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Recent advances in utilization of transition metal complexes and lanthanides as diagnostic tools

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Contents

Abstract

Based on the widely diverse coordination environment of transition metal complexes and lanthanides, and variation in the identities of coordinating ligands, synthesis of such complexes with desired molecular geometry can be realized. These compounds often possess remarkable and unique spectroscopic, photophysical and electrochemical properties which

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may be exploited in sensory and diagnostic applications. In this article, recent advances in the development and utilization of transition metal complexes and lanthanides as ion, molecular and other chemical sensors, nucleic acid probes, and other detection tools in related bioassays will be reviewed.

Keywords: Transition metal complexes; Lanthanides; Nucleic acid

1. Introduction

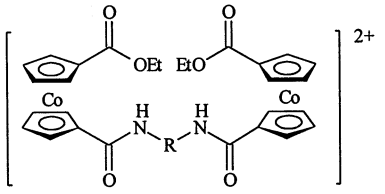
Based on the widely diverse coordination environment of transition metal complexes and lanthanides, and variation in the identities of coordinating ligands, synthesis of such complexes with desired molecular geometry can be realized. These compounds often possess remarkable and unique spectroscopic, photophysical and electrochemical properties which may be exploited in sensory and diagnostic applications. In this review article, we will introduce the recent development of transition metal complexes and the lanthanide chelates as a diagnostic tool. Instead of including examples in a specific area, we define the term "diagnostic tool" in a broad sense. In other words, we will introduce recent examples of transition metal and lanthanide complexes as cation, anion and molecular sensors. Examples describing the binding and cleavage of nucleic acids by these compounds will also be reviewed. Owing to the unique structural properties of the lanthanide complexes, we will also focus our discussion on the increasingly important role of such complexes as chiral NMR shift reagents and magnetic resonance imaging contrast agents. Furthermore, examples of lanthanide compounds as luminescent labels in various fluoroimmunoassays will also be included.

2. Ion and molecular recognition

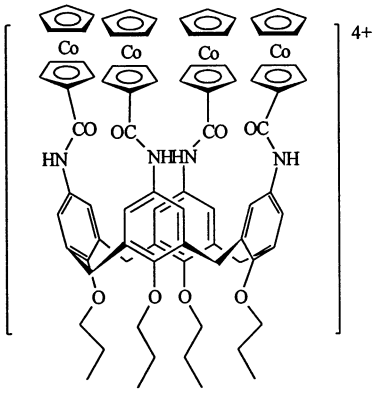
2.1. Transition metal complexes

There have been a number of excellent reviews on transition metal complexes as ion and molecular sensors [1–8]. The binding action of these complexes can be monitored by the changes in the optical properties of the systems. Besides, as many transition metal complexes can exhibit several stable oxidation states, by incorporating receptor ligands to a redox active metal centre, the binding action of the former can be revealed by the change in the electrochemical properties of the redox active centre. Beer and co-workers reported a number of receptor complexes by using the redox active ferrocene, cobaltocenium and ruthenium(II) polypyridyl unit as the reporter and crown ethers and calixarenes as the binding unit [1–3]. Recently, this research group also focused on the design of selective anion receptors [9]. The anion binding properties of most of these systems are based on the hydrogen bonding between the amide protons of the functionalized ligand and the

anionic guests. In some cases, recognition is based on the additional interaction between the Lewis acid host metal centre and the guest anions. High selectivity among common anions such as halides, nitrate and dihydrogenphosphate has been observed by NMR spectroscopy or by monitoring the change in the electrochemical and/or spectroscopic properties of the complexes. For example, a series of dinuclear cobaltocenium receptors (**1a**–**c**) show preference in binding chloride over iodide ions [10]. However, the tetranuclear cobaltocenium calix[4]arene complex (**2**) binds dihydrogenphosphate ion much more strongly than halide ions [9].



1a: R = $-(CH_2)_2-$
1b: R = $-(CH_2)_3-$
1c: R = $-(CH_2)_4-$



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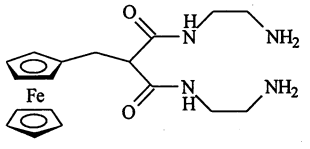

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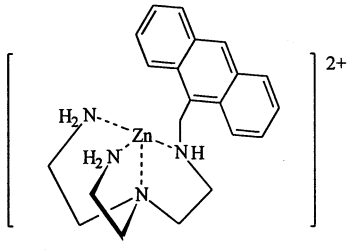

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Fabbrizzi and co-workers reported the nickel(II) ion binding properties of a water soluble ferrocene complex (**3**) with a tetraamine diketone ligand [11]. The binding abilities of the complex have been found to be significantly enhanced when the redox active unit is oxidized to the ferrocenium state. An interesting system containing Zn^{2+}-tris(2-aminoethyl)amine-anthracene (**4**) has also been synthesized [12]. While the emission intensity of the anthracene shows little changes in the presence of nitrate, thiocyanate and chloride ions, in the presence of carboxylate ions such as 4-*N*,*N*-dimethylaminobenzoate and 4-nitrobenzoate, the emission intensity of the anthracene is quenched significantly. The quenching has been ascribed to result from the

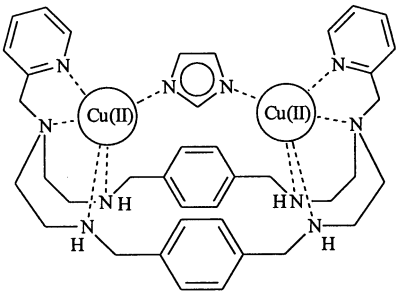
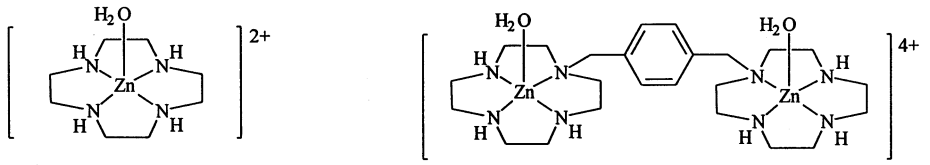


Fig. 1. Binding of an imidazole anion or imidazolate moiety of L-histidine by a dinuclear copper(II) complex. Adapted from ref. [14].

intramolecular electron transfer process between the anthracene moiety and the carboxylate ion coordinated to the Zn(II) metal centre. A similar system has also been reported recently as a sensor of imidazolate anion and the imidazolate moiety of L-histidine [13]. Interestingly, another dinuclear copper(II) complex has also been found to selectively bind imidazolate ion and imidazolate moiety of L-histidine in the presence of other amino acids (Fig. 1) [14]. The binding action is monitored by the change in the electronic absorption of the Cu(II)–N_4 chromophore.

Based on the complementary associations between a zinc(II) complex of [12]aneN_4 (1,4,7,10-tetraazacyclododecane) and N(3)-deprotonated AZT (3′-azido-3′-deoxythymidine) (Fig. 2), Kimura and co-workers reported the highly specific binding of the complex {$Zn(OH_2)$[12]aneN_4} (**5**) to thymidine and its analogues such as AZT, uridine, ftorafur and riboflavin [15]. The stability of the ternary



5 **6**

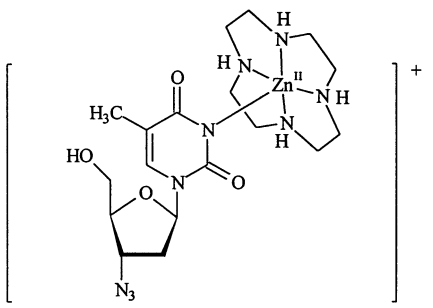
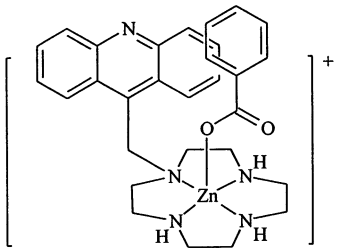
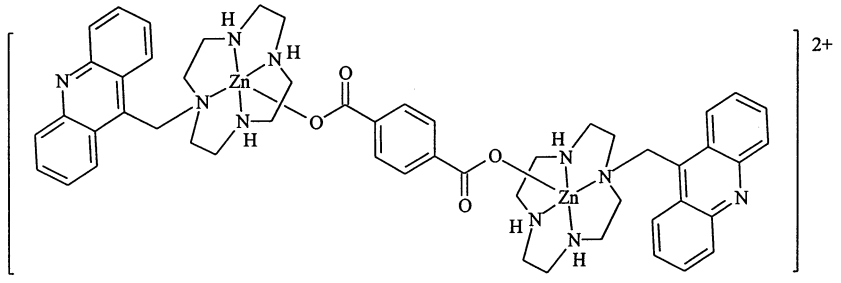


Fig. 2. A ternary complex with Zn^{II}-[12]aneN_4 and N(3)-deprotonated AZT. Adapted from ref. [15].



(a)

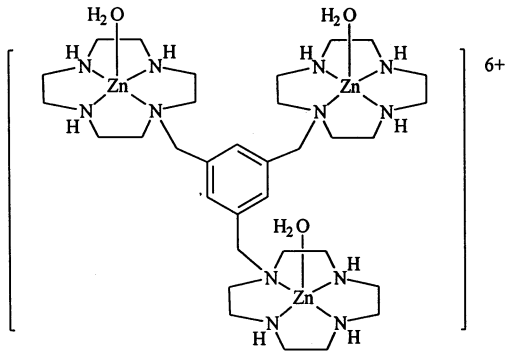


(b)

Fig. 3. Binding of (a) benzoate anion and (b) terephthalate dianion by Zn^{II}-[12]aneN_4 with an acridine pendant. Adapted from ref. [16].

complexes has been ascribed to the hydrogen bonding between the carbonyl oxygens of the thymidine and the amino groups of [12]aneN_4. Related complexes with an acridine pendant have also been found to bind strongly to benzoate anion (Fig. 3a) and terephthalate dianion (Fig. 3b) [16]. Very recently, dinuclear (**6**) [17] and trinuclear (**7**) [18] zinc(II) counterparts have been synthesized and their binding to barbital dianion and phosphate dianions, respectively, have also been investigated in detail.

With the use of phenol or pyridine pendant bipyridine ligands, Ward and co-workers synthesized a series of ruthenium(II) complexes which have been shown to function as pH sensors [19]. Ruthenium(II) terpyridine complexes with a crown ether moiety have also been reported by the same research group [20]. Besides, Sullivan and co-workers reported the synthesis of rhenium(I) complex with a 1,10-phenanthroline ligand containing an aza-15-crown-5 pendant (**8**) [21].



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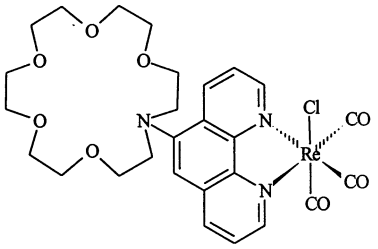

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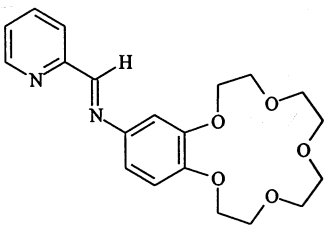
The metal-to-ligand charge-transfer emission intensity of the complex reveals a 2.7-fold increase in the presence of 15 equivalents of lead(II) ions.

Besides, a number of copper(I) [22], rhenium(I) [23] and ruthenium(II) [24] complexes with a diimine ligand in conjugation with a benzo-15-crown-5 moiety (**9**) have been synthesized by Yam and co-workers. The cation binding action of the crown unit is revealed by the change of the low energy metal-to-ligand charge-transfer/intraligand absorption band. Recently, a series of ruthenium(II) complexes containing a dipyrido[3,2-*a*:2′,3′-*c*]phenazo-15-crown-5 ligand (**10**) have also been synthesized [25]. The cation binding properties of the complexes have been investigated by electronic absorption spectroscopy, cyclic voltammetry and further confirmed by electrospray ionization mass spectrometry.



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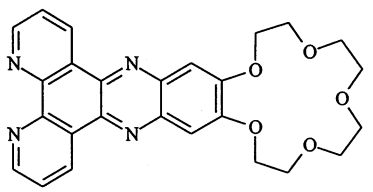

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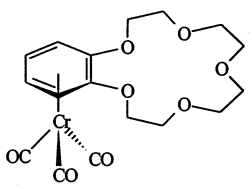

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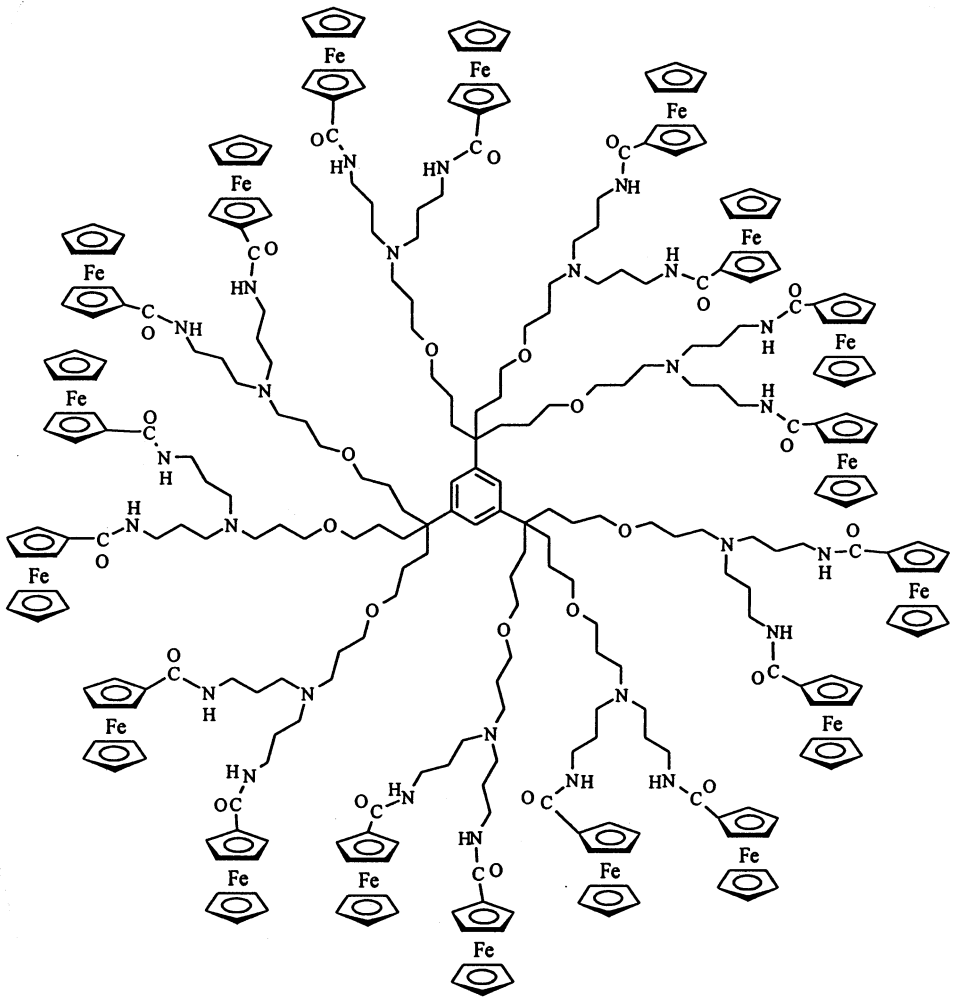
On the other hand, Anson et al. recently utilized a series of organometallic chromium(0) and iron(0) carbonyl complexes as a cation [26], aromatic [27] and pH [28] probe. The concentration of the analytes is correlated with the frequencies of the ν(CO) modes of the complexes which are detected by FTIR spectroscopy. For example, the symmetric and asymmetric ν(CO) bands of the (η^6-benzocrown ether)–$Cr(CO)_3$ complex (**11**) shift to a higher frequency in the presence of sodium and potassium ions [26].

Recently, Astruc and co-workers reported the synthesis of a series of ferrocene dendrimers such as (**12**) [29]. Electrochemical and NMR spectroscopic studies reveal that these dendrimers bind strongly towards small anions such as dihydrogenphosphate and hydrogensulfate.



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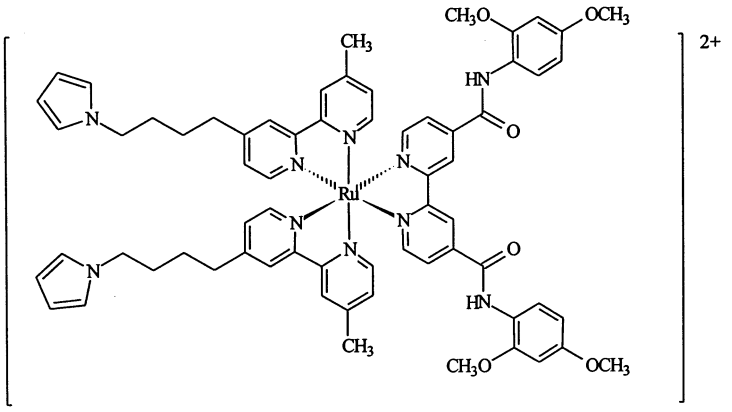
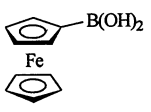
Moutet and co-workers reported the selective chloride ion binding properties of the mixed-ligand ruthenium(II) complex (**13**) by spectroscopic and electrochemical methods [30]. Platinum electrodes have also been modified by electropolymerization of the pyrrole moieties of the complex. The modified electrode reveals a shift in the first reduction of the redox active polymer film in the presence of chloride ion while iodide and bromide ions do not give rise to any significant influence.

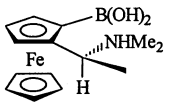


13

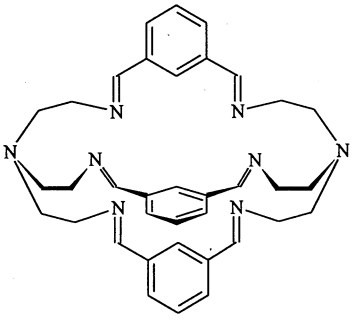
On the other hand, ferroceneboronic acid (**14a**) has been reported by Shinkai and co-workers to exhibit extraordinary selectivity in the electrochemical recognition of fluoride ions in the presence of other halides and common anions [31]. Besides, the same research group also described the attachment of a chiral tertiary amine group to ferrocenylboronic acid (**14b**) [32]. The complex shows chiral discrimination in the binding of a series of linear saccharides at pH 7. Simple



14a



14b



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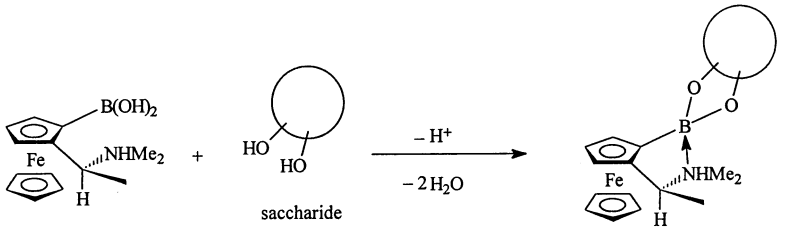
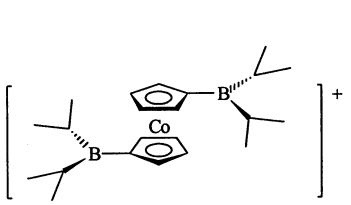


Fig. 4. Reaction of $[H^+ \cdot (\mathbf{14b})]$ and a saccharide. Adapted from ref. [32].

boronic acid–saccharide complexation is not responsible for the redox potential change in the ferrocene moiety. Instead, it is suggested that the change corresponds to conversion of $[H^+ \cdot (\mathbf{14b})]$ to [saccharide $\cdot$ (**14b**)] (Fig. 4). As the binding of F^- to (**14a**) and saccharide to $[H^+ \cdot (\mathbf{14b})]$ makes the $E_{1/2}$ more negative, the binding can be monitored by the change in the color of a dye with an $E_{1/2}$ slightly more negative than that of the ferrocenyl acceptor [33]. For example, at pH 3, the $E_{1/2}$ difference between (**14a**) and methylene blue (MB) is about 250 mV, which can be reduced by fluoride complexation. In a solution of (**14a**) (1.0×10^{-2} M) and MB (1.0×10^{-5} M), the absorption maximum of MB at 665 nm decreases at $[F^-] = 4 \times 10^{-3}$ M and reaches zero at $[F^-] = 3 \times 10^{-2}$ M. The disappearance of the blue color of MB is a result of the reduction of the dye by the fluoride-bound (**14a**).

In 1984, Martell and co-workers demonstrated the chloride and hydroxide binding properties of copper(II) containing cryptate complexes [34]. Recently, the synthesis of an octaaza macrocyclic cryptand (**15**) has also been reported [35]. Protonation constants and metal ion stability constants of the mononuclear and dinuclear copper(II) and cobalt(II) complexes of this ligand have been determined by potentiometric methods. The crystal structure of the dinuclear copper(II) cryptand reveals the presence of a bridging carbonate ion, taken up from atmospheric carbon dioxide. On the other hand, Ahlers et al. reported the recognition of cyanide ion by a dinuclear copper(II) macrocyclic complex [36]. The cyanide ion is situated within the cavity interacting with the two copper centres (Fig. 5). The complex was employed in the construction of a liquid-membrane electrode which was found to exhibit reversible near-Nernstian response to aqueous cyanide solutions at pH 10. Recently, Kitagawa and coworkers showed the anion-binding properties of dinuclear copper(I) and silver(I) complexes with the bridging phosphine ligands dpph [1,6-bis(diphenylphosphino)hexane] [37]. Crystal structures reveal that common anions such as tetrahedral perchlorate and Y-shaped nitrate ions interact strongly with the copper(I) centres.



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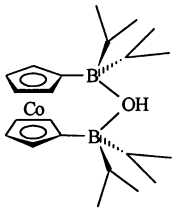

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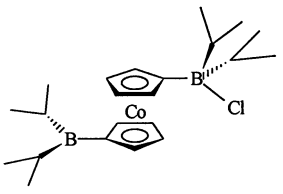

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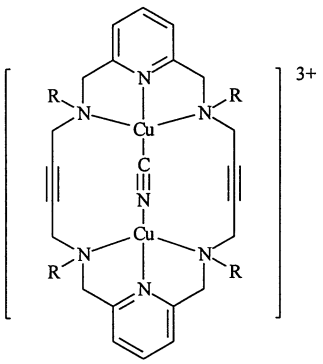
Herberich and co-workers reported the interesting anion-binding behavior of the cobaltocenium $[Co(C_5H_4BPr^i_2)_2]^+$ (**16**) cation [38]. Oxidation of the neutral cobaltocene $[Co(C_5H_4BPr^i_2)_2]$ with $Cu(OH)_2$ gave (**17**), in which a μ-hydroxide ion bridges two boron atoms on the two cyclopentadienyl rings. However, oxidation

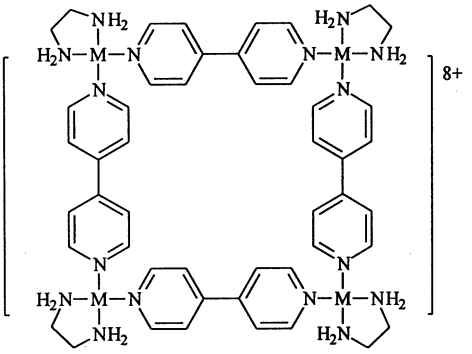


R = $C_{16}H_{33}$, CH_2Ph

Fig. 5. Binding of a cyanide ion by a dinuclear copper(II) macrocyclic complex. Adapted from ref. [36].

with C_2Cl_6 in toluene resulted in (**18**) in which a chloride ion coordinates to only one of the borons.

