa, T.; Jitsukawa, K.; Einaga, H.; Masuda, H. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 1035. (e) Gladkikh, O. P.; Polynova, T. N.; Porai-Koshits, M. A.; Poznyak, A. L. *Koord. Khim. (Russ.) (Coord. Chem.)* **1992**, *18*, 1156.
(106) Hopgood, D.; Angelici, R. J. *J. Am. Chem. Soc.* **1968**, *90*, 2508.
(107) (a) Newlin, D. E.; Pellack, M. A.; Nakon, R. *J. Am. Chem. Soc.* **1977**, *99*, 1078. (b) Dembowski, J. S.; Kurtz, D. C.; Nakon, R. *Inorg. Chim. Acta* **1988**, *152*, 209.
(108) Perrin, D. D.; Sharma, V. S. *J. Chem. Soc. A* **1967**, 724.
(109) Rabenstein, D. L.; Blakney, G. *Inorg. Chem.* **1973**, *12*, 128.
(110) Gabricevic, M.; Crumbliss, A. L. *Inorg. Chem.* **2003**, *42*, 4098.
(111) Kapanidis, A. N.; Ebright, Y. W.; Ebright, R. H. *J. Am. Chem. Soc.* **2001**, *123*, 12123.
(112) (a) Hochuli, E.; Bannwarth, W.; Dobeli, H.; Gentz, R.; Stuber, D. *Bio/Technology* **1988**, *6*, 1321. (b) Crowe, J.; Dobeli, H.; Gentz, R.; Hochuli, E.; Stuber, D.; Henco, K. *Methods Mol. Biol.* **1994**, *31*, 371.
(113) Goldsmith, C. R.; Jaworski, J.; Sheng, M.; Lippard, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 418.
(114) For related high-affinity labels for His-tagged proteins, see: Lata, S.; Reichel, A.; Brock, R.; Tampe, R.; Piehler, J. *J. Am. Chem. Soc.* **2005**, *127*, 10205.
(115) Futaki, S.; Niwa, M.; Nakase, I.; Tadokoro, A.; Zhang, Y.; Nagaoka, M.; Wakako, N.; Sugiura, Y. *Bioconjugate Chem.* **2004**, *15*, 475.
(116) Boonyarattanakalin, S.; Athavankar, S.; Sun, Q.; Peterson, B. R. *J. Am. Chem. Soc.* **2006**, *128*, 386.
(117) Kruppa, M.; Bonauer, C.; Michlova, V.; König, B. *J. Org. Chem.* **2005**, *70*, 5305.
(118) Paborsky, L. R.; Dunn, K. E.; Gibbs, C. S.; Dougherty, J. P. *Anal. Biochem.* **1996**, *234*, 60.
(119) For immobilization of NTA on a piezoelectric sensor chip for direct detection and oriented immobilization of polyhis-tagged proteins, see: Chen, H.-M.; Wang, W.-C.; Chen, S.-H. *Biotechnol. Prog.* **2004**, *20*, 1237.
(120) (a) Hart, B. R.; Shea, K. J. *J. Am. Chem. Soc.* **2001**, *123*, 2072. (b) Hart, B. R.; Shea, K. J. *Macromolecules* **2002**, *35*, 6192.
(121) NTA-functionalized graft copolymers have recently been reported as an interface for selective and reversible binding of histidine-tagged proteins: Zhen, G.; Falconnet, D.; Kuennemann, E.; Voros, J.; Spencer, N. D.; Textor, M.; Zurcher, S. *Adv. Funct. Mater.* **2006**, *16*, 243.
(122) Abad, J. M.; Mertens, S. F. L.; Pita, M.; Fernandez, V. M.; Schiffrin, D. J. *J. Am. Chem. Soc.* **2005**, *127*, 5689.
(123) (a) Liedberg, B.; Nylander, C.; Lundström, I. *Sens. Actuators* **1983**, *4*, 299. (b) Daniels, P. B.; Deacon, J. K.; Eddowes, M. J.; Pedley, D. G. *Sens. Actuators* **1988**, *16*, 11. (c) Löfas, S.; Johnsson, B. *Chem. Commun.* **1990**, 1526. (d) Nieba, L.; Nieba-Axmann, S. E.; Persson, A.; Hämäläinen, M.; Edebratt, F.; Hansson, A.; Lidholm, J.; Magnusson, K.; Karlsson, A. F.; Plückthun, A. *Anal. Biochem.* **1997**, *252*, 217.
(124) Flanagan, M. T.; Pantell, R. H. *Electron. Lett.* **1984**, *20*, 968.
(125) Sigal, G. B.; Bamdad, C.; Barberis, A.; Strominger, J.; Whitesides, G. M. *Anal. Chem.* **1996**, *68*, 490.
(126) Ferguson, A. L.; Hughes, A. D.; Tufail, U.; Baumann, C. G.; Scott, D. J.; Hoggett, J. G. *FEBS Lett.* **2000**, *481*, 281.
(127) Kröger, D.; Liley, M.; Schiweck, W.; Skerra, A.; Vogel, H. *Biosens. Bioelectron.* **1999**, *14*, 155.
(128) Hainfeld, J. F.; Liu, W.; Halsey, C. M. R.; Freimuth, P.; Powell, R. D. *J. Struct. Biol.* **1999**, *127*, 185.
(129) Conti, M.; Falini, G.; Samorì, B. *Angew. Chem., Int. Ed.* **2000**, *39*, 215.
(130) Comparison of electrochemical and chemical immobilization of Ni−NTA: Maly, J.; Di Meo, C.; De Francesco, M.; Masci, A.; Masojidek, J.; Sugiura, M.; Volpe, A.; Pilloton, R. *Bioelectrochemistry* **2004**, *63*, 271.
(131) Haddour, N.; Cosnier, S.; Gondran, C. *J. Am. Chem. Soc.* **2005**, *127*, 5752.
(132) (a) Schmitt, L.; Dietrich, C.; Tampé, R. *J. Am. Chem. Soc.* **1994**, *116*, 8485. (b) Gritsch, S.; Neumaier, K.; Schmitt, L.; Tampé, R. *Biosens. Bioelectron.* **1995**, *10*, 805. (c) Schmitt, L.; Bohanon, T. M.; Denzinger, S.; Ringsdorf, H.; Tampé, R. *Angew. Chem.* **1996**, *108*, 344.
(133) Dietrich, C.; Schmitt, L.; Tampé, R. *Proc. Natl. Acad. Sci., U.S.A.* **1995**, *92*, 9014.
(134) (a) Kubalek, E. W.; Le Grice, S. F. J.; Brown, P. O. *J. Struct. Biol.* **1994**, *113*, 117. (b) Dietrich, C.; Boscheinen, O.; Scharf, K. D.; Schmitt, L.; Tampé, R. *Biochemistry* **1996**, *35*, 1100. (c) Vénien-Bryan, C.; Balvoine, F.; Toussaint, B.; Mioskowski, C.; Hewat, E. A.; Helme, B.; Vignais, P. M. *J. Mol. Biol.* **1997**, *274*, 687. (d) Bischler, N.; Balavoine, F.; Milkereit, P.; Tschochner, H.; Mioskowski, C.; Schultz, P. *Biophys. J.* **1998**, *74*, 1522. (e) Celia, H.; Wilson-Kubalek, E.; Milligan, R. A.; Teyton, L. *Proc. Natl. Acad. Sci., U.S.A.* **1999**, *96*, 5634. (f) Kienberger, F.; Moser, R.; Schindler, H.; Blaas, D.; Hinterdorfer, P. *Single Mol.* **2001**, *2*, 99. (g) Courty, S.; Lebeau, L.; Martel, L.; Lenné, P. F.; Balavoine, F.; Dischert, W.; Konovalov, O.; Mioskowski, C.; Legrand, J. F.; Vénien-Bryan, C. *Langmuir* **2002**, *18*, 9502.
(135) (a) Lauer, S. A.; Nolan, J. P. *Cytometry* **2002**, *48*, 136. (b) Kato, K.; Sato, H.; Iwata, H. *Langmuir* **2005**, *21*, 7071.
(136) Xu, C.; Xu, K.; Gu, H.; Zhong, X.; Guo, Z.; Zheng, R.; Zhang, X.; Xu, B. *J. Am. Chem. Soc.* **2004**, *126*, 3392.
(137) Schmid, E. L.; Keller, T. A.; Dienes, Z.; Vogel, H. *Anal. Chem.* **1997**, *69*, 1979.
(138) As there are many different abbreviations of this ligand in the literature, only bpa will be used in the following chapters.
(139) (a) Kabzinska, B. *Ann. Pharm. Fr.* **1964**, *22*, 685. (b) Romary, J. K.; Barger, J. D.; Bunds, J. E. *Inorg. Chem.* **1968**, *7*, 1142.
(140) (a) Rarig, R. S.; Zubieta, J. *J. Solid State Chem.* **2002**, *167*, 370. (b) Wahnon, D.; Hynes, R. C.; Chin, J. *J. Chem. Soc., Chem. Commun.* **1994**, *12*, 1441. (c) Hartman, J. R.; Vachet, R. W.; Pearson, W.; Wheat, J. W.; Callahan, J. H. *Inorg. Chim. Acta* **2003**, *343*, 119. (d) Niklas, N.; Heinemann, F. W.; Hampel, F.; Clark, T.; Alsfasser, R. *Inorg. Chem.* **2004**, *43*, 4663. (e) Huang, G. S.; Su, C. C.; Wang, S. L.; Liao, F. L.; Lin, K. J. *J. Coord. Chem.* **2000**, *49*, 211. (f) Murali, M.; Palaniandavar, M. *Trans. Met. Chem.* **1996**, *21*, 142. (g) Nakon, R.; Rechani, P. R.; Angelici, R. J. *J. Am. Chem. Soc.* **1974**, *96*, 2117.
(141) (a) Yahiro, M.; Kaneiwa, H.; Onaka, K.; Komiyama, M. *J. Chem. Soc., Dalton Trans.* **2004**, *4*, 605. (b) Brand, U.; Burth, R.; Vahrenkamp, H. *Inorg. Chem.* **1996**, *35*, 1083.
(142) (a) Goto, M.; Koga, N.; Ohse, Y.; Kudoh, Y.; Kukihara, M.; Okuno, Y.; Kurisaki, H. *Inorg. Chem.* **2004**, *43*, 5120. (b) Rodriguez, M. C.; Lambert, F.; Morgenstern-Badarau, I. *Inorg. Chem.* **1997**, *36*, 3525. (c) Leising, R. A.; Kim, J.; Pérez, M. A.; Que, L. *J. Am. Chem.*

