

Co-ordination Chemistry with Macrocyclic Compounds

Química de Coordenação com Compostos Macrocíclicos

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A brief review from the origins of macrocycles to supramolecular chemistry was undertaken, emphasising the relevant aspects for the co-ordination chemistry and the most interesting applications. The author's work on N-acetate derivatives of macrocyclic compounds was summarised, and the analytical and medical applications of this type of ligands was stressed.

1. Macrocycles and Polymacrocycles

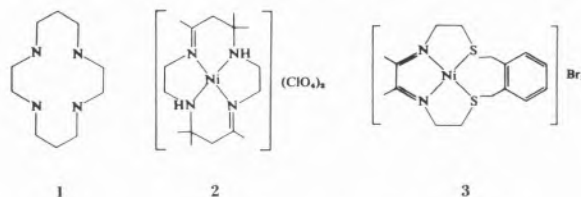
Macrocyclic compounds are synthetic or natural polydentate ligands, containing their donor atoms incorporated in a cyclic backbone or/and in substituents attached to it. They contain at least three donor atoms and the ring should have a minimum of nine atoms. The co-ordination chemistry of these compounds has now become a major subdivision of inorganic chemistry [1], while the active search for new types of macrocycles and the number of their applications has systematically increased since their discovery. Moreover, there is such a fascination for the macrocyclic compounds, that all of us who work with them become subdued by their intrinsic beauty and the unexpectedness of their structures.

The increase of data collected by Izatt *et al.* may give us an idea of the expanding scope of this field: in 1974, the 33 page article referred 197 macrocycles [2]; in 1985, the article consisted of 68 pages and 255 macrocycles are mentioned [3], and in 1991, the 364 pages of the article included the data determined for 1588 macrocycles [4]. It should be emphasised that Izatt *et al.* collected thermodynamic and kinetic data relative to macrocyclic interactions and did not mention all the macrocyclic compounds so far synthesised. Gokel and Korzeniowski report the syntheses of about 2100 macrocyclic compounds in their book published in 1982 [5]. Nowadays the future of this area still seems promising, as from the traditional Co-ordination to the Supramolecular Chemistry; it has enlarged our vision and imagination on the co-ordination chemistry field.

The earliest known examples of metal complexes of macrocyclic ligands were observed in natural sub-

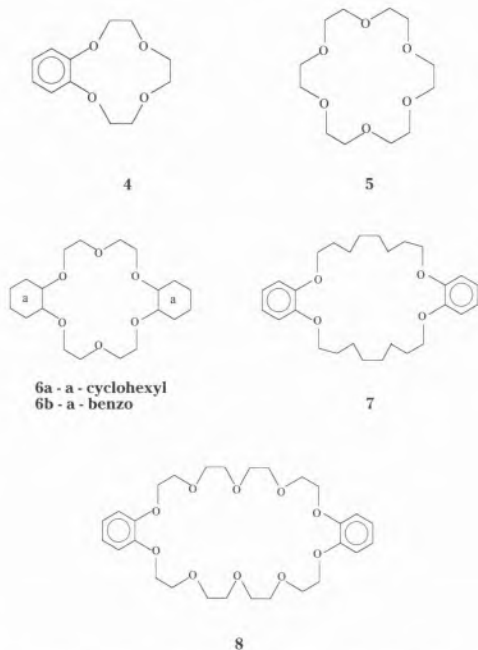
Neste artigo faz-se uma breve revisão dos compostos macrocíclicos desde a origem até à química supramolecular, salientando os aspectos significativos para a química de coordenação e as aplicações mais interessantes. Sumariza-se ainda o trabalho em que a autora tem estado envolvida no que respeita aos macrociclos com substituintes acetato nos átomos de azoto bem como as aplicações analíticas e médicas deste tipo de ligandos.

stances, such as the porphyrin ring of the iron-containing haem proteins, the related (partially reduced) chlorin complex of magnesium in chlorophyll, or the corrin ring of vitamin B₁₂ (a cobalt complex), and in some synthetic highly conjugated phthalocyanines, used mainly as dyestuffs or pigments [1,6,7]. *Cyclam* (1,4,8,11-tetraazacyclotetradecane, **1**), was obtained for the first time in 1936, as a by-product in very small yield [8], but its metal complexes were prepared and studied only in mid-1960. In the early sixties, Curtis [9] and Busch [10], working independently, synthesised some macrocyclic complexes containing nitrogen, **2**, and nitrogen and sulphur donor atoms, **3**, respectively, and new series were developed afterwards, although with restricted expansion. It was the publication of Pedersen's work on crown-ethers (macrocyclic-polyethers) in 1967, that truly opened a new era on macrocyclic chemistry [11].

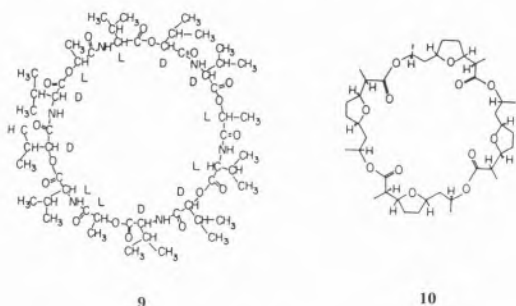


Pedersen reported syntheses of over 50 cyclic polyethers where the size of the rings, the number of oxygen atoms, and the number and type of substituent groups are varied. **4-8** are some examples. After that, a fast progress ensued in the following decades, not only for crown-ethers, but also for macrocycles containing all kind of donor atoms (mainly nitrogen, but also sulphur, phosphorus, and mixed donor atoms). New and more gene-

ral synthetic processes were reported for macrocyclic compounds containing nitrogen atoms, which strongly contributed for the interest in this field [12].



The discovery in 1964 that certain naturally occurring antibiotic ionophores such as valinomycin **9**, nonactin **10**, monensin, and others, exhibiting alkali specificity, were capable of active transport of metal ions across membranes, was a starting point for a large number of studies of alkali and alkaline-earth cations selectivity of biological and model systems. Unlike the synthetic macrocycles, these antibiotics exhibit special metal selectivity despite their very large size [7,13].



