CHAPTER 5.2

# *The Future of Biomedical Imaging: Synthesis and Chemical Properties of the DTPA and DOTA Derivative Ligands and Their Complexes*

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## 5.2.1 Introduction

The open-chain DTPA, the macrocyclic DOTA, and their derivatives are universal chelating agents, which form high stability complexes with a variety of metal ions. Over the past two decades a number of metal chelates, formed with these ligands, have become highly important in different fields of medical diagnosis and therapy. The metal ions of interest include first of all the lanthanide$^{III}$, Sc$^{III}$, Y$^{III}$, Ga$^{III}$ and In$^{III}$ ions. The biomedical use of these metal chelates is based on the special magnetic, optical or nuclear properties of the metal ions and the most important applications include magnetic resonance imaging (MRI) contrast agents, diagnostic and therapeutic radiopharmaceuticals, and optical imaging probes. Metal ions can be used in biological systems only in the form of stable complexes that practically do not dissociate in the body because both the free metal ions and ligands are toxic and the $M^{3+}(aq)$ ions readily hydrolyze at physiological pH. The M(DTPA) or M(DOTA) chelates, which keep at least partly the special properties of the $M^{3+}$ ions, must meet stringent requirements, of which high thermodynamic stability and kinetic inertness are the most important for all fields of application.

In MRI investigations the most frequently used contrast agents are the $Gd^{3+}$ complexes (Scheme 5.2.1).[1,2] The intensity of the MRI signal depends on the proton density in the body (mainly water protons), and on the longitudinal and transverse relaxation rates of protons ($1/T_1$ and $1/T_2$) which may differ in healthy and diseased tissues, leading to differences in the image contrast. This difference can be increased with the use of $Gd^{3+}$ complexes as contrast enhancing agents (CA), which are administered into the body by i.v. injection. The seven unpaired electrons of the $Gd^{3+}$ have a relatively long electronic relaxation time and their fluctuating magnetic fields increase the nuclear relaxation rates of the surrounding protons. In the $Gd^{3+}$ chelates used as CA, all the donor atoms of the octadentate ligands are coordinated. The ninth coordination site of $Gd^{3+}$ is occupied by a water molecule. The protons of this water molecule relax rapidly, and *via* the fast exchange of this inner sphere water with the bulk the relaxation effect of $Gd^{3+}$ is transferred to the surrounding molecules. For expressing the relaxation effect of CAs, the term relaxivity is used. The relaxivity ($1/T_{1,2}$, $mM^{-1}\ s^{-1}$) is the increase in the proton relaxation rate, when the concentration of the CA increases by 1 mM. All small molecular weight CAs distribute in the extracellular and intravascular space of the body in about 20–30 min after injection and the hydrophilic complexes are rapidly excreted through the kidneys ($t_{1/2} \approx 1.5$ h). The lipophilic chelates are eliminated partly through the liver and bile. Recent research of CAs is focused on the development of tissue specific agents and the so-called "smart" or responsive CAs, which can be used for the *in vivo* determination of metabolites, endogenous ligands and metal ions, pH, enzyme activity, *etc.*[1–3]

The ligands DTPA, DOTA, and their derivatives are widely used in nuclear medicine for complexation of radiometals. Although the most important diagnostic radioisotope is the $^{99m}$Tc, more recently chelates of several other metals, such as $^{47}$Sc, $^{67}$Cu, $^{67}$Ga and $^{111}$In are also used in SPECT (Single-Photon Emission Computed Tomogaraphy).[4] The other important diagnostic modality is PET (Positron Emission Tomography), where mainly $^{11}$C and $^{18}$F isotopes are used, but due to the availability of the $^{68}$Ge/$^{68}$Ga and $^{44}$Ti/$^{44}$Sc generators, there is an increasing interest in the use of $^{68}$Ga and $^{44}$Sc.[5,6] Several $\beta^-$-emitting radiometals ($^{47}$Sc, $^{90}$Y, $^{153}$Sm, $^{166}$Ho, $^{177}$Lu) are used in radiotherapy for the local treatment of cancer.[7–10] The metal ions are generally complexed with DTPA and DOTA derivative bifunctional ligands (BFCs), attached to monoclonal antibodies or smaller peptides, which deliver the radiometal to the tumor cells. Similar targeted radiopharmaceuticals, prepared with $\gamma$- or positron emitting isotopes (*e.g.* $^{67}$Ga, $^{68}$Ga, $^{67}$Cu, $^{68}$Cu, $^{88}$Y, $^{99m}$Tc, $^{111}$In), are used for tumor imaging.[7–9]

The use of $Ln^{3+}$ ions for optical imaging is based on their unique luminescence properties, characterized by the well-separated emission and absorption

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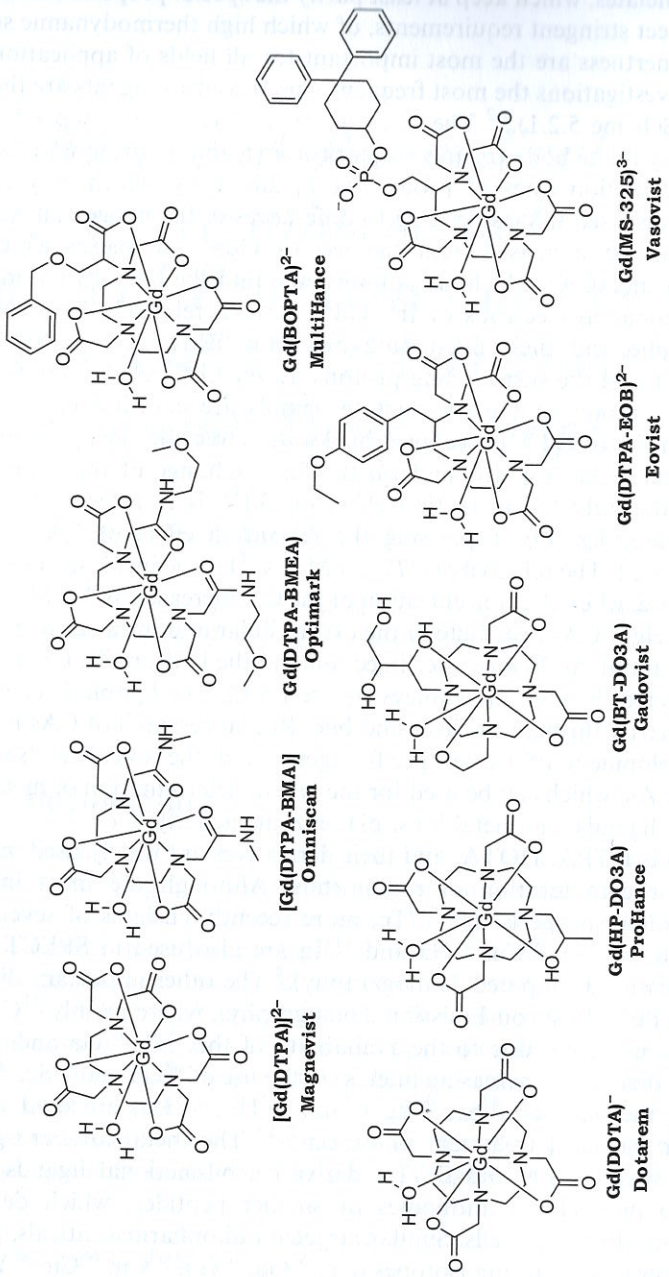



**Scheme 5.2.1** Clinically used $Gd^{3+}$ containing contrast agents.

bands, the long excited-state life-times (a few milliseconds for the $Eu^{3+}$ and $Tb^{3+}$), which allow the use of time-resolved spectroscopy and microscopy. The application of time-delay prior to detection of the emitted light eliminates the interference from the tissue fluorescence. This technique is used for immuno assays, where the detection limit is $10^{-12} - 10^{-15}$ M. For the excitation of $Ln^{3+}$ ions sensitizing chromophores are used (pyridine, bipyridine, terpyridine, triphenylene, quinoline, substituted phenyl and naphthyl, *etc.*) which are capable of transferring their excited state energies to the $Ln^{3+}$ ions. Since the interaction between the chromophores and $Ln^{3+}$ ions is generally weak, the $Ln^{3+}$ ions are bound to the chromophores in the form of chelate complexes. The chelating agents are generally DOTA derivatives, which form highly stable, inert complexes with the $Ln^{3+}$ ions. The optical probes, consisting of $Ln^{3+}$ complexes and sensitizing chromophore, have been attached to cell penetrating peptides for live cell imaging. The structure of the chelating agents has also been modified to generate sensors for bioactive ions.[11–14]

Since in all fields of application listed above, the chelating agents used for metal complexation are DTPA and DOTA derivatives, in this chapter we shall focus on the synthesis of these ligands and the equilibrium and kinetic properties of their lanthanide complexes.

## 5.2.2 Synthesis of the DTPA and DOTA Derivative Ligands and their Complexes

### 5.2.2.1 Synthesis of Substituted DTPA and DOTA Derivatives

The advent of $Gd^{III}$ complexes as MRI contrast agents has stimulated worldwide research to develop $Gd^{III}$-based agents with improved efficacy. The development of new generations of CAs has been achieved through the modification of the basic ligand structures of the two most common contrast agents, $Gd(DTPA)^{2-}$ and $Gd(DOTA)^{-}$. DTPA was first synthesized by *Frost* in 1956 by reacting diethylenetriamine (dien) with formaldehyde and sodium cyanide under alkaline conditions.[15] Since then the cyanomethylation of various polyamines (both cyclic and acyclic) and subsequent hydrolysis of the nitriles has become one of the most important synthetic routes to high purity chelating agents like EDTA or DTPA while DOTA is prepared by reacting cyclen with haloacetic acid sodium salt and NaOH in water.[16–18]

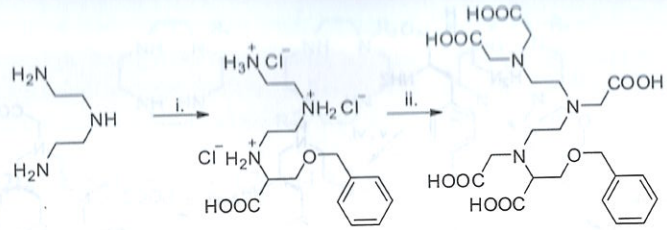
Mannich-type reaction of amines (both dien and 1,4,7,10-tetra-*aza*cyclododecane (cyclen)) with formaldehyde and phosphorous acid in acidic medium seems to be the most convenient route to derivatives with methylenephosphonate pendant arms.[19,20] Dialkyl phosphonates or alkyl phosphinates can be prepared by a similar approach using trialkyl phosphite or alkylphosphinic acids (or diethoxymethylphosphine) instead of phosphorous acid. Monoalkyl phosphonates can be obtained by partially hydrolyzing the dialyl phosphates with sodium or potassium hydroxide.[21–23]

Preparation of DTPA-*penta*amides or DOTA-*tetra*amides requires alkylation of the amines, dien and cyclen with the 2-bromo-acetamide or its derivatives.[24–28a] However, the amide bond can also be prepared by converting the DTPA or DOTA to methyl (or ethyl) esters and reacting these esters with the appropriate amines or ammonia (aminolysis). DOTA *tetra*amides were also prepared recently by activating the DOTA acetate arms with various peptide coupling agents (BOP (benzotriazol-1-yloxytris(dimethyloamino)phosphonium hexafluorophosphate) and HBTU (*N*-[(lH-benzotriazol-l-yl) (dimethylamino)-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide) *etc.*).[28b]

Preparation of functionalized ligands with mixed side arms (*N*-functionalization) has opened new directions in research areas ranging from fundamental coordination chemistry to diagnostic and therapeutic nuclear medicine.[1,2,4,10,29–36] The synthesis of mixed side arm derivatives requires the selective protection of the *N*-atoms in the polyamine. The synthetic methods that have been developed for the selective *N*-functionalization of cyclen have recently been reviewed in detail.[37] Here we will mention only the synthesis of the most important intermediates.