Soc. **1993**, *115*, 9524. (d) Fernandes, C.; Wardell, J. L.; Horn, A.; Skakle, J. M. S.; Drago, V. *Polyhedron* **2004**, *23*, 1419. (e) Hazell, A.; McKenzie, C. J.; Nielsen, L. P. *Polyhedron* **2000**, *19*, 1333. (f) Viswanathan, R.; Palaniadavar, M.; Balasubrmanian, T.; Muthiah, P. T. *J. Chem. Soc., Dalton Trans.* **1996**, *12*, 2519. (g) Thomas, K. R. J.; Velusamy, M.; Palaniandavar, M. *Acta Crystallogr., Sect. C* **1998**, *C54*, 741. (h) O'Brien, R. J.; Richardson, J. F.; Buchanan, R. M. *Acta Crystallogr., Sect. C* **1991**, *C47*, 2307. (i) Trukhan, V. M.; Gritsenko, O. N.; Nordlander, E.; Shteinman, A. A. *J. Inorg. Biochem.* **2000**, *79*, 41.

(143) (a) Choi, K. Y.; Ryu, H.; Sung, N. D.; Suh, M. *J. Chem. Cryst.* **2003**, *33*, 947. (b) For coordinates of the structure, see CCDC 196454.

(144) (a) Palaniandavar, M.; Butcher, R. J.; Addison, A. W. *Inorg. Chem.* **1996**, *35*, 467. (b) Huang, G. S.; Lai, J. K.; Ueng, C. H.; Su, C. C. *Trans. Met. Chem.* **2000**, *25*, 84.

(145) (a) Murakami, T.; Hatakeyama, S.; Igarashi, S.; Yukawa, Y. *Inorg. Chim. Acta* **2000**, *310*, 96. (b) Murakami, T.; Orihashi, Z.; Kikuchi, Y.; Igarashi, S.; Yukawa, Y. *Inorg. Chim. Acta* **2000**, *303*, 148. (c) Structure coordinates of the complexes are deposited at CCDC 162437 and CCDC 145748.

(146) Funahashi, Y.; Kato, C.; Yamauchi, O. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 415.

(147) Gultneh, Y.; Khan, A. R.; Blaise, D.; Chaudhry, S.; Ahvazi, B.; Marvey, B. B.; Butcher, R. J. *J. Inorg. Biochem.* **1999**, *75*, 7.

(148) Wirbser, J.; Vahrenkamp, H. *Z. Naturforsch.* **1992**, *47*, 962.

(149) Glerup, J.; Goodson, P. A.; Hodgson, D. J.; Michelsen, K.; Nielsen, K. M.; Weihe, H. *Inorg. Chem.* **1992**, *31*, 4611.

(150) Groβ, F.; Müller-Hartmann, A.; Vahrenkamp, H. *Eur. J. Inorg. Chem.* **2000**, *12*, 2363.

(151) Mandon, D.; Nopper, A.; Litrol, T.; Goetz, S. *Inorg. Chem.* **2001**, *40*, 4803.

(152) Butcher, R. J.; Addison, A. W. *Inorg. Chim. Acta* **1989**, *158*, 211.

(153) Mito-oka, Y.; Tsukiji, S.; Hiraoka, T.; Kasagi, N.; Shinkai, S.; Hamachi, I. *Tetrahedron Lett.* **2001**, *42*, 7059.