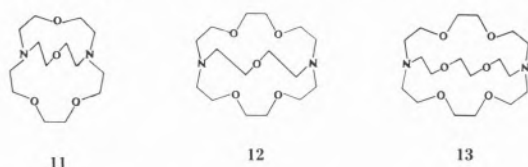
The intense interest on macrocyclic compounds was related to their unusual affinity for certain metal ions, their ability to bind selectively some cations in preference to others, the inertia to decomposition of some complexes, and the capacity of some of those ligands to solubilize inorganic salts in non-aqueous media [11]. Macrocyclic ligands typically contain central hydrophilic cavities with electronegative or electropositive binding atoms and an exterior flexible skele-

ton exhibiting a hydrophobic behaviour. Their hydrophobic exteriors allow them to solubilize ionic substances in non-aqueous solvents and in membrane media. The most spectacular fact, at that time, was the dissolution of potassium permanganate in benzene by an 18-crown-6, **5**, reported by Pedersen [11]. Particularly interesting is the strong affinity shown by the crown-ethers for alkali and alkaline-earth metal ions and for transition metal ions by polyazamacrocycles. The selective binding of some of their metal complexes allows them to be attractive for many applications, such as ion storage and transport *in vivo*, solvent extraction of metals, the development of metal-ion selective reagents for analytical applications or of new chromatographic materials for separation of metal ions. Macrocyclic compounds in general offer a unique way of carrying out many ionic reactions in non-aqueous solvents, as for example: oxidation of olefins to carboxylic acids in benzene or toluene at room temperature by complexed potassium permanganate with the cyclic polyether dicyclohexyl-18-crown-6, **6a**; more efficient saponification of esters by complexed potassium hydroxide with a crown-ether in toluene than by potassium hydroxide itself in propanol; or by phase transfer catalysis, which allows the development of reactions between reagents contained in two different phases [1,13-15].

In the first studies on the co-ordination chemistry of macrocyclic ligands, crown-ethers and tetraazamacrocycles, it was recognised that these ligands co-ordinate most strongly those metal ions whose ionic crystal radius best matches the size of the cavity formed by the ring upon complexation. This complementarity rule means that bond energies between cation and ligand will be greatest when all donor atoms can fully participate. In this case, the macrocyclic structure restricts ligand adaptation so that the macrocycle would exhibit a so-called *peak selectivity* for size [15]. If the macrocyclic ring is too large, the metal ion would "fall through" the cavity and not take full advantage of the available binding interactions, while if the ring is too small the metal ion would achieve only limited penetration or require some distortion of the ligand away from its equilibrium conformation with consequent energy-loss [1,13-15]. This behaviour is the basis of the *macrocyclic effect*, which can be quantified by the ratio between the stability constants of the complexes of the macrocyclic ligand over an analogous acyclic ligand bearing the same set of binding sites, with a given cation, in a given solvent, at a given temperature. However, the *hole-size selectivity rule* often does not hold [13,15]. Complementarity (size and shape) provides the minimal requirement for strong affinity [16]. Other effects are also important in determining selectivity and stability patterns. Differences in stability depend also upon the level of *preorganisation* of the ligand into its optimal binding conformations [15]. In Lehn's words "this level of preorganization is related to both the solvation state and the equilibrium solution conformation, which are

functions of each other and are difficult to predict" [15]. Or, in Busch's words, "an additional stereochemical contribution to the complexation affinity... is determined by topological and rigidity constraints. Topological and rigidity constraints are the design factors available for arbitrarily enhancing affinity" [16]. In general, when no problems of complementarity exist, the increase of topological and flexibility constraint leads to increases in binding affinities.

It follows from the above considerations that the introduction of other bridges on simple or mono-macrocycles should present certain advantages if more selective ligands were required. This point was soon confirmed by the work of Lehn *et al.* [17] through the synthesis of macrobicyclic polyethers containing three polyether strands joined by two bridgehead nitrogen atoms, forming a bicyclic ligand (**11-13** are some examples), a logical extension of crown-ether chemistry.



These compounds having three-dimensional cavities, which can accommodate metal ions of suitable size, were called *cryptands* and the corresponding complexes,

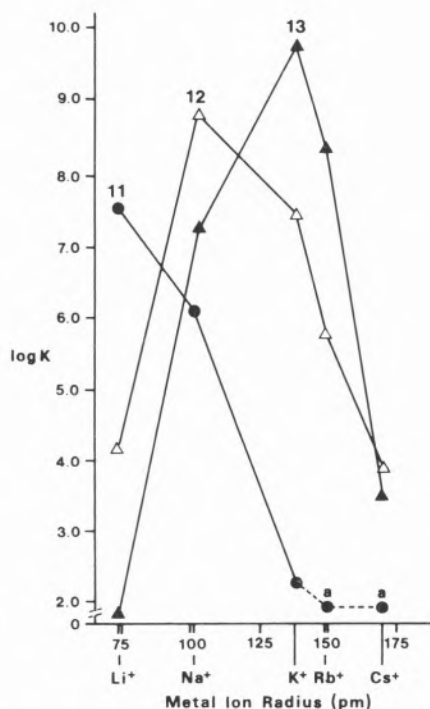
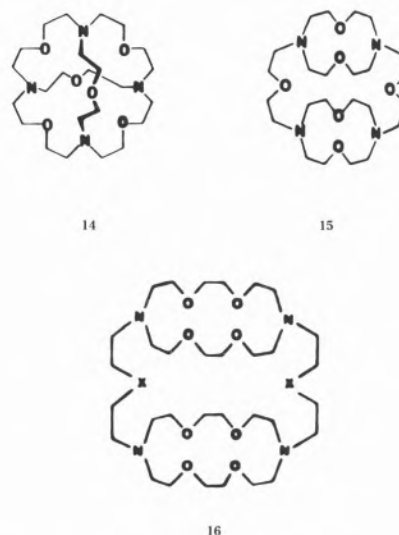


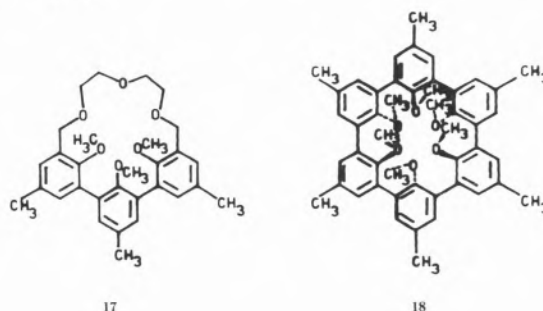
Figure 1. Selectivity of *cryptands*: stability constants ($\log K$) of some *cryptands* (**11-13**) with alkali metal ions *versus* cation radius. **a** - values reported to be less than 2.0.

cryptates. It was found that on proceeding across a series of these ligands of ever increasing size, each of the alkali metal ion is in turn preferentially bound, the cation located in each selectivity peak having an ionic radius very close to the ligand cavity size (Figure 1) [18].