### 5.2.2.2 Synthesis of the Most Important DTPA Based Intermediates and CAs

The large gap between the protonation constants of the amine nitrogen atoms of certain polyamines (cyclen, piperazine) provides a very convenient access to selectively functionalized derivatives. In these examples, the proton itself acts as a "protecting group" since the protonated nitrogens of the polyamine will not react. The protonation sequence of dien and cyclen differ considerably, which in turn are responsible for the large differences in their functionalization chemistry. The protonation constants of cyclen were found to be: log $K_1^H = 10.65$, log $K_2^H = 9.64$, log $K_3^H = 1.4$, and log $K_4^H$ probably being even lower.[38] The first and the second protonation constants do not differ considerably and this may be responsible for the difficulties observed during mono alkylation (the 1,7-diprotected products are also formed). However the difference between the second and third protonation constants is reasonably high, which can be exploited to selectively obtain 1,7-diprotected cyclen derivatives.[39–41] The protonation constants determined for dien are as follows: log $K_1^H = 9.84$, log $K_2^H = 9.02$, and log $K_3^H = 4.25$. The first proton protonates the central nitrogen and when the second proton protonates one of the terminal nitrogen atoms, the proton from the central nitrogen moves to the other terminal nitrogen in order to achieve better charge separation. The protonation constants are very close and because of the delocalization of the protons pH-controlled protection of the dien is not feasible. However, monoalkylation of terminal nitrogens can be accomplished with a large dien excess. This allows the preparation of mono- and tetra-substituted DTPA based ligands through regioselective alkylation with α-haloacids.[42] The preparation of the ligand BOPTA is a good example of such a protection procedure (Scheme 5.2.2).[43]




**Scheme 5.2.2** Preparation of the BOPTA ligand (i) 2-chloro-3-(phenylmethoxy)-propanoic acid in $H_2O$ followed by ion exchange on Amberlite IRA 400; (ii) bromoacetic acid, NaOH (pH = 10) followed by ion exchange on Amberlite IR 120.

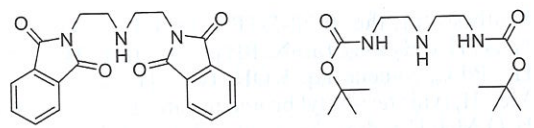
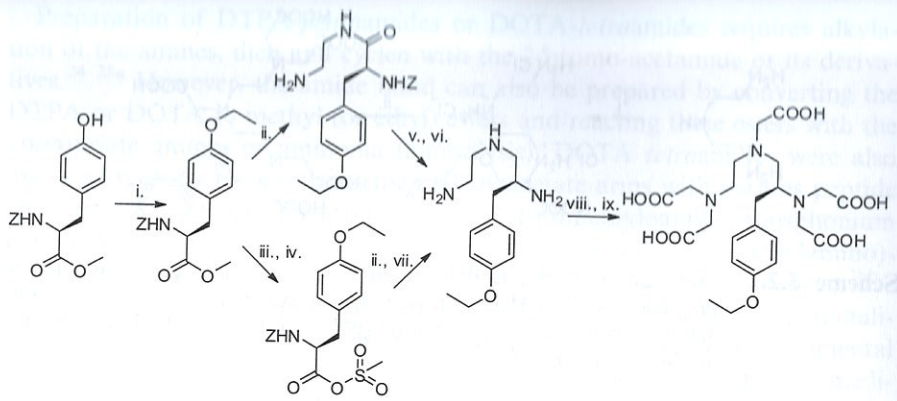



**Figure 5.2.1** Structures of Bis(phthaloyl)- and *N,N'*-Boc- protected diethylenetriamine.

DTPA-*N*-monoamides were prepared according to a similar scheme while bis-amides including two ligands used for the preparation of commercial CAs, DTPA-BMA and DTPA-BMEA are obtained from DTPA-bis(anhydride), which is commercially available or can be prepared from DTPA by reacting it with acetic anhydride.[44] For instance, the cyclic 15-DTPA-EAM ligand was prepared using this method.[45] Selective functionalizaton of dien at the central *N*-atom has been achieved through the protection of the two terminal nitrogens with Boc or phthaloyl groups (Figure 5.2.1).[46,47] A large number of DTPA-*N'*-substituted derivatives including DTPA-*N'*-monoamides and some bifunctional ligands were prepared from dien in this way.[48]

The ligand EOB-DTPA, a precursor to the hepatospecific MRI contrast agent Gd(EOB-DTPA)$^{2-}$, has been prepared following two synthetic routes, which differ in the preparation of the chiral triamine 1-*N*-(2-amino-ethyl)-3-(4-ethoxy-phenyl)-propane-1,2-diamine. (Scheme 5.2.3).[49] First, aminolysis of the benzyloxycarbonyl-protected amino acid methyl ester, prepared from tyrosine with an excess of ethylendiamine, was performed. Addition of HCl to the reaction mixture afforded the [1-(2-amino-ethylcarbamoyl)-2-(4-ethoxy-phenyl)-ethyl]-carbamic acid benzyl ester hydrochloride in good yield. Catalytic hydrogenation and reduction of the amide bond resulted in the *C*-derivatized chiral triamine. The second scenario was developed in order to circumvent the use of diborane. The 2-benzyloxycarbonylamino-3-(4-ethoxy-phenyl)-propionic acid methyl ester was reduced to the corresponding alcohol which in turn was transformed to the mesylate. The reaction of the latter compound with an excess of ethylenediamine and subsequent catalytic hydrogenation of the product afforded the key triamine in the form of the crystalline dihydrochloride. Alkylation of the triamine with *t*-butyl bromoacetate in THF/$H_2O$ in the



**Scheme 5.2.3** Synthesis of the EOB-DTPA ligand (i) EtI, $K_2CO_3$, DMF; (ii) $NH_2CH_2CH_2NH_2$; (iii) $NaBH_4$, THF; (iv) $CH_3SO_3Cl$, $Et_3N$, THF; (v) $H_2$, Pd/C, toluene–aq. KOH; (vi) $BH_3 \cdot THF$, THF; (vii) $H_2$, Pd/C, MeOH; (viii) *tert*-butyl bromoacetate, $THF/H_2O$, $K_2CO_3$; (ix) NaOH, $H_2O$/MeOH reflux, Amberlite IR 120 ($H^+$ form).

presence of $K_2CO_3$ afforded the pentaester, which was deprotected in the presence of NaOH and purified by ion-exchange chromatography.

The backbone of the MS-325 ligand (Figure 5.2.1), which shows strong binding ability to human serum albumin, was synthesized by a similar procedure.[50]

### 5.2.2.3 N-functionalization of DOTA Derivative Macrocyclic Ligands

Synthesis of DOTA-based ligands is of great importance in the field of CA research, because of the high thermodynamic stability and kinetic inertness of the lanthanide complexes formed with this type of ligand. *N*-derivatization has been accomplished by either direct derivatization or a protection-derivatization-deprotection sequence. Several 1-mono-, 1,7-disubstituted, and 1,4,7-trisubstituted key intermediates (Figure 5.2.2) were synthesized for the convenient modification of the basic cyclen skeleton. In some cases more than one procedure has been developed to introduce the same type of functionality/pendant arm. Hence, it may be difficult to choose the right procedure to solve an actual synthetic problem.

The two most important monosubstituted cyclen derivatives are *N*-formyl and *N*-benzyl cyclen. The synthesis of the former compound is surprisingly easy and straightforward. The reaction between *N,N*-dimethylformamide dimethylacetal and cyclen results in a triprotected (tricyclic) compound which can be hydrolyzed in a mixture of water and alcohol at low temperature, to form quantitatively the monoprotected *N*-formyl cyclen.[51] Benzyl protected cyclen can be prepared either by introducing the benzyl protection before the cyclization step (with the use of 4-benzyl-1,7-ditosyl-1,4,7-diethylenetriamine) or by alkylating cyclen

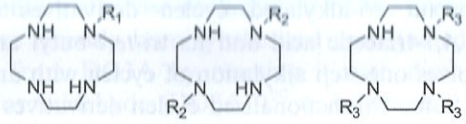


**Figure 5.2.2** Structure of 1-, 1,7- and 1,4,7-substitiuted protected cyclens and intermediates ($R_1$ = CHO, $CH_2C_6H_5$ or $COOCH_2C_6H_5$, $R_2$ = $COOCH_2C_6H_5$ or $CH_2SO_3H$ while $R_3$ = CHO, $CH_2COO^tBu$, $COO^tBu$ or in some cases $CH_2COOH$.

with benzyl–bromide. *Dischino et al.*,[51] have tried several synthetic approaches to this product according to the first scenario, but the desired compound could only be obtained in low yields. Monoalkylation of the cyclen with benzyl–bromide can be achieved in the presence of a large excess of cyclen.[52–54] However, cyclen is relatively expensive and using it in large excess is not economical. Although some of the latest attempts to prepare mono alkylated products by direct alkylation of cyclen have been very promising, the tris-Boc or tris-formyl intermediates are still frequently used to prepare monoalkylated cyclens.[55–58]

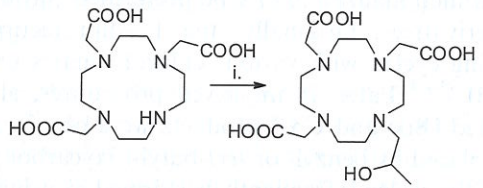
In the case of diprotection, the formation of two regioisomers, the 1,4- and 1,7-substituted cyclen derivatives, is expected.[59,60] The 1,7-dialkylated cyclen derivatives are more useful in the synthesis of CA's and two intermediates in particular were found to be of great importance. Mannich-type reaction of cyclen with the bisulfite adduct of formaldehyde affords 1,7-disulfomethylated cyclen. This intermediate can be used to synthesize di- (or even mono-) substituted cyclen derivatives (acetates, phosphonates or phosphinates).[61] On the other hand, 1,7-difunctionalization can be performed through the 1,7-Boc or Cbz protected derivatives. Originally, the 1,7-diprotection of cyclen was achieved by reacting cyclen with various chloroformates under acidic conditions (pH $\approx$ 2 – 3).[62–64] Later, in improved procedures, almost quantitative yields of diprotected (Boc and Cbz) products were obtained when the chloroformates were replaced by benzyl- or *tert*-butyl-(oxycarbonyl) succinimides.[39]