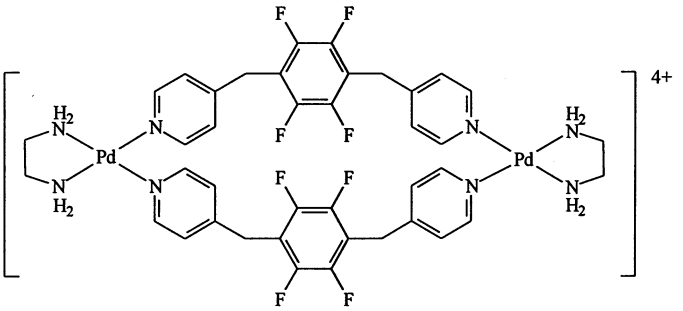
Fujita and co-workers reported the self-assembly of a number of molecular squares containing four Group 10 metal centres [Ni(II), Pd(II) and Pt(II)] and four 4,4′-bipyridines (**19**) [39]. The molecular recognition properties of these complexes



M = Ni, Pd, Pt

19

have also been studied. For example, addition of the tetranuclear Ni(II) complex into a D_2O solution of 1,3,5-trimethoxybenzene causes upfield shifts in the ^{1}H-NMR signals of the phenyl and methoxy protons. The association constant between the complex and the organic guest has been determined to be 7.5×10^2 M^{-1} at 298 K [40]. By using different bridging ligands, the research group has synthesized a series of dinuclear (**20**) [41], trinuclear (**21**) [42] and tetranuclear [2]catenane (**22**) [43] palladium(II) macrocyclic complexes. The dinuclear complex



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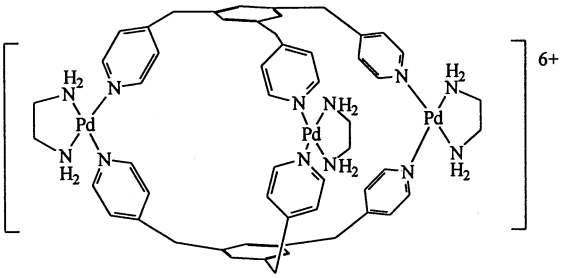
(**20**) has also been found to recognize electron-rich aromatic compounds with high shape specificity [41]. Polymethoxybenzenes are bound specifically over other derivatives such as *p*-dicyanobenzene and *p*-dinitrobenzene. The specific recognition of electron-rich benzene derivatives has been ascribed to the electronic environment in the cavity surrounded by electron-deficient tetrafluorophenylene and coordinated pyridyl groups.



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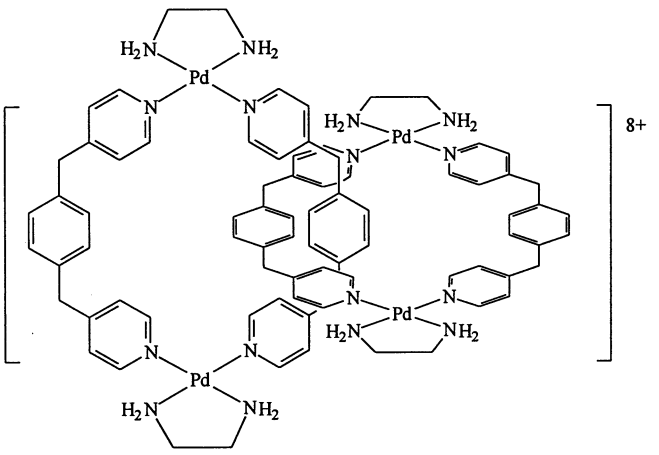

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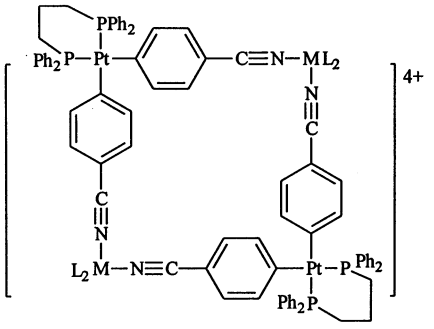
Stang and co-workers recently reported the related organometallic molecular squares using benzonitrile (**23**) and 4-ethynylpyridine (**24**) as the bridging unit [44]. The alkynyl moieties of the 4-ethynylpyridine squares have been found to be capable of binding two equivalents of silver(I) ions (**25**). The binding has been studied by different spectroscopic methods and confirmed by FAB-MS. Besides, crown ether and calix[4]arene units have also been attached to a series of related platinum molecular squares [45].



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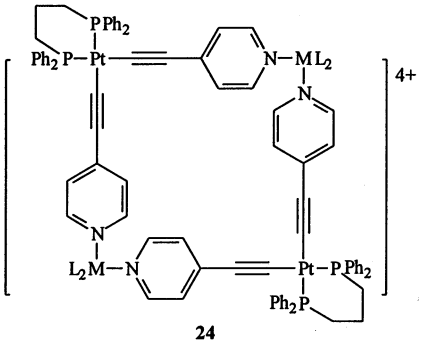

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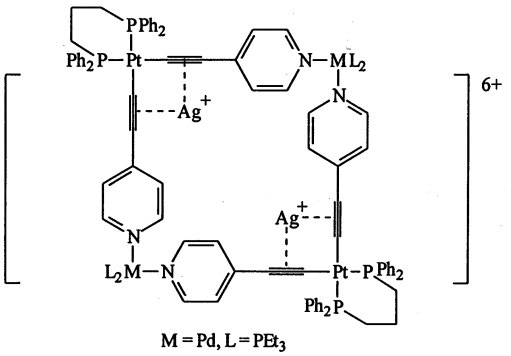
M = Pt or Pd

$L_2 = Ph_2P(CH_2)_3PPh_2$ or L = PEt_3



M = Pd, L = PEt_3

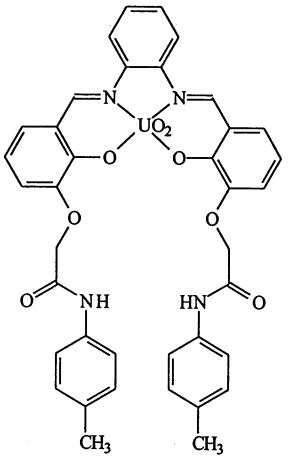
M = Pt, $L_2 = Ph_2P(CH_2)_3PPh_2$

25

On the other hand, Hupp and co-workers recently reported the synthesis of a similar molecular square containing two Pd(II) and two Re(I) metal centres with 4,4′-bipyridine as the bridging ligand [46]. Th emission intensity of the complex at 625 nm in acetone exhibits enhancement in the presence of tetraethylammonium perchlorate. The change in the emission intensity is assigned to the binding of the perchlorate anion by the tetracationic square host. An association constant of 900 M^{-1} has been determined.

Interestingly, Lehn and co-workers recently reported the self-assembly of a circular double helicate containing five iron(II) centres [47]. The central cavity of the complex has been found to be occupied by a chloride anion.

Reinhoudt and co-workers described a series of neutral mononuclear uranyl salen complexes containing amido side chains [48–50]. An example is illustrated as (**26**). The complexes have been found to bind dihydrogen-phosphate anion. The recognition is based on the coordination between the anion and the uranyl cation and the hydrogen bonding between the substrate and the host. Recently, dinuclear uranyl macrocyclic complexes (**27a**) and (**27b**) have also been reported to bind dianions such as terephthalate, succinate and fumarate [51].



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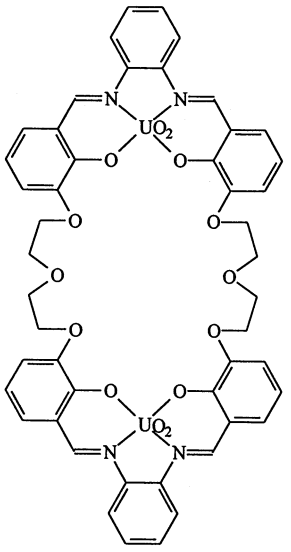
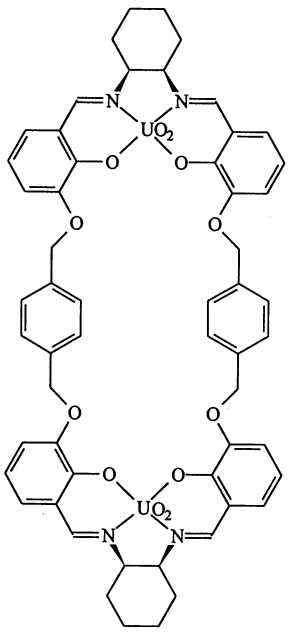


27a



27b

Besides, Gokel and co-workers recently described a series of ferrocene derivatives as diamine receptors [52]. On the other hand, the reversible binding of C_{60} to the dendrimers containing $Ir(CO)Cl(PPh_2R)_2$ {PPh_2R = [3,5-bis(benzyloxy)benzyl] diphenylphosphine (**28a**) or (3,5-bis{[3,5-bis(benzyloxy)benzyl]oxy}benzyl)diphenylphosphine (**28b**)} complexes has also been reported by Catalano and co-workers (Fig. 6) [53]. Thermodynamic data on the binding have been obtained by line width analysis of the $^{31}P\{^{1}H\}$-NMR spectra.

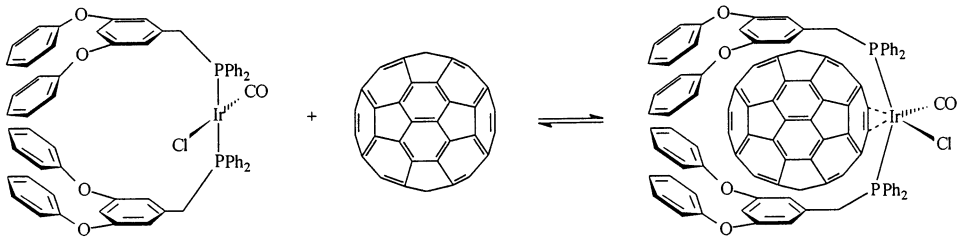
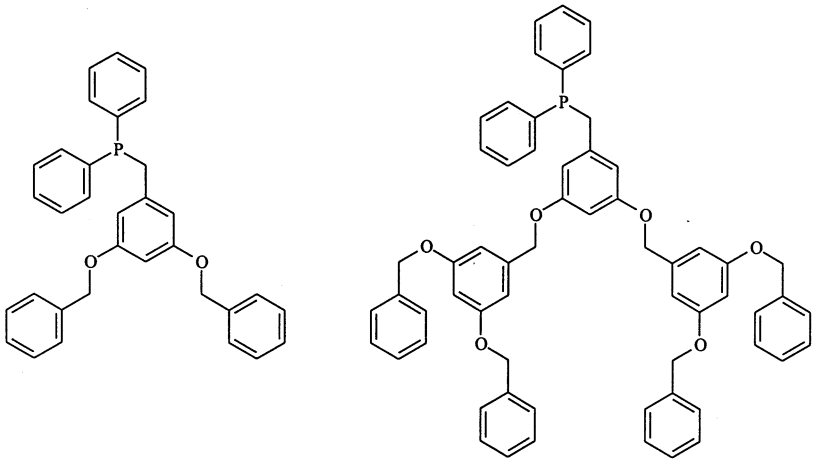
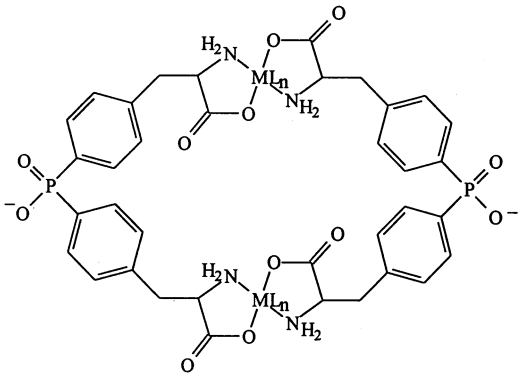


Fig. 6. Reversible binding of C_{60} to [Ir(CO)Cl(**28a**)$_2$]. Adapted from ref. [53].



28a **28b**

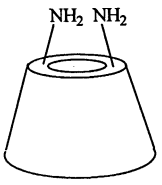


M = Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}

29

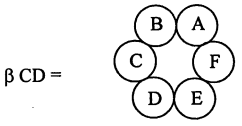
Schwabacher and co-workers reported the synthesis of a series of dinuclear transition metal complexes with the ligand 4,4′-(hydroxyphosphinylidene)bis-L-phenylalanine (**29**) [54]. The Co(II) and Ni(II) complexes have been found to bind pyrene effectively.

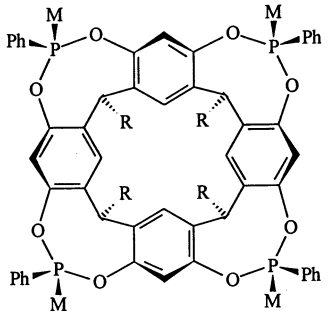
On the other hand, the copper(II) complex with 6-difunctionalized β-cyclodextrin (6^A,6^B-diamino-6^A,6^B-dideoxy-β-cyclodextrin) (A,B-$CDNH_2$) (**30**) has been shown by Rizzarelli and co-workers to exhibit chiral recognition properties towards different amino acids [55]. It has been suggested that the recognition is based on the inclusion of the aromatic side chains of the amino acids in the CD cavity. However, the other two copper(II)-containing regioisomers (6^A,6^X-diamino-6^A,6^X-dideoxy-β-cyclodextrin) (X = C and D) do not show any chiral selectivities.



30

A,B-$CDNH_2$





R = CH_2CH_2Ph

31: M = AuCl

32: M = $PtCl_2(SMe_2)$

Loeb and co-workers reported the binding of small molecules such as water, ammonia, amines, hydrazine and the hydrazinium ions to palladium complexes with a thiacyclophane ligand [56]. An adduct of water is shown in Fig. 7. The guest molecules have been found to interact with the palladium centre and in hydrogen bonding with the peripheral crown ether oxygens. Selective binding of primary amines over secondary and tertiary amines can be accounted for by the number of hydrogen bonds between the amine and the oxygen atoms.

Recently, Puddephatt and co-workers have shown that bowl-shaped gold(I) (**31**) and platinum(II) (**32**) rimmed calixresorcinarene complexes can bind amines such as Bu^nNH_2, Pr^nNH_2, Bu^iNH_2 and Pr^iNH_2 [57]. The binding of alkali metal cations and ammonium ion has also been studied. On the other hand, different diamines have also been recognized by a copper(II) bridged bis-calix[4]arene (**33**) reported by Shinkai and co-workers [58].

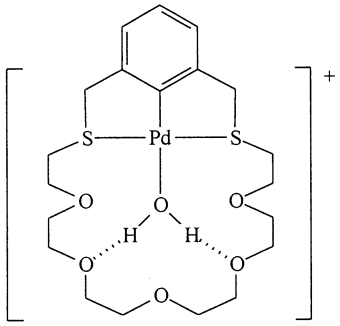
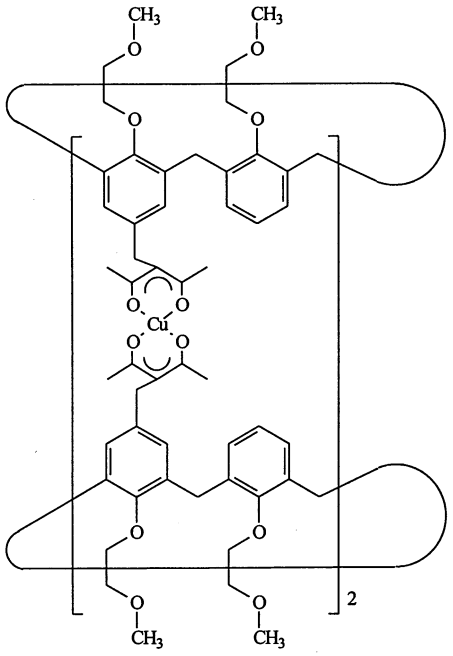


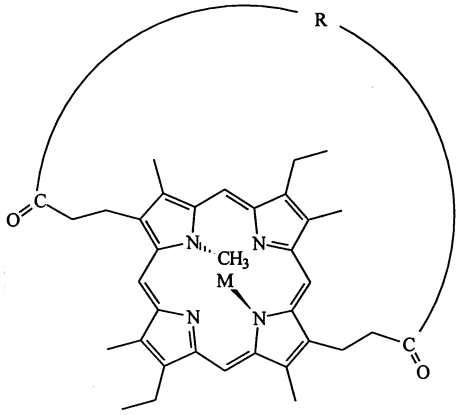
Fig. 7. Binding of a water molecule by an organopalladium(II) complex with a thiacyclophane ligand. Adapted from ref. [56].



33

Lehn and co-workers reported the synthesis of a series of porphyrins containing two uracil recognition groups [59]. The self assembly products of these porphyrins with alkyl substituted triaminopyrimidine have been investigated (Fig. 8).

Inoue and co-workers reported the synthesis of a chiral zinc strapped *N*-methylated porphyrin (**34**) [60]. The metalloporphyrin exhibits excellent enantioselective



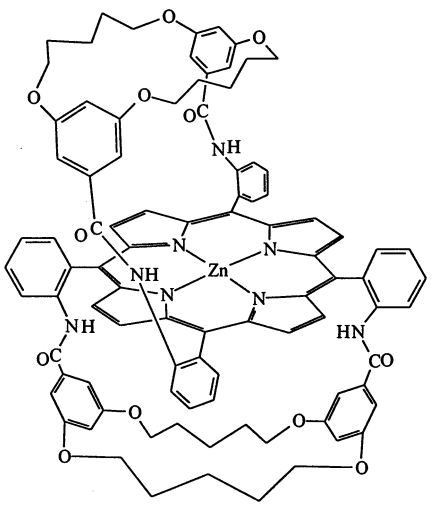
34: R = $NHCH_2$-*p*-C_6H_4-CH_2NH

M = ZnOAc

binding towards carboxylate anions of *N*-benzyloxycarbonyl, *tert*-butoxycarbonyl, 3,5-dinitrobenzoyl and acetyl amino acids. The hydrogen bonding interactions between the NHCO moieties of the chiral amino acids and receptor play an important role in the enantioselectivity of the system.

Recently, D'Souza showed that the hydroquinone pendant zinc(II) porphyrin could function as a sensor for quinones [61]. The fluorescence of the porphyrin has been found to decrease significantly in the presence of quinones. An electron transfer mechanism has been suggested to account for the quenching (Fig. 9). The strong interaction of the hydroquinone moiety with the guest has also been evidenced by electrochemical studies.

By using different side chains and macrocycles, Kyuno and co-workers designed a series of zinc(II) porphyrins with significantly enhanced binding towards substrates such as pyridines and amines [62,63]. Selected examples are illustrated as (**35**) and (**36**).



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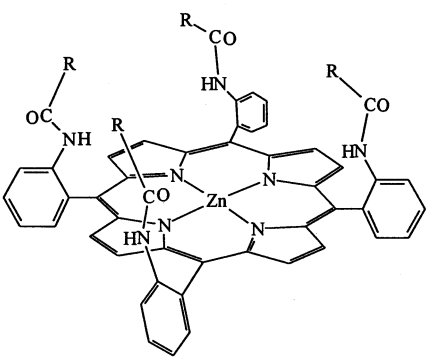


R = $C(CH_3)_3$, $CH(CH_3)_2$, $CH_2C(CH_3)_3$, $(CH_2)_3CH_3$

36

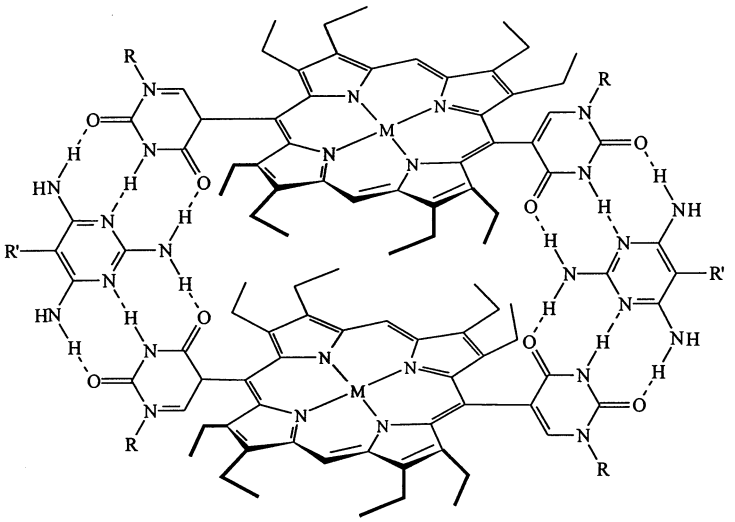


Fig. 8. Recognition of alkyl substituted triaminopyrimidines by uracil-pendant (metallo)porphyrins. Adapted from ref. [59].

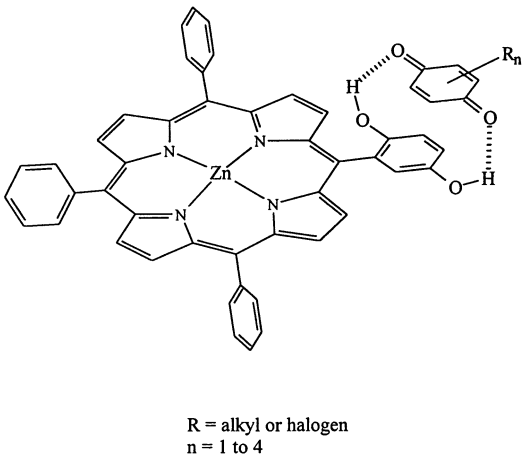
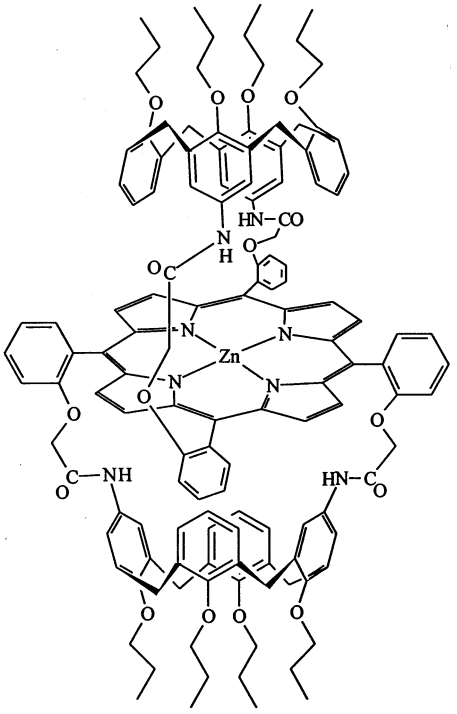


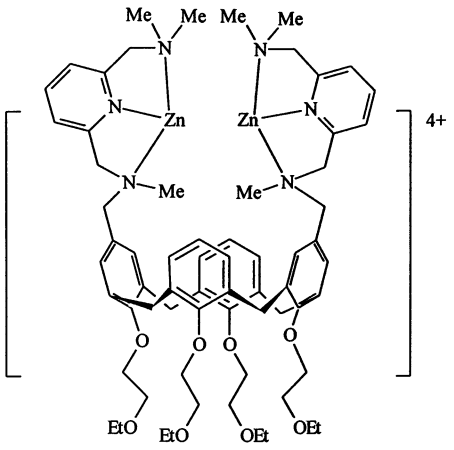
Fig. 9. Binding of quinones to a hydroquinone pendant Zn(II) porphyrin. Adapted from ref. [61].

Reinhoudt and co-workers reported the synthesis of a bis-calix[4]arene zinc(II) porphyrin (**37**) [64]. The receptor has been found to bind pyridine and its analogues



37

more efficiently due to the shielding and encapsulation of the substrates from the environment by the action of the calix[4]arene moieties. Recently, the same research group reported a system in which two zinc(II) metal centres are attached to the upper rim of a calix[4]arene (**38**) [65]. The complex shows strong binding to the phosphate diester 2-(hydroxypropyl)-*p*-nitrophenyl phosphate and a high catalytic transesterification of the substrate has also been observed.