(154) Ojida, A.; Miyahara, Y.; Kohira, T.; Hamachi, I. *Biopolymers* **2004**, *76*, 177.

(155) (a) Ojida, A.; Mito-oka, Y.; Inoue, M.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 6256. (b) Ojida, A.; Mito-oka, Y.; Inoue, M.; Hamachi, I. *J. Am. Chem. Soc.* **2003**, *125*, 10184.

(156) Ojida, A.; Park, S.; Mito-oka, Y.; Hamachi, I. *Tetrahedron Lett.* **2002**, *43*, 6193.

(157) Ojida, A.; Mito-oka, Y.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 2454.

(158) Yoshimura, I.; Miyahara, Y.; Kasagi, N.; Yamane, H.; Ojida, A.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 12204.

(159) Ojida, A.; Kohira, T.; Hamachi, I. *Chem. Lett.* **2004**, *33*, 1024.

(160) Wongkongkatep, J.; Miyahara, Y.; Ojida, A.; Hamachi, I. *Angew. Chem., Int. Ed.* **2006**, *45*, 665−668.

(161) Kinoshita, E.; Takahashi, M.; Takeda, H.; Shiro, M.; Koike, T. *J. Chem. Soc., Dalton Trans.* **2004**, *8*, 1189.

(162) Han, M. S.; Kim, D. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1079.

(163) Lee, D. H.; Kim, S. Y.; Hong, J. I. *Angew. Chem.* **2004**, *116*, 4881.

(164) For a related macrocyclic Zn(II)-based receptor for ATP binding, see: Bazzicalupi, C.; Bencini, A.; Bianchi, A.; Danesi, A.; Giorgi, C.; Lodeiro, C.; Pina, F.; Santarelli, S.; Valtancoli, B. *Chem. Commun.* **2005**, 2630.

(165) Han, M. S.; Kim, D. H. *Bull. Korean Chem. Soc.* **2004**, *25*, 1151.

(166) Hanshaw, R. G.; Hilkert, S. M.; Jiang, H.; Smith, B. D. *Tetrahedron Lett.* **2004**, *45*, 8721.

(167) Koulov, A. V.; Stucker, K. A.; Lakshmi, C.; Smith, B. D. *Cell Death Differ.* **2003**, *10*, 1357.

(168) Laksmi, C.; Hanshaw, R. G.; Smith, B. D. *Tetrahedron* **2004**, *60*, 11307.

(169) da Mota, M. M.; Rodgers, J.; Nelson, S. M. *J. Chem. Soc. A* **1969**, *13*, 2036.

(170) (a) Xu, J. Y.; Bian, H. D.; Gu, W.; Yan, S. P.; Cheng, P.; Liao, D. Z.; Jiang, Z. H.; Shen, P. W. *J. Mol. Struct.* **2003**, *646*, 237. (b) Yang, L. Y.; Peng, Y.; Bian, F.; Yan, S. P.; Liao, D. Z.; Cheng, P.; Jiang, Z. H. *J. Coord. Chem.* **2003**, *56*, 961. (c) Szajna, E.; Dobrowolski, P.; Fuller, A. L.; Arif, A. M.; Berreau, L. M. *Inorg. Chem.* **2004**, *43*, 3988. (d) Ito, M.; Kawano, H.; Takeuchi, T.; Takita, Y. *Chem. Lett.* **2000**, *29*, 372.

(171) (a) Failes, T. W. *Acta Crystallogr.* **2004**, *E60*, m781. (b) Failes, T. W. *Acta Crystallogr.* **2003**, *E59*, m616. (c) Otter, C. A.; Hartshirn, R. M. *J. Chem. Soc., Dalton Trans.* **2004**, *1*, 150. (d) Mandel, J. B.; Maricondi, C.; Douglas, B. E. *Inorg. Chem.* **1988**, *27*, 2990.

(172) (a) Zheng, H.; Que, L. *Inorg. Chim. Acta* **1997**, *263*, 301. (b) Neubrand, A.; Thaler, F.; Körner, M.; Zahl, A.; Hubbard, C. D.; van Eldik, R. *J. Chem. Soc., Dalton Trans.* **2002**, *6*, 957. (c) Mukhopadhyay, U.; Bernal, I.; Massoud, S. S.; Mautner, F. A. *Inorg. Chim. Acta* **2004**, *357*, 3673.

(173) Madden, D. P.; Mota, M. M.; Nelson, S. M. *J. Chem. Soc. A* **1970**, 790.

(174) (a) Zang, Y.; Que, L. *Inorg. Chem.* **1995**, *34*, 1030. (b) Zheng, H.; Zang, Y.; Dong, Y.; Young, V. G.; Que, L. *J. Am. Chem. Soc.* **1999**, *121*, 2226. (c) Corsi, D. M.; Murthy, N. N.; Young, V. G.; Karlin, K. D. *Inorg. Chem.* **1999**, *38*, 848. (d) Kryatov, S. V.; Nazarenko, A. Y.; Robinson, P. D.; Rybak-Akimova, E. V. *Chem. Commuun.* **2000**, *11*, 921. (e) Mandon, D.; Machkour, A.; Goetz, S.; Welter, R. *Inorg. Chem.* **2002**, *41*, 5364. (f) Viswanathan, R.; Palaniandavar, M.; Balasubramanian, T.; Muthiah, T. P. *Inorg. Chem.* **1998**, *37*, 2943. (g) Kim, J.; Dong, Y.; Larka, E.; Que, L. *Inorg. Chem.* **1996**, *35*, 2369.

(175) Karlin, K. D.; Hayes, J. C.; Juen, S.; Hutchinson, J. P.; Zubieta, J. *Inorg. Chem.* **1982**, *21*, 4108.

(176) (a) Zhu, L.; dos Santos, O.; Koo, C. W.; Rybstein, M.; Pape, L.; Canary, J. W. *Inorg. Chem.* **2003**, *42*, 7912. (b) For structure coordinates, see CCDC 225929.

(177) Allen, C. S.; Chuang, C. L.; Cornebise, M.; Canary, J. W. *Inorg. Chim. Acta* **1995**, *239*, 29.

(178) Ito, M.; Fujita, K.; Chitose, F.; Takeuchi, T.; Yoshida, K.; Tacita, Y. *Chem. Lett.* **2002**, *6*, 594.

(179) Adams, H.; Bailey, N. A.; Fenton, D. E.; He, Q. Y. *J. Chem. Soc., Dalton Trans.* **1995**, *4*, 697.

(180) Mao, Z. W.; Hang, Q. W.; Tang, W. X.; Liu, S. X.; Wang, Z. M.; Huang, J. L. *Polyhedron* **1996**, *15*, 321.

(181) Whittlesey, B. R.; Pang, Z.; Holwerda, R. A. *Inorg. Chim. Acta* **1999**, *284*, 124.

(182) (a) Merkel, M.; Pascaly, M.; Wieting, M.; Duda, M.; Rompel, A. *Z. Anorg. Allg. Chem.* **2003**, *629*, 2216. (b) For structure coordinates, see CCDC 213827−213829.