Triprotection of the cyclen is frequently performed by using a carbamate-type protecting group. The triprotected intermediates are subject to further functionalization followed by removal of the protecting groups. This step can be performed under mild conditions, which makes these carbamate protecting groups very convenient in various synthetic procedures. The tri-*N*-benzyloxycarbonyl (tri-*N*-Cbz-cyclen) can be obtained by reacting the cyclen with 3.2 equiv. of benzyl chloroformate in the presence of $Et_3N$ (6.4 equiv.) in dichloromethane. The synthesis of tri-Boc-cyclen was accomplished according to a similar procedure. Switching from $CH_2Cl_2$ to $CHCl_3$ as solvent and addition of 3.0 equiv. $Et_3N$ base resulted in almost quantitative yield of the tri-Boc-cyclen.[55,65–67] The tris-formyl protected cyclen was prepared initially in excellent yields by reacting the cyclen with the excess chloral hydrate in hot EtOH.[68] Difficulties associated with the removal of the formyl protecting group (alkaline hydrolysis, oxidative or reductive deprotection) may limit its use, although acid hydrolysis was found to be an efficient route for deprotection.[57,69–71]

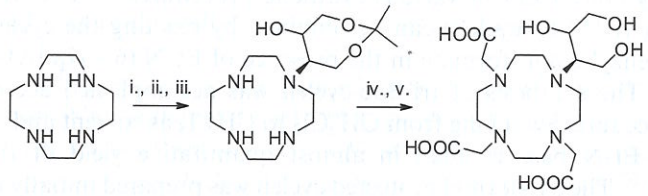
The most important tri-alkylated cyclen derivatives are 1,4,7,10-tetra-*aza*cyclododecane-1,4,7-triacetic acid and its tri-*tert*-butyl and tribenzyl esters. Their synthesis involves one-step alkylation of cyclen with an appropriate alkylating agent.[51,72–74] Some trifunctionalized cyclen derivatives could also be prepared without using any kind of protection. The earlier syntheses suffered from low yields and time-consuming column chromatography to separate the di-, tri- and tetra-substituted cyclen derivatives formed under these conditions.[75–77] However, the synthetic procedure was improved recently by using slightly larger excess of the alkylating agents and considerably larger amount of the base (triethylamine).[73]

These important building blocks have been used in CA research (MRI, optical, X-ray and PET) and some of them are now available commercially. Starting from these intermediates and protected cyclen derivatives (Figure 5.2.2) a large number of DOTA derivatives have been synthesized. For instance, derivatization of DO3A was performed by reacting it with 2-Methyl-oxirane (alkylating agent) in aqueous NaOH which resulted in a derivative known as HP-DO3A, whose $Gd^{III}$-complex is currently used as a CA in MRI (Scheme 5.2.4).[51]

The ligand BT-DO3A, used for preparation of the nonionic contrast agent Gd(BT-DO3A), was synthesized starting from a triprotected tricyclic intermediate as a nucleophile in a ring opening reaction with 4,4-dimethyl-3,5,8-trioxabicyclo[5,1,0]octane. The resulting product was hydrolyzed in aqueous MeOH and the formyl protection was removed subsequently in aqueous KOH. The triacid was obtained by reacting the compound obtained in the previous step, with chloroacetic acid in water at pH = 9 – 10, followed by hydrolysis to give BT-DO3A (Scheme 5.2.5).[78,79]




**Scheme 5.2.4** Synthesis of the HP-DO3A (i) 2-Methyl-oxirane, $NaOH/H_2O$ than anion-exchange chromatography.




**Scheme 5.2.5** Synthesis of the BT-DO3A ligand (i) *N,N*-dimethylformamide dimethyl acetal and 4,4-dimethyl-3,5,8-trioxabicyclo[5,1,0]octane, 120 °C; (ii) $H_2O$/MeOH, 20 °C; (iii) KOH, $H_2O$/MeOH, 80 °C; (iv) $ClCH_2COOH$, NaOH, $H_2O$ 60 °C, pH = 9 – 10; (v) HCl and ion-exchange.

Ligands representing stepwise replacement of the acetates by phosphonate pendant arms have recently been synthesized and studied. The synthesis was performed either from DO3A by reacting it with diethyl phosphite in the presence of paraformaldehyde (DO3AP)[80] or by reacting the 1,7-DO2A-*tert*-butyl ester with triethyl phosphite in the presence of paraformaldehyde (DO2A2P).[81] The DO3P and DOA3P ligands were prepared with the use of similar procedures and conditions and studied recently.[82,83]

Pyclen is an interesting cyclen derivative in which a pyridine ring is fused to the tetra*aza*cyclododecane macrocycle. Pyclen was first isolated by *Stetter*,[84] and its triacetate derivative, PCTA, was later synthesized by *Aime et al.*,[85,86] by alkylating the macrocycle with chloroacetic acid in the presence of sodium carbonate. The free acid form of PCTA was obtained after acidifying the reaction mixture. Ln complexes of PCTA have reasonably high thermodynamic stability and satisfactory kinetic inertness for biomedical applications.[85,87] These chelates contain two inner sphere water molecules, which cannot be replaced by bioligands. In addition, the pyridine chromophore can act as an antenna for Ln sensitization in the UV-vis (Nd Eu, Tb and Yb). Several other pyclen based ligands, including the monophosphonate and a bifunctional ligand derived from the PCTA, have also been prepared and studied.[88,89] The synthesis of PCTA derivatives has recently been reviewed in detail by Guy *et al.*[90,91]

### 5.2.2.4 Structure and Synthesis of Bifunctional Ligands Derived from DTPA and DOTA

The term "bifunctional chelating agent" (BFC) has been used to denote ligands that can perform two different functions: they can sequester a therapeutic or diagnostic metal ion in a thermodynamically stable and kinetically inert complex, and besides metal binding they also possess a reactive functional group (anchor) that can be used for the covalent attachment of the complex to a targeting vector (peptides, proteins, dendrimers, nano-particles, or even another complex). The targeting vector is attached to the chelate through a spacer that is often an aliphatic or aromatic moiety (Figure 5.2.3).

The targeting molecules may contain different groups available for conjugation, hence the bifunctional ligands synthesized and investigated to date can be very different as far as their reactive groups are concerned. BFCs may be

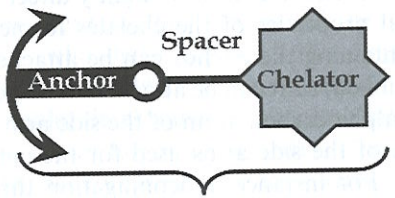



**Figure 5.2.3** Schematic representation of a bifunctional ligand.

classified by the functional groups of the targeting vector they will react with. The most frequently used functionalities of biomolecules for bioconjugation include amines (*e.g.* lysine or other reactive amines), carboxylic acids (glutamic acid, aspartic acid or other carboxylic acids), thiols (cysteine or other reactive thiols) or alcohols.

Vectors containing carboxylate groups may be derivatized by converting them into amides (any amide bond forming reaction) or through an active ester such as hydroxysuccinimide active ester (NHS-ester) or reactive carbonyl intermediates (with the use of carbodiimide coupling agents such as EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride), DCC (dicyclohexyl carbodiimide) or DIC (diisopropyl carbodiimide). In these reactions the carboxylate group acts as an acylating agent toward the group to be modified. The activated intermediates can be reacted with alcohols (the product of the reaction will be an ester), thiols (the product will be a thioester), amines (an amide bond will be formed) or even hydrazide derivatives.[92]

For bioconjugation purposes the modification of alcohols is less important than the modification of any other functional groups discussed earlier, but at the same time not impossible, as these derivatives can be alkylated or acylated quite easily. In addition, the aromatic alcohols (phenols) may be targeted by Mannich condensation reactions or electrophilic reagents such as iodine or diazonium ions.

The maleimide group reacts specifically and efficiently with the sulfhydryl group (–SH moiety) of the biomolecule in the Michael-type addition reaction and has been widely used to form protein conjugates through thioether linkage. Conjugation reactions involving sulfhydryl groups may also involve simple alkylation or acylation reactions to form stable thioethers and relatively unstable thioesters, respectively.

Amines can be derivatized quite easily using alkylation and acylation reactions. It is worth noting that BFCs with an aryl isothiocyanate group can also be used to achieve conjugation to primary and secondary amines. Although several functional groups (carboxylic acids, OH, SH, amines) available for bioconjugation are discussed above, it should be emphasized that DOTA and DTPA complexes are almost exclusively conjugated to peptides and proteins through an amino group and the most commonly used bifunctional ligands are the isothiocyanato derivatives.

The site where the spacer and the anchor is attached to the bifunctional ligand seems to be an important issue, as it may slightly affect the complexation and other physico-chemical properties of the chelates formed by the bifunctional ligand. The spacer containing the anchor can be attached to the backbone of the ligand (backbone linked) or it can be attached to one of the side arms on the ligand (usually to the alpha carbon atom of the side arm – side arm linked). In the third scenario one of the side arms used for the complexation acts as an anchor (Figure 5.2.4). For instance, bioconjugation through one of the carboxylate groups often gives rise to an amide functionality, which is still capable of coordinating to metal ions. Among these options the last two are more cost effective than the first one and therefore, they are more frequently used.

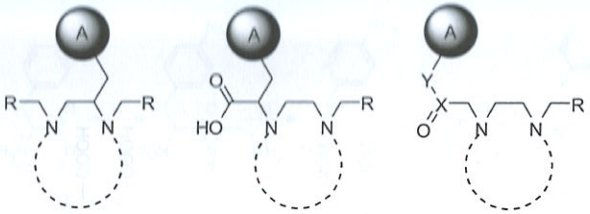



**Figure 5.2.4** Places available for the attachment of the "anchor" to the chelator (A denotes anchor while R is a group with a donor atom capable of coordination, X can be a C atom (Y = NH) or P-OH moiety (Y = $CH_2$)).

In the past three decades there has been an increasing demand for bifunctional ligands, particularly for nuclear medicine applications. Several bifunctional versions of well-known chelating scaffolds, such as EDTA, DTPA and DOTA, have been synthesized and studied in detail. These ligands offer polyaminocarboxylate type binding for a variety of metal ions with donor atoms ranging from 6 (EDTA) to 8 (DTPA and DOTA) and they also provide acyclic (EDTA and DTPA) or macrocyclic (DOTA) encapsulation for the selected metal ion.

BFCs based on EDTA ligands were first synthesized by *Meares* and coworkers,[93–95] however the EDTA based BFCs do not offer enough donor atoms for complexation of metal ions with coordination number (CN) larger than six ($Y^{III}$, $In^{III}$ and lanthanide$^{III}$ ions). As a result, DTPA based BFCs were designed and synthesized because they provide a better match between the number of donor binding groups and the CN for larger ions such as $Y^{III}$, $In^{III}$ and $Ln^{III}$ ions. The structure of the most important DTPA based BFCs is shown in Figure 5.2.5.