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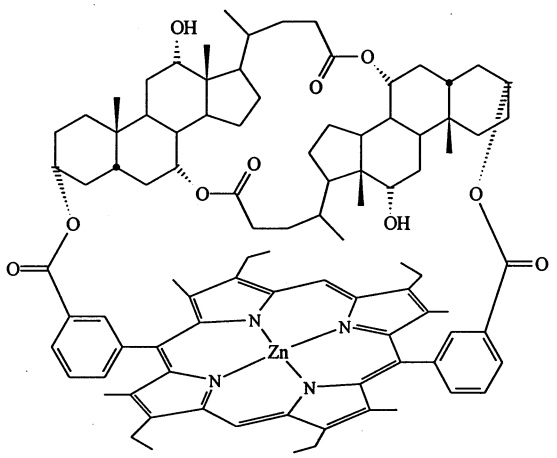
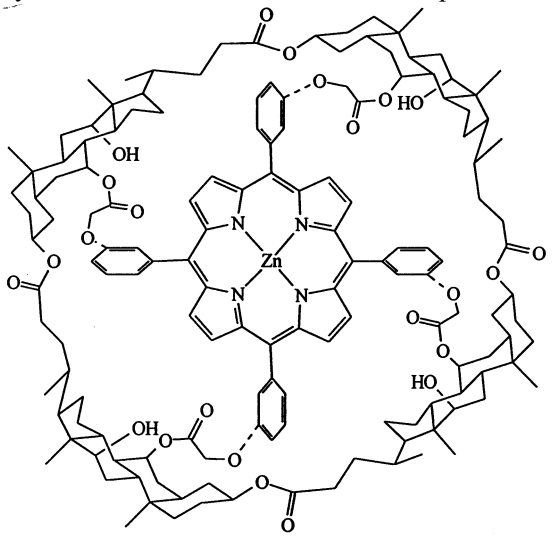


39

Bonar-Law and Sanders reported a series of steroid-capped porphyrin systems [66,67]. For example, the zinc(II) porphyrin bridged by a steroidal diol (**39**) has been found to complex alcohols, polyols and pyranoside derivatives in nonpolar solvents by means of Lewis acid coordination and hydrogen bonding [67]. The steroid roof and the porphyrin floor both play a crucial role in the recognition of the substrates. Two or more molecules of alcohols or small diols can bind inside the spacious receptor cavity. Besides, addition of water and methanol has been found to increase and modulate pyranoside binding by filling in the gaps between the

receptor and a misfit ligand. On the other hand, a molecular bowl has also been constructed using a zinc(II) porphyrin as the floor and four cholates as the walls (**40**) [68]. The system has been found to bind morphine with a much enhanced



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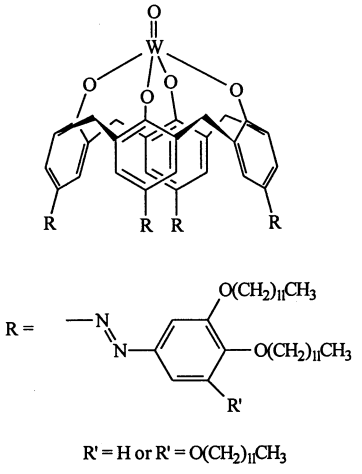
efficiency compared to the reference porphyrin without the cyclocholate component. The recognition has been attributed to the nitrogen–zinc(II) interaction as well as the hydrogen bonding between the substrate and the hydroxyl groups on the cyclocholate inner-walls.

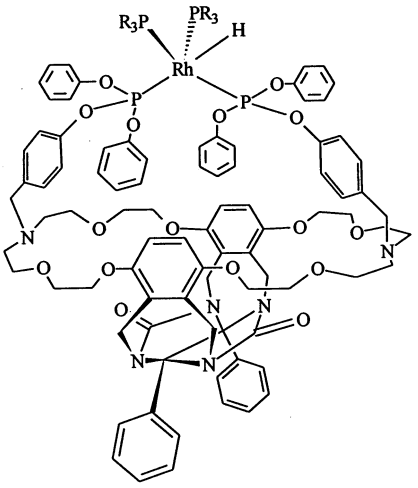
Floriani and co-workers reported a very interesting crystal structure of a molybdenum(VI) oxo group connected to the upper rim of a calix[4]arene [69]. The complex has been found to interact with a free molecule of calix[4]arene with a nitrobenzene molecule trapped in the cavity (Fig. 10).



R' = H or R' = $O(CH_2)_{11}CH_3$

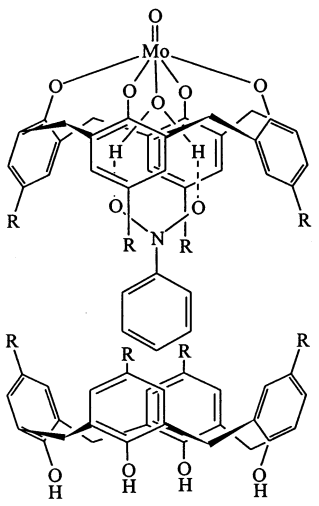
41

Swager and co-worker utilized a tungsten(VI) oxo calixarene complex (**41**) as a special class of rigid bowlic liquid crystal system [70]. The same research group also reported the spectroscopy, photophysical properties, and electronic structures of the tungsten(VI) and molybdenum(VI) oxo calix[4]arene complexes [71]. The binding of pyridine to the complexes has also been studied.



R = OPh

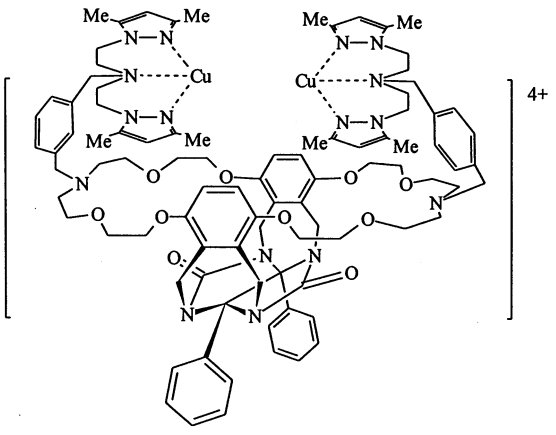
42



$R = C(CH_3)_3$

Fig. 10. Encapsulation of a nitrobenzene molecule by an oxomolybdenum(VI) calix[4]arene and a free calix[4]arene. Adapted from ref. [69].

By connecting a receptor to a catalyst, selective catalytic reactions can be carried out. Nolte and co-workers recently reported a supramolecular catalyst consisting of a basket-shaped receptor and a rhodium(I) metal centre (**42**) [72]. The catalyst can selectively hydrogenate and isomerize allyl substituted dihydroxyarene substrates bound in its cavity to the corresponding propyl derivatives and β-methylstyrenes. The rate of hydrogenation of 1-allyl-3,4-dimethoxybenzene has been found to be delayed while those of 4-allyl-1,2-benzenediol and 5-allyl-1,3-ben-zenediol significantly accelerated compared with the model catalyst $HRh[P(OPh)_3]_4$. Besides, the hydrogenation/isomerization selectivity ratio for the first substrate decreases whereas those for the other two increases. It has been suggested that the hydrogenation reaction takes place preferentially inside the cavity of the metallohost while the faster isomerization reaction occurs outside. The hydrogen bonding between the substrate and the urea moieties of the supramolecule plays a key role in the substrate recognition. A similar design of metallohost–catalyst has also been reported recently where a basket-shaped receptor is connected to two Cu(II) pyrazole units (**43**) [73]. Dihydroxybenzene can



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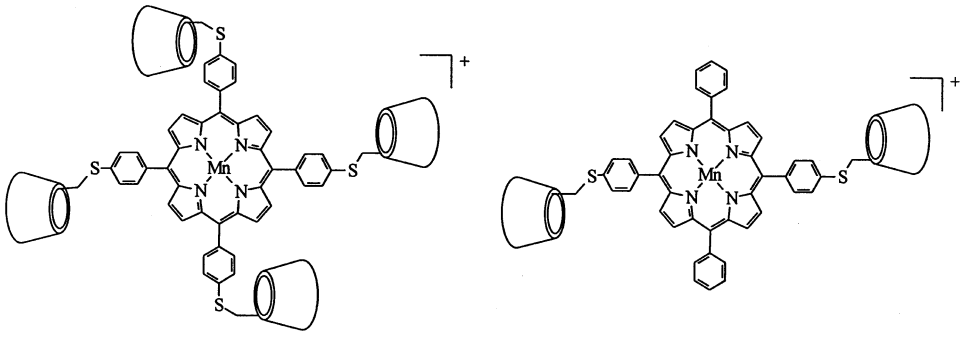
be bound inside the cavity. In the presence of benzylic alcohols, the copper(II) centres are reduced to copper(I) and the bound alcohols oxidized to aldehydes. 3-Hydroxybenzyl and 3,5-dihydroxybenzyl alcohols have been found to be oxidized extremely effectively. The enhanced efficiency is attributed to the fact that these alcohols are bound in the cavity of the metallohost and oriented in the correct position by means of the hydrogen bonding between the hydroxyl groups on the benzene ring and the urea groups of the host. Recently, related copper(I) metallohosts have also been found to bind molecular oxygen [74].

Breslow and co-workers recently reported an interesting class of manganese(III) porphyrins carrying two or four cyclodextrin units (**44**) [75]. Selective



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epoxidations of stilbene substrates of appropriate length with two hydrophobic ends have been observed. In the presence of 1-adamantanecarboxylate, the selectivity increases. It has been suggested that the coordination of the carboxylate ion to the manganese centre would render the oxo group to be on the side facing the double bond of the bound substrate, thereby increase the oxidation selectivity (Fig. 11). However, excess 1-adamantanecarboxylate ions will compete with the substrate for binding to the cyclodextrin rings of the host and the selectivity of the epoxidation decreases. Recently, the selective catalytic hydroxylation of a steroid by the manganese porphyrin with four cyclodextrin moieties has been reported [76]. Besides, the same research group also constructed a β-cyclodextrin dimer with a linking bipyridine unit (**45**) [77]. The receptor binds both ends of ester substrates

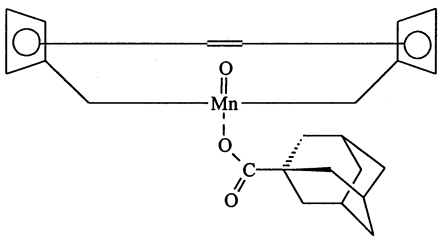
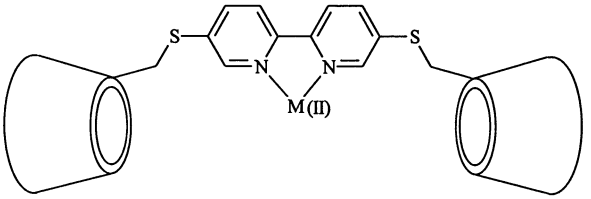


Fig. 11. Coordination of 1-adamantanecarboxylate to the metal centre of a manganese porphyrin enhances the selectivity of epoxidation. Adapted from ref. [75].



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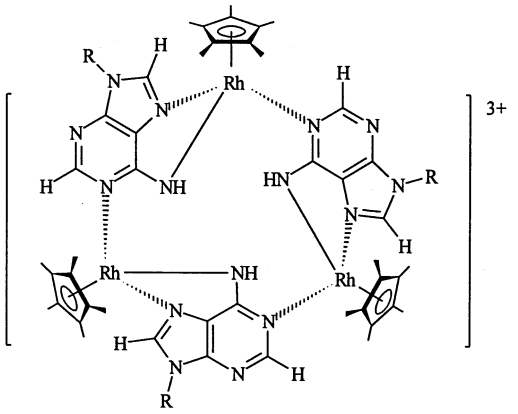
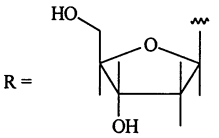
and hold the ester carbonyl group right above a metal ion such as Cu(II), Zn(II) and Ni(II) coordinated to the bipyridine moiety. The ester hydrolysis is performed with turnover and very high rate accelerations.

Recently, Fish and co-workers reported a novel series of trimeric Cp^*Rh–DNA/RNA cyclic complexes as a bioorganometallic host [78,79]. The complexes have been found to recognize aromatic amino acid guests L-tryptophan and L-phenylalanine in aqueous media at pH 7 [79]. The strongest interaction is observed between (**46**) and L-tryptophan, with an estimated association constant of 607 M^{-1}. It has been suggested that the aromatic rings of L-tryptophan are bound inside the host cavity of (**46**), with the aromatic plane being parallel to the C_3 axis of the organometallic host.





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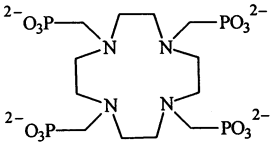
2.2. Lanthanides

For an introduction on the structural and photophysical properties of lanthanide complexes, one could be referred to a number of excellent reports and review articles [80–89]. Lanthanide ions in aqueous solutions usually do not luminesce as a result of the efficient deactivation pathway associated with the surrounding water molecules. Besides, the low extinction coefficients of free lanthanide ions result in very inefficient light absorption. However, the luminescence properties of lanthanides can be improved by means of encapsulation of the ions in suitably designed ligands which are highly light absorbing. The lanthanide ion can therefore be shielded from the solvent environment and the electronic energy in the form of light absorption of the ligands can also be transferred to the metal ion. The emissions of lanthanide complexes are very long-lived, with a lifetime in the millisecond scale. In view of the intense and long-lived luminescence of lanthanide complexes, they are ideal candidates for the application as sensory materials.

The effect brought about by anions such as iodide and phosphate ions on a series of lanthanide cryptates has been investigated [90]. It has been found that addition of iodide anions to aqueous solution of $[Eu \subset 2.2.1]^{3+}$ {2.2.1 = 4,7,13,16,21-pentaoxa-1,10-diazabicyclo[8.8.5]tricosane} results in the quenching of the luminescence of the complex. The quenching mechanism has been suggested to be electron transfer in nature. However, in the case of phosphate ions, ion-pair formation between Eu^{3+} or Tb^{3+} cryptates and the anion has been found to occur.

Sherry and co-workers reported the solution properties such as stability and protonation of a series of lanthanide complexes with the ligand $[DOTP]^{8-}$ (**47**) [H_8DOTP = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylenephosphonic acid)] by a variety of spectroscopic methods [91]. The binding of alkali metal cations by $[TmDOTP]^{5-}$ has also been studied in detail. It has been found that tetraethyl-



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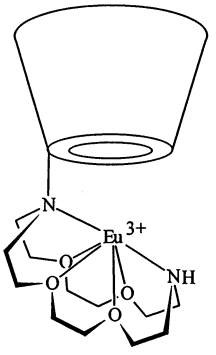
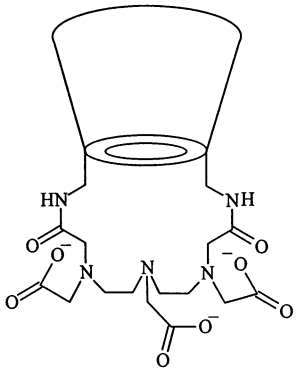


48

ammonium and tetramethylammonium ions cannot compete effectively with sodium ions for the binding sites on $[TmDOTP]^{5-}$. However, potassium and ammonium ions compete more effectively while cesium and lithium ions less effectively.

Nocera and co-workers have designed a very interesting molecular sensing system in which a cyclodextrin is modified with a $Eu^{3+} \subset$ azacrown (azacrown = 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane) unit (**48**) [92]. The photophysical properties of the system are identical to the model complex ($Eu^{3+} \subset$ azacrown). However, in the presence of benzene, the europium emission of the cyclodextrin complex is enhanced. The excited state of benzene is too short-lived for any bimolecular energy transfer to occur. Therefore, the emission enhancement has been suggested to originate from a unimolecular absorption–energy-transfer–emission process from the benzene guest in the cavity of cyclodextrin to the Eu^{3+} ion in the azacrown unit [92,93]. However, owing to the 3+ charge of the appended europium cradle at the bottom of the cyclodextrin ring, the cavity is less hydrophobic and strong association of the substrate is prevented. Recently, the same research group designed a related supramolecular sensor for aromatic hydrocarbons in which a cyclodextrin cup is appended with a derivative of diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetate moiety (**49**) which neutralizes the charge of the lanthanide ion [94]. The emission of the supramolecule containing a terbium ion at the acetate units is significantly enhanced in the presence of aromatics such as 1,2,4,5-tetramethylbenzene and naphthalene. The emission enhancement has also been ascribed to indirect excitation of the ^7F_J excited state manifold of the lanthanide ion by a unimolecular absorption–energy-transfer–emission process from the aromatic guest to the Tb^{3+} coordinated to the acetate moieties.



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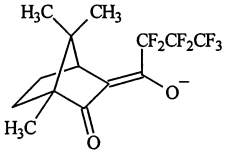

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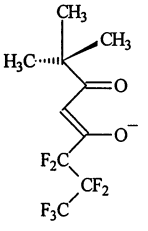


51

In 1991, Willner and co-workers reported that a series of lanthanide complexes such as $Yb(hfc)_3$, $Eu(hfc)_3$, $Pr(hfc)_3$, [hfc = 3-(heptafluoropropylhydroxymethylene)camphorate (**50**)], $Eu(fmod)_3$ and $Pr(fmod)_3$ [fmod = 6,6,6,7,7,8,8-heptafluoro-2,2-dimethyloctane-3,5-dionate (**51**)] can act as effective carriers for dyes such as safranine-O, methylene blue, thionin and proflavine [95]. The carrier properties of the complexes have been attributed to the interaction between the peripheral amino groups of the dyes and the lanthanide metal centres. Besides, $Eu(fmod)_3$ has also been found to be a carrier for amino acids such as tryptophan, *o*-tyrosine, phenylalanine and *p*-tyrosine in liquid–liquid membrane transport systems [95].

Tsukube and co-workers reported the binding properties of a series of lanthanide complexes in the presence of an armed crown ether [96]. The complexes have been found to transport protonated ester salts of amino acids such as histidine and lysine. The binding is based on the interaction between the ammonium cation of the substrate and the crown ether. The ester group of the substrate also interacts with the $Dy(fmod)_3$ settled on the sidearm of the crown ether (Fig. 12). On the other hand, some lanthanide tris(β-diketonate) complexes have also been found to bind organic carboxylate anions with potassium ions [97]. Recently, the same research group reported the enantioselective binding and extraction of zwitterionic amino acids by lanthanide complexes with a chiral camphor-derived ligand [98]. An enantiomeric excess of 49% has been determined between the ytterbium complex with (+)-camphor-derived β-diketonates and L-phenylglycine.

Sammes and co-workers reported an interesting luminescent europium complex with two cooperative ligands [99]. The bis-acetate derivative of 1,7-diaza-4,10,13-trioxacyclopentadecane (DATOCPD) (**52a**) forms a tight complex with europium ions. The coordination sphere of the ion is not completely occupied. Therefore, another ligand, 1,10-phenanthroline-2,9-dicarboxylate (PDA) (**52b**) can also bind to the same ion to act as the photosensitizer. Eu^{3+}·DATOCPD does not show any

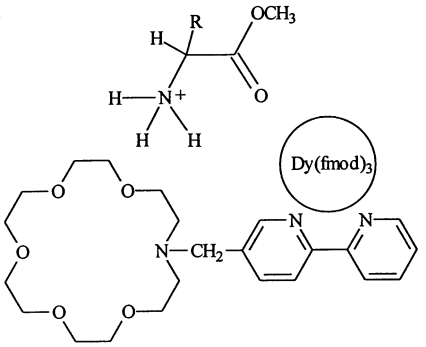
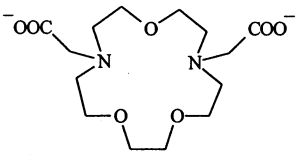
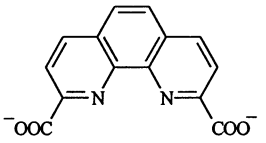


Fig. 12. Binding of protonated ester salts of amino acids to a crown ether-pendant $Dy(fmod)_3$ complex. Adapted from ref. [96].



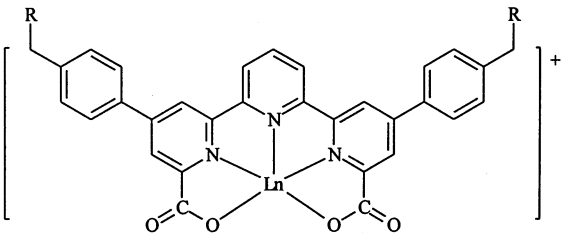
52a



52b

luminescence due to the lack of a strong light absorbing unit. Eu^{3+}·PDA shows only weak emission due to the effective deactivation of the excited state by vibronic energy transfer to the surrounding water molecules in the coordination sphere. Strong luminescence can only be observed when all three components co-exist. The complex DATOCPD·Eu^{3+}·PDA shows a remarkable dependence on pH. In highly alkaline medium, the lanthanide forms insoluble hydroxide complex. However, in highly acidic medium, protonation of the carboxylate groups lowers their affinity for the metal ion and therefore no emission can be detected.

Recently, de Silva and co-workers reported the design of pH sensors which contain a lanthanide–terpyridine unit with amino groups on the side-chains (**53**) [100]. The emission intensities of the complexes show significant enhancement in low pH medium without dissociation of the complexes. The proton-switched emission enhancement has been ascribed to the protonation of the amino groups on the side-chains of the ligand.



R = $N(CH_2CH_3)_2$, $N[(CH_2)_2]_2O$

Ln = Eu, Tb

53

Transesterification of the 2-(4-nitrophenylphosphate) ester of propylene glycol has been found by Morrow and co-workers to be promoted by a series of transition metal and lanthanide cations [101]. Recently, simple lanthanide cryptates have also been found by Park and co-workers to possess catalytic hydrolysis properties of

phosphate diesters [102]. For example, cryptate (2.2.1) [2.2.1 = 4,7,13,16,21-pentaoxa-1,10-diazabicyclo[8.8.5]tricosane] complexes of La^{3+}, Ce^{3+} and Eu^{3+} are catalysts for the hydrolysis of bis(4-nitrophenyl)phosphate. It has been suggested that the lanthanide metal centres act as a site for substrate binding and a source of nucleophilic metal hydroxides.

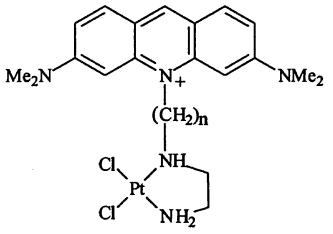
Chin and co-workers showed that the efficiency of oxidative hydrolysis of bis(4-nitrophenyl)phosphate promoted by $La(ClO_4)_3$ is increased in the presence of hydrogen peroxide [103]. The enhancement is attributed to the formation of a lanthanum peroxide monomer and at least one dimer or higher order aggregates. Recently, Breslow and co-workers also synthesized a La^{3+}–bipyridine complex with two cyclodextrin pendants [104]. In the presence of hydrogen peroxide, the complex reveals enhanced catalytic hydrolysis properties towards bis(4-nitrophenyl)phosphate. However, it has been suggested that the transition state only involves one La^{3+} and one H_2O_2. The enhanced reaction rate at low concentrations of La^{3+} is ascribed to the recognition of the nitrophenyl units by the cyclodextrins.

Besides, luminescent europium ions have been used to probe the metal-binding sites of different proteins [105–109]. The most extensively studied system is based on the substitution of lanthanide ions into calcium binding sites in proteins owing to the similar ionic radii of calcium (1.06 Å) and lanthanide ions (from 0.85 to 1.06 Å). Inter-metal energy transfer can be used to probe the metal–metal site distances of protein. Luminescence lifetime measurements and site-selective Eu(III) excitation spectroscopy can probe the metal ion coordination differences. Recently, the metal-binding sites of different calcium-binding proteins have been probed by measuring the circular polarized luminescence of bound lanthanide ions [110,111].

3. Nucleic acid binding and cleavage

3.1. Transition metal complexes

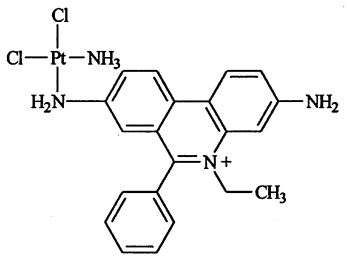
The most widely used chemotherapeutic agent is *cis*-diamminedichloroplatinum(II) (*cis*-platin). It has been suggested that the therapeutic effects of the drug arise from its covalent interaction with DNA, thereby blocking DNA and RNA syntheses. There have been a number of studies on the interaction of related platinum(II) complexes with DNA [112–124].