Macrocyclic compounds containing two bridges, like **14**, would be expected to induce even more rigidity and to show enhanced peaks of selectivity and affinity for metal ions than the *criptand* series. This macrotricyclic presents a spherical cavity, though larger than would be necessary for alkaline metal ions. It will play however an important role in supramolecular chemistry, owing to special affinity for NH_4^+ , as will be shown in the following section. Other macrotricycles have been synthesised as well (**15** and **16**, are examples) which can bind more than one metal ion forming di- or polynuclear complexes, or be suitable receptors for molecular recognition.



An entirely different design of macrocyclic ligands has been developed [19] with the same objectives, namely to get rigid compounds: cavitands, spherands and carcerands, **17**, **18**. These ligands exhibit rigid cavities in tridimensional arrays approaching spherical geometry. For example, **18** has a small cavity and does bind strongly to lithium and sodium, among alkali metal ions [19].

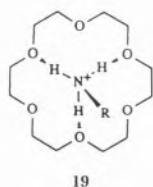


2. Supramolecular Chemistry

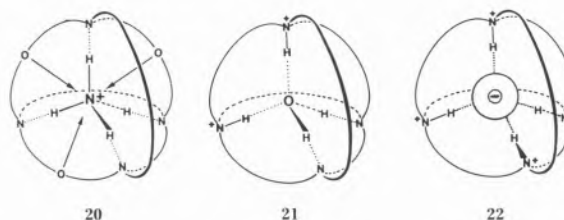
The association of two or more species by non-covalent bonds constituted what has been designated by *supramolecular chemistry* or *host-guest chemistry*, and extends the Fischer's "lock and key" concept from steric fit to other molecular properties [15,20, 21]. Among macrocycles there is a vast number of molecules that can act as *receptors* or *host* molecules for the complexation of inorganic or organic anions and cations or neutral molecules - *substrate* or *guest* molecule. Some of them are able to modify significantly the properties of the substrates upon complexation, as found in many natural systems like enzymes. The structures of the associates are governed by relatively weak forces such as ion-dipole and dipole-dipole interactions, hydrogen-bonds, van der Waals interactions, etc. [1,14-16,21-24]. The association is characterised by similar thermodynamic and kinetic constants as used in classical co-ordination chemistry. Supramolecular chemistry may be considered generalised co-ordination chemistry. It extends the classical field of metal co-ordination chemistry to all kinds of substrates or guests: cationic, anionic and neutral species of an inorganic, organic or biological nature [16,20,21]. For the "recognition" and binding of the receptor to a potential substrate, the two species must complement each other in size, shape and binding sites. This behaviour has been observed for many natural molecules, including enzymes and drugs and also for smaller natural products, such as cyclodextrins and antibiotics. Such selective complexation of ions is the basis for the extraction of ions or neutral molecules and, if accompanied by transport of the complex across a lipophilic membrane, provides the basis for ion-selective electrodes, which have been developed not only for metal ions but also for many other ionic species. If, in addition to the binding sites, the receptor bears reactive sites that may transform the bound substrate, it could be an interesting catalytic reagent. Therefore, the functional properties of such an association cover molecular recognition, catalysis and transport. Other areas of interest are model systems for enzymes, including the activation of small molecules like O_2 , CO , CO_2 and N_2 [1,14-16,21-24].

As it is not my goal in this paper to develop this theme, but only to mention this important and active field of the macrocyclic chemistry, I will summarise this point using some examples from the literature [1,14,15]:

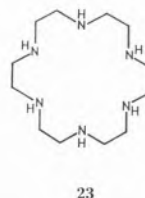
- The alternating oxygen atoms of an 18-membered crown-ether ring are appropriately spaced to form hydrogen bonds with each of the three N-H bonds of an ammonium ion, **19**.



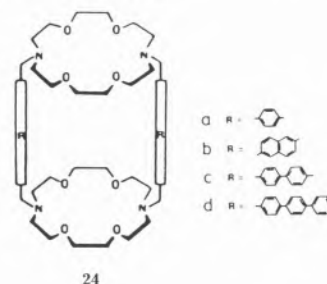
- The receptor **14** presents a spherical recognition site with an octahedron of oxygen sites superimposed on a tetrahedron of nitrogen sites in appropriate positions for NH_4^+ , **20**. The complementary between cavity size and the nature and arrangement of the co-ordination sites gives this ligand the highest affinity for NH_4^+ and among the alkali metal ions, for Rb^+ . The cavity of this macrocycle in its diprotonated form is also adequate for the complexation of a neutral molecule, like H_2O **21**, or of anions (halides) in its tetraprotonated form, **22**. A remarkable Cl/Br selectivity $>10^3$ was observed in this last case.



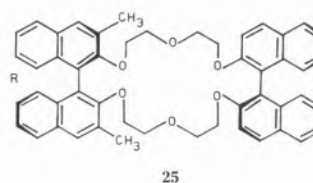
- The hexacyclen **23** complexes a large variety of anions, its triprotonated form co-ordinate polycarboxylate anions (citrate³⁻, succinate²⁻, malonate²⁻, etc.) and its tetraprotonated form complexes inorganic anions (Cl^- , NO_3^- , Br^- , ClO_4^- , IO_3^- , etc.).



- The architecture of the macrotricyclic **24** displays good length selectivity for linear diammonium $\dot{N}H_3 - (CH_2)_n - \dot{N}H_3$.

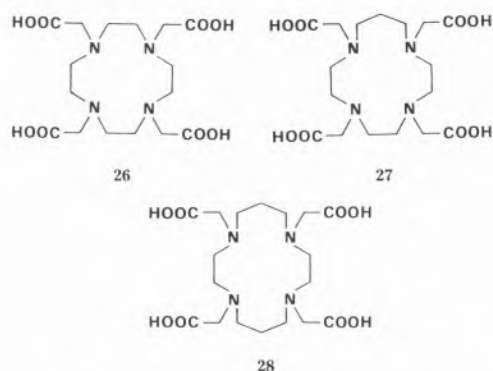


- Crown-ethers incorporating chiral binaphthyl units **25**, show high chiral discrimination on interaction with particular racemic amine salts.