DTPA-bis anhydride is often considered as the first DTPA based bifunctional ligand, and it was frequently used for bioconjugation in the early days of MRI contrast agent research. Depending upon the molar ratio of the amines selected for the reaction, one or two amide bonds will be present in the product as a result of the conjugation. This can be a disadvantage, since the cross-linking may considerably affect the biological activity of the conjugate. In addition, the thermodynamic stability (and probably the kinetic inertness) of the resultant DTPA-mono- and bis- amide complexes will be significantly lower than those of the corresponding DTPA chelates.[96–101]

Considerable improvement in the stability of the chelates was achieved by *Brechbiel et al.*,[102] who have reported the synthesis of l-(*p*-isothiocyanatobenzyl)-DTPA (1B-DTPA), a bifunctional ligand that can be conjugated to the biomolecule through a thiourea linkage. Inclusion of the *p*-isothiocyanatobenzyl group not only provides a reactive functionality for bioconjugation but the backbone substitution increases the stability of the resulting complexes. An isomeric form of 1B-DTPA in which the protein-linking functional group is attached to one of the terminal carboxylate arms was synthesized somewhat later by *Westberg* and *Keana*.[103–104] The synthetic

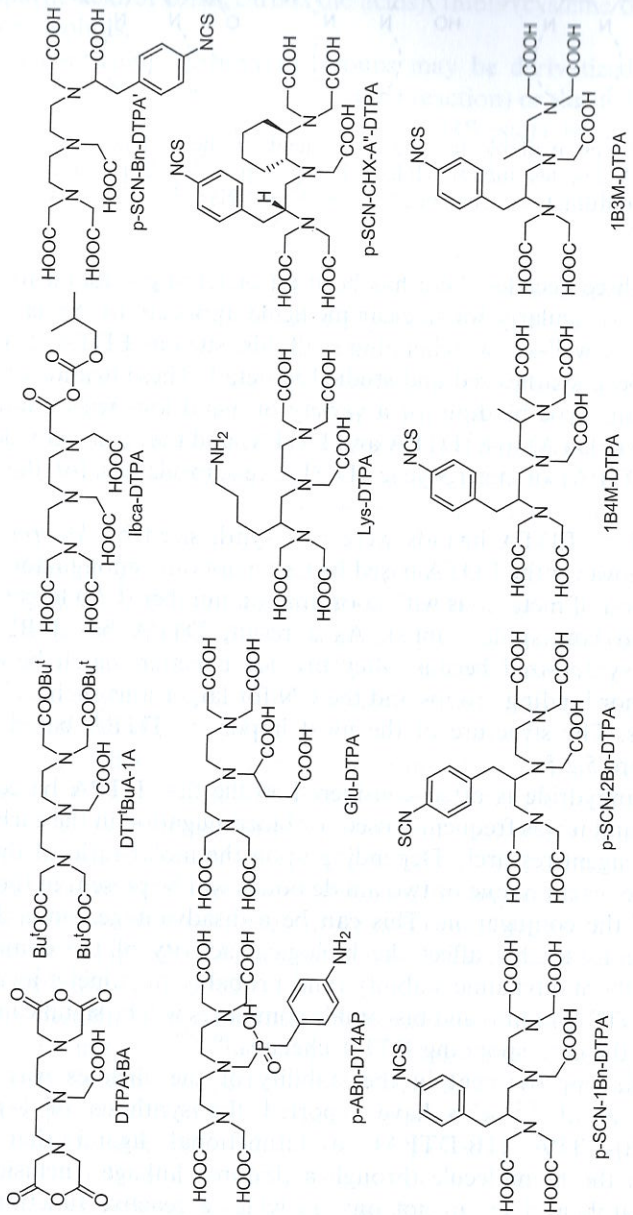
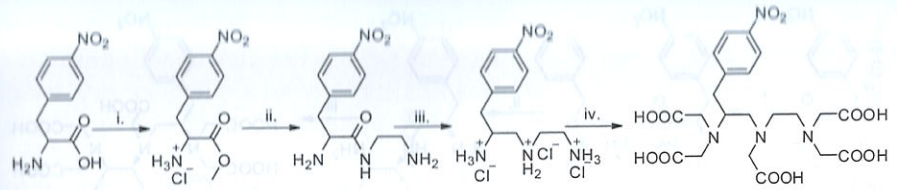


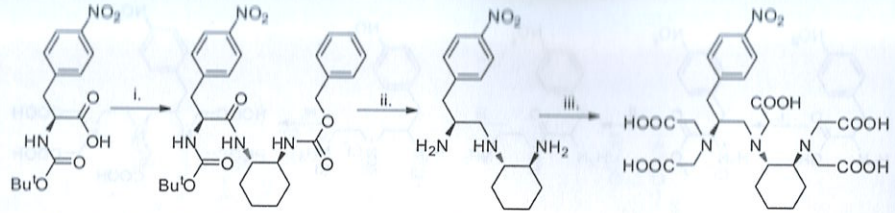

**Figure 5.2.5** Selection of DTPA based BFC ligands.



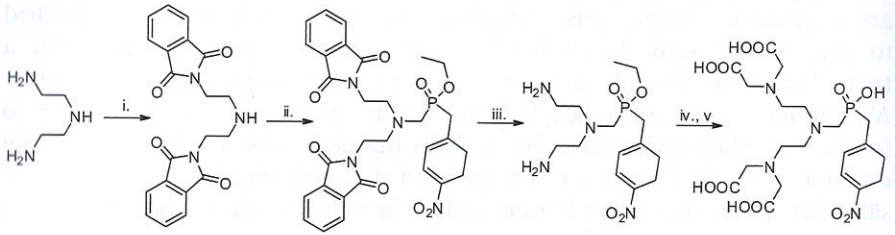

**Scheme 5.2.6** Synthesis of the *p*-$NO_2$-Bn-DTPA ligand (i) HCl/MeOH; (ii) $Et_3N$, MeOH, $NH_2CH_2CH_2NH_2$; (iii) $BH_3 \cdot THF$ in THF than HCl in EtOH; (iv) $BrCH_2COOH/C_6H_5CH_3$, $KOH/H_2O$ than ion-exchange on AG50W-X8 followed by HPLC.

procedure developed by *Brechbiel et al.*,[102] is shown in Scheme 5.2.6 and it is used very often when backbone modified (*C*-functionalized) DTPA derivatives are synthesized. Using this procedure, *p*-nitrophenylalanine was esterified to give methyl ester hydrochloride. The free base was liberated with a triethylamine (TEA) and reacted with ethylene diamine in methanol to generate *N*-(2-aminoethyl)-p-nitrophenylalanine amide. Reduction of the amide to (*p*-nitrobenzyl)diethylenetriamine (*p*-$NO_2$-Bn-dien) was accomplished using excess borane–THF complex. The product was isolated as the hydrochloride salt after decomposing the borane adduct in refluxing ethanol saturated with HCl gas. Alkylation of the amine under standard conditions with bromoacetic acid and aqueous KOH resulted in 1-(*p*-nitrobenzyl)diethylenetriaminepentaacetic acid (*p*-$NO_2$-Bn-DTPA), which was purified by ion exchange and HPLC chromatographic techniques (note: the *p*-$NO_2$-Bn-DTPA is the precursor of *p*-NCS-Bn-DTPA.). Reduction of the nitro group with Pd/C under basic conditions afforded the amino derivative *p*-$NH_2$-Bn-DTPA, which was converted to isothiocyanate (BFC) with thiophosgene (these steps are not indicated in the scheme).

Considerable improvements in the thermodynamic and *in vivo* stability of the complexes were achieved by replacing one of the flexible ethane-1,2-diamine units of DTPA with the more rigid propane-1,2-diamine (1B4M-DTPA) or cyclohexane-1,2-diamine (CHX-A or CHX-B ligands) units.[105,106] Synthesis of the 1B4M-DTPA was performed following a route that is very similar to the one reported for *p*-$NO_2$-Bn-DTPA (Scheme 5.2.6), but propane-1,2-diamine was used instead of ethylene diamine.[107] The first step in synthesis of *p*-$NO_2$-Bn-CHX-A-DTPA (Scheme 5.2.7) involved the reaction carbamate-protected (−)-*p*-nitrophenylalanine with *N*-benzyloxycarbonyl-(*R,R*)-*trans*-cyclohexane-1,2-diamine using a carbodiimide coupling agent. The carbamate protecting groups were removed with HBr in acetic acid and the resulting amide was reduced with borane–THF complex. Alkylation of the triamine (*p*-$NO_2$-Bn-CHX-A-triene) was achieved with *tert*-butyl bromoacetate. Treatment of the penta-ester with trifluoroacetic acid resulted in the BFC precursor. HPLC purification of the crude product afforded an analytically pure sample. This synthetic procedure was reported to give better yields and was used to prepare diastereoisomers of 1B4M-DTPA.[108]



**Scheme 5.2.7** Synthesis of the *p*-$NO_2$-Bn-CHX-A"-DTPA bifunctional ligand precursor (i) *N*-benzyloxycarbonyl-cyclohexane-1,2-diamine, HOBT, EDC, EtOAc, DMF, 25 °C; (ii) HBr, AcOH; (iii) $BH_3 \cdot THF$, THF, 50°C than HCl in dioxane; (iv) *tert*-butyl bromoacetate, $Na_2CO_3$, DMF; 80°C; (v) TFA, r.t. than HPLC and ion-exchange chromatography.



**Scheme 5.2.8** Synthesis of the *p*-$NO_2$-Bn-DT4AP BFC precursor (i) phtalic anhydride, toluene, reflux; (ii) ethyl *p*-nitrobenzylphosphinate, $(CH_2O)n$, toluene, reflux; (iii) $N_2H_4 \cdot H_2O$, EtOH, reflux; (iv) ethyl bromoacetate, $K_2CO_3$, DMF, rt; (v) aq. HCl, reflux).

From a synthetic chemical point of view, modification through the central nitrogen of the 1-*N*-(2-amino-ethyl)-ethane-1,2-diamine backbone is also interesting, since this approach provides access to a large number of side arm linked BFC's. The synthesis requires simultaneous blocking of both of the terminal nitrogens by protecting them with phthalic anhydride or Boc protecting group.[109,110] For example, the phthaloyl-protected diethylenetriamine was reacted with *p*-nitrobenzyl-phosphinic acid ethyl ester in a Mannich-type reaction to give the ethyl *N,N''*-bis(phthaloyl)diethylenetriamine-*N'*-methylene(*p*-nitrobenzyl)phosphinate intermediate. Removal of the phthaloyl protective groups with hydrazine hydrate followed by alkylation of the resultant diamine with ethyl bromoacetate gave an ester intermediate that was converted in one hydrolysis step to the BFC precursor, *p*-$NO_2$-Bn-DT4AP (Scheme 5.2.8).

Despite the successes achieved with acyclic DTPA based BFC's, the macrocyclic ligands have been shown to form more stable complexes. The insufficient kinetic inertness of chelates formed with radionuclides could be potentially harmful to the body. To overcome this disadvantage, a large number of macrocyclic BFCs were designed and synthesized. A selection of DOTA based BFCs to complex radionuclides such as $^{86/90}Y$, $^{111}In$, the radiolanthanides, $^{212}Pb$, $^{213}Bi$, and $^{225}Ac$ is shown in Figure 5.2.6.