(183) Norman, R. E.; Yan, S.; Que, L.; Backes, G.; Ling, J.; Sanders-Loehr, J.; Zhang, J. H.; O'Connor, C. *J. Am. Chem. Soc.* **1990**, *112*, 1554.

(184) Ménage, S.; Zang, Y.; Hendrich, M. P.; Que, L. *J. Am. Chem. Soc.* **1992**, *114*, 7786.

(185) Umakoshi, K.; Tsuruma, Y.; Oh, C. E.; Takasawa, A.; Yasukawa, H.; Sasaki, Y. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 433.

(186) For similar studies with the TPA related ligand system hydrotris-(3,5-phenylmethylpyrazolyl)borate, see: (a) Jacobsen, F. E.; Cohen, S. M. *Inorg. Chem.* **2004**, *43*, 3038. (b) Puerta, D. T.; Lewis, J. A.; Cohen, S. M. *J. Am. Chem. Soc.* **2004**, *126*, 8388.

(187) Baek, H. K.; Holwerda, R. A. *Inorg. Chem.* **1983**, *22*, 3452.

(188) Mareque Rivas, J. C.; Prabaharan, R.; de Rosales, R. T. M. *Chem. Commun.* **2004**, *1*, 76.

(189) Mareque Rivas, J. C.; de Rosales, R. T. M.; Parsons, S. *Chem. Commun.* **2004**, *5*, 610.

(190) (a) Tobey, S. L.; Anslyn, E. V. *Org. Lett.* **2003**, *5*, 2029. (b) Tobey, S. L.; Jones, B. D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 4026. (c) Tobey, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 14807.

(191) Rasmussen, S. E. *Acta Crystallogr. Scand.* **1959**, *13*, 2009.

(192) (a) McLachlan, G. A.; Muller, J. G.; Rokita, S. E.; Burrows, C. J. *Inorg. Chim. Acta* **1996**, *251*, 193. (b) Escuer, A.; Font-Bardia, M.; Massoud, S. S.; Mautner, F. A.; Penalba, E.; Solans, X.; Vicente, R. *New J. Chem.* **2004**, *28*, 681. (c) Huang, J.; Li, S.-A.; Li, D.-F.; Yang, D.-X.; Sun, W.-Y.; Tang, W.-X. *Eur. J. Inorg. Chem.* **2004**, *9*, 1894. (d) Ouyang, X.-M.; Li, Z.-W.; Okamura, T.; Li, Y.-Z.; Sun, W.-Y.; Tang, W.-X.; Ueyama, N. *J. Solid State Chem.* **2004**, *177*, 350. (e) Bolos, C. A.; Papzisis, K. T.; Kortsaris, A. H.; Voyatzi, S.; Zambouli, D.; Kyriakidis, D. A. *J. Inorg. Biochem.* **2002**, *88*, 25. (f) Willet, R. D. *Acta Crystallogr.* **2001**, *E57*, m605. (g) Ullah, M. R.; Bhattacharya, P. K.; Venkatasubramanian, K. *Polyhedron* **1996**, *15*, 4025. (h) Liu, S.-J.; Su, C.-C. *Polyhedron* **1996**, *15*, 1141. (i) Biagini-Cingi, M.; Manotti-Lanfredi, A. M.; Ugozzoli, F.; Haasnoot, J. G. *Inorg. Chim. Acta* **1994**, *227*, 181. (j) Mori, W.; Sakurai, T.; Nakahara, A.; Nakao, Y. *Inorg. Chim. Acta* **1987**, *132*, 247. (k) San Felices, L.; Vitoria, P.; Gutierrez-Zorrilla, J. M.; Reinoso, S.; Etxebarria, J.; Lezama, L. *Chem.—Eur. J.* **2004**, *10*, 5138. (l) Patel, R. N.; Singh, N.; Shukla, K. K.; Chauhan, U.K. *Spectrochim. Acta* **2005**, *A61*, 287.

(193) (a) Boujday, S.; Lambert, J.-F.; Che, M. *J. Phys. Chem. B* **2003**, *107*, 651. (b) Boujday, S.; Lambert, J.-F.; Che, M. *ChemPhysChem* **2004**, *5*, 1003. (c) Muga, I.; Gutierrez-Zorilla, J. M.; Vitoria, P.; Roman, P.; Lezama, L.; Beitia, J. I. *Eur. J. Inorg. Chem.* **2004**, *9*, 1886. (d) Paharova, J.; Cernak, J.; Boca, R.; Zak, Z. *Inorg. Chim. Acta* **2003**, *346*, 25.

(194) (a) Costas, M.; Anda, C.; Llobet, A.; Parella, T.; Evans, H. S.; Pinilla, E. *Eur. J. Inorg. Chem.* **2004**, *4*, 857. (b) Zhang, Y. H.; Vahrenkamp, H. *Inorg. Chim. Acta* **2003**, *351*, 201. (c) Yang, D.; Li, S.; Li, D.; Xia, J.; Yu, K.; Tang, W. *J. Chem. Soc., Dalton Trans.* **2002**, *21*, 4042. (d) Fabbrizzi, L.; Licchelli, M.; Marcotte, N.; Stomeo, F.; Taglietti, A. *Supramol. Chem.* **2002**, *14*, 127. (e) Koner, S.; Ghosh, A.; Chaudhuri, N. R. *Therm. Acta* **1990**, *159*, 55. (f) Cova, G.; Galizzioli, D.; Giusto, D.; Morazzoni, F. *Inorg. Chim. Acta* **1972**, *6*, 343. (g) Mondal, A.; Mostafa, G.; Ghosh, A.; Chaudhuri, N. R.;

Wong, W.-T. *Polyhedron* **1998**, *17*, 1217. (h) Alzuet, G.; Ferrer-Llusar, S.; Borras, J.; Martinez-Manez, R. *Polyhedron* **2000**, *19*, 725.

(195) (a) Del Piero, S.; Ghezzi, L.; Melchior, A.; Tine, M. R.; Tolazzi, M. *Helv. Chim. Acta* **2005**, *88*, 839. (b) Jiang, H.; Xie, Y.-S.; Zhou, Z.-Y.; Xu, X.-L.; Liu, Q.-L. *J. Coord. Chem.* **2003**, *56*, 825. (c) Browne, R. J.; Buckingham, D. A.; Clark, C. R.; McKeon, J. A.; Jackson, W. G. *Helv. Chim. Acta* **2003**, *86*, 13. (d) Browne, R. J.; Buckingham, D. A.; Blackman, A. G.; Clark, C. R. *Acta Crystallogr.* **2002**, *E58*, m125. (e) Bhat, G. R.; Dubey, K. P.; Segal, B.; Pierpont, C. G. *Acta Crystallogr.* **1988**, *C44*, 999. (f) Kapon, M.; Reisner, G. M. *Acta Crystallogr.* **1990**, *C46*, 621.