3. Polyazamacrocycles with N-acetate Arms - Cyclic Complexones

My interests in the world of macrocyclic chemistry started with my PhD work when my supervisor - Prof. Fraústo da Silva - suggested me to repeat the work published three years earlier by Stetter and Frank [25]. Their work consisted on the synthesis and the stability constants determination of the metal complexes (alkaline-earth and divalent first-row transition metal ions) of N-acetate derivatives of three tetraazamacrocycles, DOTA **26**, TRITA **27** and TETA **28**. The astonishing though not unlikely fact for macrocyclic compounds



stated on the Stetter *et al.* very short paper was an inversion of the usual order of stability constants of Ca^{2+} and Sr^{2+} TRITA complexes. It was to be expected for the complexes of these metal ions, held together mainly by electrostatic interactions, that stability would follow the order of the ionic potential. The published values were 8.06 for the Ca^{2+} and 11.7 for the Sr^{2+} complexes, in log units, respectively [25]. If confirmed, this fact would be very important for the removal of radioactive ^{89}Sr and ^{90}Sr from the human body. Since ^{90}Sr is one of the most dangerous and abundant fission products, having a radioactive half-life of 28 years and a long biological half-life, it is readily assimilated into the body lodging in the bones. Nevertheless, although it were important to verify that these N-tetraacetate tetraazamacrocycles could exhibit specific selectivity for the alkaline-earth metal ions, we soon became aware that another type of macrocycles managed to invert the usual stability order of the complexes of these metal ions. In fact, the *cryptand*, **13**, presents the following stability constants for the complexes: Ca^{2+} , 4.4, Sr^{2+} , 8.0 and Ba^{2+} , 9.5 (values in log units, determined in aqueous solution at 25 °C and ionic strength 0.1 M) [26]. It is one of those cases where the hole-size selectivity rule does hold and certainly one fact that strongly contributed for the Nobel Prize awarded to Jean-Marie Lehn in 1987 (the prize was shared with two other big names of the macrocyclic field, C. J. Pedersen and D. J. Cram). Also, it was confirmed in experiments made with rats, that 80% of ^{85}Sr was

removed 72 hours after injection of the radioactive element using the *cryptant* **13**, while the remaining radioactivity was found in the skeleton [27].

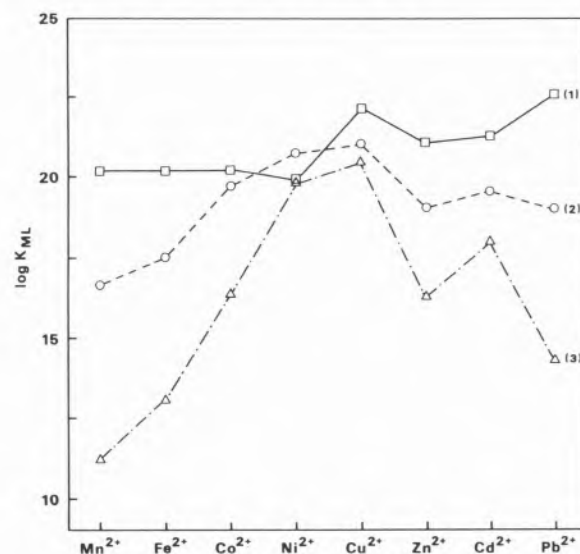


Figure 2. Stability constants of the metal complexes of N-tetraacetate tetraazamacrocycles (log K) along the first row transition metal ions, Cd^{2+} and Pb^{2+} : (1) DOTA; (2) TRITA and (3) TETA [28b].

In spite of our lack of confirmation of the most intriguing data included in the Stetter and Frank report, our work was not in vain. In fact, it allowed the determination of a series of more accurate thermodynamic data, namely, protonation constants [28a,b] and protonation sequence of the ligands [29], stability constants of metal complexes for a series of mono- and divalent metal ions (alkali and alkaline-earth, first-row transition metal ions, Cd^{2+} and Pb^{2+}) [28a,b], enthalpy changes for the metal complexations [30] and some structural studies in aqueous solution by EPR and NMR spectroscopic techniques [31,32], very useful for the later development of many analytical and medical applications.

The appending of arms on the macrocyclic compounds, specially when additional ligating groups are introduced such as acetates, can greatly modify the properties of the metal complexes of the macrocycles, namely the stability, the selectivity, the solubility, the kinetics of formation or dissociation of complexes and the reactivity. In fact, in the case of the DOTA-TETA metal complexes, all the acetate groups are located on the same side of the plane formed by the four nitrogen atoms of the ring, building up a kind of cage where the metal ions will be encapsulated. This cage is quite rigid for the macrocycle of small size, DOTA, and to fit inside, the metal ions must be first stripped off of their hydration shells, before co-ordinating to the nitrogen and carboxylate oxygen atoms of the macrocycle [28a,b, 30].

All these ligands lead to stable complexes with both alkaline-earth and transition metal ions and, also, with

lanthanide ions [33,34] and other trivalent metal ions, like Ga^{3+} , Fe^{3+} and In^{3+} [35], particularly DOTA which forms by far the most stable Ca^{2+} and lanthanide complexes. The stability of the complexes of the alkaline-earth ions decreases abruptly with the increase in the size of the tetraaza ring of the ligands, none being particularly favoured by the increase of the size of the cavity of the macrocycle. However, the complexes of the divalent transition metal ions have very close stabilities, which are not specially high when compared with linear complexes. The rigid cage of DOTA exhibits such a conformation that the size of the transition metal ion does not make much difference, which means that there is no selectivity for these metal ions, all the complexes having constants between *ca.* 20 (for Mn^{2+} or Fe^{2+}) and *ca.* 22 (for Cu^{2+} or Pb^{2+}), values in log units. The increased flexibility of TRITA and TETA allows a better discrimination of the metal ions, TETA having a behaviour almost as a non-cyclic ligand following the Irving-Williams series, *cf.* Figure 2. These observations were interpreted and later confirmed by NMR studies in solution [32,36-38] and by some crystal X-ray structure determinations [38-44], as arising from the co-ordination of all the donor atoms in the complexes of the alkaline-earth metal ions, while not all the donor atoms of the macrocycle are involved in the complexes of the transition metal ions.