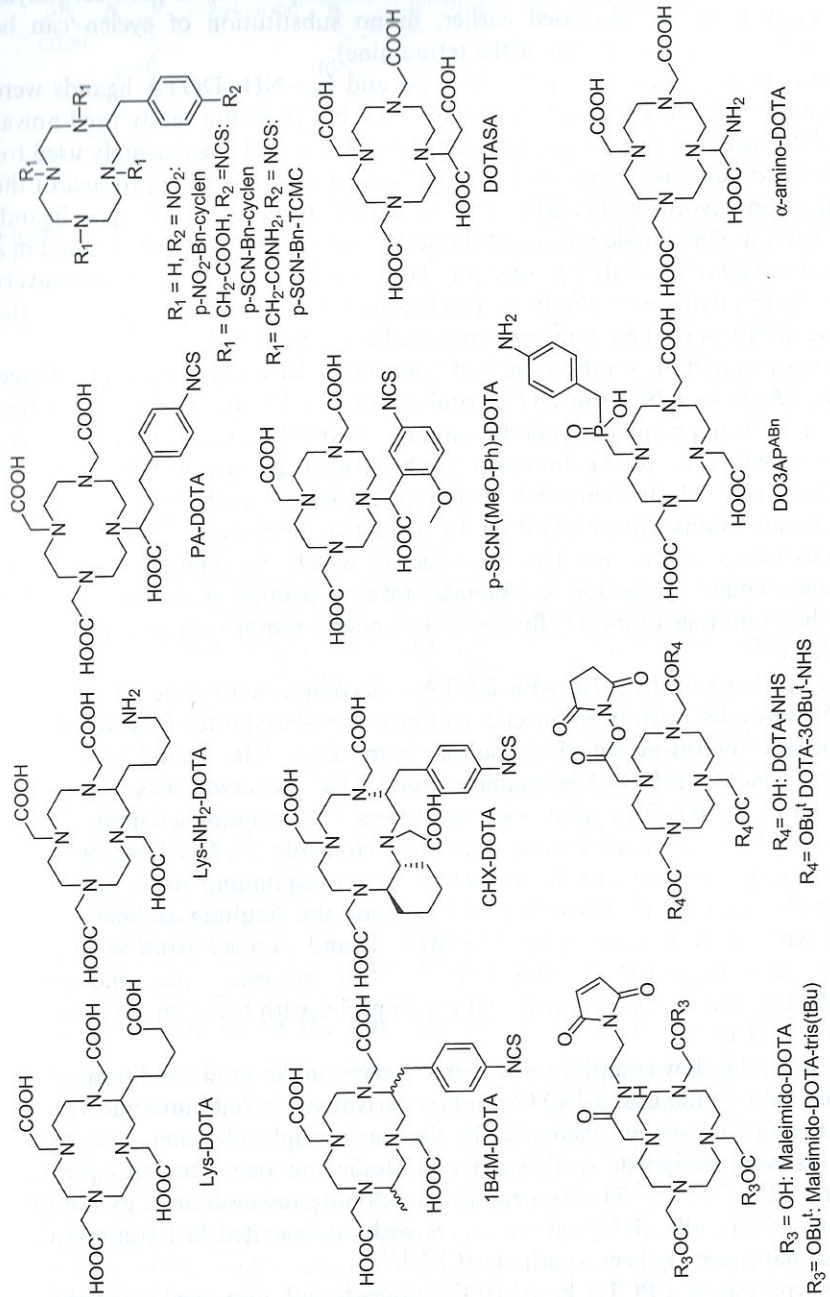



**Figure 5.2.6** DOTA based BFC ligands (1B4M-DOTA201, CHX-DOTA201 and α-amino-DOTA202 ligands are not discussed in detail in the text).

The side arm linked BFCs can be prepared either by alkylating DO3A (or DO3A-tris-*tert*-butyl ester) or cyclen with the reactive functional group precursor containing the appropriate side arm (usually an alpha-halo carboxylic acid derivative). As discussed earlier, mono substitution of cyclen can be achieved using a large excess of the tetraamine).

In the family of DOTA BFCs the Lys- and Lys-$NH_2$-DOTA ligands were among the first DOTA based BFCs prepared, but these are rarely used nowadays.[111] The commercially available DOTA-NHS ester is increasingly used for side arm conjugation, however, it should be mentioned that the product of the conjugation involving this BFC will be a DOTA-mono amide type ligand. Substitution of an amide for one of the acetate side arms in DOTA resulted in a slight decrease of the stability constant of complexes. In spite of the successively lower thermodynamic stability of DOTA-mono-, bis- and tetra- amides, the kinetic inertness of their complexes is not affected negatively.[112–115]

The key step in the synthesis of backbone linked BFC chelating agents based on DOTA (both *p*-$NO_2$-Bn-DOTA and *p*-$NO_2$-Bn-TCMC ligands) is the formation of their precursor cyclic tetraamine, *p*-$NO_2$-Bn-cyclen. Published synthetic procedures for *p*-$NO_2$-Bn-cyclen can be divided into three groups: 1) peptide based synthesis, which often uses high dilution cyclization methods;[116–119] 2) use of a Richman-Atkins type cyclization to form the macrocycle;[116,120,121] and 3) a combination of the first two methods, in which the intermediate for the Richman-Atkins cyclization is obtained from a peptide precursor. The first published synthesis of *p*-$NO_2$-Bn-cyclen is a good example of this combination approach.[122]

The synthesis of the *p*-$NO_2$-Bn-DOTA is accomplished by the alkylation of *p*-$NO_2$-Bn-cyclen with bromoacetic acid or its *tert*-butyl ester followed by the subsequent hydrolysis of the resultant tetra-ester. The ligand *p*-$NO_2$-Bn-TCMC, which is a DOTA-tetraamide based BFC precursor, was synthesized by reacting *p*-$NO_2$-Bn-cyclen with an excess of 2-bromo-acetamide in the presence of excess triethylamine base in acetonitrile.[123] The isothiocyanato functionality was obtained by reducing the corresponding nitro derivatives with hydrogen over Pd/C catalyst and reacting the resulting aniline (*p*-$NH_2$-Bn-TCMC) with thiophosgene. The BFC ligand derived from *p*-$NO_2$-Bn-TCMC was suggested to sequester $^{203/212}Pb$ isotopes, since the parent DOTAM ligand forms extremely stable complexes with heavy metal ions such as $Pb^{2+}$ or $Cd^{2+}$.[27,124]

Owing to the slow complex formation observed under mild conditions (25 °C and pH = 4 – 5) the use of DOTA and its dérivatives in radioimmuno-therapy or -imaging may be problematic. In the past couple of years, new ligand scaffolds were designed, synthesized and studied to overcome this problem (Figure 5.2.7).[87,125–127] This is a rapidly developing research area, as the BFC versions of virtually all ligand structures with documented fast complexation kinetics have already been synthesized.[128–131]

The synthesis of a PCTA based BFC precursor with improved complexation kinetics was accomplished by a Richman-Atkins type cyclization as shown in Scheme 5.2.9. The *p*-toluenesulfonyl protected tritosyl-nitrobenzyl-pyclen was



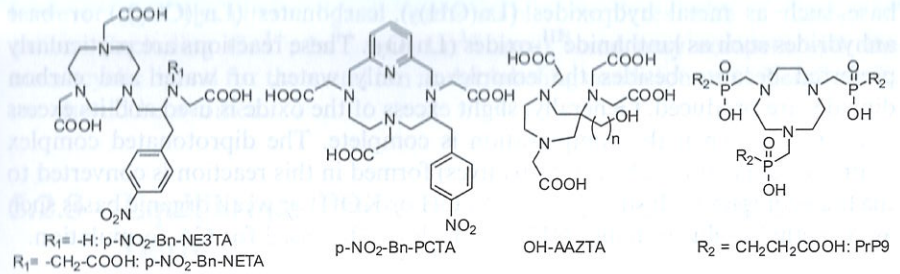
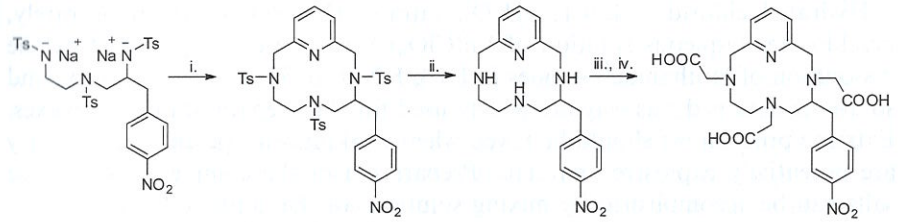



**Figure 5.2.7** BFC ligands with advanced complex formation kinetics (OH-AAZTA ligand structures with n = 1,[203,204] and n = 2,[205] were prepared recently).




**Scheme 5.2.9** Synthesis of *p*-$NO_2$-Bn-PCTA, BFC precursor with accelerated complexation kinetics (i) 2,6-bis(chloromethyl)pyridine, DMF, 55 °C; (ii) $H_2SO_4$, 180 °C; (iii) *tert*-butyl bromoacetate, anhydrous $K_2CO_3$, MeCN; (iv) HCl, $H_2O$ than HPLC.

obtained by reacting the disodium salt of *N,N',N''*-tritosyl-(*S*)-2-(*p*-nitrobenzyl)-diethylenetriamine with 2,6-bis(chloromethyl)pyridine in DMF. The tosyl groups of the product were removed in concentrated sulfuric acid at elevated temperatures and the free amine was alkylated with *tert*-butyl bromoacetate. The resulting triester was hydrolyzed in aqueous hydrochloric acid to afford the final product as an HCl salt. An analytically pure sample of *p*-$NO_2$-Bn-PCTA was obtained by HPLC purification.[131]

### 5.2.2.5 Synthesis of the Complexes

The general method of preparing the complexes depends on the form of the ligand that is accessible for the complexation (in acidic (protonated $H_xL$) or basic (deprotonated L) form). It should be noted that the kinetics of complexation of $Ln^{III}$ ions with open chain ligands (DTPA and DTPA based ligands) is nearly instantaneous in solution while it can be exceedingly slow for DOTA derivatives. Hence, complexations with DOTA derivatives are often carried out at elevated temperatures and the base used to neutralize the protons is added slowly, over a period of several hours.