(196) (a) Shoukry, M. M.; Kenawy, I. M.; El-Haj, I. H. *Trans. Met. Chem.* **1991**, *16*, 637. (b) Sheldrick, W. S.; Heeb, G. *Inorg. Chim. Acta* **1991**, *190*, 241. (c) Canovese, L.; Cattalini, L.; Uguagliati, P.; Tobe, M. L. *J. Chem. Soc., Dalton Trans.* **1990**, *11*, 3271.

(197) Goto, M.; Koga, N.; Ohse, Y.; Kudoh, Y.; Kukihara, M.; Okuno, Y.; Kurosali, H. *Inorg. Chem.* **2004**, *43*, 5120.

(198) (a) Anzellotti, A. I.; Ma, E. S.; Farrell, N. *Inorg. Chem.* **2005**, *44*, 483. (b) van Zutphen, S.; Kraus, M.; Driessen, C.; van der Marel, G. A.; Overkleeft, H. S.; Reedijk, J. *J. Inorg. Biochem.* **2005**, *99*, 1384. (c) Wheate, N. J.; Day, A. I.; Blanch, R. J.; Arnold, A. P.; Cullinane, C.; Collins, J. G. *Chem. Commun.* **2004**, *12*, 1424. (d) Kalayda, G. V.; Jansen, B. A. J.; Molenaar, C.; Wielaard, P.; Tanke, H. J.; Reedijk, J. *J. Biol. Inorg. Chem.* **2004**, *9*, 414. (e) Anorbe, M. G.; Lueth, M. S.; Roitzsch, M.; Cerda, M. M.; Lax, P.; Kampf, G.; Sigel, H.; Lippert, B. *Chem.—Eur. J.* **2004**, *10*, 1046. (f) Favero, G.; Peloso, A.; Vettori, U. *Inorg. Chim. Acta* **1990**, *168*, 189. (g) Canovese, L.; Cattalani, L.; Gemelli, L.; Tobe, M. L. *J. Chem. Soc., Dalton Trans.* **1988**, *4*, 1049. (h) Höpp, M.; Erxleben, A.; Rombeck, I.; Lippert, B. *Inorg. Chem.* **1996**, *35*, 397. (i) Admiraal, G.; Alink, M.; Altona, C.; Dijt, F. J.; van Garderen, C. J.; de Graaff, R. A. G.; Reedijk, J. *J. Am. Chem. Soc.* **1992**, *114*, 930.

(199) Lundin, N. J.; Hamilton, I. G.; Blackman, A. G. *Polyhedron* **2004**, *23*, 97.

(200) (a) Valdes-Martinez, J.; Toscano, R. A.; Del Rio-Ramirez, M. *Acta Crystallogr.* **2004**, *E60*, m45. (b) For structure coordinates, see CCDC 214721.

(201) Gonzalez-Alvarez, M.; Alzuet, G.; Borras, J.; Macias, B.; Monetjo-Bernardo, J. M.; Garcia-Granda, S. *Z. Anorg. Allg. Chem.* **2003**, *629*, 239.

(202) (a) Castillo, O.; Muga, I.; Luque, A.; Gutierrez-Zorrilla, J. M.; Sertucha, J.; Vitoria, P.; Roman, P. *Polyhedron* **1999**, *18*, 1235. (b) For structure coordinates, see CCDC 102007.

(203) (a) Subramanian, P. S.; Suresh, E.; Shukla, R. S. *Inorg. Chim. Acta* **2005**, *358*, 2651. (b) For structure coordinates, see CCDC 249081 and 249082.

(204) Bolos, C. A.; Christidis, P. C.; Triantafyllou, S. T. *Polyhedron* **1998**, *17*, 2719.

(205) (a) Song, A.-R.; Song, Y.; Ouyang, X.-M.; Li, Y.-Z.; Sun, W.-Y. *Inorg. Chem. Commun.* **2005**, *8*, 186. (b) For structure coordinates, see CCDC 262783.

(206) (a) Vicente, M.; Bastida, R.; Macias, A.; Valencia, L.; Geraldes, C. F. G. C.; Brondino, C. D. *Inorg. Chim. Acta* **2005**, *358*, 1141. (b) Price, J. R.; Fainerman-Melnikova, M.; Fenton, R. R.; Gloe K.; Lindoy, L. F.; Rambusch, T.; Skelton, B. W.; Turner, P.; White, A. H.; Wichmann, K. *Dalton Trans.* **2004**, 3715.

(207) (a) Nesterova, O. V.; Petrusenko, S. R.; Kokozay, V. N.; Skelton, B. W.; Bjernemose, J. K.; Raithby, P. R. *Inorg. Chim. Acta* **2005**, *358*, 2725. (b) Muga, I.; Vitoria, P.; Reinoso, S.; Gutierrez-Zorilla, J. M. *Acta Crystallogr.* **2003**, *E59*, m576. (c) For structure coordinates, see CCDC 242580.

(208) (a) Callejo, L. M.; Muga, I.; Vitoria, P.; Reinoso, S.; Roman, P.; Gutierrez-Zorrilla, J. M. *Acta Crystallogr.* **2003**, *E59*, m684. (b) Muga, I.; Gutierrez-Zorrilla, J. M.; Vitoria, P.; Roman, P.; Lloret, F. *Polyhedron* **2002**, *21*, 2631.

(209) Razak, I. A.; Raj, S. S.; Fun, H.-K.; Tong, Y.-X.; Lu, Z.-L.; Kang, B.-S. *Acta Crystallogr.* **2000**, *C56*, 291.

(210) Dapporto, P.; Formica, M.; Fusi, V.; Giorgi, L.; Micheloni, M.; Paoli, P.; Pontellini, R.; Rossi, P. *Inorg. Chem.* **2001**, *40*, 6186.

(211) Gupta, D.; Huelsekopf, M.; Cerda, M. M.; Ludwig, R.; Lippert, B. *Inorg. Chem.* **2004**, *43*, 3386.

(212) (a) Zhu, T.; Jackson, W. G. *Inorg. Chim. Acta* **2003**, *343*, 147. (b) Zhu, T.; Qian-jiang, Z.; Sai-feng, X.; Jackson, W. G.; Zhong-Yuan, Z.; Xiang-Ge, Z. *J. Mol. Struct.* **2004**, *703*, 83. (c) For structure coordinates, see CCDC 186174.

(213) Zhu, T.; Zhu, Q.-J.; Xie, S.-F.; Jackson, W. G.; Zhou, Z.-Y.; Zhou, X.-G. *Polyhedron* **2003**, *22*, 1603.

(214) (a) Barth, M.; Kästele, X.; Klüfers, P. *Eur. J. Inorg. Chem.* **2005**, 1353. (b) For structure coordinates, see CCDC 226511.

(215) Patel, R. N.; Singh, N.; Shukla, K. K.; Gundla, V. L. N. *Spectrochim. Acta* **2005**, *A61*, 1893.

(216) Szilagyi, I.; Labadi, I.; Hernadi, K.; Palinko, I.; Nagy, N. V.; Korecz, L.; Rockenbauer, A.; Kele, Z.; Kiss, T. *J. Inorg. Biochem.* **2005**, *99*, 1619.