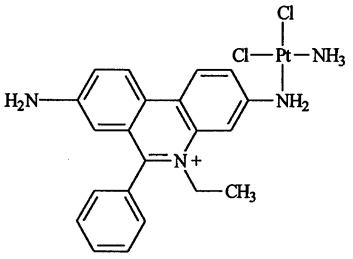
n = 3, 6

54

Lippard and co-workers reported the syntheses of (1,2-diaminoethane) platinum(II) complexes linked to an acridine orange (AO) intercalator with a polymethylene chain, $[Pt\{AO(CH_2)_n en\}Cl_2]Cl$ ($n = 6$ and 3) (**54**) [125]. The complexes are designed in such a way that each of the platinum and the acridine can interact freely with DNA while being close enough to experience the structural pertubations of one another on the duplex. It has been found that the platinum moiety of $[Pt\{AO(CH_2)_6 en\}Cl_2]Cl$ binds covalently to the DNA while the acridine orange part is intercalated one or two base pairs away. Recently, a series of related platinum(II) complexes containing an ethidium moiety have also been synthesized [126,127]. The ethidium is coordinated to the platinum through one of the exocyclic amino groups (*N*3 and *N*8). Selected examples are illustrated as (**55a**) and (**55b**). The unwinding of closed circular supercoiled DNA by these platinum–intercalator complexes has been investigated in detail.

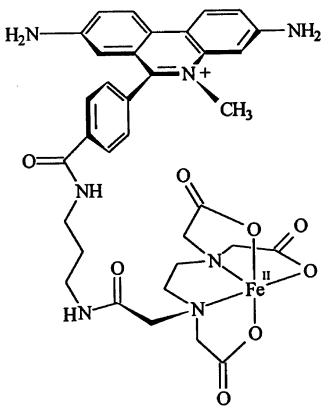


55a

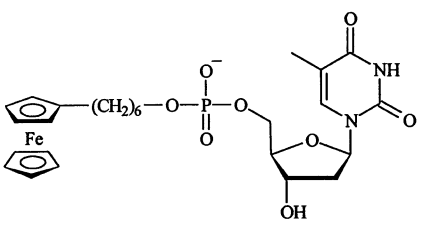


55b

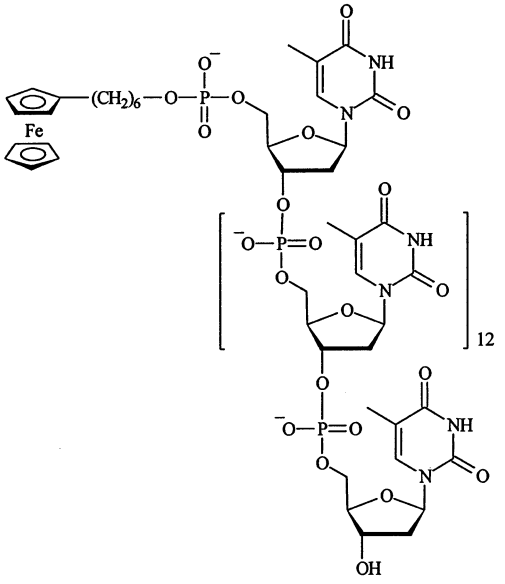
A pioneering synthetic DNA cleavage agent based on transition metal chemistry was reported by Dervan and co-workers in 1982 [128]. The complex includes a methidium intercalator linked to a [Fe(EDTA)] moiety (**56**). The methidium brings the complex to close proximity of the DNA where the iron(II) centre promotes the cleavage of the biopolymer by the production of hydroxyl radicals.



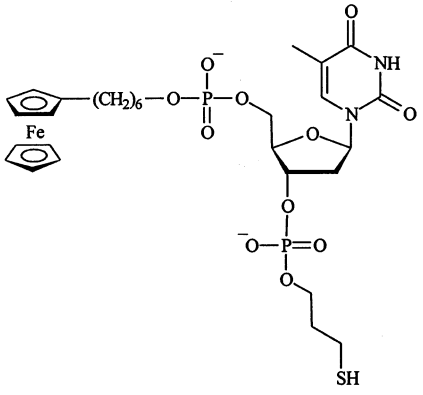
56



57a



57b



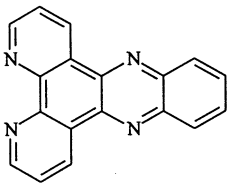
57c

In the late 1980s, Bard and co-workers reported a series of electrochemical studies of the interaction of transition metal complexes such as $[Co(phen)_3]^{3+}$ and $[Fe(bpy)_3]^{2+}$ with DNA [129–133]. Recently, the same research group also reported a new method to monitor the hybridization of DNA by detection of the electrogenerated chemiluminescence of $[Ru(N–N)_3]^{2+}$ (N–N = bpy or phen) labels [134]. On the other hand, Mirkin, Letsinger and co-workers recently described the attachment of a ferrocene to the 5′-end of a nucleotide (**57a**) and an oligonucleotide (**57b**) [135]. In another example, a ferrocene and a thiol have been connected to the 5′- and 3′-ends of a nucleotide, respectively (**57c**) [135]. The electrochemistry of a monolayer of (**57c**) adsorbed on a gold thin film electrode has also been studied. A reversible wave with $E_{1/2} = 220$ mV vs. Ag/AgCl has been observed [135].

Basically, octahedral transition metal complexes offer an additional characteristic that they can exhibit stereoisomerism and the binding of the stereoisomers towards chiral DNA can be more specific. Barton and co-workers first reported the chiral discrimination between the Δ and Λ forms of $[Zn(phen)_3]^{2+}$, $[Co(phen)_3]^{3+}$, $[Co(dip)_3]^{3+}$ and $[Ru(phen)_3]^{2+}$ towards B-form double stranded DNA [136]. The

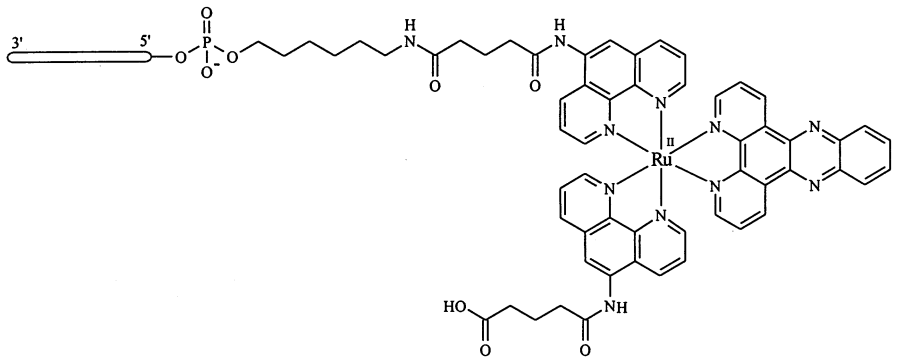
Δ-isomer, being associated with a right-handed chirality, showed predominant binding towards B form (right handed) DNA duplex. The enantioselectivity arises from the steric repulsions between the non-intercalated phenanthrolines of the Λ-isomer and the phosphate oxygen atoms of the right-handed DNA.

In fact, the interaction of ruthenium(II) complexes with DNA has been widely studied. Ruthenium(II) polypyridine complexes are usually stable in aqueous solution. Besides, they exhibit intense low energy metal-to-ligand charge-transfer (MLCT) absorption. The MLCT emissive excited state of Ru(II) diimine complexes is well known to be sensitive to local surrounding. All these properties make ruthenium(II) polypyridine complexes an ideal candidate as spectroscopic probe for DNA.



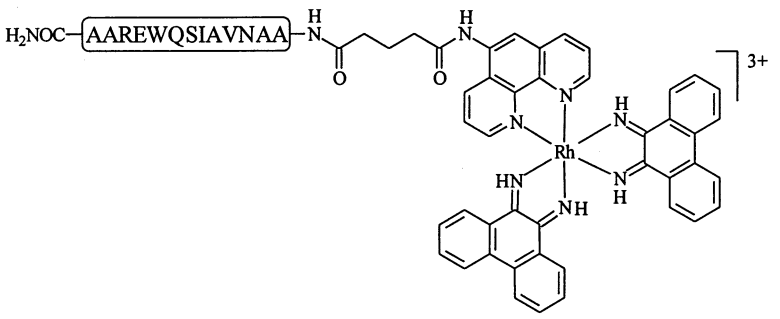
58

The complex $[Ru(bpy)_2(dppz)]^{2+}$ with an extended planar dppz (**58**) ligand has been termed as a molecular light switch for double stranded DNA [137]. The complex does not show any luminescence in aqueous buffer. However, with intercalation into the DNA double helix and the protection of the phenazine ring from quenching by interaction with water, intense luminescence is observed. The syntheses, photophysical and DNA-binding properties of related ruthenium(II) dppz derivatives have also been reported [138]. An interesting Ru(II)–dppz complex containing an oligonucleotide tethered to one of the ancillary 1,10-phenanthroline ligands (**59**) has been utilized as a sequence-specific sensor for single stranded DNA [139]. In the presence of the complementary strand, the complex can intercalate into the DNA hybrid and luminescence is observed. The emission intensity is dependent on the number as well as the locaton of mismatches between the probe and a series of single stranded DNA samples.



59

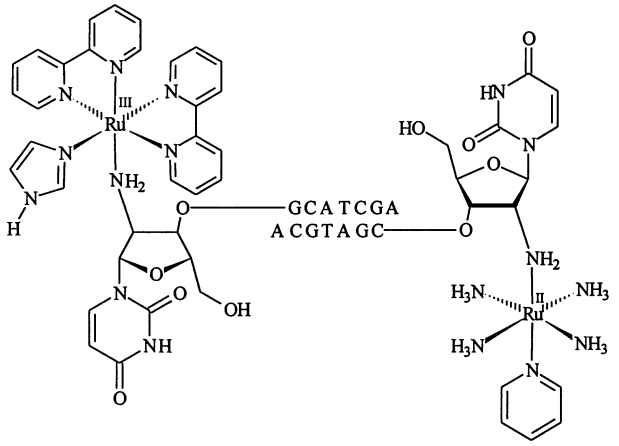
The photocleavage of DNA by a series of rhodium(III) metallointercalators have been reported [140–144]. The rhodium(III) complex $[Rh(phi)_2(phen')]^{3+}$ [phi = 9,10-phenanthrenequinone diimine, phen′ = 5-(amidoglutaryl)-1,10-phenanthroline] has been linked to a series of short oligopeptides (**60**) [145]. The complexes have been found to intercalate into DNA with the site specificity dependent on the peptide side-chain functional groups. A single glutamate at position 10 has been found to be essential in directing DNA site-recognition to the sequence 5′-CCA-3′. Site specific photocleavage of DNA by these complexes has also been observed.



60

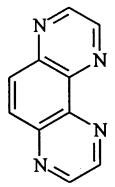
Recently, Barton and co-workers demonstrated the long range photoinduced electron transfer reactions through DNA duplex [146,147]. The donor $[Ru(phen)_2(dppz)]^{2+}$ and the acceptor $[Rh(phi)_2(phen)]^{3+}$ are linked to the ends of a 15-base pair DNA duplex [148]. The electron transfer is so efficient that a lower limit of 3×10^9 s^{-1} has been estimated for the reaction rate. Ultrafast electron transfer reactions mediated by DNA between non-covalently linked but intercalating donor and acceptor have also been reported [149,150]. Recently, long range photoinduced oxidative DNA damage [151] and oxidative thymine dimer repair [152] have also been reported. However, Lincoln and co-workers suggested that the fast electron transfer reactions observed for the non-covalently linked model are due to the cooperative binding of the donor and acceptor to the DNA [153].

On the other hand, Meade and co-workers reported the synthesis of an 8-base pair DNA containing the acceptor $[Ru(bpy)_2(imidazole)]^{3+}$ and the donor $[Ru(NH_3)_4(py)]^{2+}$ at the two ends (**61**) [154]. The rate of intramolecular electron transfer in the duplex is determined to be $1.6(4) \times 10^6$ s^{-1}.

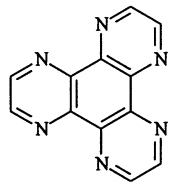


61

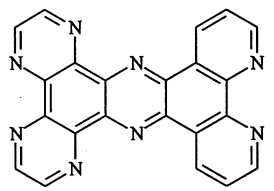
Kirsch-De Mesmaeker and co-workers demonstrated the remarkable interactions and photoreactions of the ruthenium(II) complexes containing the ligands tap (tap = 1,4,5,8-tetraazaphenanthrene) (**62a**) or hat (hat = 1,4,5,8,9,12-hexaazatriphenylene) (**62b**) with DNA [155–162]. For example, it has been shown that



62a



62b



63

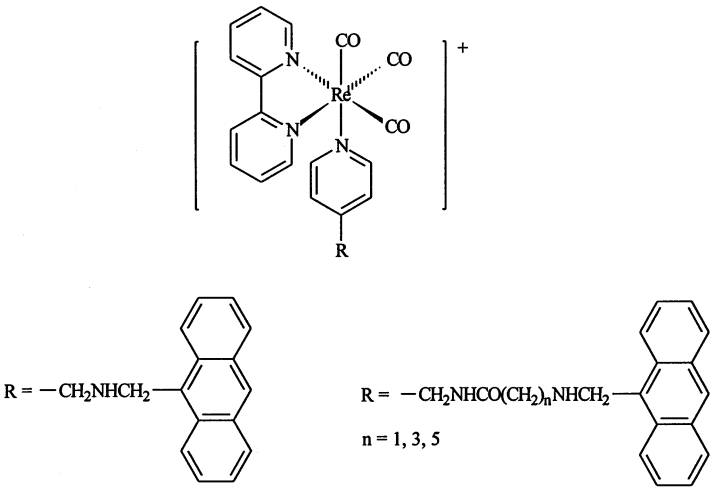
the metal-to-ligand charge-transfer excited state of $[Ru(tap)_3]^{2+}$ is so oxidizing that it can carry out effective photocleavage of the DNA backbone [155,162]. In addition, the complex has also been found to form photoadduct with DNA [160,162]. The photoreactions of this series of complexes with DNA have been attributed to the oxidation of the guanine of DNA by the excited complexes. Recently, the same research group reported a novel complex $[Ru(phen)_2(PHEHAT)]^{2+}$ {PHEHAT = 1,10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene (**63**)} which possesses similar molecular light switch properties of $[Ru(phen)_2(dppz)]^{2+}$ for DNA [163]. The excited complex has been shown to be able to oxidize guanosine-5′-monophosphate.

On the other hand, the binding of the Δ- and Λ-enantiomers of $[Ru(phen)_3]^{2+}$, $[Ru(phen)_2(dppz)]^{2+}$ and those of the benzodipyridophenazine analogue $[Ru(phen)_2(dppn)]^{2+}$ with DNA have also been studied in detail by Nordén, Lincoln and co-workers using linear and circular dichroism spectroscopy and other spectroscopic methods [164–167].

Thorp and co-workers demonstrated that high valent transition metal complexes are capable of oxidizing DNA. The electron transfer reaction of the photoexcited rhenium(V) species $[ReO_2(py)_4]^{+}$* to methyl viologen has been found to result in the oxidative cleavage of ΦX174 double stranded DNA [168]. Recently, isomerization of ΦX174 DNA by *trans*-dioxoruthenium(VI) complexes such as *trans*-$[Ru(bpy)_2O_2]^{2+}$, *trans*-$[Ru(tpy)(OH_2)O_2]^{2+}$ and *trans*-$[Ru(tmc)O_2]^{2+}$ (tmc = 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane) has also been reported by Thorp and co-workers [169]. Besides, a series of electrogenerated ruthenium(IV) species have also been found to be active in DNA cleavage [170–175]. For example, the complex $[Ru^{IV}O(dppz)(tpy)]^{2+}$ has been shown to have a high affinity in the binding and cleavage of double stranded DNA [173]. The kinetics and reaction products of $[Ru^{IV}O(bpy)(tpy)]^{2+}$ with a series of sugars and nucleotides have also been studied in detail [174]. The effects of steric hindrance on the oxidation of DNA by these polypyridyl oxoruthenium(IV) complexes have been investigated recently [175]. Besides, the complex $[(bpy)_2(OH_2)Ru^{III}–O–Ru^{III}(OH_2)(bpy)_2]^{4+}$ has been found to exhibit interesting interactions with DNA [176]. In the presence of DNA, the oxo-bridged dimer is hydrolyzed to a monomeric Ru(III) complex and strand scission of supercoiled ΦX174 DNA has been observed. The cleavage is promoted by magnesium ions. However, linearization of the plasmid prior to treatment with the complex does not lead to further fragmentation.

As an extension on the ruthenium(II) systems, osmium(II) polypyridine complexes have also been recently utilized as a luminescent DNA probe. For example, the synthesis, photophysics and DNA binding properties of the complex $[Os(phen)_2(dppz)]^{2+}$ have been reported by Barton and co-workers [177]. The complex mimics the Ru(II) counterpart but exhibits a lower-energy and shorter-lived emission. The electron transfer reaction between the excited osmium(II) donor and the rhodium(III) acceptor $[Rh(phi)_2(bpy)]^{3+}$ in the presence of DNA has also been reported [177]. On the other hand, Kirsh-De Mesmaeker and co-workers reported a new osmium(II) complex $[Os(tap)_3]^{2+}$ (tap = 1,4,5,8-tetraazaphenanthrene) [178]. The excited complex has been found to oxidize guanosine 5′-monophosphate. Moreover, the formation of a photoadduct for this electron transfer reaction has also been observed.

On the other hand, while the interactions of ruthenium(II) complexes with DNA have been well documented, studies on the corresponding d^6 rhenium(I) counterparts have received relatively less attention. Thorp and co-workers reported the synthesis, crystal structure and photophysical properties of a rhenium(I) complex containing a guanine moiety [179]. Schanze and co-workers reported a series of interesting chromophore–quencher complexes as a probe for DNA (**64**) [180,181]. The chromo-



64

phore [Re(bpy)$(CO)_3$] is covalently connected to an anthracene moiety by a flexible spacer. The low energy metal-to-ligand charge-transfer MLCT [$d\pi(Re) \rightarrow \pi^*(bpy)$] emission is effectively quenched by the tethered anthracene unit via an energy transfer mechanism. However, in the presence of calf thymus DNA, the anthracene intercalates strongly into the base pairs of the duplex and the MLCT emission intensity is increased. The luminescence enhancement is attributed to the decrease of ($Re \rightarrow An$) energy transfer quenching caused by the conformation change in the flexible linker after the anthracene moiety binds to the biopolymer. The DNA binding properties of rhenium(I) complexes containing the dppz ligand have been reported independently by Schanze and co-workers [182] and Yam and co-workers [183,184]. The complexes $[Re(dppz)(CO)_3(py)]^+$ and $[Re(dppn)(CO)_3(py)]^+$ are reported by Yam and co-workers to bind to double stranded calf thymus DNA by intercalation. The complexes reveal different luminescent properties in the presence of oligonucleotides such as poly(dA)·poly(dT) and poly(dG)·poly(dC). Besides, the complexes have also been found to promote photocleavage of supercoiled pBR322 DNA [184].

Interestingly, Co(III) and Ni(II) complexes with the dppz ligand, $[Co(phen)_2(dppz)]^{3+}$ and $[Ni(phen)_2(dppz)]^{2+}$, have also been reported recently [185]. Results from absorption titrations, thermal denaturation and differential pulse voltammetric experiments suggest that these complexes bind strongly to calf thymus DNA. The cobalt(III) complex has also been found to cleave supercoiled pBR322

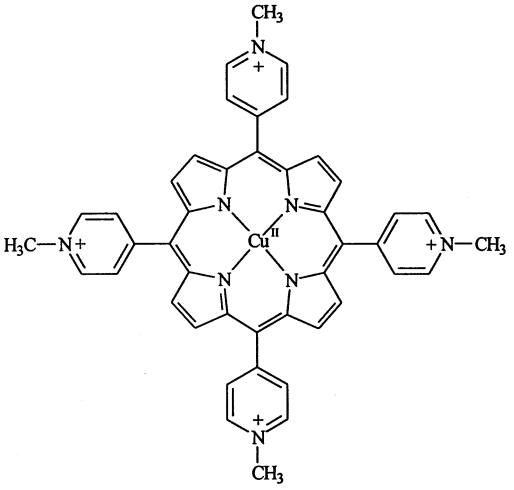
DNA upon photoexcitation. In addition, the synthesis, crystal structure, and DNA binding and cleavage properties of the organometallic complexes $[(\eta^5\text{-}C_5Me_5)Ru(NO)(N\text{–}N)]^{2+}$ (N–N = bpy and dppz) have also been reported [186].

While there have been a lot of interest on the covalent interaction of *cis*-platin with DNA, luminescent platinum(II) complexes have also been used as a probe for DNA. Arena et al. reported the DNA binding properties of an organoplatinium(II) complex $[Pt(tpy)CH_3]^+$ [187]. The complex has been found to be inert towards substitution in aqueous solution. At high concentration, dimer or larger aggregations have been observed. However, at low concentration, the complex binds to DNA by intercalation. McMillin and co-workers described that the bifunctional platinum(II) complex $[Pt(tpy)OH]^+$ (tpy = 2,2′:6′2″-terpyridine) binds to DNA by competitive covalent and intercalative mode [188]. The intercalated form of the complex exhibits emission enhancement. The hydroxide ligand acts as a good leaving group and covalent adduct formation between the complex and DNA has also been found. Che and co-workers reported the DNA binding properties of a luminescent Pt(II) complex $[Pt(5,5'\text{-}Me_2bpy)(4\text{-}ampy)_2]^{2+}$ (5,5′-Me_2bpy = 5,5′-dimethyl-2,2′-bipyridine; 4-ampy = 4-aminopyridine) [189]. The structured metal-to-ligand charge-transfer MLCT [$d(Pt) \rightarrow \pi^*$ (5,5′-Me_2bpy)] emission is enhanced in the presence of calf thymus DNA. It has been proposed that the complex intercalates into the duplex with the 5,5′-dimethyl-2,2′-bipyridine moiety stacking in between the base pairs. Recently, a cyclometallated Pt(II) [190] complex has also been utilized as luminescent probes for DNA. In addition, the strongly luminescent complex $[Pt_2(P_2O_5H_2)_4]^{4-}$ has been found to cause photoinduced oxidative cleavage of DNA [191–193]. On the other hand, the interaction of nucleic acids with nickel(II) complexes [194] and amino terminal Cu(II) and Ni(II) binding motifs of proteins and peptides [195] has also been reviewed recently.

For the late transition metals, apart from platinum, copper is the most extensively studied system. Sigman and co-workers first discovered the nuclease activity of 1,10-phenanthroline-copper during the course of investigating the inhibition of *Escherichia coli* DNA polymerase by 1,10-phenanthroline [196]. The untargeted tetrahedral complex $[Cu(phen)_2]^+$ has been found to bind reversibly to the minor groove of B-DNA and is subjected to the one-electron oxidation by hydrogen peroxide. According to detailed investigation and isolation of the oxidation products such as 3′- and 5′-phosphomonoester termini, free bases and 5-methylene-2(5*H*)-furanone, it has been suggested that the attack at the C-1 hydrogen of the deoxyribose is the initial step of the chemical scission [197–200]. A recent isotope labeling study indicates that the carbonyl oxygen of the 5-methylenefuranone is derived from water and the phosphodiester backbone is cleaved before the attack of water [201].

On the other hand, McMillin and co-workers also carried out studies on the interactions of copper(I) complexes $[Cu(N\text{–}N)_2]^+$ [N–N = dmp (2,9-dimethyl-1,10-phenanthroline), dmpp (2,9-dimethyl-4-phenyl-1,10-phenanthroline) and bcp (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline)] with DNA [202–207]. The methyl groups at the 2- and 9-positions of the ligands render the cuprous complexes less reducing and therefore the nuclease activities are reduced. Complexes of this kind show intense metal-to-ligand charge-transfer [$d(Cu) \rightarrow \pi^*(N\text{–}N)$] absorptions. Luminescence has also been observed in non-coordinating solvents. However, in donor solvents, the complexes reveal negligible emissions as a result of the exciplex formation between the pseudo copper(II) centre and the solvent [208]. The binding of $[Cu(dmp)_2]^+$ to DNA has been suggested to occur by an external mode [205].