Crystal structural data for alkaline-earth metal complexes are not available, nevertheless the related structures involving lanthanide ions should give transferable information, as lanthanide ions also form complexes through mainly non-directional electrostatic interactions, while the ionic radius of Ca^{2+} is of the same order of magnitude of that of some trivalent lanthanides. The X-ray structure of the Eu^{3+} complex of DOTA, $\text{Na}[\text{Eu}(\text{DOTA})(\text{H}_2\text{O})]\cdot 4\text{H}_2\text{O}$ [39] shows a nine co-ordinate metal ion linked to the four nitrogens of the macrocycle, and its four carboxylate oxygens atoms, as well as to one water molecule. The co-ordination polyhedron is a distorted capped square antiprism, Figure 3. The metal ion lies between the two planes of the four nitrogen and the four oxygens, which are nearly parallel to each other. NMR studies in solution were in good agreement with the structure, suggesting that the lantha-

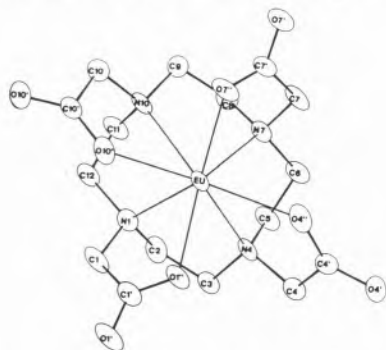


Figure 3. Crystal X-ray structure of $\text{Na}[\text{Eu}(\text{DOTA})(\text{H}_2\text{O})]\cdot 4\text{H}_2\text{O}$ [39].

nide DOTA complexes are unusually rigid. The crystal structure analysis of the Tb^{3+} complex of TETA, $\text{Na}[\text{Tb}(\text{TETA})]\cdot 6\text{H}_2\text{O} \cdot 1/2\text{NaCl}$ [40], has shown that this ligand also wraps itself around the lanthanide ion but in a different manner than that described for the DOTA complex. The TETA ring is more flexible and can wrap in a more effective, although more distorted way. The Tb^{3+} ion is eight co-ordinated, being also linked to the four nitrogen atoms and the four carboxylate oxygen atoms of the ligand, and the co-ordination polyhedron in this case is a severely distorted dodecahedron, Figure 4.

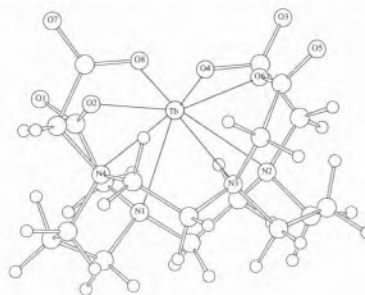


Figure 4. Crystal X-ray structure of $\text{Na}[\text{Tb}(\text{TETA})]\cdot 6\text{H}_2\text{O} \cdot 1/2\text{NaCl}$ [40, 88].

X-ray structures of $[\text{Ni}(\text{H}_2\text{DOTA})]$ and $[\text{Cu}(\text{H}_2\text{DOTA})]$ [38] have shown that the four N-atoms of the macrocycle and two of the carboxylate oxygen atoms are bound to the metal ion, whereas the other two carboxylates are protonated and not involved in the co-ordination. Two nitrogen and two oxygen atoms define a plane in which the metal ions is located. The other two nitrogen atoms of the ring are in axial positions. A *cis*-octahedral geometry is observed, the macrocycle being folded along the axis passing through two opposed nitrogen atoms and takes a *trans-I* conformation according to the nomenclature of Bosnich [45], Figure 5. The Cu^{2+}

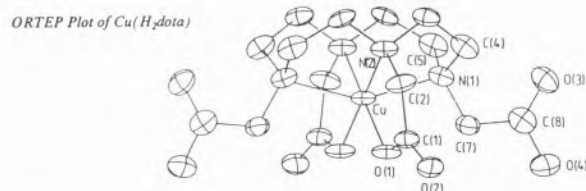
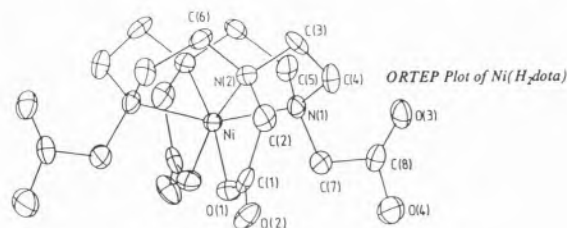


Figure 5. Crystal X-ray structures of $[\text{Ni}(\text{H}_2\text{DOTA})]$ and $[\text{Cu}(\text{H}_2\text{DOTA})]$ [38].

complex is more distorted than the Ni^{2+} one, which is only slightly distorted. Studies in solution carried out by Kaden et al. [38] have also shown that the co-ordination geometry for $[\text{Cu}(\text{DOTA})]^{2-}$ or $[\text{Ni}(\text{DOTA})]^{2-}$ are very similar to those of the corresponding diprotonated species. The structure of a $[\text{Cu}(\text{TETA})]^{2-}$ (Figure 6) complex has also shown two

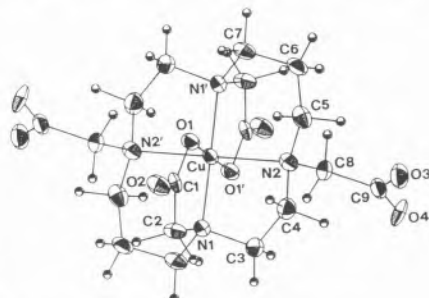
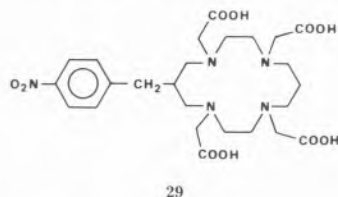


Figure 6. Crystal X-ray structure of $\text{Ba}[\text{Cu}(\text{TETA})]$ [44].

non-co-ordinated acetate groups: the Cu^{2+} has a distorted octahedral environment with the four amino nitrogen atoms in a plane and two apical acetate oxygen donors [44]; the Cu atom is situated exactly in the N_4 plane of the macrocycle, which thus takes a *trans*-III configuration, according to the Bosnich nomenclature [45]. In this case the 14-membered ring is able to encompass the metal ion and does not need to fold as happens with the 12-membered ring. However, this structure is in contrast with that of a Cu^{2+} complex of a C-substituted analogue of TETA **29** [43] in which the metal ion is situated in a N_2O_2 plane, and the two nitrogen atoms linked to the two non-co-ordinated acetate groups are in axial positions.



DOTA and TETA also form binuclear complexes with transition metal ions, M_2L , whose stability constants have been determined [28b] and crystal structures of some Cu and Ni complexes have been determined [41,42,43], but their description is not essential for the continuation of this discussion.