(217) Kurzak, B.; Bogusz, K.; Kroczewska, D.; Jezierska, J. *Polyhedron* **2001**, *20*, 2627.

(218) Tsiveriotis, P.; Hadjiliadis, N.; Stavropoulos, G. *Inorg. Chim. Acta* **1997**, *261*, 83.

(219) (a) Knobloch, B.; Sigel, R. K. O.; Lippert, B.; Sigel, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 3793. (b) Carlone, M.; Marzilli, L. G.; Natile, G. *Inorg. Chem.* **2004**, *43*, 584. (c) van Boom, S. S. G. E.; Chen, B. W.; Teuben, J. M.; Reedijk, J. *Inorg. Chem.* **1999**, *38*, 1450. (d) Song, B.; Zhao, J.; Griesser, R.; Meiser, C.; Sigel, H.; Lippert, B. *Chem.—Eur. J.* **1999**, *5*, 2374. (e) Kampf, G.; Lüth, M. S.; Kapinos, L. E.; Müller, J.; Holy, A.; Lippert, B.; Sigel, H. *Chem.—Eur. J.* **2001**, *7*, 1899.

(220) Guo, Z.; Sadler, P. J.; Zang, E. *Chem. Commun.* **1997**, 27.

(221) Sheldrick, W. S.; Neumann, D. *Inorg. Chim. Acta* **1994**, *223*, 131.

(222) Wirth, W.; Blotevogel-Baltronat, J.; Kleinkes, U.; Sheldrick, W. S. *Inorg. Chim. Acta* **2002**, *339*, 14.

(223) Gasowska, A.; Jastrzab, R.; Bregier-Jarzebowska, R.; Lomozik, L. *Polyhedron* **2001**, *20*, 2305.

(224) Liu, Y.; You, C.-C.; Li, B. *Chem.—Eur. J.* **2001**, *7*, 1281.

(225) For a fluorescent chemosensor for organic guests and Cu(II) ions based on dansyldiethylenetriamine-modified β-cyclodextrin, see: Corradini, R.; Dossena, A.; Galaverna, G.; Marchelli, R.; Panagia, A.; Sartor, G. *J. Org. Chem.* **1997**, *62*, 6283.

(226) Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1994**, *33*, 1032.

(227) Jurek, P. E.; Martell, A. E.; Motekaitis, R. J.; Hancock, R. D. *Inorg. Chem.* **1995**, *34*, 1823.

(228) Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1992**, *31*, 5534.

(229) (a) Li, S.; Li, D.; Li, Y.; Huang, J.; Yu, K.; Tang, W. *Chem. Commun.* **2003**, 880. (b) Yang, D.-X.; Li, S.-A.; Li, D.-F.; Chen, M.; Huang, J.; Tang, W.-X. *Polyhedron* **2003**, 925. (c) Li, D.; Li, S.; Yang, D.; Yu, J.; Huang, J.; Li, Y.; Tang, W. *Inorg. Chem.* **2003**, *42*, 6071.

(230) Coughlin, P. K.; Martin, A. E.; Dewan, J. C.; Watanabe, W.-I.; Bulkowski, J. E.; Lehn, J.-M.; Lippard, S. J. *Inorg. Chem.* **1984**, *23*, 1004.

(231) Fabbrizzi, L.; Pallavicini, P.; Parodi, L.; Perotti, A.; Taglietti, A. *Chem. Commun.* **1995**, 2439.

(232) Hortala, M. A.; Fabbrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *J. Am. Chem. Soc.* **2003**, *125*, 20.

(233) (a) Lamarque, L.; Navarro, P.; Miranda, C.; Aran, V. J.; Ochoa, C.; Escarti, F.; Garcia-Espana, E.; LaTorre, J.; Luis, S. V.; Miravet, J. F. *J. Am. Chem. Soc.* **2001**, *123*, 10560. (b) Miranda, C.; Escarti, F.; Lamarque, L.; Garcia-Espana, E.; Navarro, P.; LaTorre, J.; Lloret, F.; Jimenez, H. R.; Yunta, M. J. R. *Eur. J. Inorg. Chem.* **2005**, 189.

(234) (a) Striegler, S.; Dittel, M. *Anal. Chim. Acta* **2003**, *484*, 53. (b) Striegler, S. *Macromolecules* **2003**, *36*, 1310.

(235) Striegler, S. *Tetrahedron* **2001**, *57*, 2349.

(236) Striegler, S. *Anal. Chim. Acta* **2005**, *539*, 91.

(237) (a) Flay, M.-L.; Comte, V.; Vahrenkamp, H. *Z. Anorg. Allg. Chem.* **2003**, *629*, 1147. (b) Ballester, L.; Gutierrez, A.; Perpinan, M. F.; Sanchez, A. E.; Azcondo, M. T.; Gonzalez, M. J. *Inorg. Chim. Acta* **2004**, *357*, 1054. (c) Lu, Z.-L.; Duan, C.-Y.; Tian, Y.-P.; Wu, Z.-W.; You, J.-J.; Zhou, Z.-Y.; Mak, T. C. W. *Polyhedron* **1996**, *16*, 909. (d) Xu, X.-Y.; Zheng, T.; Ma, W.-X.; Zhang, M.-X.; Liu, Q.-L.; Gruguric, S. R.; Dinovic, V. M.; Kaluderovic, G. N.; Sabo, T. J. *J. Coord. Chem.* **2002**, *55*, 711. (e) Stibrany, R. T.; Ptoenza, J. A.; Schugar, H. J. *Acta Crystallogr.* **1993**, *C49*, 1561. (f) Morrow, J. R.; Trogler, W. C. *Inorg. Chem.* **1988**, *27*, 3387. (g) Diaz, G.; Bustos, C.; Shepherd, R. E. *Inorg. Chim. Acta* **1987**, *133*, 23. (h) Siddiqui, S.; Shepherd, R. E. *Inorg. Chem.* **1986**, *25*, 3869. (i) Duggan, M.; Ray, N.; Hathaway, B.; Tomlinson, G.; Brint, P.; Pelin, K. *J. Chem. Soc., Dalton Trans.* **1980**, *8*, 1342. (j) Alberin, G.; Bordignon, E.; Orio, A. A. *Inorg. Chem.* **1975**, *14*, 1411. (k) Cayley, G. R.; Kelly, I. D.; Knowles, P. F.; Yadav, K. D. S. *J. Chem. Soc., Dalton Trans.* **1981**, 2370.

(238) (a) Komiyama, K.; Furutachi, H.; Nagatomo, S.; Hashimoto, A.; Hayashi, H.; Fujinami, S.; Suzuki, M.; Kitagawa, T. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 59. (b) Navon, N.; Cohen, H.; Paoletti, P.; Valtancoli, B.; Bencini, A.; Meyerstein, D. *Ind. Eng. Chem. Res.* **2000**, *39*, 3536.