For $[Cu(dmpp)_2]^+$ and $[Cu(bcp)_2]^+$, the MLCT emissions have been observed in the presence of DNA, indicative of a stronger binding of the complexes to the biopolymer, resulting in the protection of the metal centre from being attacked by the solvent molecules. However, $[Cu(dmpp)_2]^+$ has been suggested not to be a classical intercalator as no change in the specific viscosity is observed in the presence of DNA [206]. For $[Cu(bcp)_2]^+$, the increase in the viscosity is so great that it has been proposed that the complex can actually extend the effective chain length of the biopolymer by bridging between two or more DNA molecules [205]. Besides, the interaction between copper(II) porphyrins such as (**65**) and DNA has also been investigated [209]. The complexes exhibit little luminescence in aqueous solution due to the effective quenching by solvent coordination at the axial positions. Emissions have been observed in the presence of DNA and it has been suggested to result from the intercalation of the complexes into the base pairs of the biopolymer, thereby preventing the coordination of solvent molecules at the axial positions.



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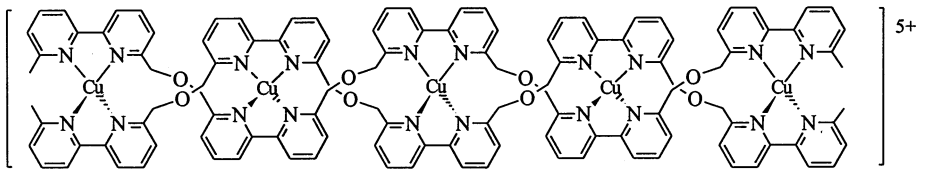

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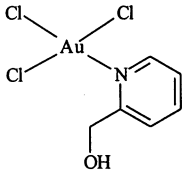
Lehn and co-workers reported the DNA binding and photocleavage properties of a series of copper(I) polypyridine double helical complexes of nuclearity from two to five [210]. The pentanuclear complex is illustrated as (**66**). From the spectroscopic and DNA melting studies, it has been found that the complexes bind to the DNA in an external mode and the binding strength increases with the number of metal centres in the complexes. Besides, binding of the complexes to pBR322 plasmid DNA can inhibit the enzymatic actions of *Ssp* I and *Eco*R V. Upon excitation in the MLCT absorption region, the complexes can cause photocleavage of pBR322 DNA. It has been proposed that the cleavage proceeds through degradation of the helicate metal complexes.

Biological activities of gold compounds have received immense attention [211–214]. The interactions of gold complexes with DNA have also been investigated. Dabrowiak and co-workers reported the DNA binding specificity of the gold(III) complex $(CH_3CH_2)_3PAuBr_3$ [215]. The complex has been found to bind to the *Hin*d III/ *Nci* I, a 139-base pair restriction fragment from pBR322 DNA. The gold(III) complexes $AuCl_3$(Hpm) (**67a**) and $AuCl_2$(pm) (**67b**) (Hpm = 2-pyridylmethanol) have been found by Calamai and co-workers to bind rapidly and tightly to polynucleotides and calf thymus DNA as revealed by circular dichroism spectroscopy [216].



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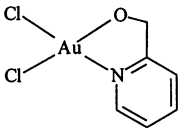

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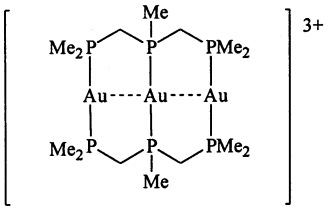

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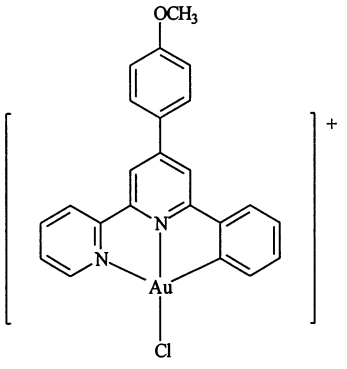


69

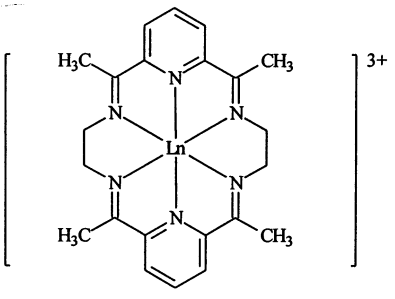
Recently, Yam and co-workers reported that the trinuclear gold(I) complex $[Au_3(dmmp)_2]^{3+}$ (**68**) [dmmp = bis(dimethylphosphinomethyl)methylphosphine] can carry out photocleavage of supercoiled pBR322 plasmid DNA [217]. The excited complex is so reducing that it can reduce molecular oxygen followed by the formation of singlet oxygen which has been suggested to be the reactive species for the DNA cleavage. The interaction between a cyclometallated Au(III) complex (**69**) and DNA has also been reported by Che and co-workers [218].

3.2. Lanthanides

In this section, studies on the interaction between lanthanides and DNA, the cleavage of RNA by lanthanide ions and the utilization of lanthanide complexes in DNA hybridization assays will be described.

Reports on the direct interactions between lanthanide ions and DNA are comparatively rare. Formoso and co-workers first reported that the luminescence of terbium ions is enhanced in the presence of some nucleotides and yeast RNA [219]. It has been suggested that the luminescence enhancement is a result of the energy transfer from the DNA or RNA to the lanthanide ions. Recently, Klakamp and co-workers carried out detailed studies on the binding of lanthanide ions with a series of nucleotides and single stranded oligomers [220,221]. It has been shown that there are two classes of binding sites in the oligonucleotides such as oligo$(dG)_{10}$ and d-GMP. It has also been suggested that six to seven atoms from the oligomers coordinate to the europium ion strongly in the first site while there are only one or two atoms coordinated to the europium ion in the second site. The occurrence of the first site corresponds to the interstrand association of europium ion with the oligomers forming dimeric or polymeric structures. It has also been suggested that the coordination of Eu^{3+} in guanine and non-guanine containing oligomers are different.

Cleavage of RNA by the lanthanide ions have received enormous attention. In 1991, Breslow and co-workers reported the catalytic ability of europium ions in the cyclization and cleavage of 3′,5″-uridyluridine to form 2′,3′-cyclic uridylic acid [222]. Imidazole has also been found to activate these cleavage reactions. Morrow and co-workers reported the stability and the catalytic RNA cleavage of a series of lanthanide macrocyclic complexes (**70**) at 37°C under neutral pH conditions [223].

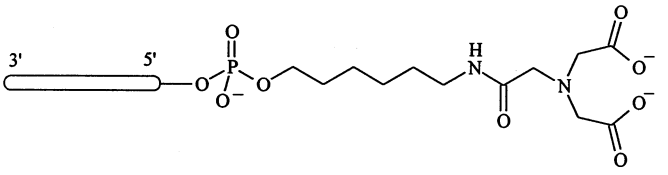


Ln = La, Eu, Gd, Tb, Lu

70

The cleavage is a result of transesterification of the phosphate diester linkage of the RNA. It has been proposed that the positive charge on the complexes is important in the cleavage reaction. Among the complexes, the europium complex is the most efficient in promoting transesterification of the RNA oligomers.

Komiyama and co-workers also reported the hydrolysis of RNA dinucleoside monophosphates ApA and UpU by the lanthanide ions [224]. The same research group also described the attachment of an iminodiacetate (IDA) moiety at the 5′-end of a 15-mer DNA (**71**) [225]. In the presence of lanthanide ions, the



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DNA-IDA causes scission of a 39-mer RNA selectively at the 3′-side of its 15-mer sequence, which is complementary with the DNA (Fig. 13). The selective hydrolysis has been attributed to the catalysis by the lanthanide ions linked to the DNA-IDA moiety. A similar system promoting site-specific RNA cleavage has also been described by Magda and co-workers at about the same time [226]. A europium texaphyrin has been connected to a synthetic oligodeoxyribonucleotide (**72**). Upon hybridization with an RNA, the modified DNA can induce cleavage of the RNA at a position near the location of the europium texaphyrin complex.

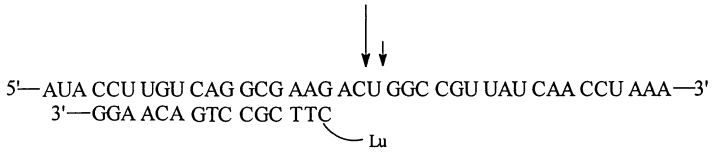
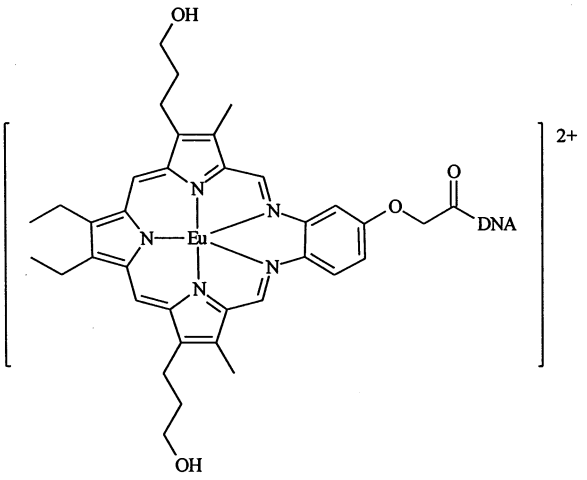


Fig. 13. Cleavage of a 39-mer RNA by a 15-mer DNA with a lanthanide-IDA moiety at the 5′-end. Adapted from ref. [225].



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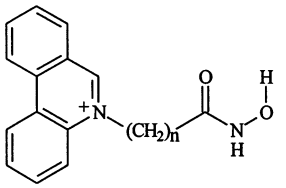


n = 3 - 7

73

Recently, a series of phenanthridine-linked hydroxamic acids with alkyl chain of different lengths (**73**) have been synthesized [227]. DNA cleavage by the complexes between the hydroxamic acids and divalent transition metal ions or lanthanide ions has been observed. The cleavage efficiency of lutetium(III) ions increases with increasing pH and an optimum Lu:hydroxamic acid ratio of 2:1 has been found. It has been suggested that the coordination of a lanthanide ion to the hydroxamic acid serves to provide the ligated hydroxide nucleophile in close proximity to the DNA. A second lanthanide ion then acts as a Lewis acid by binding to the phosphorus oxygen and promotes the nucleophilic attack of hydroxide at the phosphorus atom.

On the other hand, different DNA-labeling procedures using europium chelates have been reported. In 1991, Hurskainen and co-workers described a chemical method for labeling DNA directly [228]. An aliphatic primary amine group is introduced onto

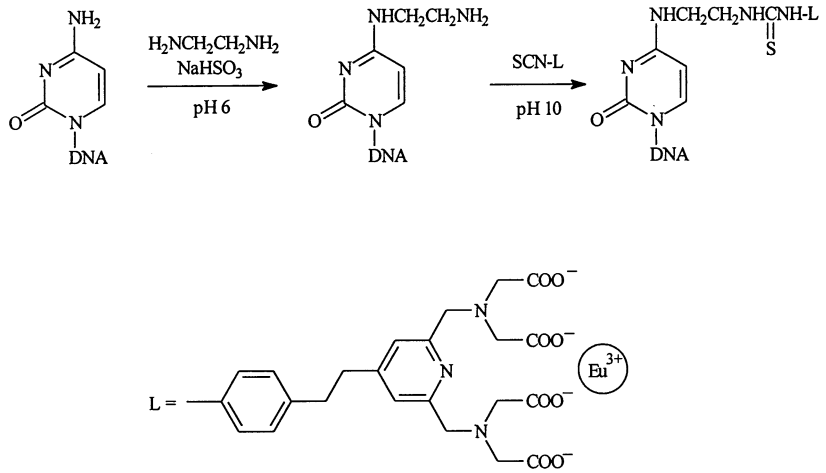
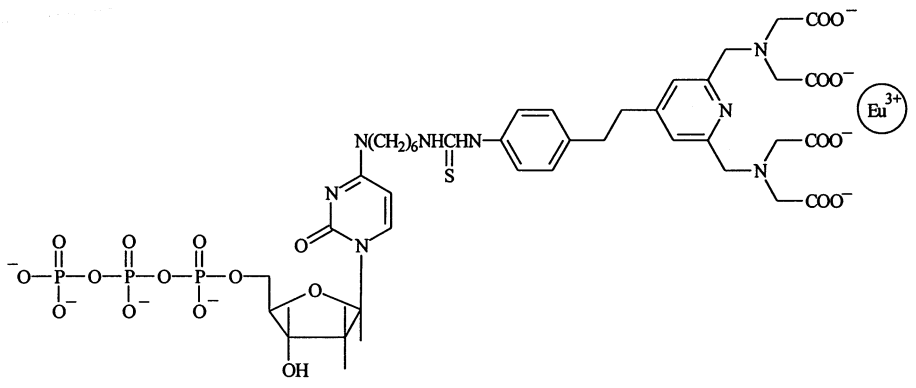


Fig. 14. Labeling of cytosine by an isothiocyanate derivative of a europium chelate. Adapted from ref. [228].



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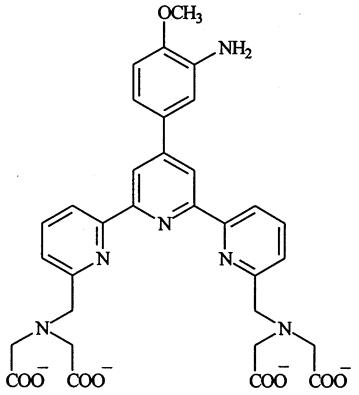
the cytosine group. The DNA is then reacted with an isothiocyanate derivative of a europium chelate (Fig. 14). A viral DNA has been labeled by this method [229]. Besides, DNA can also be labeled enzymatically by using a labeled deoxynucleoside triphosphate. For example, a europium-labeled 2′-deoxycytidine 5′-triphosphate (Eu-dCTP) (**74**) has been developed [230]. Enzymatic europium labeling of DNA is then performed by nick translation or random priming reactions. However, although the europium is strongly chelated by the ligand, owing to the lack of sensitizer, the luminescence detection procedure has to be performed after the europium is released from the analyte chelate and recomplexed in an enhancement solution (see Section 4.3).



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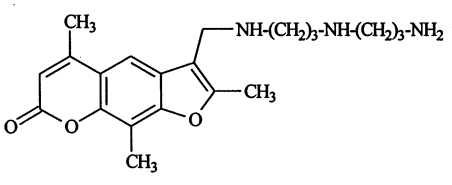
Saha and co-workers described a bifunctional terpyridine chelate 4′-(3-amino-4-methoxyphenyl)-6,6″-bis[*N*,*N*-bis(carboxymethyl)aminomethyl]-2,2′:6′,2″-terpyridine (**75**) [231], which forms a very stable complex with europium ion with a large association constant. In addition, the chelate absorbs strongly in the UV region, and the luminescence of the europium complex can be detected directly. The 3-amino substituent on the phenyl ring of the ligand can be readily converted to the isothiocyanate group which can then be linked to the primary amino group of protein and DNA samples.

On the other hand, Oser et al. described a photochemical labeling method in 1990 [232]. The 5′-end of a single stranded DNA sample is prolonged by a 16-base long alternating A–T sequence (Fig. 15). The DNA is first denatured and then the poly(A–T) tail is folded back under reannealing conditions to form a partly double stranded oligonucleotide. An amino substituted psoralen derivative (**76**) is then added to the DNA sample. The planar psoralen moiety intercalates into the double stranded region of the DNA. As thymine undergoes photochemical reaction with psoralen, the double stranded region of the DNA is covalently crosslinked upon UV irradiation. An amino-specific terbium chelate containing diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetate and 4-aminosalicylate with long-lived luminescence is then attached to the primary amino groups of the intercalated psoralen molecules (Fig. 16).



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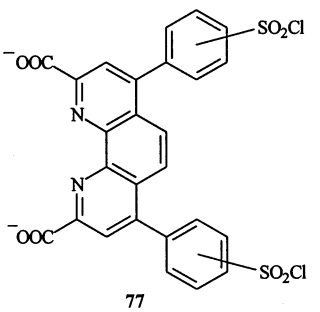

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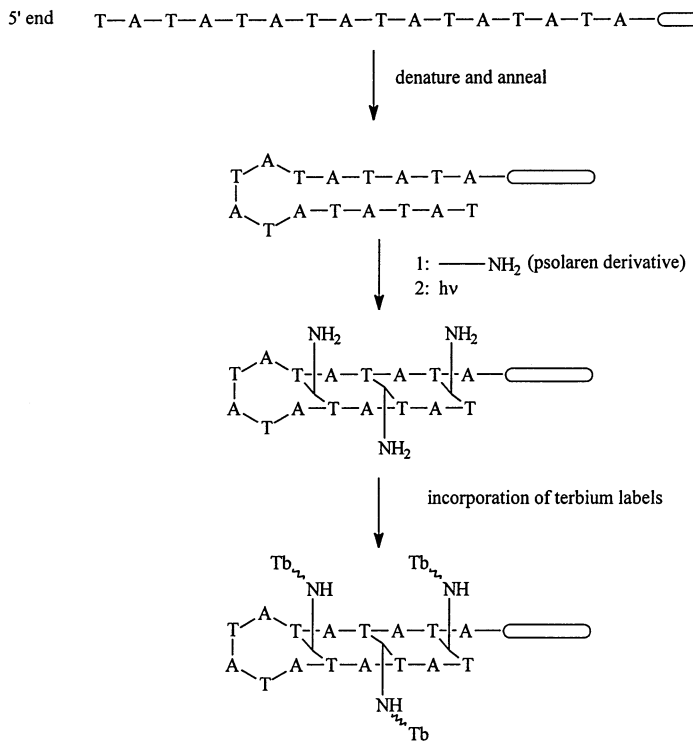
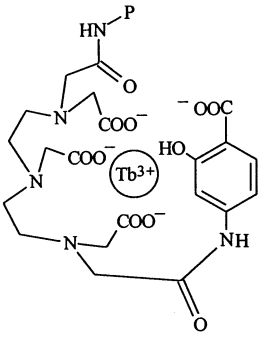


Fig. 15. A photochemical method to label single-stranded DNA by an amino substituted psoralen derivative. Adapted from ref. [232].

DNA can also be labeled indirectly with luminescent lanthanide chelates. For example, Mathis and co-workers described a heterogeneous DNA hybridization assay (Fig. 17) in which the target DNA is first immobilized on a nitrocellulose



P = psoralen moiety

Fig. 16. A luminescent terbium chelate attached to the amino derivative of psoralen. Adapted from ref. [232].

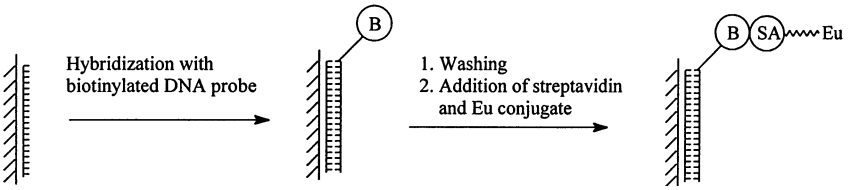


Fig. 17. A heterogeneous DNA hybridization assay in which the DNA probe is labeled with a luminescent europium chelate based on the recognition of biotin and streptavidin. Adapted from ref. [233].

membrane [233]. The biotinylated DNA probe is then hybridized with the target. Biotin is a small molecule (vitamin H) which binds very strongly to the protein streptavidin (SA). After the washing procedures, the biotinylated DNA is then recognized by a luminescent europium tris(bipyridine) cryptate attached with a streptavidin.

On the other hand, Diamandis and co-workers employed a streptavidin-based macromolecular complex labeled with the europium chelate of 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylate (**77**) as a luminescent label for biotinylated DNA present on nitrocellulose filters [234]. The molecule can be represented by the formula $SA(TG)_{3.3}(BCPDA)_{480}$, where SA stands for streptavidin, TG bovine thyroglobulin and BCPDA 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylate. Owing to the presence of a number of luminescent labels for each streptavidin unit, the detection limit of this system reaches 10 pg of target DNA. The fluorescent spots or bands can be observed under UV illumination, photographed by instant camera photography or quantified by using a high resolution time-resolved fluorometric scanner. The complex has also been used in different fluoroimmunoassays (see Section 4.3).

Homogeneous DNA hybridization assays have been focussed on with increasing attention. In this respect, Selvin and co-workers utilized the principle of Förster resonance energy transfer [235]. A europium chelate containing a light absorbing dye carbostyril 124 is connected to the 5′-end of a single stranded DNA [236]. The 5′-end of the complementary DNA strand is linked to an energy accepter CY-5. Upon hybridization, the europium chelate donor is in close proximity to the CY-5 acceptor (Fig. 18). The lifetime and quantum yield of the luminescence of the europium chelate decrease as a result of resonance energy transfer quenching. Remarkably, the sensitized emission of the acceptor can be measured without interfering background such as the donor emission by monitoring the emission of the acceptor at a region the donor emission is negligible. The interference due to the direct acceptor fluorescence can also be avoided as it is too short-lived compared with the sensitized emission which is in a millisecond time-scale. A similar system based on luminescence resonance energy transfer between a terbium chelate donor and a rhodamine acceptor has also been described (Fig. 19) [237].

On the other hand, Valet and co-workers reported a homogeneous DNA hybridization assay employing a pair of oligonucleotide probes [238]. One of the oligonucleotides is linked to a salicylate group which serves as the light absorbing unit while the other probe is attached with a terbium chelate. The oligonucleotide

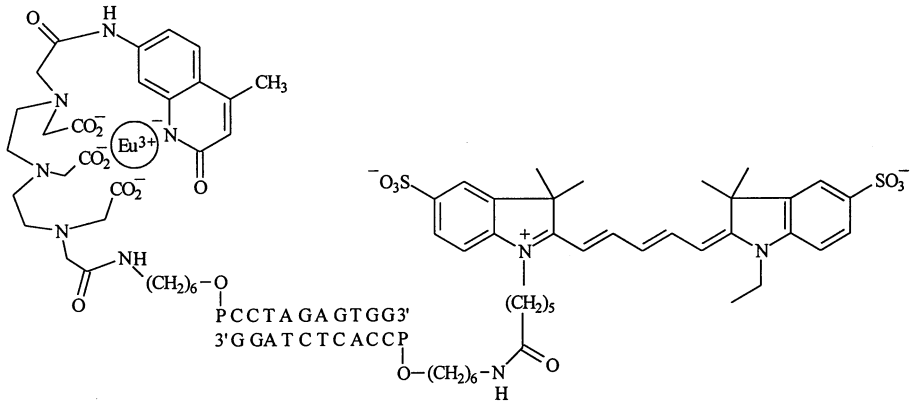


Fig. 18. Double-stranded DNA with europium-carbostyril 124 (donor) at one 5′-end and CY-5 (acceptor) at the other 5′-end. Adapted from ref. [236].

sequences are designed in such a way that, after hybridization to the complementary DNA target, the energy donor and acceptor are in close proximity (Fig. 20). Upon irradiation, the salicylate group of one oligonucleotide probe transfers the excitation energy to the terbium ion of the other oligonucleotide probe and the long-lived and intense luminescence of the terbium ion is observed.

Sammes and co-workers recently described a very interesting system in which the 5′-end of the DNA probe is linked to a europium chelate (**78**) [239]. On the other

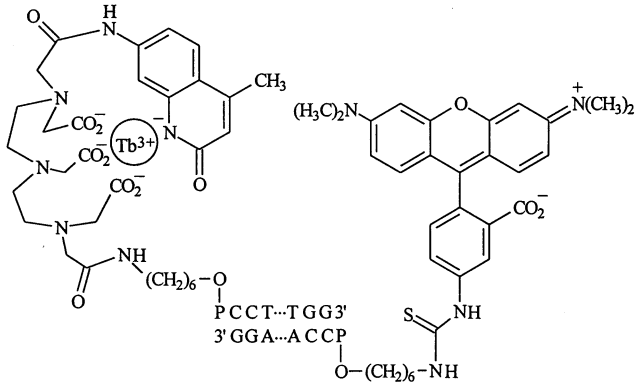


Fig. 19. Double-stranded DNA with terbium-carbostyril 124 (donor) at one 5′-end and a rhodamine derivative (acceptor) at the other 5′-end. Adapted from ref. [237].