4. Applications of N-tetraacetate tetraazamacrocyclic ligands

Analytical applications

The most interesting applications of those macrocyclic compounds are found in analytical and medical fields. The selectivity shown by TETA for some alkaline-earth metal ions, owing to the very low stabi-

ties of the Mg^{2+} and Ba^{2+} complexes, allows the use of this ligand for the semimicro determination of calcium in the presence of other alkaline-earth metals in natural and synthetic water samples; the end-point detection can be achieved amperometrically with $\text{Zn}(\text{en})_3^{2+}$ as indicator. When strontium is present in concentrations similar to that of calcium, two distinct end-point are obtained, but, if the concentrations are substantially different, calcium and strontium appear to be titrated together [46] (constants are 1.97 for Mg^{2+} , 8.32 for Ca^{2+} , 5.73 for Sr^{2+} and 3.85 for Ba^{2+} , in log units [28a]). DOTA will be an interesting alternative to EGTA for the simultaneous potentiometric direct titration of calcium and magnesium [28a]. The very high molar absorptivity of the Cu^{2+} complex of TRITA, the stability of the colour of the complex and the wide range of pH at which the complex formation is completed make this ligand a convenient reagent for the fast and easy spectrophotometric determination of copper in metal alloys, without interference of nickel and cobalt [31].

Medical applications

More interesting, however, has been the use of those ligands in medical and pharmaceutical applications. In fact, in contrast with the analytical ones, the medical applications only require small amounts of ligands, which is more convenient, considering the long and tedious synthesis of these macrocycles. They are used, in the metal complexes form, as contrast-enhancing agents in nuclear magnetic resonance imaging (MRI), and as radiopharmaceuticals for diagnostic or therapeutic uses.

Lanthanides or radionuclides used in those experiments are toxic elements, so for safety reasons they should preferably be administered intravenously as a co-ordination complex and its premature release in the body should be prevented, as it leads to their accumulation in the liver, bone, and bone marrow. The biodistribution of the complex will principally be determined by its shape, charge, lipophilicity and redox properties. The most important condition for this kind of experiments is the stability of the complex *in vivo* and its integrity in the biological medium. Some of the properties requested for the complexes are:

- 1) They should have high stability constants, because the complex may be administered in very diluted concentrations if radiopharmaceuticals are used (about 10^{-10} M or less) and should not dissociate or transfer to thermodynamic competing ligands present in biological media, such as albumin or transferrin. Serum proteins will be present in great stoichiometric excess over the artificial ligand, so metal ions can be lost by mass action effects alone; also metal ions present *in vivo*, like Fe^{3+} , can compete with the radioactive metal for the ligand.
- 2) Kinetic inertia or very slow dissociation, and resistance to acid and cation promoted dissociation *in*

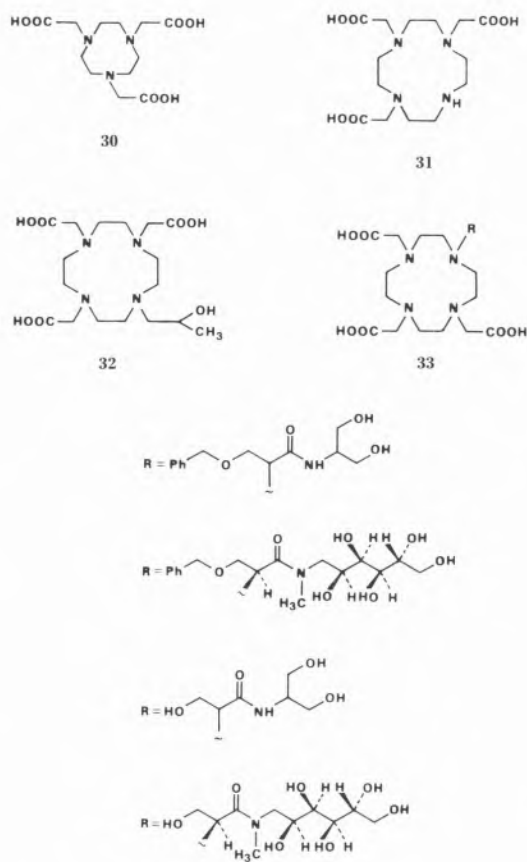
vivo, particularly important as the complex need to cross low pH regions, such as the stomach and the liver, and some cations like, Ca^{2+} and Zn^{2+} , exist in relatively high concentrations.

- 3) Fast rate formation of the complex when radionuclides of short half-life are used.
- 4) Other important conditions are: the oxidation state of the metal in the complex should be stable to resist to electron transfer; the complex should be neutral for best passive diffusion into cells and a combination of charge neutrality, sufficient lipophilicity and low molecular weight is desired, if the complex needs to penetrate the blood-brain barrier, or even the nuclear membrane of a cell [47-50].

MRI contrast agents

In recent years MRI has been recognised as a powerful diagnostic tool in clinical practices. It is a tomographic technique giving three-dimensional images in the form of slices of tissues [50] and relies on detecting the spatially localised NMR signals of water protons. The signal intensity of these protons depends on many factors such as the values of the water relaxation times, T_1 and T_2 and the type of pulse sequence. Paramagnetic molecules catalyse the proton relaxation of aqueous solutions in which they are dissolved. The relaxation time of the water molecules decreases of a factor of the order of 10^6 when the water oxygen atom is co-ordinated to a highly paramagnetic ion. This in turn perturbs the signal intensity: decreases in T_1 enhance the signal intensity, while decreases in T_2 decrease the signal intensity [49,51]. Contrast agents, which provide contrast between diseased and normal tissues, are usually paramagnetic metal ions complexed with a chelating substance, which are capable of enhancing the signal intensity of NMR images. It is necessary that it efficiently reduces the water proton relaxation rate or enhances the relaxivity (the increase of the water proton relaxation rate per unit concentration of paramagnetic contrast agent) of the water protons compared to the relaxivity induced in such water protons by the paramagnetic substance alone, free in solution. Complexes of Gd^{3+} are the most used owing to the largest magnetic moment, with seven unpaired electrons, and long electron spin relaxation times of this lanthanide. Some contrast agents can bind non-covalently to plasma proteins (*eg.* human serum albumin) through van der Waals interactions, by hydrophobic domains, or by electrostatic interactions [51].