(239) (a) Lever, A. B. P.; Paoletti, P.; Fabbrizzi, L. *Inorg. Chem.* **1979**, *18*, 1324. (b) Misra, T. K.; Chen, L.-H.; Lin, Y.-C.; Chung, C.-S. *Polyhedron* **2002**, *21*, 2045. (c) Flay, M.-L.; Vahrenkamp, H. *Z. Anorg. Allg. Chem.* **2004**, *630*, 2508. (d) Castro, I.; Calatayud, M. L.; Sletten, J.; Lloret, F.; Julve, M. *J. Chem. Soc., Dalton Trans.* **1997**, *5*, 811. (e) Fun, H.-K.; Yip, B.-C.; Lu, Z.-L.; Duan, C.-Y.; Tian, Y.-P.; You, X.-Z. *Acta Crystallogr.* **1996**, *C52*, 509. (f) Colpas, G. J.; Kumar, M.; Day, R. O.; Maroney, M. J. *Inorg. Chem.* **1990**, *29*, 4779. (g) Ihara, Y.; Sakura, M. *Thermochim. Acta* **1988**, *136*, 279.

(240) (a) Interrante, L. V. *Inorg. Chem.* **1968**, *7*, 943. (b) Canary, J. W.; Xu, J.; Castagnetto, J. M.; Rentzeperis, D.; Marky, L. A. *J. Am. Chem. Soc.* **1995**, *117*, 11545. (c) Marzotto, A.; Clemente, D. A.; Valle, G. *Acta Crystallogr.* **1994**, *C50*, 1451.
(241) (a) Kimura, E.; Young, S.; Collman, J. P. *Inorg. Chem.* **1970**, *9*, 1183. (b) Saha, M. K.; Mukhopadhyay, U.; Bernal, I. *Dalton Trans.* **2004**, 1466. (c) Hartshorn, R. M. *J. Chem. Soc., Dalton Trans.* **2002**, 3214. (d) Barfod, R.; Eriksen, J.; Golding, B. T.; Hammershoi, A.; Jacobsen, T. A.; Langkilde, A.; Larsen, S.; Monsted, O.; Sargeson, A. M.; Sorensen, H. O. *Dalton Trans.* **2005**, 491. (e) Chun, H.; Salinas, B. J.; Bernal, I. *Eur. J. Inorg. Chem.* **1999**, *4*, 723. (f) Chun, H.; Bernal, I. *Eur. J. Inorg. Chem.* **1999**, *4*, 717. (g) Brasch, N. E.; Buckingham, D. A.; Clark, C. R.; Rogers, A. J. *Inorg. Chem.* **1998**, *37*, 4865. (h) Yonemura, T.; Shibuya, K.; Okamoto, K.; Ama, T.; Kawaguchi, H.; Yasui, T. *Inorg. Chim. Acta* **1997**, *260*, 119. (i) Brasch, N. E.; Buckingham, D. A.; Clark, C. R.; Simpson, J. *Inorg. Chem.* **1996**, *35*, 7728. (j) Tafesse, F.; Massoud, S. S.; Milburn, R. M. *Inorg. Chem.* **1993**, *32*, 1864. (k) Nakano, Y.; Yoshikawa, Y.; Kondo, H. *J. Am. Chem. Soc.* **1986**, *108*, 7630. (l) Jackson, W. G.; McLaughlin, G. M.; Sargeson, A. M.; Watson, A. D. *J. Am. Chem. Soc.* **1983**, *105*, 2426. (m) Mitsui, Y.; Watanabe, J.; Harada, Y.; Sakamaki, T.; Iitaka, Y.; Kushi, Y.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1976**, *20*, 2095. (n) Mitsui, Y.; Watanabe, J.; Iitaka, Y.; Kimura, E. *J. Chem. Soc., Chem. Commun.* **1975**, *8*, 280. (o) Massoud, S. S.; Milburn, R. M. *Inorg. Chim. Acta* **1988**, *154*, 115.
(242) Krotz, A. H.; Kuo, L. Y.; Barton, J. K. *Inorg. Chem.* **1993**, *32*, 5963.
(243) Nair, V. S.; Hagen, K. S. *Inorg. Chem.* **1994**, *33*, 185.
(244) (a) Brudenell, S. J.; Crimp, S. J.; Higgs, J. K. E.; Moubaraki, B.; Murray, K. S.; Spiccia, L. *Inorg. Chim. Acta* **1996**, *247*, 35. (b) Barnes, C. M.; Petoud, S.; Cohen, S. M.; Raymond, K. N. *J. Biol. Inorg. Chem.* **2003**, *8*, 195.
(245) Lu, Z.-L.; Duan, C.-Y.; Tian, Y.-P.; You, X.-Z.; Fun, H.-K.; Yip, B.-C. *Polyhedron* **1996**, *15*, 1769.
(246) Lu, Z.-L.; Duan, C.-Y.; Tian, Y.-P.; You, X.-Z.; Fun, H.-K.; Yip, B.-C.; Hovestreydt, E. *Polyhedron* **1997**, *16*, 187.
(247) Lu, Z.-L.; Duan, C.-Y.; Tian, Y.-P.; You, X.-Z.; Huang, X.-Y. *Inorg. Chem.* **1996**, *35*, 2253.
(248) Su, C.-C.; Lu, W.-S.; Hui, T.-Y.; Chang, T.-Y.; Wang, S.-L.; Liao, F.-L. *Polyhedron* **1993**, *12*, 2249.
(249) Lu, Q.; Luo, Q. H.; Dai, A. B.; Zhou, Z. Y.; Hu, G. Z. *J. Chem. Soc., Chem. Commun.* **1990**, 1429.
(250) Marzotto, A.; Ciccarese, A.; Clemente, D. A.; Valle, G. *J. Chem. Soc., Dalton Trans.* **1995**, 1461.
(251) (a) Salam, M. A.; Aoki, K. *Inorg. Chim. Acta* **2001**, *314*, 71. (b) For structure coordinates, see CCDC 154386.
(252) (a) Ochs, C.; Hahn, F. E.; Lügger, T. *Eur. J. Inorg. Chem.* **2001**, 1279. (b) Marzotto, A.; Clemente, D. A.; Valle, G. *Acta Crystallogr.* **1993**, *C49*, 1252. (c) For structure coordinates, see CCDC 149397.
(253) (a) Fu, H.; Chen, W.-L.; Fu, D.-G.; Tong, M.-L.; Chen, X.-M.; Ji, L.-N.; Mao, Z.-W. *Inorg. Chem. Commun.* **2004**, *7*, 1285. (b) For structure coordinates, see CCDC 239958.
(254) Mao, Z.-W.; Liehr, G.; van Eldik, R. *J. Chem. Soc., Dalton Trans.* **2001**, 1593.
(255) (a) Cai, J.; Hu, X.; Bernal, I.; Ji, L. N. *Polyhedron* **2002**, *21*, 817. (b) For structure coordinates, see CCDC 164478.
(256) (a) Herdin, S.; Klüfers, P.; Kunte, T.; Piotrowski, H. *Z. Anorg. Allg. Chem.* **2004**, *630*, 701. (b) For structure coordinates, see CCDC 227974.
(257) Ibrahim, M. M.; Ichikawa, K.; Shiro, M. *Inorg. Chem. Commun.* **2003**, *6*, 1030.
(258) Fu, H.; Liu, X.-W.; Zhou, Z.-Y.; Mao, Z.-W.; Zheng, K.-C.; Chen, X.-M. *Inorg. Chem. Commun.* **2004**, *7*, 906.