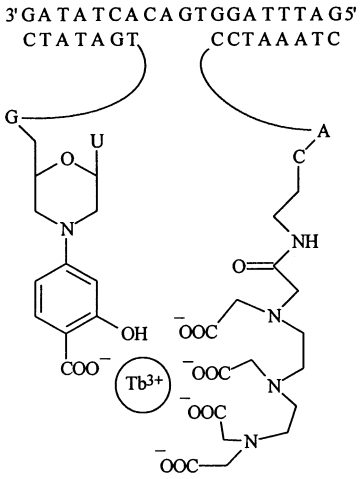
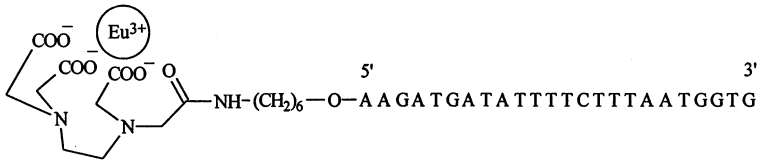


Fig. 20. A homogeneous DNA hybridization assay with a salicylate and a terbium chelate as the co-operative probes. Adapted from ref. [238].

hand, the sensitizer, 1,10-phenanthroline-2,9-dicarboxylate, is linked to an intercalator phenanthridinium (**79**). The binding constant of the sensitizing ligand for the



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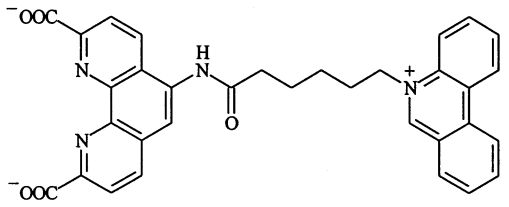

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chelated europium ion is in the range of 10^6–10^7 M^{-1}. When these two components are present in low concentrations ($< 10^{-7}$ M), little association occurs. In the

presence of the target DNA with a complementary sequence to the probe, the sensitizer is brought near to the DNA hybrid as a result of the intercalation of the phenanthridinium to the duplex. Binding of the sensitizer to the metal ion is effectively promoted and the water molecules surrounding the europium are displaced and an enhancement in the luminescence of the europium is detected (Fig. 21). A related system has been shown to detect single point mutations in targets such as those present in some mutations associated with cystic fibrosis [240].

Owing to the recent development of polymerase chain reaction (PCR), amplification of target DNA sequences is possible. Luminescent lanthanide complexes have been used to detect PCR products. For example, Mathis and co-workers reported a method in which a luminescent europium cryptate is used to detect PCR-amplified sequences (Fig. 22) [241]. The sequence of interest is first amplified using a pair of outer primers (OP). A second PCR is carried out on the diluted product from the first PCR using biotinylated and 2,4-dinitrophenol (DNP) labeled primers. The hybrid is incubated on microtiter wells coated with streptavidin (SA). After washing, the PCR products are recognized with an anti-DNP antibody labeled with a europium–tris(bipyridine) cryptate complex.

On the other hand, Diamandis and co-workers reported an interesting method to detect PCR amplified product [242]. The 5′-end of a PCR primer is labeled with the chelate 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylate (**77**). The PCR is carried out in the presence of another untreated primer. After the reaction, the PCR product is separated by agarose gel electrophoresis and the gel is then immersed into a solution containing europium ions. The europium ion diffuses into the gel and forms a highly luminescent complex with the chelates. Quantification of the PCR products is carried out using a time-resolved fluorometric reader. The detection limit for this assay is about 5 ng DNA.

Besides, Liukkonen and co-workers described another assay for detecting point mutations by utilizing PCR and europium–cryptate labeled allele-specific oligonucleotide (ASO) probes [243]. The procedure is described in Fig. 23. The europium labeled ASO probe is hybridized simultaneously with an adjacent biotinylated

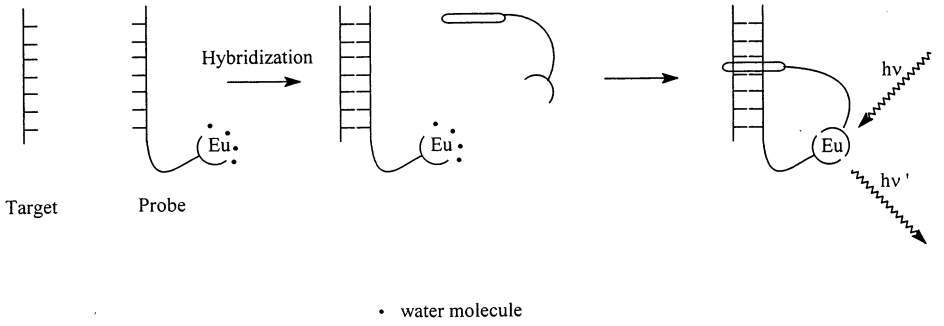


Fig. 21. A homogeneous DNA hybridization assay with a europium chelate-DNA as the probe and an intercalator connected to a sensitizing ligand as the energy donor. Adapted from ref. [239].

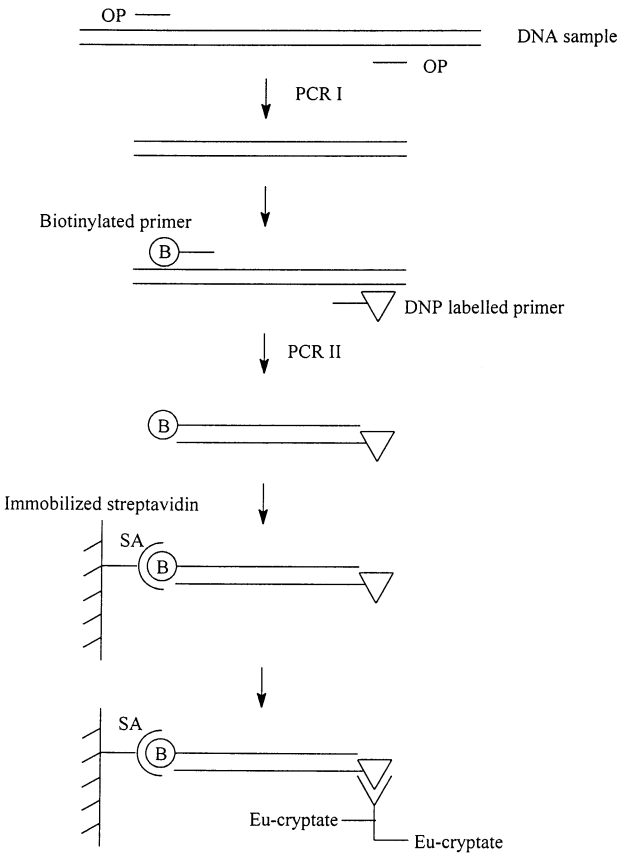


Fig. 22. Detection of PCR products by a luminescent europium cryptate. Adapted from ref. [241].

26-mer to the PCR amplified product. The hybrids are attached onto microtiter wells coated with streptavidin. The well is then washed stringently and the luminescence of the remaining bound europium cryptate probe is then measured in a time-resolved fluorometer. The method has been employed to detect a point mutation in the α_1-antitrypsin gene.

4. Miscellaneous

4.1. Chiral nuclear magnetic resonance shift reagents

Addition of a lanthanide shift reagent to an organic compound may result in shifts of resonance to a different frequency. The observation is attributed to the formation of a weak addition complex between the lanthanide and the organic compound in fast exchange with the unbound counterpart on the NMR time-scale

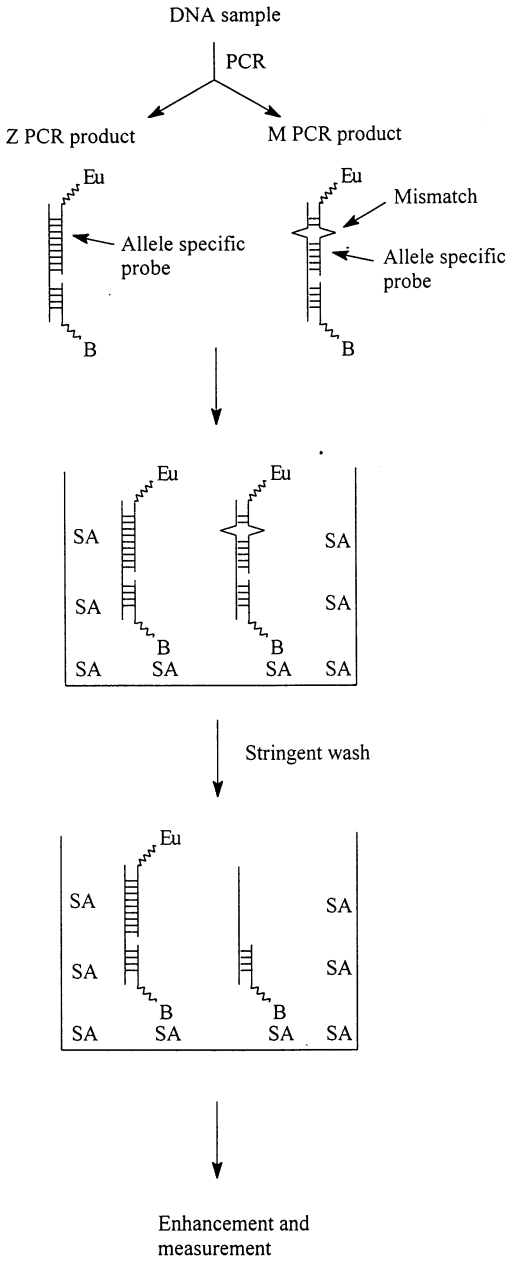
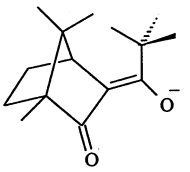


Fig. 23. An assay for the detection of a point mutation by PCR. Adapted from ref. [243].

[244–248]. In 1970, Whitesides and co-workers first utilized the camphor-based chelate $Eu(pvc)_3$ [pvc = pivaloyl-*d*-camphorate (**80**)] as a chiral NMR shift reagent [249].

Reuben described the NMR spectral resolution of enantiomeric protons of α-hydroxycarboxylates by lanthanide ions in aqueous solution [250]. It has been found that 1:1 Ln complexes of chiral hydroxycarboxylates can exhibit spectral resolution of enantiomeric mixtures and of enantiotopic protons of other hydroxycarboxylates. Moreover, spectral resolution is observed for complexes of a 3:1 mixed ligand/metal stoichiometry but not in the 2:1 complexes [251]. It has been suggested that the rigidity of the 3:1 complexes prevents the averaging out of the chemical-shift differences between the enantiomeric nuclei.



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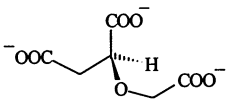

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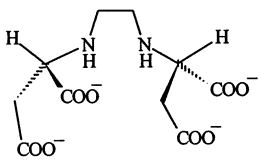

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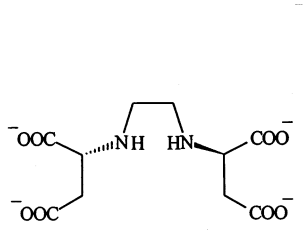
Besides, Peters and co-workers used the chelate (*S*)-[(carboxymethyl) oxy]succinate (CMOS) (**81**) of europium and ytterbium ions to resolve the enantiomer protons of chiral α-amino acids and carboxylic acids [252]. The proton spectral resolution of the two enantiomers in racemic oxydilactate and of the enantiotopic CH_2 protons of oxydiacetate and nitrilotriacetate has been observed with the complex Eu-(*S*)-CMOS.

Brittain and co-workers showed that the europium chelate Eu-(*S*,*S*)-EDDS [(*S*,*S*)-EDDS = (*S*,*S*)-ethylenediamine-*N*,*N*′-disuccinate (**82**)] can also be used as a chiral NMR shift reagent for a series of enantiomeric amino acids in aqueous solution [253]. The workable pH range of the complex is between 9 and 11. An enantiomeric difference $\Delta\Delta\delta$ of 0.4 ppm between the α-protons of D,L-phenylglycine is observed at an [Eu-(*S*,*S*)-EDDS]:[substrate] ratio of 0.3.

Recently, Feringa and co-workers reported the synthesis of a series of chiral *N*,*N*′-disuccinate ligands (**83a**–**c**) [254]. The europium complexes of these ligands

have been shown to be effective chiral NMR shift reagents for the enantiomeric spectral resolution of amino acids in aqueous solutions.



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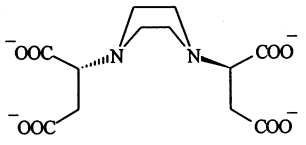

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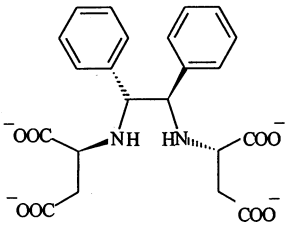
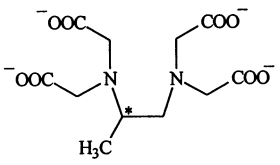


83c

Kabuto and Sasaki focussed on the europium chelate with a chiral ligand (*R*/*S*)-pdta (pdta = 1,2-propanediaminetetraacetate) (**84**) [255–257]. The complex $[Eu\{(R)\text{-pdta}\}(H_2O)_3]^-$ has been shown to be a useful chiral NMR shift reagent for amino acids and hydroxycarboxylic acids [255]. However, the shifts show a strong pH dependence. For example, the separation of signals of lactic acid is optimum at a pH of ca. 4 but is not observed at pH 12. In contrast, for amino acids, better resolutions occur at pH of ca. 12. The difficulty of pH limitation has recently been overcome. In a recent report, the same research group described the synthesis and crystal structure of a new chiral lanthanide shift reagent $[EuCl_2\{(R)\text{-tppn}\}]^+$ [(*R*)-tppn = (*R*)-*N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)propylenediamine (**85**)] [258]. The chelate has been found to resolve the enantiomer signals of a series



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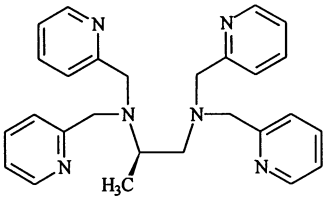

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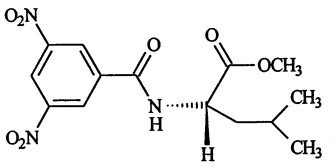
of α-amino acids in aqueous solution at neutral pH. The resolving ability of the chelate and the different formation constants with the enantiomeric amino acids have been ascribed to the twisting of the propylene moiety. For example, the α-proton signals of an enantiomeric mixture (D:L = 1:2) of valine are resolved into a pair of signals ($\Delta\Delta\delta = 0.30$, pH at 7.5, [reagent]:[substrate] = 0.44) with the L-isomer proton occurring more upfield.

On the other hand, in 1975, Pirkle and co-workers first demonstrated that addition of achiral and paramagnetic $Eu(fod)_3$ (fod = 6,6,7,7,8,8,8-heptafluorooctane-3,5-dionate) into a mixture of chiral aryl perfluoroalkylcarbinols (solvating

agents) and sulfoxide enantiomers (substrates) can also cause an enhancement in enantiomeric resolution [259]. A prerequisite is that the enantiomers exhibit association with the resolving agents to different extents. It is also important that the association between the substrate and the lanthanide should be stronger than that between the resolving agent and the lanthanide.

The organic host cyclodextrins are optically active and chiral discrimination with enantiomeric organic guests may be present within the cavity of the receptor. Utilization of cyclodextrins and chiral donor–acceptor compounds such as the methyl ester of *N*-(3,5-dinitrobenzoyl)-L-leucine (**86**) as resolving agents has been described by Wenzel and co-workers [260]. Lanthanide chelates such as $Eu(fod)_3$, $Pr(fod)_3$, $[Pr(TTHA)]^{3-}$ (fod = 6,6,7,7,8,8,8-heptafluorooctane-3,5-dionate, TTHA = triethylenetetraaminehexaacetate) have been found to enhance the enantiomeric resolution of substrates. Recently, the same research group designed a system in which a cyclodextrin is covalently linked to a diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetate moiety (**87**) [261]. Addition of dysprosium(III) ion to the modified



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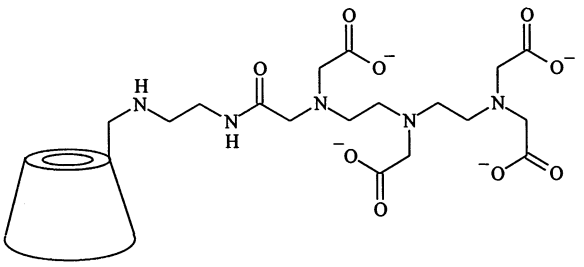

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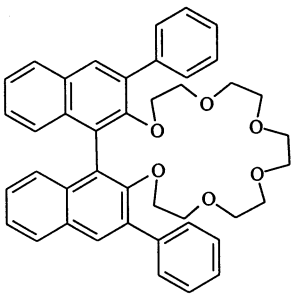
cyclodextrins enhances the enantiomeric resolution in the ^{1}H-NMR spectra of a series of organics such as carbinoxamine maleate, doxylamine succinate, pheniramine maleate, propranolol hydrochloride and tryptophan. The observed shifts have also been used to elucidate the geometry of the cyclodextrin–substrate complexes.

On the other hand, two chiral crown ethers, 2,3:4,5-bis[1,2-(3-phenylnaphtho)]-1,6,9,12,15,18-hexaoxacycloeicosa-2,4-diene (**88**) and 1,2:5,6-di-*O*-isopropylidene-3,4-[(*tert*-butylbenzenediyl)bis(oxyethoxy)ethyl]-D-mannitol (**89**) have been used as NMR resolving agents for protonated amino acid esters, amines and amino alcohols [262]. The enantiomeric resolution is enhanced in the presence of $[Ln(fod)_4]^-$ [Ln = Eu, Pr; fod = 6,6,7,7,8,8,8-heptafluorooctane-3,5-dionate] generated by the reaction of $Ln(fod)_3$ and Ag(fod).



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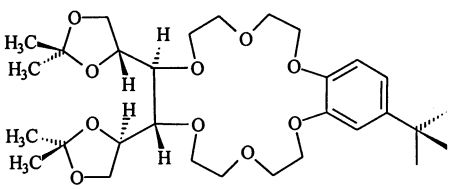

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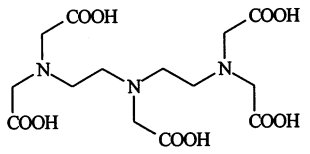
4.2. Magnetic resonance imaging

Magnetic resonance imaging (MRI) is based on the measurement of the ^{1}H-NMR signal (essentially water protons) in different encoded volume elements in the body. The image contrast results from the different relaxation rates of water protons in different tissues. Paramagnetic ions can enhance the contrast by increasing the relaxation rates in surrounding water. There have been a lot of excellent monographs on this fast-growing subject [263–272]. Since the introduction of MRI as a powerful diagnostic tool in medical science, there has been a demand for the development of effective contrast agents. The contrast of the NMR images depends on the proton density and the proton relaxation times T_1 (longitudinal or spin-lattice relaxation time) and T_2 (transverse or spin–spin relaxation time). Usually, the effect of a paramagnetic agent in NMR imaging is to increase the signal intensity of the tissue containing the agent by decreasing T_1 because T_2 is very short and cannot be significantly decreased by low concentration of the contrast agent. The efficiency of a MRI contrast agent is described by its relaxivity which contains two components, the inner-sphere and the outer-sphere relaxivity. The inner-sphere relaxivity is based on the interactions between the unpaired electron spins and the protons of the coordinated water molecules while the outer-sphere relaxivity is determined by the interactions between paramagnetic metal ion and the bulk water.

Gadolinium(III) ion, with seven unpaired electrons, is the most commonly used paramagnetic species on the basis of its high magnetic moment and the long electronic relaxation time. However, the free ion is too toxic to be used clinically. In order to reduce the toxicity, the ion must be chelated by a ligand, forming a stable complex. However, the complex must involve the coordination of at least one water molecule to allow the proton nuclear relaxation of the lanthanide ion-bound water to be transferred to the bulk aqueous solution. The most widely used contrast agents are the gadolinium(III) chelates $[Gd(DTPA)(H_2O)]^{2-}$ [H_5DTPA = diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid (**90a**)] and $[Gd(DOTA)(H_2O)]^{-}$ [H_4DOTA = 1,4,7,10 - tetrakis (carboxymethyl) - 1,4,7,10 - tetraazacyclododecane (**90b**)]. These complexes are soluble in water and inert to dissociation [264].



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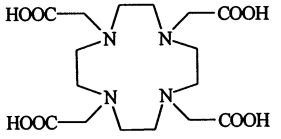

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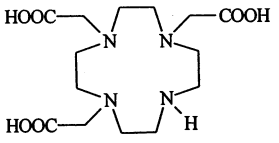

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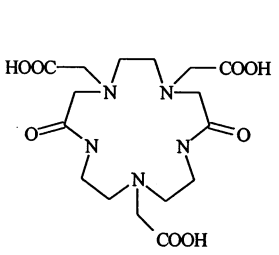
However, these complexes both carry a negative charge that makes the concentrated injectable solutions hyperosmolar with respect to blood and most body fluids. In view of this, Tweedle and co-workers reported the synthesis of a series of Gd(III) complexes with the ligand $DO3A^{3-}$ [H_3DO3A = 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (**91**)] and its analogues [273]. The neutral complexes $[Gd(DO3A)(H_2O)]$ are highly water soluble, stable and suitable as proton relaxation catalysts, revealing their potential as effective MRI contrast agents.

DeW. Horrocks, Jr. and co-workers reported the synthesis of a series of amide-based macrocycles as potential MRI contrast agents [274]. The solution complexes of the ligands $EDTA\text{-}DAM^{3-}$[H_3EDTA-DAM = 4,10,13-tris(carboxymethyl)-8,15-



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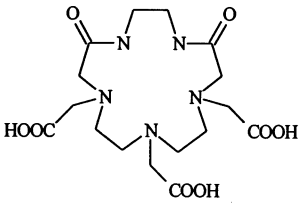

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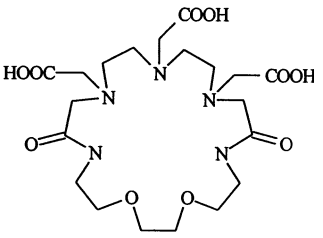

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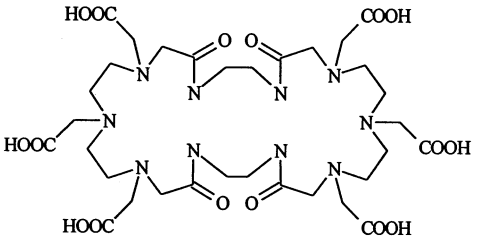
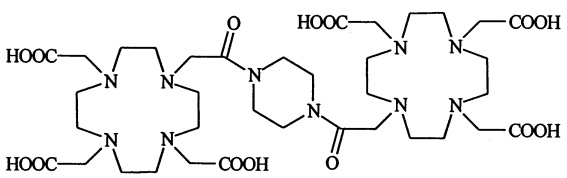


92d

dioxo-1,4,7,10,13-pentaazacyclopentadecane (**92a**)], DTPA-EAM^{3-} [H_3DTPA-EAM = 1,4,7-tris(carboxymethyl)-9,14-dioxo-1,4,7,10,13-pentaazacyclopentadecane (**92b**)], DTPA-OAM^{3-} [H_3DTPA-OAM = 1,4,7-tris(carboxymethyl)-9,20-dioxo-13,16-dioxa-1,4,7,10,19-pentaazacycloheneicosane (**92c**)], bis(DTPA-EAM)$^{6-}$ [H_6 bis(DTPA-EAM) = 1,4,7,16,19,22-hexa(carboxymethyl)-9,14,24,29-dioxo-1,4,7,10, 13,16,19,22,25,28-decaazacyclotriacontane (**92d**)] and PIP-bis(DO3A)$^{6-}$ [H_6PIP-bis(DO3A) = 1,4-bis{[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl}piperazine (**92e**)] have been characterized by europium luminescence spectroscopy. While EDTA-DAM^{3-}, DTPA-EAM^{3-} and DTPA-OAM^{3-} form



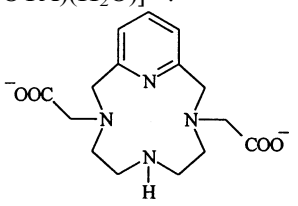
92e

1:1 stoichiometric metal complexes with europium, the ligands bis(DTPA-EAM)$^{6-}$ and PIP-bis(DO3A)$^{6-}$ form 2:1 metal–ligand complexes. The number of coordi-

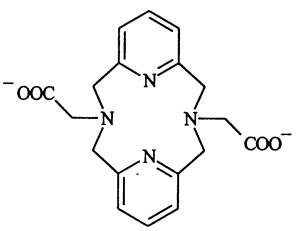
nated water molecules have also been determined for the Eu^{3+} complexes of the ligand. The composition of the first coordination sphere of the europium ion has been determined by means of molecular mechanics calculations.