The first compound used as a contrast agent in MRI was $[\text{Gd}(\text{DTPA})]^{2-}$, but soon the higher thermodynamic and kinetic stability of $[\text{Gd}(\text{DOTA})]^{-}$ was currently preferred. Macrocyclic ligands, such as DOTA and TETA, have the great advantage of forming stable complexes, but their slow complexation rates constitute a serious setback in their use as contrast agent. All the complexes mentioned are water soluble and stable, but they are charged complexes and their solutions have



high osmolality when injected intravenously, so some other ligands which could form neutral complexes were developed: NOTA, **30**, or NOTA derivatives [52-56] or DOTA and its derivatives as **31 - 33** [56-61]. In general, the search for new contrast agents is directed to functionalized derivatives of macrocycles, generally DOTA or NOTA, supposing that the stability constants of Gd^{3+} complexes are not altered in comparison to that of the parent macrocycles.

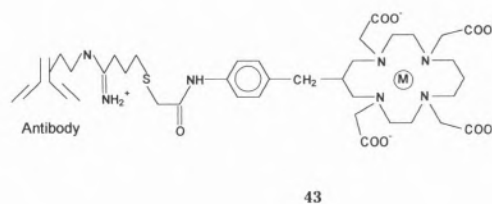
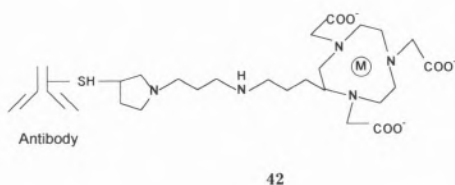
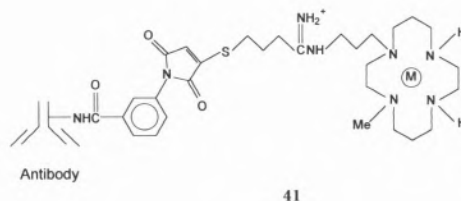
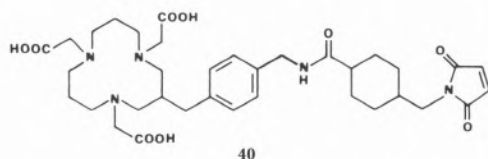
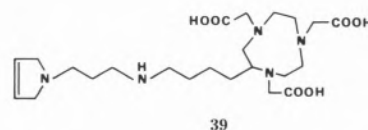
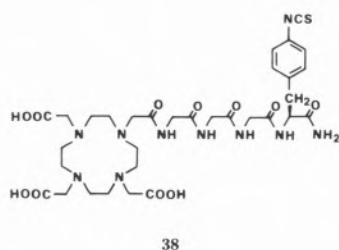
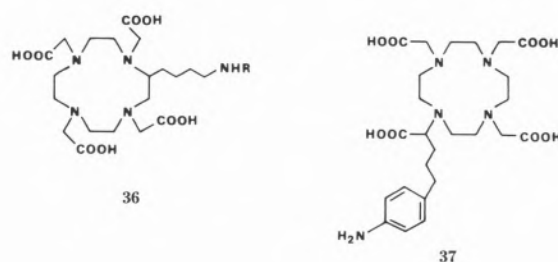
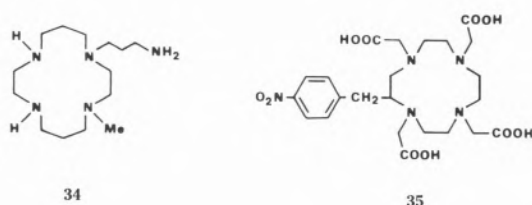
Nuclear medicine - radiopharmaceuticals

The radioisotopes used in nuclear medicine vary according to their purpose: the diagnosis (radioimmunosintigraphy) or the therapy (radioimmunotherapy). In diagnosis, the location of the radionuclide in the body is detected by extremely sensitive devices, available today, for which low radiation doses are needed. But if the therapy is the aim, a high dose of radiation is necessary, and consequently highly selective agents are needed which rapidly bind at the target site, and quickly clear from the body if unabsorbed. Isotopes used for imaging should emit only penetrating radiation (γ or β^+) of a single energy (with energies greater than 80 KeV). The half-lives must be sufficiently long to synthesise the radiopharmaceutical, inject the drug into the patient, and get images. Although $^{99\text{m}}\text{Tc}$ (γ emitter; $t_{1/2}$ 6.02 h; 141 keV) is the most widely used radioisotope in diagnostic,

it has a very short half-life which limits its use; best candidates are ^{111}In (γ emitter; $t_{1/2}$ 2.83 d; 171, 247 keV), ^{67}Ga (γ emitter; $t_{1/2}$ 3.25 d; 184 keV) and ^{64}Cu ($t_{1/2}$ 12.8 h; 511 keV). Radioisotopes used for therapy should emit only non-penetrating radiations (β , α , Auger e^-) to deliver a radiation dose sufficient to cause cell death through multiple double-strand cleavage of cellular DNA and usually have half-life in the range of 1-10 days to permit transportation to the tumour site. The best candidates are β^- emitting radioisotopes: ^{161}Tb ($t_{1/2}$ 166 h; 0.45-0.58 MeV) has good nuclear properties but is difficult to obtain; ^{67}Cu ($t_{1/2}$ 52 h; 0.40-0.58 MeV) with lower β^- energy may be suited to the elimination of small metastases or leukemias but is very expensive, and the best choice seems to be ^{90}Y (β^- ; $t_{1/2}$ 64 h; 2.25 MeV) [47,48].

The majority of the radiopharmaceutical applications requires the attachment of the radioactive metal ions to a ligand by complexation to control biodistribution. The first ligands used were naturally occurring molecules which exhibited specific *in vivo* properties, like cyanocobalamin. Nowadays, the most promising vehicles for