Preparation of the complexes is often performed in a neutralization reaction. The complexation occurs between two reactants – the protonated ligand and a

base such as metal hydroxides ($Ln(OH)_3$), carbonates ($Ln_2(CO_3)_3$) or base anhydrides such as lanthanide$^{III}$-oxides ($Ln_2O_3$). These reactions are particularly preferred because besides the complexes, only water, or water and carbon dioxide, are produced. Generally, slight excess of the oxide is used and its excess is filtered off when the complexation is complete. The diprotonated complex (monoprotonated for DOTA derivatives) formed in this reaction is converted to the final complex with strong bases (NaOH or KOH) or weak organic bases such as *N*-methyl-D-glucosamine (NMG), which is often used for CA formulation.

$$LN_2O_3 + 2H_5DTPA = 2H_2\{Ln(DTPA)\} + 3H_2O \quad (5.2.1)$$

$$\{H_2(LnDTPA)\} + 2NMG = \{H(NMG)^+\}_2 \cdot \{Ln(DTPA)\}^{2-} \quad (5.2.2)$$

Hydrated chlorides ($LnCl_3 \cdot 6H_2O$), nitrates ($Ln(NO_3)_3 \cdot 6H_2O$) or rarely, perchlorates (aqueous solution of $Ln(ClO_4)_3$), can be easily prepared by the dissolution of lanthanide$^{III}$-oxides in HCl, $HNO_3$ or $HClO_4$, respectively, and solutions obtained this way are widely used for the preparation of complexes. Extreme precautions should be taken when working with perchlorates as they are potentially explosive materials. Preparation of the complexes with these salts can be accomplished by mixing solutions of the lanthanide salt and the ligand in the acidic form followed by raising the pH to about 6.0 (pH = 9 – 10 for phosphonate ligands such as DOTP) to scavenge the protons released during the complex formation.[132] As a result, the solution of the desired complex also contains by-products (salts such as $NaNO_3$, NaCl *etc.*), which can be removed with desalting columns or HPLC purification of the complexes if salt-free compounds are required.

There are some special cases when either the ligand or the complex formed is not soluble in water, the complex hydrolyses in aqueous media due to its low stability, or the complexes are simply not formed below the pH where the hydrolysis of the metal ions starts.[133–135] Nevertheless, it is still possible to prepare the complexes in anhydrous organic solvents, such as pyridine, alcohols (*e.g.* methanol, ethanol), acetonitrile or even a mixture of solvents (*e.g.* $CHCl_3$/EtOH/$H_2O$).[136,137] For this purpose $Ln^{III}$ triflates (trifluoromethanesulfonates, $Ln(CF_3SO_3)_3$) are the best choice. In this procedure the $Ln^{III}$ complexes are prepared by refluxing $Ln(CF_3SO_3)_3$ and the ligand (usually a macrocyclic chelator) in dry organic solvent for several hours. The complexes are often obtained by crystallization or by precipitation after adding another solvent.

Preparation of $Ga^{3+}$ and $In^{3+}$ complexes requires slightly different conditions than those applied for lanthanide complex preparations, because these metal ions hydrolyze at a much lower pH.[138] A recent paper suggests that $Ga^{3+}$ complexes should be prepared at pH = 3.5 in the presence of HEPES buffer, which is also suitable for human use.[139] Radiolabeling of ligands conjugated to biological vectors is often performed in buffered solutions, most commonly in acetate-based buffer systems such as sodium acetate–acetic acid or ammonium acetate–acetic acid. In some reports, sodium or ammonium citrate buffer was used for preparations involving radioisotopes. It should be emphasized,



however, that the citrate ligand forms stable complexes with a number of cations (including $Ga^{3+}$, $In^{3+}$ and $Ln^{3+}$ ions)[38] and may compete with the macrocyclic ligand for the metal ion under labeling conditions and therefore, its use should be avoided.

## 5.2.3 Equilibrium Properties of the DTPA and DOTA Derivative Complexes

The formation equilibria of the metal complexes are characterized by their thermodynamic stability constants as defined by the Equation 5.2.3:

$$K_{ML} = \frac{[ML]}{[M][L]} \quad (5.2.3)$$

where [ML], [M] and [L] are the equilibrium concentration of the complex, the metal ion and deprotonated ligand, respectively (the charges of the species are omitted for simplicity). Because of the multidentate nature of ligands, one or more donor atoms can be protonated at low pH values, when protonated complexes are formed. By considering the protonation equilibria, the protonation constants of complexes are defined as follows:

$$K_{MH_iL} = \frac{[MH_iL]}{[MH_{i-1}L][H^+]} \quad (5.2.4)$$

where i = 1, 2, . . . , n, and where $[H^+]$, $[MH_{i-1}L]$ and $[MH_iL]$ are the equilibrium concentration of $H^+$, $MH_{i-1}L$ and $MH_iL$ species, respectively.

To calculate the stability constants, the protonation constants of the ligands must be known, which are defined by Equation 5.2.5.

$$K_i^H = \frac{[H_iL]}{[H_{i-1}L][H^+]} \quad (5.2.5)$$

The conditional stability constants ($K^c_{ML}$), which reflect the competition between the metal ion and protons for the ligand, can be used to compare the behaviour of metal complexes at physiological pH:

$$K^c_{ML} = \frac{[ML]}{[M][L]_t} = \frac{[ML]}{[M][L]\alpha_H} \quad (5.2.6)$$

where $[L]_t = [L] + [HL] + [H_2L] + \ldots + [H_nL]$ $\alpha_H = 1 + K_1[H^+] + K_1K_2[H^+]^2 + \ldots + K_1K_2 \ldots K_n[H^+]^n$ and so $K^c_{ML} = K_{ML}/\alpha_H$.

In biological systems, the free ligand L can be protonated or it can form complexes with the endogenous metal ions ($Ca^{2+}$, $Zn^{2+}$ and $Cu^{2+}$), while the $M^{z+}$ metal ion can interact with endogenous ligands A, B, . . . (citrate, phosphate, carbonate, etc.). In addition, the metal complex can be protonated and it

can also form ternary complexes with the endogenous ligands. By taking into account all the possible side-reactions, a more general conditional stability constant ($K^*$) can be defined.[140]

$$K^* = \frac{[ML]_t}{[M]_t[L]_t} = \frac{[ML]}{[M][L]}\frac{\alpha_{ML}}{\alpha_M\alpha_L} = K_{ML}\frac{\alpha_{ML}}{\alpha_M\alpha_L} \qquad (5.2.7)$$

where:

$[M]_t = [M] + [MA] + [MB] + \ldots$

$[L]_t = [L] + [HL] + [H_2L] + \ldots + [H_nL] + [CaL] + [CaHL] + \ldots + [ZnL] + [ZnHL] + \ldots$

$[ML]_t = [ML] + [MHL] + [MLA] + [MLB] + \ldots$

$\alpha_M = 1 + K_{MA}[A] + K_{MB}[B] + \ldots$

$\alpha_L = 1 + K_1[H^+] + K_1K_2[H^+]^2 + \ldots + K_1K_2\ldots K_n[H^+]^n + K_{CaL}[Ca^{2+}] + K_{CaHL}[H^+] + \ldots$

$\alpha_{ML} = 1 + K_{MHL}[H^+] + K_{MLA}[A] + K_{MLB}[B] + \ldots$

The stability and protonation constants of the complexes, the conditional stability constant ($K^*$), and the concentration of the free metal ion ($[M^{z+}]$) can be calculated using the protonation constants of the ligand. It is generally assumed that the toxicity of metal complexes is related to the concentration of the "free" metal ion ($[M^{z+}]$), which can be expressed by the p$M$ value ($-\log[M^{z+}] = pM$). The p$M$ values are calculated for the special condition as proposed by *Raymond et al.*:[141] $[M]_t = 10^{-6}$ M, $[L]_t = 10^{-5}$ M, physiological concentration of the $[Ca^{2+}]$, $[Zn^{2+}]$ and $[Cu^{2+}]$, pH = 7.4.

### 5.2.3.1 Experimental Methods and Computer Programs used for the Characterization of Complexation Equilibria

*a. Methods and conditions*:

The stability constants of the complexes and the protonation constants of the ligands are defined by the concentration of the species formed in the equilibrium (Equations 5.2.3 – 5.2.5). In order to keep the activity coefficients at a constant value during the measurements, an inert electrolyte is used in relatively high concentration, which maintains a constant ionic strength. The inert electrolyte is generally KCl, $KNO_3$, $Me_4NCl$, $Me_4NNO_3$, NaCl or $NaClO_4$. Their concentration is most often 0.1 M or 1.0 M, depending on the concentration of the ligand L and metal ion $M^{z+}$ being investigated. The cations of the salts are prone to form weak complexes with the multidentate ligands, the stability of which decreases in the following order: $Na^+ > K^+ > Me_4N^+$.

In the complex formation reactions, there is generally a competition between the metal and $H^+$ ions for the donor atoms of ligands, which results in a change in the $[H^+]$ of the solutions. To study the complexation, the most widely used method is the pH-potentiometric titration of the ligand in the presence and absence of the metal ion. The instrumental set for the pH-potentiometric titration consists of a glass and a reference electrode (or a combined electrode), a pH-meter, and an autoburette. Before the pH-potentiometric titration is performed, the electrode system must be calibrated with two or three standard buffer solutions. In the practice of pH-potentiometric titrations, the pH meters are frequently used to measure the electromotive force ($E$), but measuring the pH values ($pH_r$) is also quite common. Both of these methods give $H^+$ ion activities from the titration data; however, for the calculation of the protonation or stability constants, $H^+$ concentrations ($[H^+]$) are needed. The simplest procedure to convert the measured $pH_r$ values to $[H^+]$ using the relationship between $pH_r$ and the activity coefficient ($f$) of the background electrolyte solution: $p[H^+] = pH_r + \log f$.[142]

When the electromotive force is measured, the $E$ values are related to the $H^+$ concentration as:[143]

$$E = E'_0 + Q\log[H^+] + j_H[H^+] + j_{OH}\frac{K_W}{[H^+]} \qquad (5.2.8)$$

where $E'_0$ contains the standard potential, the activity coefficients and liquid-junction potential of the inert electrolyte, $Q$ is the *Nernstian* slope, $K_w$ is the stoichiometric water ionic product, and $j_H[H^+]$ and $j_{OH}[OH^-]$ express the contribution of the $H^+$ and $OH^-$ ions to the liquid-junction potential. The $H^+$ concentrations are calculated with the Equation (5.2.8), but the $E'_0$, $Q$, $K_w$, $j_H$ and $j_{OH}$ values must be determined by titrating a strong acid with the same strong base that is used in the titration experiments.

The other frequently used method to obtain $[H^+]$ from the measured $pH_r$ values was proposed by *Irving et al.*[144] A strong acid (HCl or $HNO_3$) of known concentration is titrated with the base used for the titration experiments and the differences between the measured and calculated pH values ($A$) are determined in acidic solutions. The $A$ values are used to calculate the $[H^+]$ from the measured $pH_r$ values ($pH_r = pH + A$). Using the term $A$, the $K_w$ is calculated from the titration data of the strong acid obtained in basic solutions.[144] A prerequisite of pH-potentiometric titration techniques is that the equilibrium must be attained rapidly (in a few minutes) after the addition of the titrant. The metal: ligand concentration ratios are generally 1:1 but for some metal complexes, titrations are performed at other metal: ligand ratios. The volume of samples is generally 2 – 20 mL and the temperature of the samples must be kept constant (25 °C). The solutions are stirred and $N_2$ or Ar is bubbled through to prevent absorption of the $CO_2$ during the titration.