(259) Ibrahim, M. M.; Shimomura, N.; Ichikawa, K.; Shiro, M. *Inorg. Chim. Acta* **2001**, *313*, 125.
(260) Stibrany, R. T.; Potenza, J. A.; Schugar, H. J. *Acta Crystallogr.* **1993**, *C49*, 1561.
(261) (a) Chun, H.; Jackson, W. G.; McKeon, J. A.; Somoza., F. B., Jr.; Bernal, I. *Eur. J. Inorg. Chem.* **2000**, 189. (b) For structure coordinates, see CCDC 118583.
(262) (a) Cai, J.; Hu, X.-P.; Feng, X.-L.; Shao, W.-Y.; Ji, L.-N.; Bernal, I. *Eur. J. Inorg. Chem.* **2000**, *10*, 2199. (b) Hu, X.-P.; Cai, J.; Chen, C.-H.; Chen, X.-M.; Ji, L.-N. *Cryst. Eng.* **2001**, *4*, 141. (c) Yamanari, K.; Fuyuhiro, A. *Chem. Lett.* **1991**, 1551. (d) Yamanari, K.; Fuyuhiro, A. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 2543. (e) Yamanari, K.; Fuyuhiro, A. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1289. (f) Butler, P. A.; Crane, C. G.; Golding, B. T.; Hammershoi, A.; Hockless, D. C.; Petersen, T. B.; Sargeson, A. M.; Ware, D. C. *Inorg. Chim. Acta* **2002**, *331*, 318. (g) Cai, J.; Hu, X.; Cai, H.; Ji, L. N. *Acta Crystallogr.* **2001**, *C57*, 394.
(263) Okamoto, K.; Konno, T.; Hidaka, J. *Acta Crystallogr.* **1990**, *C46*, 216.
(264) Torres, L. M.; Marzilli, L. G. *J. Am. Chem. Soc.* **1991**, *113*, 4678.
(265) Liu, Y.; Sletten, E. *J. Inorg. Biochem.* **2003**, *93*, 190.
(266) For complexes of metal-free protonated Tren ligands and phosphate anions, see: Hossain, M. A.; Liljegren, J. A.; Powell, D.; Bowman-James, K. *Inorg. Chem.* **2004**, *43*, 3751.
(267) Tobey, S. L.; Jones, B. D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 4026.
(268) Fabbrizzi, L.; Licchelli, M.; Parodi, L.; Poggi, A.; Taglietti, A. *Eur. J. Inorg. Chem.* **1999**, 35.
(269) For a Tren ligand with coumarine chromophore and its Zn(II) complex, see: Dakanali, M.; Roussakis, E.; Kay, A. R.; Katerinopoulos, H. E. *Tetrahedron Lett.* **2005**, *46*, 4193.
(270) De Santis, G.; Fabbrizzi, L.; Licchelli, M.; Poggi, A.; Taglietti, A. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 202.
(271) Tobey, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 10963.
(272) Binding of orotic acid by ATMCA (anthrylmethyl-2,4,6-triamino-1,3,5-trimethoxycyclohexane), a Tren related ligand system, is reported: Fabbrizzi, L.; Licchelli, M.; Mancin, F.; Pizzeghello, M.; Rabaioli, G.; Taglietti, A.; Tecilla, P.; Tonellato, U. *Chem.—Eur. J.* **2002**, *8*, 94.
(273) Binding histidine by a Tren related ligand can be found: Fabbrizzi, L.; Foti, F.; Patroni, S.; Pallavicini, P.; Taglietti, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5073.
(274) Dagnall, S. P.; Hague, D. N.; Moreton, A. D. *J. Chem. Soc., Dalton Trans.* **1986**, 1505.
(275) For a recent review on thermolecular devices based on transition metals, see: Amendola, V.; Fabbrizzi, L.; Foti, F.; Licchelli, M.; Mangano, C.; Pallavicini, P.; Poggi, A.; Sacchi, D.; Taglietti, A. *Coord. Chem. Rev.* **2006**, *250*, 273.
(276) Fabbrizzi, L.; Francese, G.; Licchelli, M.; Perotti, A.; Taglietti, A. *Chem. Commun.* **1997**, 581.
(277) For "naked-eye" detection of histidine by a related acyclic Cu(II) complex, see: Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V. *Chem.—Eur. J.* **2005**, *11*, 5319.
(278) Fabbrizzi, L.; Licchelli, M.; Perotti, A.; Poggi, A.; Rabaioli, G.; Sacchi, D.; Taglietti, A. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2108.
(279) (a) Bag, B.; Bharadwaj, P. K. *Inorg. Chem.* **2004**, *43*, 4626. (b) Darbost, U.; Zeng, X.; Rager, M.-N.; Giorgi, M.; Jabin, I.; Reinaud, O. *Eur. J. Inorg. Chem.* **2004**, 4371. (c) Izzet, G.; Douziech, B.; Prange, T.; Tomas, A.; Jabin, I.; Le Mest, Y.; Reinaud, O. *Proc. Natl. Acad. Sci.* **2005**, *102*, 6831.
(280) Darbost, U.; Rager, M.-N.; Petit, S.; Jabin, I.; Reinaud, O. *J. Am. Chem. Soc.* **2005**, *127*, 8517.
(281) Amendola, V.; Fabbrizzi, L.; Mangano, C.; Pallavicini, P.; Poggi, A.; Taglietti, A. *Coord. Chem. Rev.* **2001**, *219−221*, 821.
(282) Amedola, V.; Fabbrizzi, L.; Mangano, C.; Paalvicini, P.; Zema, M. *Inorg. Chim. Acta* **2002**, *337*, 70.
(283) (a) Bonizzoni, M.; Fabbrizzi, L.; Piovani, G.; Taglietti, A. *Tetrahedron* **2004**, *60*, 11159. (b) Boiocchi, M.; Bonizzoni, M.; Fabbrizzi, L.; Piovani, G.; Taglietti, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 3847.
(284) Sharma, S.; Agarwal, G. P. *Anal. Biochem.* **2001**, *288*, 126.
(285) Wright, A. T.; Anslyn, E. V. *Chem. Soc. Rev.* **2006**, 35, 14.
(286) Wright, A. T.; Anslyn, E. V.; McDevitt, J. T. *J. Am. Chem. Soc.* **2005**, *127*, 17405.
(287) Wright, A. T.; Griffin, M. J.; Zhong, Z.; McCleskey, S. C.; Anslyn, E. V.; McDevitt, J. T. *Angew. Chem., Int. Ed.* **2005**, *44*, 6375.

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