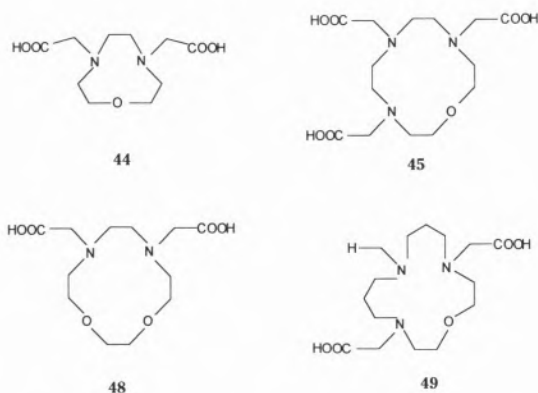
tumour therapy are conjugates or bifunctional ligands, which are expanding fast, since advances in genetic engineering have made possible not only to generate antibodies for specific antigens, but also to allow the incorporation of additional amino acids or amino acids sequences into the antibodies. The complex carrying the radioisotope is linked directly to the lysine residues of the antibody by simple reactions [47,48,50]. Until the middle-eighties the emphasis had been on the synthesis of C-functionalized derivatives of EDTA and DTPA, but their anionic complexes were not sufficiently stable at low pH or in the presence of serum cations to allow their successful use. More recently, functionalized macrocyclic ligands have been used, owing to their metal binding properties, forming more stable antibody conjugates. Some of those considered are derivatives bearing a C-substituted functional group or having one of the N-acetate groups modified for antibody attachment, such as derivatives of tetraazamacrocycles **34** [62], of DOTA **35-38** [63-70], of TETA **29** [65, 71-74], and of NOTA **39**, or other triazamacrocyclic derivatives **40** [66, 75,76]. **41**



[62], **42** [72,73] and **43** [75] are examples of some conjugate complexes. Recently, experimental therapy in tumour-bearing animals with a ^{90}Y , ^{212}Bi and ^{67}Cu labelled conjugate, by D. Parker *et al.* [64,68], O Gansow *et al.* [77,78] and DeNardo *et al.* [72,73] have obtained promising results for immunotherapy.

5. Present and future research

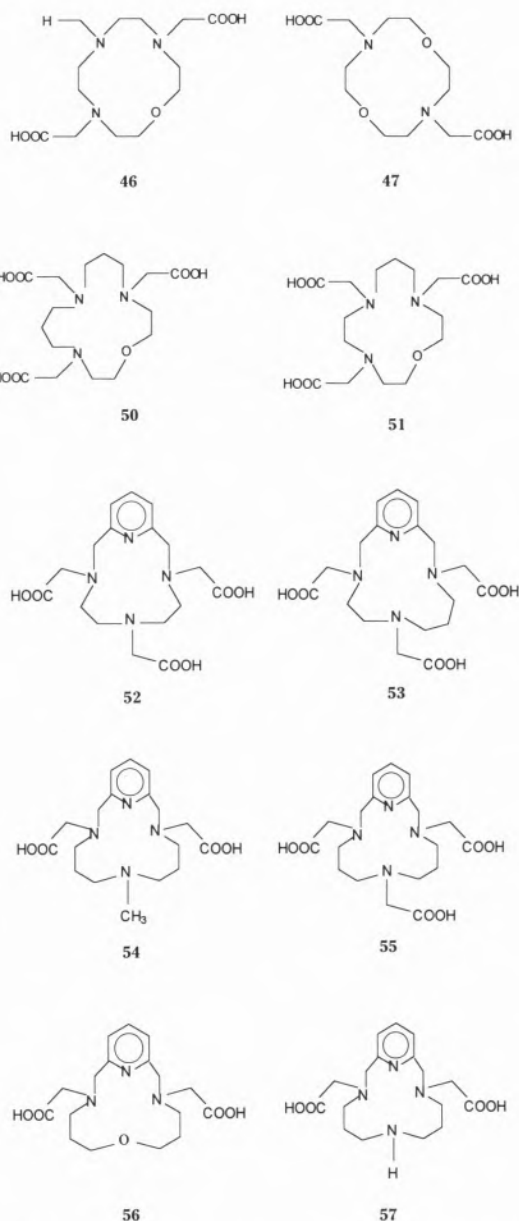
As mentioned several times in the precedent section, the principal drawback for the use of DOTA-TETA ligands and their derivatives in nuclear medicine is the slow complexation rates of their metal complexes. Stability constants of those complexes are in general higher than necessary for those applications. So the logic pursuit of new ligands having faster kinetics of formation of complexes led us to synthesise two new series: the first one, by the replacement of one or more of the nitrogen atoms of the tetraazamacrocyclic series by oxygen atoms, **44-51**; the second one, by the replacement of one of the nitrogen atoms of the tetraazamacrocyclic series by a pyridine ring, **52-57**.



The studies with the first ligand series are almost finished and, as a conclusion, we can say that there is a considerable decrease in the stabilities of the metal complexes formed as each nitrogen is replaced by one oxygen atom, as expected, and there are faster reaction kinetics; nevertheless, the gain in kinetics, which is not really exciting, does not compensate for the decrease in stability of the majority of the complexes [79-82].

The studies of the other series containing a pyridine in the macrocycle, are still in progress, but the results are more promising. All the ligands have been synthesised, but stability constants are not yet completely determined [83]. Other N-substituted groups were introduced, such as methyl and methylpyridine groups, to test the effect on complexation [84].

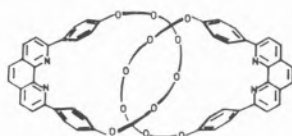
In general, the established conclusions for similar linear amines, like complexones EDTA, DTPA and so on, should not bear on macrocyclic compounds due the many constraints imposed by the macrocycle on



complexation. In fact, stability constants data of macrocyclic complexes are difficult to interpret without the support of structural data. So, in the last years, not only X-ray diffraction studies have been carried out [82,85] or are in course of publication [86], but also several spectroscopic studies in solution have been made using Uv-vis-near IR, EPR and NMR techniques [82,83,85]. Such a small research group has no possibilities to pursue enough studies in order that generalisations could be made, so lately some theoretical studies including molecular mechanics calculations came to our help too [86].

This chemistry field is so marvellous, that in general anyone who is introduced to it does not give it up. It is also so vast, if natural macrocycles and supramolecu-

lar chemistry are included, that we can move on it for ever! The aim would be fundamental co-ordination chemistry, practical applications, or the simple artistic pleasure of designing beautiful molecular architectures. Sauvage *et al.* [87] have written "The search for aesthetically attractive molecules has been a concern going back to the origin of chemistry. The criteria for beauty have obviously changed with time, being connected to analytical and synthetic tools. The interest in catenanes (58) and related systems originates to a large extent in their aesthetic appeal. In this respect, graphic arts and chemistry find a link".



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Note - Names of linear ligands abbreviated in the this text:

- EGTA - Ethylenegbis (oxyethylenenitrilo) tetraacetic acid;
- DTPA - Diethylenetrinitripentaaetic acid;
- EDTA - Ethylenedinitritettraacetic acid.

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