The complex formation reactions of the macrocyclic ligands are generally slow at low pH values and so for determining the stability constants the "out-of-cell" (or batch) method is used.[145] Several samples are prepared in the pH range where complexation equilibria exist and the closed samples are kept until the equilibria are reached. Reliable protonation and stability constants can be obtained by pH-potentiometry in the pH range of about 1.7 – 12. To determine the stability constants, competition reactions can also be used if the complexation equilibria exist outside of the well-measurable pH range (*e.g.* at pH 2). For the competition, another metal ion (or another ligand) can be used if the

stability constants of their complexes are known and in their presence the equilibria are shifted into the well-measurable pH range.

The protonation of ligands and the formation of complexes can be studied by spectrophotometry if the metal ions or ligands have absorption band/s in the UV-vis range.[146] The absorbance values at each wavelength can be expressed by Equation 5.2.9:

$$A = \sum_{1}^{n} \varepsilon_i x_i l \qquad (5.2.9)$$

where $l$ is the path length of the cell, and $\varepsilon_i$ and $x_i$ are the molar absorptivity and molar fraction of the species $i$, respectively. Equation 5.2.9 can be used to calculate the protonation constants of the ligand or the stability constants of complexes if the molar fractions are expressed with protonation or the stability constants and spectrophotometric measurements are performed at different pH values.[146] Spectrophotometric studies can be carried out at higher $H^+$ or $OH^-$ concentrations, where pH-potentriometry cannot be used.

Multinuclear NMR spectroscopy is a convenient method to study the protonation of ligands and the formation of complexes. To obtain information about the protonation sites and protonation constants of the free ligand, the chemical shifts of the non-labile NMR active nuclei ($^1H$, $^{13}C$, $^{31}P$, *etc.*) are followed as a function of $H^+$ or $OH^-$ concentration. The NMR "titration curve" displays sharp changes at different pH values, which are related to the protonation/deprotonation of the ligand. The protonation sites and protonation sequence of the ligand can be identified by the assignments of the signals. Since the protonation/deprotonation of the ligand is generally fast on the NMR time scale, the chemical shifts of the observed signals represent a weighted average of the shifts of the different $H_iL$ species involved in a specific protonation step (Equation 5.2.10).[147]

$$\delta_{obs} = \sum_{1}^{n} x_{H_iL} \delta^{H_iL} \qquad (5.2.10)$$

where $i = 0, 1, 2, \ldots, n$, $\delta_{obs}$ is the observed chemical shift of a given signal, and $x_{H_iL}$ and $\delta^{H_iL}$ are the molar fraction and the chemical shift of the involved species, respectively. After expressing the molar fraction with the $[L]_t$ and the protonation constants, the obtained equation is suitable for the calculation of protonation constants and $\delta^{H_iL}$ values of the ligand.[147]

*b. Calculation of the equilibrium constants*:
To calculate the equilibrium constants (protonation and stability constants), several computer programs (MINIQUAD, PSEQUAD, SUPERQUAD, HYPERQUAD OPIUM, *etc.*) are available, which generally operate on the basis of nonlinear least squares principle.[148–150]

In the calculations a chemical model is presumed, which involves the chemical reactions for the formation of species ($M_pH_qL_r$) from the components



(M, L, H) characterized by the cumulative formation constants $\beta_{pqr}$. The programs calculate a series of datapoints (*e.g.* titration volume, chemical shift, *etc.*) from the analytical concentrations of the components and series of measured data (*e.g.* pH values) using the model. The differences between the measured and calculated data (the residuals) are used for further calculations, when the minimum of the square sum of residuals is obtained by the variation of the $\beta_{pqr}$ values.

$$\beta_{pqr} = \frac{[M_pH_qL_r]}{[M]^p[H^+][L]^r} \qquad (5.2.11)$$

The programs calculate the statistical data characterizing the goodness of fitting ($\chi^2$ or the standard deviation of fitted data) and the reliability of the estimated $\beta_{pqr}$ constants. The calculations can be repeated with different models and the model that has the best statistical parameters is accepted. The individual stability constants are calculated from the log $\beta_{pqr}$ values with the related protonation and equilibrium constants. The standard deviation values calculated for the stability constants originate from random experimental error and reflect only a part of the total uncertainty. The true errors of the calculated equilibrium constants can be evaluated by comparing the constants obtained with different methods, or obtained in independent laboratories.

### 5.2.3.2 Protonation Sequence and Protonation Constants of the DTPA and DOTA Derived Ligands

The protonation constants of the DTPA and DOTA based ligands, defined by Equation 5.2.5, are listed in Table 5.2.1 and 5.2.2.

The basicity of the donor atoms of DTPA and DOTA derived ligands are affected by several factors, such as the strength of H-bonds between the non-bonding electron pairs of the donor atoms and the protonated donor atoms, the electrostatic repulsion between the protonated donor atoms, and the electron donating or withdrawing effects of the neighbouring groups. The contributions of the different effects determine the protonation constant values.

The data in Table 5.2.1 show that the first three protonation constants of the DTPA derivatives are markedly influenced by the nature of the substituent/s (for the structures of the ligands please refer to Schemes 5.2.10 and 5.2.11). The processes related to the variation of the protonation constants were identified by pH dependent NMR studies of the ligands.[147] Starting with the deprotonated ligand, the protonation sequence of the DTPA derivatives generally follows this trend: the first protonation takes place at the central nitrogen atom. The second protonation occurs at one of the terminal nitrogens with the concurrent shift of proton from the central nitrogen to the other terminal nitrogen due to the electrostatic repulsion between the two protons. The third protonation occurs at the central nitrogen (with the partial protonation of the carboxylate attached to the central nitrogen). Further protonations take place at

**Table 5.2.1** Protonation constants of the DTPA derivate ligands at 25 °C.

| *Ligands* | *Electrolyte* | $logK_1^H$ | $logK_2^H$ | $logK_3^H$ | $logK_4^H$ | $logK_5^H$ | *Method* | *Ref.* |
|---|---|---|---|---|---|---|---|---|
| DTPA | 0.1 M KCl | 10.41 | 8.37 | 4.09 | 2.51 | 2.04 | pH | 206 |
| BOPTA | 0.1 M KCl | 10.71 | 8.27 | 4.35 | 2.83 | 2.07 | pot.(20 °C) | 43,207 |
| | – | 10.51 | 8.17 | 4.19 | 1.94 | 1.5 | NMR | |
| EOB-DTPA | 0.1 M KCl | 10.95 | 8.62 | 4.23 | 2.80 | 2.06 | pH | 208 |
| MS-325 | 0.1M $Me_4NCl$ | 11.15 | 8.62 | 4.51 | 2.96 | 2.37 | pH | 209 |
| *p*-$NO_2$-Bn-DTPA | 0.1M $Me_4NCl$ | 11.16 | 8.30 | 4.44 | 2.75 | 2.53 | pH | 105 |
| CHX-DTPA | 0.1M $Me_4NCl$ | 12.3 | 9.24 | 5.23 | 3.32 | 2.18 | pH | 105 |
| *p*-$NO_2$-Bn-CHX-DTPA | 0.1M $Me_4NCl$ | 12.3 | 8.99 | 4.99 | 2.84 | 2.35 | pH | 105 |
| DTPA-N-MA | 0.1 M KCl | 10.18 | 6.19 | 3.55 | 2.0 | – | pH | 48 |
| DTPA-N'-MA | 0.1 M KCl | 10.04 | 8.41 | 2.73 | 1.94 | – | pH | 48 |
| DTPA-BMA | 0.1 M NaCl | 9.37 | 4.38 | 3.31 | 1.43 | – | pot. | 140b |
| DTPA-BMEA | 0.1 M $NaClO_4$ | 9.26 | 4.54 | 3.40 | 2.09 | – | pH | 210,99 |
| | 0.1 M $NaClO_4$ | 9.33 | 4.36 | – | – | – | NMR | |
| DTPA-B(BbuA) | 0.1 M KCl | 9.77 | 6.72 | 4.08 | – | – | pH | 158 |
| DTPA-BBzA | 0.1 M KCl | 9.39 | 4.57 | 3.54 | – | – | pH | 101 |
| DTPA-BAMA | 0.1 M KCl | 9.35 | 4.85 | 3.73 | – | – | pot. | 211 |
| DTPA-TrA | 0.1 M KCl | 8.50 | 6.53 | 2.82 | – | – | pH | 158 |
| 15-DTPA-EAM | 0.1M KCl | 9.45 | 4.21 | 3.39 | 1.96 | – | pot. | 45, 212 |
| EPTPA | 0.1 M $Me_4NNO_3$ | 10.60 | 8.92 | 5.12 | 2.80 | – | pot. | 159 |
| *p*-$NO_2$-Bn-EPTPA | 0.1 M $Me_4NCl$ | 10.86 | 8.91 | 4.70 | 3.25 | 2.51 | pH | 160 |

the carboxylate groups of the terminal nitrogen atoms. The protonation sequence is modified by the replacement of the central carboxylate with an amide group. For DTPA-*N'*-MA both the first and second protons protonate the terminal nitrogens. The third proton presumably protonates the central nitrogen.[48] The lower basicity of the central nitrogen of the DTPA-*N'*-MA and the terminal nitrogen of the DTPA-*N'*-MA can be interpreted by the electron withdrawing effect of the amide group and the formation of weak H-bonds between the amine nitrogen and the amide hydrogens, respectively. The replacement of two carboxylates with two amides at the terminal nitrogen atoms (*e.g.* DTPA-BMA, DTPA-BMEA, DTPA-BBzA, 15-DTPA-EAM) results in further lowering of the basicity of the nitrogens. The substitution of both amide hydrogens with alkyl (or aryl) groups increases the basicity of the