On the other hand, the design and modelling of gadolinium complexes as effective MRI contrast agents have also been investigated with molecular mechanics force fields calculation methods as demonstrated by Cundari and co-workers [275].

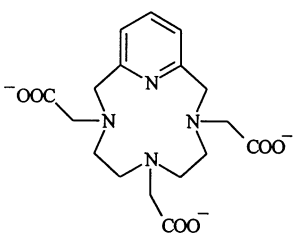
Sherry and co-workers prepared a series of gadolinium complexes with the macrocyclic ligands (**93a**–**d**) [276]. The number of inner-sphere water molecules for [Gd(**93a**)]$^{+}$, [Gd(**93b**)]$^{+}$, [Gd(**93c**)], [Gd(**93d**)]$^{-}$ have been determined to be 3.5, 3.3, 2.4 and 0.2, respectively. Tissue biodistribution results using radioactive ^{153}Sm and ^{159}Gd complexes in rats reveal that the cationic [^{153}Sm(**93a**)]$^{+}$ and [^{153}Sm(**93b**)]$^{+}$ complexes accumulate essentially in the bone tissue while the neutral [^{153}Sm(**93c**)] and anionic [^{153}Sm(**93d**)]$^{-}$ complexes appear to have renal clearances similar to those of other low molecular weight contrast agents such as [Gd(DTPA)(H_2O)]$^{2-}$ and [Gd(DOTA)(H_2O)]$^{-}$.



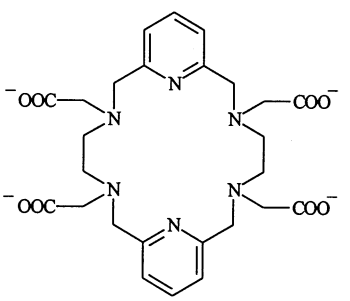
93a



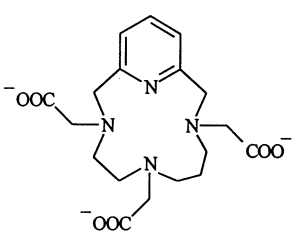
93b



93c



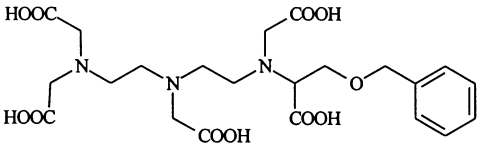
93d



94

Moreover, Aime et al. reported the synthesis of an asymmetric macrocyclic triacetate ligand (**94**) [277]. The ligand forms neutral complexes with europium, ytterbium and gadolinium ions. The relaxivity of the gadolinium chelate is determined to be 6.3 mmol dm^{-3} s^{-1} (20 MHz; 298 K), about 35% higher than that of $[Gd(DOTA)(H_2O)]^-$. The value is consistent with the presence of two coordinated water molecules. It has been suggested that the complex and its analogues represent a new class of MRI contrast agents.

Uggeri and co-workers reported the synthesis of the Gd(III), La(III) and Lu(III) complexes with the ligand $BOPTA^{5-}$ [H_5BOPTA = 4-carboxy-5,8,11-tris(carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatridecan-13-oic acid (**95**)] [278]. The X-ray



95

crystal structure of the gadolinium complex reveals a coordinated water molecule. The coordination of a water molecule is also shown by relaxation studies. The potential of the gadolinium complex as a MRI contrast agent has also been examined. Besides, Powell and co-workers recently carried out a detailed simultaneous analysis of ^{17}O-NMR, EPR and NMRD (Nuclear Magnetic Resonance Dispersion) on a series of gadolinium complexes such as $[Gd(H_2O)_8]^{3+}$, $[Gd(DTPA)(H_2O)]^{2-}$, $[Gd(DOTA)(H_2O)]^-$ and their analogues [279]. The parameters affecting the proton relaxivity of gadolinium complexes have been investigated in detail.

On the other hand, Bednarski and co-workers described the synthesis and characterization of a novel class of polymerized liposome particles with lanthanide chelates as head groups [280]. By incorporating Gd(III)–DTPA-conjugated lipids into liposomes, it is expected that *in vivo* distribution of the liposomes can be visualized using MRI. Organic lipids are also incorporated into the polymer to control the surface metal density. The R_1 molar relaxivities of the particles are essentially dependent on the linker length and the surface metal density but not on the size of the particle. A biotinylated lipid has also been incorporated into the particle without affecting R_1 relaxivities for use as a marker for histochemical studies. An example is illustrated in Fig. 24.

Merbach and co-workers reported the water exchange and rotational dynamics of a series of macrocyclic gadolinium complexes based on dendrimers such as [G3(N{CS}N-bz-Gd{DO3A}{H_2O})$_{23}$] (**96**) [281]. It has been found that

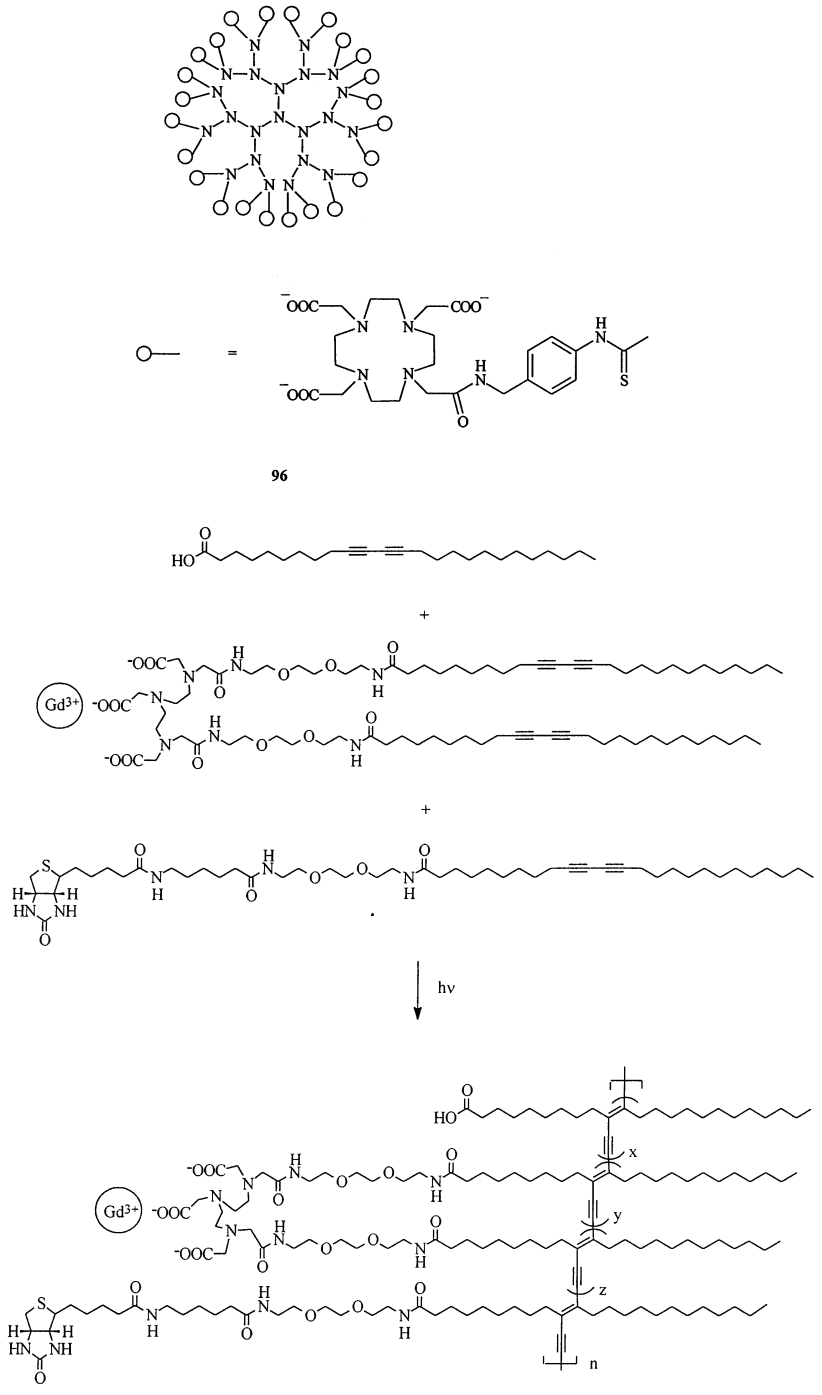
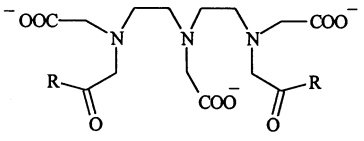
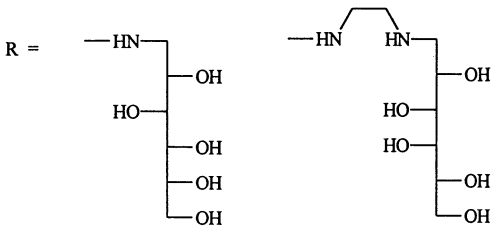


Fig. 24. Formation of paramagnetic polymerized liposomes. Adapted from ref. [280].

owing to the rigid structures of the dendrimers, their rotational correlation times are 4 to 8 times longer than that for the monomeric or dimeric Gd(III) poly(amino carboxylates). The relaxivities of these dendrimers are therefore higher than those of the simpler complexes. However, owing to the low water exchange rates, higher relaxivities cannot be obtained.

Another approach to increase the relaxivity by slowing down the rotation motion is to couple the gadolinium chelate to high molecular weight polysaccharides [282]. Recently, Lammers et al. reported the synthesis of a series of lanthanide complexes of sugar-based DTPA-bis(amides) such as DTPA-BGLUCA^{3-} {*N*,*N*″-bis[*N*-(D-*gluco*-2,3,4,5,6-pentahydroxyhexyl)carbamoylmethyl]diethylenetriamine-*N*,*N*′,*N*″-triacetate} (**97a**) and DTPA-BENGALAA^{3-} {*N*,*N*″-bis[*N*-(3-aza-D-*galacto*-5,6,7,8,9-pentahydroxynonyl)carbamoylmethyl]diethylenetriamine-*N*,*N*′,*N*″-triacetate} (**97b**). It has been found that replacement of the alkyl functions in the



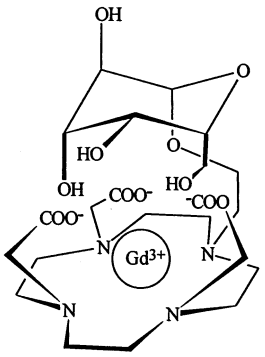


97a **97b**

lanthanide complexes of DTPA-bis(alkylamides) by polyhydroxylated ones has no significant influence on the structure of the complex. While the coordination sphere remains the same, the water exchange rate is typically one order of magnitude smaller than that of $[Gd(DTPA)(H_2O)]^-$. The observation has been ascribed to the bulkiness of the amide groups.

As the inner-sphere relaxivity of a gadolinium chelate is related to the number of coordinated water molecules, Meade and co-workers designed a novel gadolinium chelate EGad {EGad = [4,7,10-tris(carboxymethyl)-1-(2-β-galactopyranosylethoxy)-

1,4,7,10-tetracyclododecane)gadolinium]} (**98**) whose relaxivity can be controlled by enzymatic activity [283]. In this complex, eight coordination sites of Gd^{3+} are



98

coordinated by a DOTA derivative while the remaining coordination site is blocked by a galactopyranose unit. Upon exposure to the enzyme β-galactosidase (β-gal), the blocking unit is irreversibly removed and water exchange with the bulk is therefore enhanced. The number of water molecules in fast exchange with the blocked EGad and unblocked Gad complexes has been determined to be 0.7 and 1.2, respectively. Upon the enzymatic action, the relaxivity of the contrast agent increases and the MRI signal of the nearby water protons are therefore enhanced.

4.3. Other bioassays

Conventional fluoroimmunoassay methods using organic fluorescent compounds as labels cannot achieve the sensitivity of radioimmunoassays, owing to the short lifetime of the fluorescence and the high background fluorescence. Based on the strong and long-lived luminescence of lanthanide chelates, the problem of detection sensitivity can be solved and such complexes have widely been utilized as a luminescent label for bio-molecules [284–291]. In this section, important fluoroimmunoassays employing lanthanide chelates will be described. In general, there are two main types of time-resolved fluoroimmunoassays based on lanthanide complexes—the dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) [292] and the CyberFluor fluoroimmunoassay [293].

For the indirect DELFIA method (Fig. 25), in a heterogeneous system, one of the specific binding reagents is immobilized on a solid support. After the immunoreaction between the immobilized binding reagent and the analyte, another

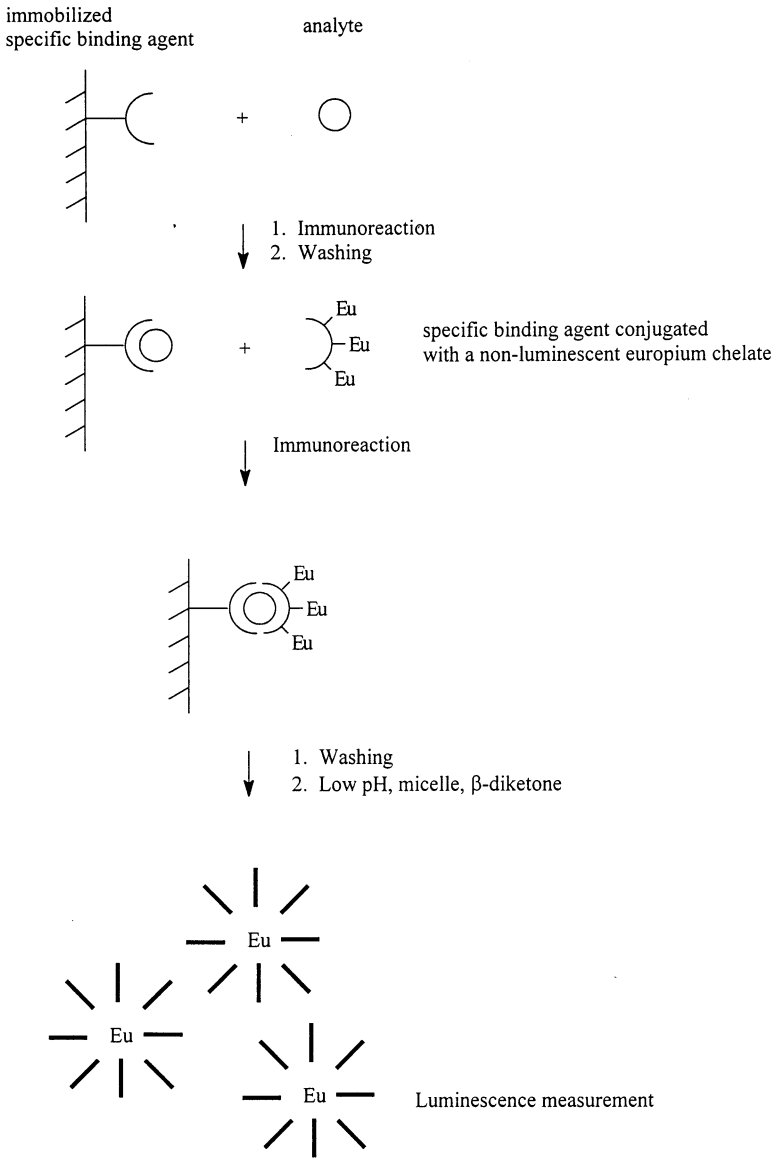
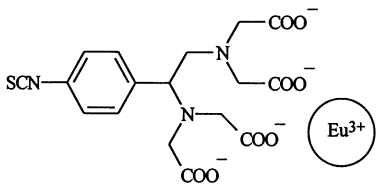


Fig. 25. Dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA).

specific binding reagent labeled with a nonluminescent lanthanide chelate such as isothiocyanatophenyl–EDTA–europium (**99**) is allowed to react with the immobilized analyte. After washing procedures, the lanthanide ion is dissociated from



99

the nonluminescent chelate. This is done by lowering the pH of the solution below 3, which reduces the chelating capability of the chelate. The enhancement solution also contains energy transfer reagents such as aromatic β-diketones and therefore strongly luminescent lanthanide complexes are formed.

In 1983, Siitari et al. described an immunoassay of hepatitis B surface antigen [294]. Immunoglobulin is first coated onto polystyrene tubes. The plasma specimen is then incubated in the coated tube in the presence of the antibody labeled with an EDTA–europium complex. The tube is washed with saline solution. Europium fluorescence is developed in the presence of 2-naphthoyltrifluoroacetone and trioctylphosphine oxide (TOPO). Dakubu and co-workers also reported a related fluoroimmunoassay for the detection of rabbit immunoglobulin G (IgG) [295]. The anti-rabbit IgG is labeled either with diazophenyl–EDTA–europium or isothiocyanatophenyl–EDTA–europium. After the immunoreaction and washings, the luminescence of europium is developed in a buffer at pH 7–8 in the presence of β-diketone, TOPO and 0.2% Tween 29. Alternatively, the luminescence enhancement can be developed by dissociating the europium from the solid phase antibody at pH 3–3.5 in a buffered solution containing 0.1–0.2% Triton X-100, β-diketone and TOPO. The non-ionic detergent Triton X-100 or Tween 20 is added to solubilize the β-diketone and the synergistic agent POTO and to provide a suitable hydrophobic micellar environment for luminescence measurement.

Quantification of the steroids testosterone and cortisol has been performed in a competitive assay [296]. For example, testosterone is immobilized by physical adsorption as a testosterone-3-(carboxymethyloxime)-ovalbumin conjugate on the solid phase (Fig. 26). The steroid sample is added immediately before the addition of the europium labeled antigen. The immobilized and free steroids will compete for the binding sites on the labeled antibody. After washing and enhancement procedures, the luminescence of europium is detected. The luminescence intensity is inversely proportional to the concentration of steroid in the sample. Besides, related immunoassays of human thyrotropin and luteotropin have also been carried out [296].

In view of the strong interaction between biotin and streptavidin, they have been

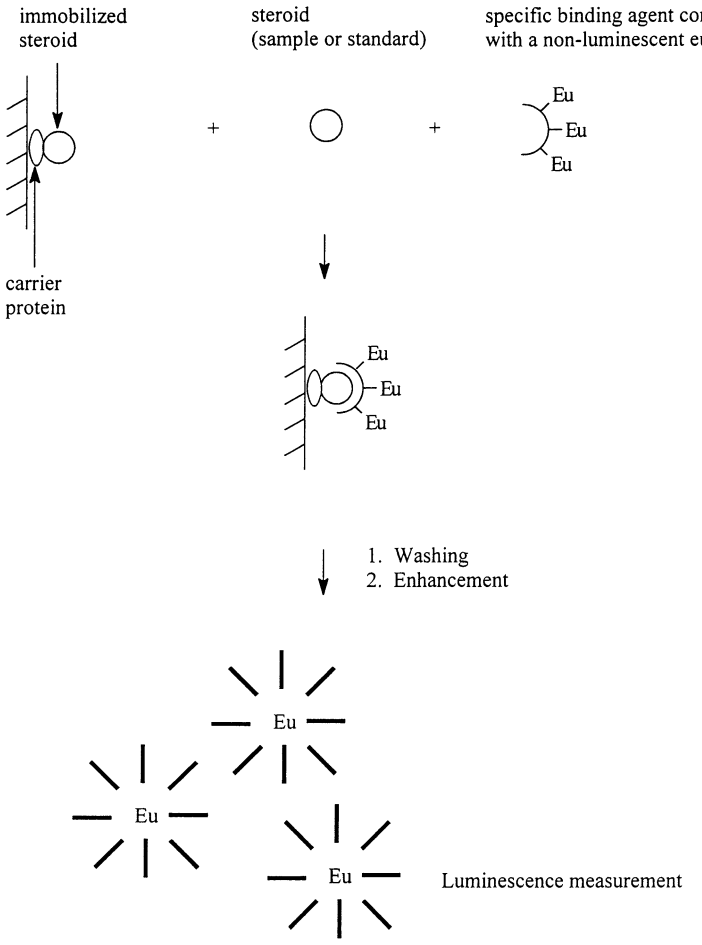
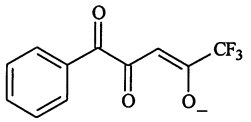


Fig. 26. A competitive assay of steroids.

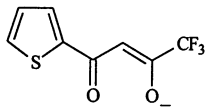
extensively employed to connect bio-molecules and luminescent labels in fluoroimmunoassays. While over-labeling decreases the affinity and binding specificity of antibodies, conjugation of all the free 13 amino groups of streptavidin with europium chelates does not affect significantly the binding capacity of the protein or its affinity towards biotin. The recognition of a biotinylated antibody by the streptavidin multiply labeled with europium chelates can provide a higher detection sensitivity in the assay (Fig. 27). Indirect time-resolved fluoroimmunoassays of human follicle and thyroid stimulating hormones developed by Hemmilä and co-workers are based on such a method [297].

On the other hand, under certain colloidal conditions in the presence of some other lanthanide ions or yttrium ions, the luminescence intensities of the β-diketo-

nates of Eu^{3+} and Sm^{3+} ions can be increased significantly. For example, in the presence of 1,10-phenanthroline and excess Y^{3+}, the luminescence intensities of Eu^{3+} and Sm^{3+} chelated with benzoyltrifluoroacetonate (BTA) (**100a**) or thenoyltrifluoroacetonate (TTA) (**100b**) are increased significantly. This co-fluorescence



100a



100b

phenomenon has led to the development of dual-label immunoassays of luteinizing hormone (LH) and follicle stimulating hormone (FSH) [298]. The monoclonal anti-β-LH and anti-β-FSH antibodies are labeled with Eu^{3+} and Sm^{3+} labeling reagents, respectively. Microtiter strip wells are coated with monoclonal antibodies against the α-subunits of LH and FSH (Fig. 28). In the assay, the strips are first incubated with LH and FSH. After washing, they are further incubated with a mixture of Eu-anti-β-LH and Sm-anti-β-FSH. Then, the strips are washed and the labels are dissociated with an acidic dissociative solution containing β-diketonates, Y^{3+} and Triton X-100 in an acidic buffer at pH 3.1. The luminescence is enhanced by addition of another solution containing 1,10-phenanthroline and a tris buffer which can increase the pH. As the signal levels are improved, the co-fluorescence enhancement system can provide a high sensitivity.

In general, for the indirect method, there is a possibility of contamination by lanthanide ions in the enhancement process. Another most common detection methodology for heterogeneous assays, the CyberFluor system, employs a luminescent lanthanide chelate as a label. Instead of measuring the luminescence of released lanthanide ions in the enhancement solution, the luminescence from the label is directly detected from the surface of the solid support for immobilization (Fig. 29). The chelate must form a highly luminescent and stable complex with the lanthanide ions. Such a ligand is difficult to obtain but the chelate 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylate (BCPDA) (**77**) can satisfy the requirements as a good chelator and sensitizer [299]. Moreover, the ligand contains two sulfonyl chloride groups through which it can react with the amino groups of the protein to be labeled under mild conditions. The optimal conditions of labeling a range of proteins such as streptavidin, avidin, monoclonal and polyclonal antibodies with BCPDA have been investigated [300].

Diamandis and co-workers reported a heterogeneous competitive time-resolved fluoroimmunoassay of serum cortisol [301]. Cortisol sample competes with immobilized cortisol (in the form of cortisol–thyroglobulin conjugate) for binding to a monoclonal anti-cortisol biotinylated antibody (Fig. 30). The microtiter strips are then washed and incubated with a working reagent containing BCPDA–Eu^{3+}

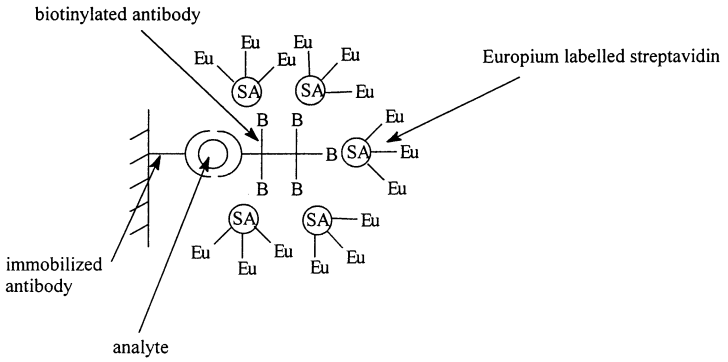


Fig. 27. Recognition of a biotinylated antibody by streptavidins multiply labeled with europium chelates.

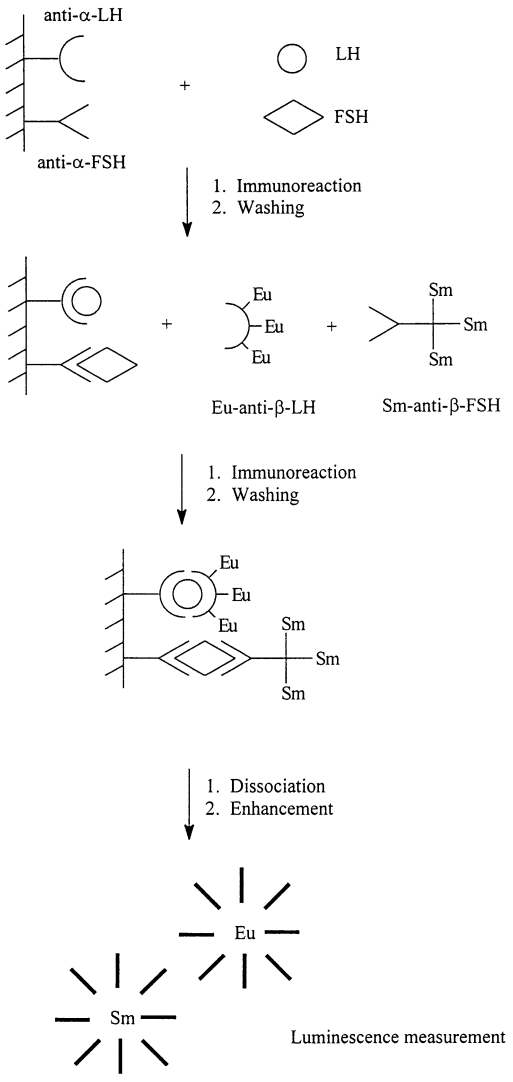


Fig. 28. A dual-label immunoassay of luteinizing hormone (LH) and follicle stimulating hormone (FSH).

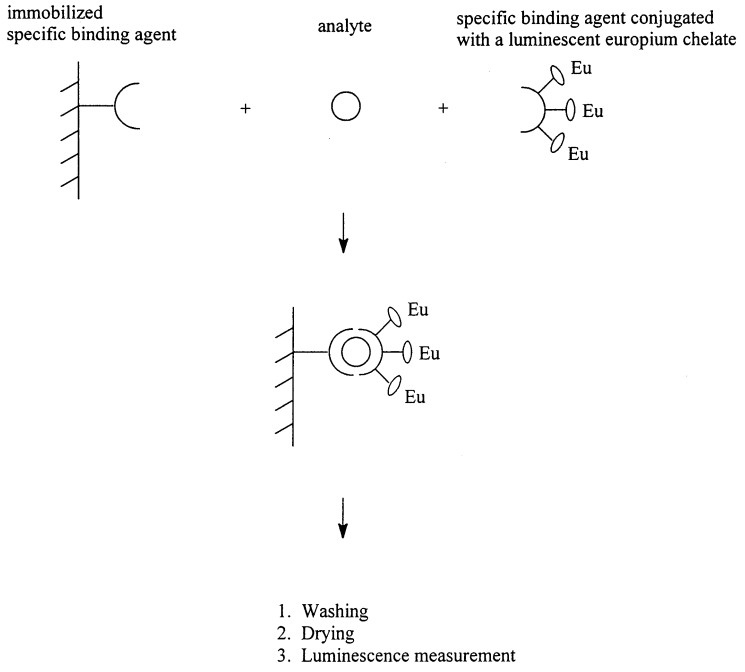


Fig. 29. CyberFluor fluoroimmunoassay.

labeled streptavidin. After washing and drying procedures, the surface luminescence of the labels is measured. A similar competitive assay in which BCPDA is linked to the anti-cortisol antibody via covalent linkages with the bovine serum albumin has also been described [302].

In 1989, Diamandis and co-workers developed a new time-resolved fluoroimmunoassay based on multiple-fluorescence labeling with BCPDA-europium chelates [303]. A total of 150 BCPDA molecules are incorporated into one thyroglobulin molecule (TG) by the reaction between the sulfonyl chloride groups of the chelate and the amino groups on the protein. In the immunoreaction, the biotinylated antibody is recognized by a streptavidin which is covalently coupled to the BCPDA labeled thyroglobulin (Fig. 31). Highly sensitive time-resolved heterogeneous fluoroimmunoassays of α-fetoprotein [303] and digoxin [304] in serum have been developed using such a multiple labeling strategy.

Furthermore, it has been found that incubation of the multiply BCPDA labeled streptavidin, SA-TG-$(BCPDA)_{150}$, [SA = streptavidin, TG = thyroglobulin,

BCPDA = 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylate] with BCPDA-labeled thyroglobulin, TG-$(BCPDA)_{150}$, in the presence of europium ions produces a macromolecular complex with a molecular weight of about 3×10^6 (Fig. 32) [305]. As the streptavidin is indirectly labeled with a large number of luminescent chelates, the detection sensitivity of the reagent shows a significant enhancement in different fluoroimmunoassays. The complex has also been used as a luminescent label to stain and quantify proteins blotted or spotted on nitrocellulose [306].

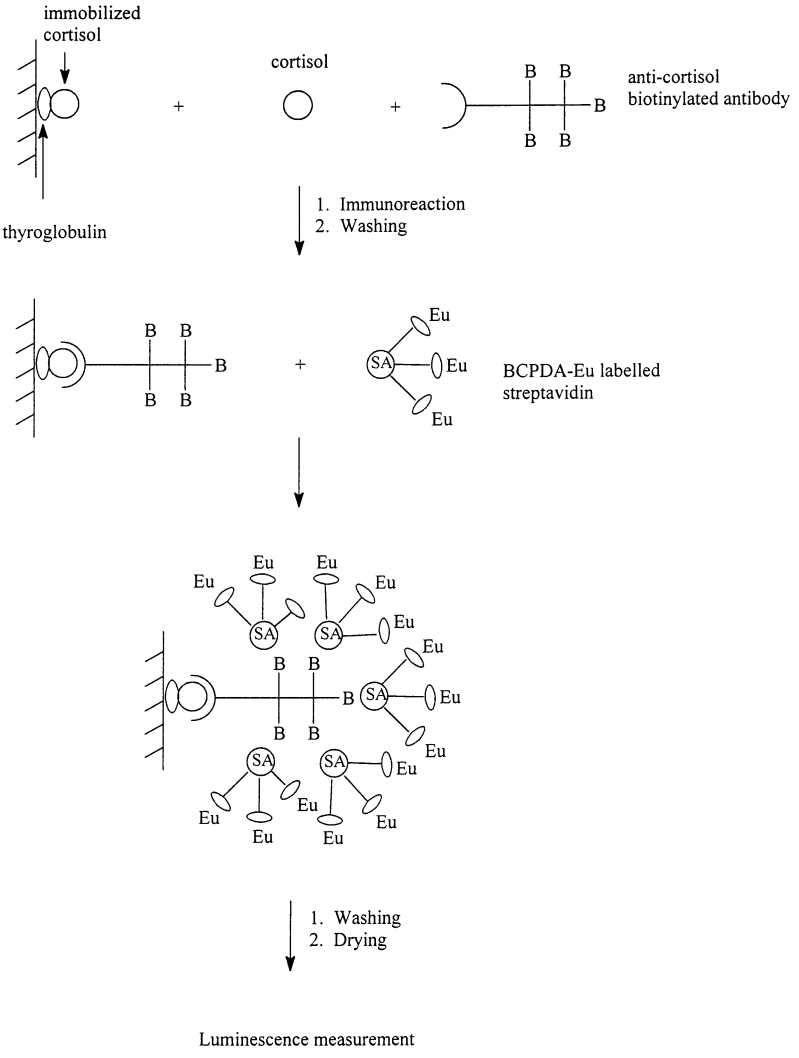


Fig. 30. A heterogeneous competitive fluoroimmunoassay of serum cortisol.

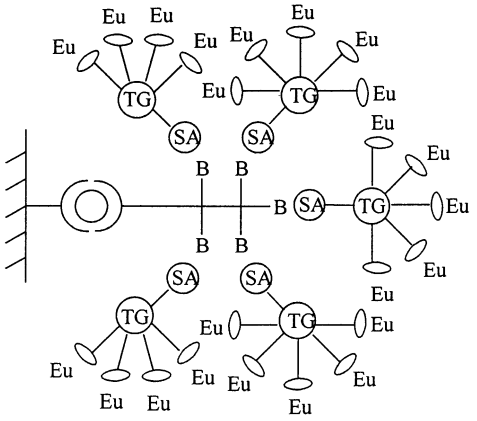


Fig. 31. Recognition of a biotinylated antibody by streptavidins multiply labeled with BCPDA-Eu via thyroglobulin.

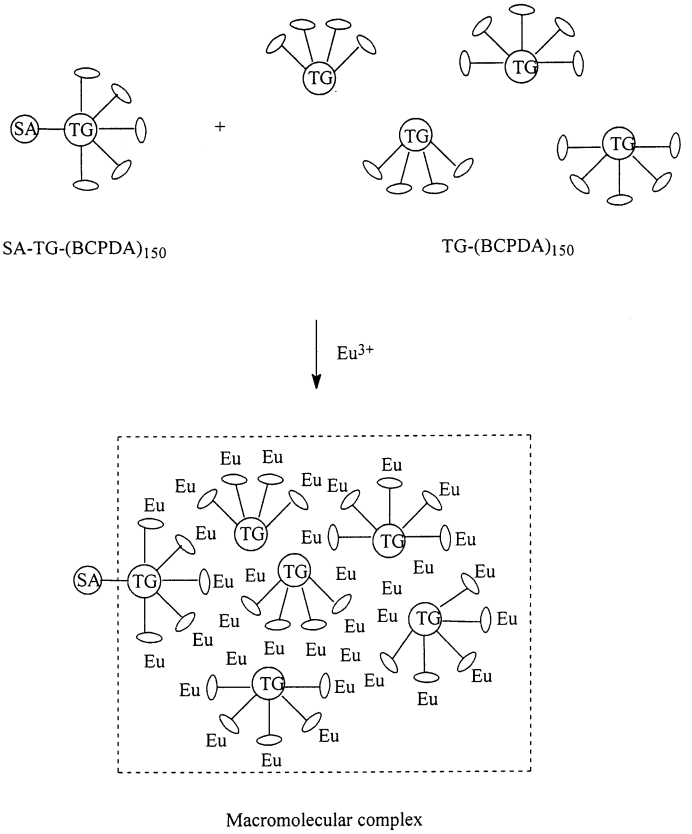
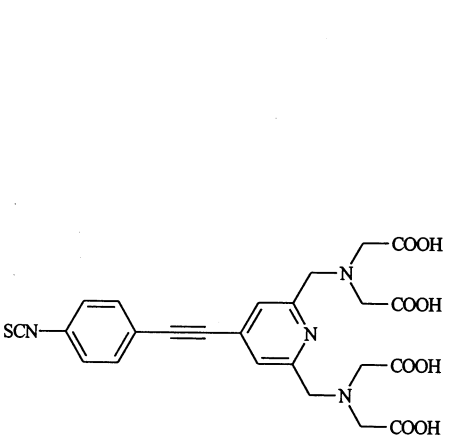


Fig. 32. Formation of a macromolecular complex which contains a streptavidin multiply labeled with BCPDA-Eu. Adapted from ref. [305].

Simultaneous measurement of two analytes by means of dual-label immunoassay has also been described recently [307–309]. For example, Lövgren and co-workers reported a dual-label one-step immunoassay of free and total prostate-specific antigen in serum [308]. The glycoprotein prostate-specific antigen (PSA) has been found to increase in prostate cancer patients and it is present in serum predominantly in complex with the serine protease inhibitor α_1-antichymotrypsin (ACT). The monoclonal antibody recognizing free PSA is labeled with a europium chelate of SCN-PEAP^{4-} {H_4SCN-PEAP = 4-[2-(4-isothiocyanatophenyl)ethynyl]-2,6-bis{[*N*,*N*-bis(carboxymethyl)amino]methyl}pyridine (**101**)} while the monoclonal antibody recognizing both the free and complexed form of PSA is labeled with a terbium chelate of SCN-PAAZ^{4-} {H_4SCN-PAAZ = 3-{6′-[*N*,*N*-bis(carboxymethyl)aminomethyl]-4′-(*p*-isothiocyanatobenzyl)-2′-pyridyl}-5-{6″-[*N*,*N*-bis(carboxymethyl)aminomethyl]-2″-pyridyl}-1,2,4-triazole (**102**)} [308].



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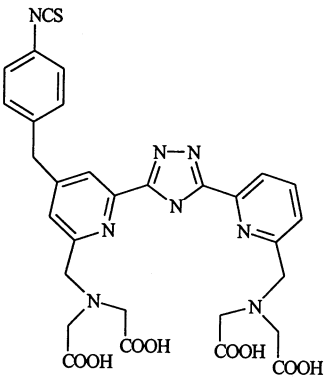

102

The recognition of the complexed and free forms of PSA is illustrated in Fig. 33. Capturing monoclonal antibodies recognizing both the free and complexed form of PSA are first immobilized on microtiter plates. The serum samples are then added to the plate, followed by europium-labeled and terbium-labeled monoclonal antibodies. After incubation and washings, a buffer solution which can release the bound antigens and labeled antibodies from the solid capturing antibodies is then added. The luminescence of the europium and terbium chelates is measured and hence the free and complexed forms of PSA can be assayed simultaneously.

On the other hand, in 1991, Evangelista and co-workers introduced the idea of enzyme-amplified lanthanide luminescence in bioanalytical assays [310]. Signal generation is performed by enzymatically transforming a substrate into a product

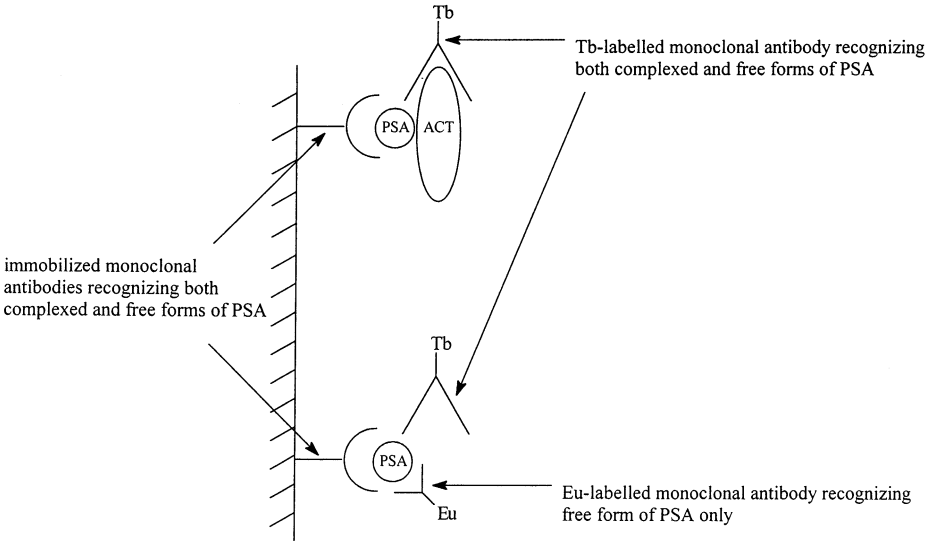

Fig. 33. Recognition of the complexed and free forms of PSA. Adapted from ref. [308].

which forms a strongly luminescent complex with lanthanide ions. For example, detection of the enzyme xanthine oxidase has been carried out by using the substrate salicylaldehyde. The enzyme catalyzes the oxidation of the substrate into salicylic acid (Fig. 34) which forms a highly luminescent complex with terbium–EDTA at high pH. Besides, the quantification of the enzyme β-galactosidase is possible by using the substrate salicyl-β-galactoside which is also converted to salicylic acid. On the other hand, it has been found that in the presence of hydrogen peroxide and light, 1,10-phenanthroline-2,9-dicarboxylic acid dihydrazide (PDAdh) is converted to 1,10-phenanthroline-2,9-dicarboxylic acid (H_2PDA) which forms a highly luminescent complex with europium at high pH (Fig. 35). The hydrogen

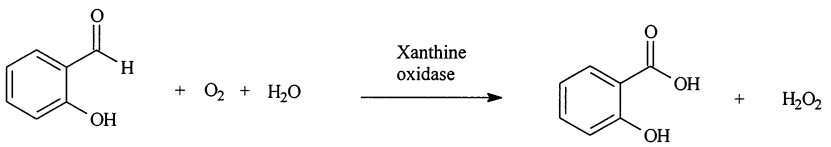

Fig. 34. Oxidation of salicylaldehyde to salicylic acid catalyzed by xanthine oxidase.

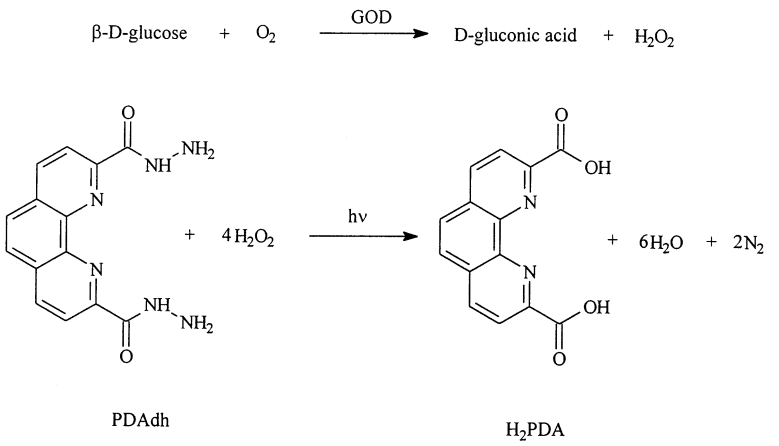

Fig. 35. Reaction of PDAdh and H_2O_2 to form H_2PDA.

peroxide can be produced from the catalytic oxidation of the substrate β-D-glucose by the enzyme glucose oxidase (GOD). Therefore, detection of the enzyme is possible by measuring the Eu-PDA luminescence which can only be observed when PDAdh, Eu^{3+}, β-D-glucose and GOD are present.

Besides, Diamandis and co-workers described an fluoroimmunoassay of α-feto-protein (AFP) (Fig. 36) in which the biotinylated antibody is recognized by streptavidin labeled with the enzyme alkaline phosphatase (ALP) [311]. The enzyme dephosphorylates the substrate 5-fluorosalicylic acid phosphate ester (FSAP) to produce 5-fluorosalicyclic acid (FSA). Terbium–EDTA forms a strongly emissive complex with the deprotonated form of FSA and the luminescence is then measured. Furthermore, the potential of different organic compounds as a substrate in enzyme-amplified fluoroimmunoassay has also been investigated [312–314]. It is interesting to note that the chelate 4-methylumbelliferyl phosphate (4-MUP), which forms a highly luminescent complex with europium ions, is converted by alkaline phosphatase into 4-methylumbelliferone (4-MU), which does not form luminescent complexes with europium ions (Fig. 37). This pair works in the opposite way to the previous FSAP/FSA example. Utilization of related substrates in the immunoassays for thyroid stimulating hormone and thyroxine in human serum has been described [312].

On the other hand, homogeneous immunoassays offer the advantage that immobilization and washing steps in heterogeneous counterparts are not necessary. Mathis and co-workers demonstrated homogeneous fluoroimmunoassays based on Förster's resonance energy transfer (FRET) principles. In an assay of prolactin in human sera, two monoclonal antibodies are labeled with the europium tris-bipyridine cryptate (Eu-TBP) donor and the phycobiliprotein allophycocyanin (APC) acceptor, respectively [315]. After the immunoreaction, the donor and acceptor are in close proximity to each other. Long-lived emission of APC as a result of the energy transfer from the lanthanide cryptate is observed (Fig. 38).

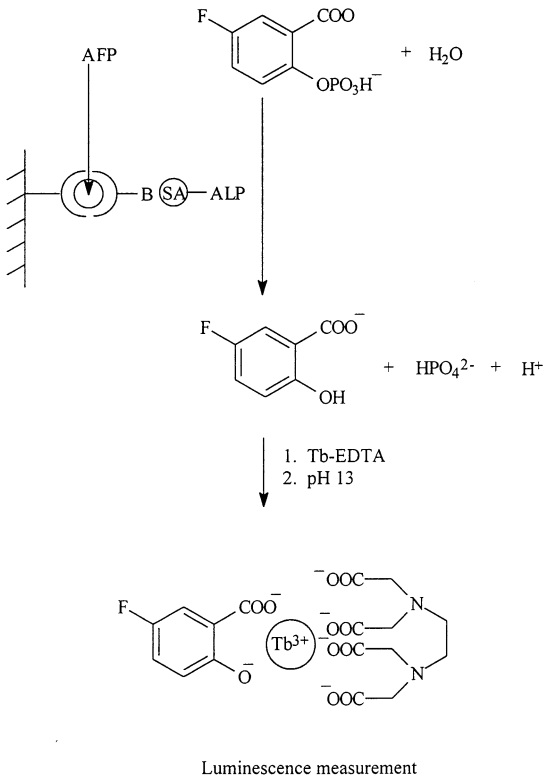

Fig. 36. Principle of Tb-chelate-based enzymatically amplified fluoroimmunoassay of α-fetoprotein (AFP). Adapted from ref. [311].

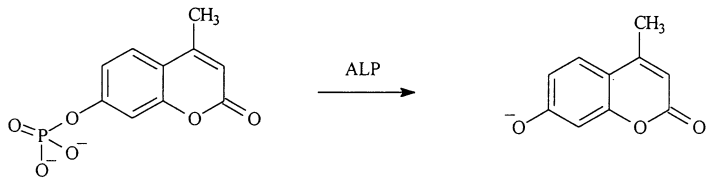

Fig. 37. Conversion of 4-methylumbelliferyl phosphate to 4-methylumbelliferone catalyzed by alkaline phosphatase (ALP).

5. Concluding remarks

In this review, it has been shown that transition metal and lanthanide complexes can be employed as a sensor in different areas such as ion and molecular recognition, nucleic acid probing and cleavage. The utilization of lanthanide complexes as chiral NMR shift reagents and magnetic resonance imaging contrast reagents have been discussed. Besides, the employment of luminescent lanthanide complexes as labels in different fluoroimmunoassays have also been introduced. It

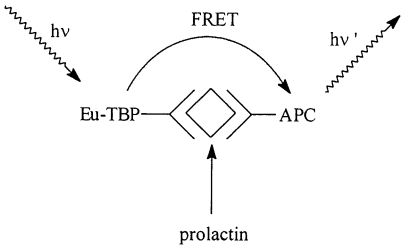

Fig. 38. Homogeneous fluoroimmunoassay of prolactin based on Forster's resonance energy transfer principles.

is anticipated that with the structural flexibility provided by these complexes, as well as their unique photophysical and electrochemical properties, they will continue to play a key role in various diagnostic areas.

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