**Table 5.2.2** Protonation constants of the DOTA derivative ligands at 25 °C.

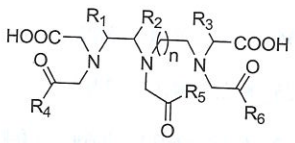
| *Ligands* | *Electrolyte* | $log\ K_1^H$ | $log\ K_2^H$ | $log\ K_3^H$ | $log\ K_4^H$ | $log\ K_5^H$ | $log\ K_6^H$ | $log\ K_7^H$ | *Method* | *Ref.* |
|---|---|---|---|---|---|---|---|---|---|---|
| DOTA | 0.1M $Me_4NCl$ | 12.6 | 9.70 | 4.50 | 4.14 | 2.32 | – | – | [b]pH | 182 |
| *p*-$NO_2$-Bn-DOTA | 1.0M $Me_4NCl$ | 10.93 | 9.14 | 4.44 | 4.19 | 2.33 | 1.4 | – | [b]pH | 198 |
| DO3A | 0.1M $Me_4NCl$ | 11.59 | 9.24 | 4.43 | 3.48 | – | – | – | [b]pH | 197, 213 |
| HP-DO3A | 0.1M $Me_4NCl$ | 11.96 | 9.43 | 4.30 | 3.26 | – | – | – | [b]pH | 197, 213 |
| BT-DO3A | 0.1M $Me_4NCl$ | 11.75 | 9.23 | 4.13 | 2.97 | – | – | – | [b]pH | 79 |
| P-730 | 0.1 M $Me_4NCl$ | 12.22 | 9.18 | 6.29 | 5.69 | 5.04 | 4.80 | 4.17 | [a]pot. | 214 |
| DO2A | 0.1M $Me_4NCl$ | 10.94 | 9.55 | 3.85 | 2.55 | – | – | – | [b]pH | 215 |
| DO3AP | 0.1M $Me_4NCl$ | 13.83* | 10.35 | 6.54 | 4.34 | 3.09 | 1.63 | 1.07 | [a]pot. | 155 |
| DO2A2P | 1.0 M KCl | 12.6 | 11.43 | 5.95 | 6.15 | 2.88 | 2.77 | – | [b]pH | 82 |
| | 1.0 M KCl | 12.8 | 11.7 | 5.93 | 6.15 | – | – | – | [c]NMR | |
| DOA3P | 1.0 M KCl | 13.6* | 11.42 | 7.69 | 6.33 | 5.13 | 2.73 | 1.62 | [b]pH | 83 |
| DOTP | 0.1 M $Me_4NNO_3$ | 14.65* | 12.4* | 9.28 | 8.09 | 6.12 | 5.22 | – | [b]pH | 152,153 |
| $DO3AP^{ABn}$ | 0.1 M $Me_4NCl$ | 12.55 | 9.60 | 5.11 | 4.11 | 2.71 | 1.54 | – | [a]pot. | 154 |
| DOTEP | 0.1 M $KNO_3$ | 10.94 | 8.24 | 3.71 | – | – | – | – | [b]pH | 216 |
| | 1.0 M KCl | 10.87 | 8.21 | – | – | – | – | – | [c]NMR | |
| PCTA | 1.0 M KCl | 11.36 | 7.35 | 3.83 | 2.12 | 1.29 | – | – | [b]pH | 87 |
| *p*-$NO_2$-Bn PCTA | 1.0 M KCl | 11.29 | 6.70 | 3.96 | 2.08 | 1.82 | – | – | [b]pH | 131 |
| DOTAM | 1.0 M KCl | 9.08 | 6.44 | – | – | – | – | – | [b]pH | 113 |
| DTMA | 1.0 M KCl | 9.56 | 5.95 | – | – | – | – | – | [b]pH | 113 |
| TRITA | 0.1 M $Me_4NCl$ | 11.81 | 9.21 | 4.03 | 2.62 | – | – | – | [a]pot. | 145,217 |

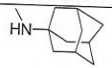
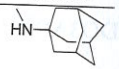
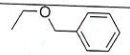
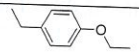
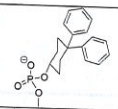
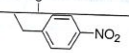
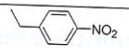
[a]potentiometry.
[b]pH-potentiometry.
[c]NMR titration.

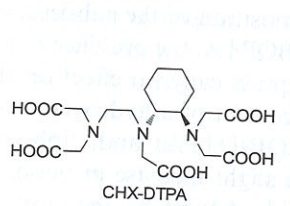
terminal nitrogen atoms because of the formation of fewer hydrogen bonds (DTPA-B(BBuA) and DTPA-BMA).[99] The effect of the substituents attached to the ligand backbone is mainly controlled by the position of the substitution and the properties of the substituent. In the case of BOPTA, the presence of the benzyl-oxy-methyl group on the pendant arm has practically no effect on the basicity of the nitrogen atoms. However, the replacement of a hydrogen atom of the ligand backbone by the etoxy-benzyl- (EOB-DTPA) and diphenyl-cyclohexyl-phosphine groups (MS-325) results in a slight increase in the basicity of the central nitrogen atom which is probably caused by the electron donating behaviour of the substituents. The replacement of an ethylene group of the DTPA with cyclohexyl moiety results in an increase of the basicity of the

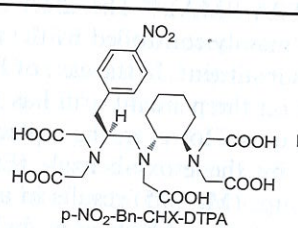
central and the terminal nitrogen atoms because of the electron donating behaviour of the alkyl ring and the favourable position of the nitrogen atoms for hydrogen bond formation.[105]

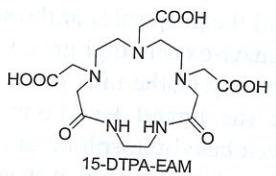
DOTA based ligands most commonly contain amino nitrogen, carboxylate oxygen, phosphonate or phosphinate oxygen, and amide oxygen (and nitrogen) donor atoms. The NMR titration of DOTA indicates that the first and second



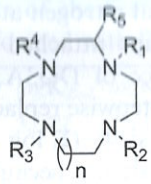
| Ligands | n | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ |
|---|---|---|---|---|---|---|---|
| DTPA | 1 | -H | -H | -H | -OH | -OH | -OH |
| DTPA-N-MA | 1 | -H | -H | -H | -OH | -OH | -NH-Me |
| DTPA-N'-MA | 1 | -H | -H | -H | -OH | -NH-Me | -OH |
| DTPA-BMA | 1 | -H | -H | -H | -NH-Me | -OH | -NH-Me |
| DTPA-BMEA | 1 | -H | -H | -H | -NH-Et-O-Me | -OH | -NH-Et-O-Me |
| DTPA-B(BbuA) | 1 | -H | -H | -H | $-N(n\text{-}Bu)_2$ | -OH | $-N(n\text{-}Bu)_2$ |
| DTPA-BBzA | 1 | -H | -H | -H | -NH-Bn | -OH | -NH-Bn |
| DTPA-BAMA | 1 | -H | -H | -H |  | -OH |  |
| DTPA-TrA | 1 | -H | -H | -H | $-N(n\text{-}Bu)_2$ | -NH-Me | $-N(n\text{-}Bu)_2$ |
| BOPTA | 1 | -H | -H |  | -OH | -OH | -OH |
| EOB-DTPA | 1 | -H |  | -H | -OH | -OH | -OH |
| MS-325 | 1 |  | -H | -H | -OH | -OH | -OH |
| $p\text{-}NO_2\text{-}Bn\text{-}DTPA$ | 1 |  | -H | -H | -OH | -OH | -OH |
| EPTPA | 2 | -H | -H | -H | -OH | -OH | -OH |
| $p\text{-}NO_2\text{-}Bn\text{-}EPTPA$ | 2 |  | -H | -H | -OH | -OH | -OH |

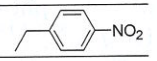
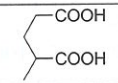
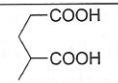
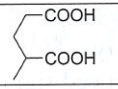
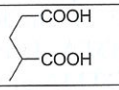
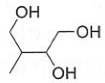
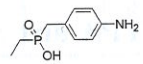

CHX-DTPA

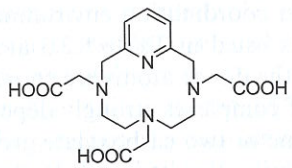

p-$NO_2$-Bn-CHX-DTPA

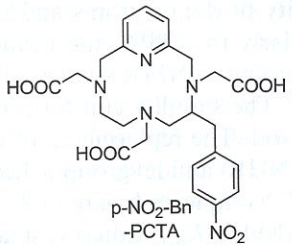

15-DTPA-EAM

**Scheme 5.2.10** Structure of the DTPA derivative ligands discussed in the current Chapter.



| Ligands | n | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|---|
| DOTA | 1 | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | -H |
| $p\text{-}NO_2\text{-}Bz\text{-}DOTA$ | 1 | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ |  |
| P730 | 1 |  |  |  |  | -H |
| HP-DO3A | 1 |  | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | -H |
| BT-DO3A | 1 |  | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | -H |
| DO3A | 1 | -H | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | -H |
| DO2A | 1 | -H | $-CH_2\text{-}COOH$ | -H | $-CH_2\text{-}COOH$ | -H |
| DO3AP | 1 | $-CH_2\text{-}PO_3H_2$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | -H |
| $DO3AP^{ABn}$ | 1 |  | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | -H |
| DO2A2P | 1 | $-CH_2\text{-}PO_3H_2$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}PO_3H_2$ | $-CH_2\text{-}COOH$ | -H |
| DOA3P | 1 | $-CH_2\text{-}PO_3H_2$ | $-CH_2\text{-}PO_3H_2$ | $-CH_2\text{-}PO_3H_2$ | $-CH_2\text{-}COOH$ | -H |
| DOTP | 1 | $-CH_2\text{-}PO_3H_2$ | $-CH_2\text{-}PO_3H_2$ | $-CH_2\text{-}PO_3H_2$ | $-CH_2\text{-}PO_3H_2$ | -H |
| DOTEP | 1 | $-CH_2\text{-}PO_2H\text{-}CH_2\text{-}CH_3$ | $-CH_2\text{-}PO_2H\text{-}CH_2\text{-}CH_3$ | $-CH_2\text{-}PO_2H\text{-}CH_2\text{-}CH_3$ | $-CH_2\text{-}PO_2H\text{-}CH_2\text{-}CH_3$ | -H |
| DOTAM | 1 | $-CH_2\text{-}CO\text{-}NH_2$ | $-CH_2\text{-}CO\text{-}NH_2$ | $-CH_2\text{-}CO\text{-}NH_2$ | $-CH_2\text{-}CO\text{-}NH_2$ | -H |
| DTMA | 1 | $-CH_2\text{-}CO\text{-}NH\text{-}CH_3$ | $-CH_2\text{-}CO\text{-}NH\text{-}CH_3$ | $-CH_2\text{-}CO\text{-}NH\text{-}CH_3$ | $-CH_2\text{-}CO\text{-}NH\text{-}CH_3$ | -H |
| TRITA | 2 | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | $-CH_2\text{-}COOH$ | -H |


PCTA


p-$NO_2$-Bn-PCTA

**Scheme 5.2.11** Structure of the macrocyclic ligands discussed in the current Chapter.

protonations occur at two diagonal nitrogen atoms of the ring followed by the protonation of carboxylate groups attached to the non-protonated nitrogens.[151] The protonation sequence of DO3A, HP-DO3A and BT-DO3A is similar to that of DOTA. By the stepwise replacement of the acetate arms with phosphonate groups, the total basicity ($\Sigma$ log $K_i^H$), the log $K_1^H$ and log $K_2^H$ values of the ligands increase gradually because of the presence of the more basic phosphonate/s and the formation of a strong hydrogen bond between the protonated nitrogen atoms and phosphonate group/s. The protonation scheme of the phosphonate derivatives is somewhat different from that of DOTA.[81,83,132,152–155]

The presence of amide groups also decreases the basicity of the amine nitrogens of DOTA derivatives.[99,156] The first two protonation constants of DOTAM and DTMA are indeed significantly lower than those of DOTA. The incorporation of a pyridine ring into the macrocycle increases the rigidity of the ligand and decreases the basicity of the donor atoms due to the electron withdrawing effect of the aromatic ring. The protonation schemes of the PCTA and DOTA are slightly different. The first protonation of PCTA occurs at the nitrogen atom opposite to the pyridine ring. The addition of a second proton results in the protonation of the tertiary nitrogen atoms, positioned *trans*- to each other. The third protonation occurs at the carboxylate pendant of the non-protonated nitrogen atom. Further protonations of PCTA occur at the non-protonated carboxylate groups.[85]

### 5.2.3.3 Complexation Equilibria of the DTPA and DOTA Based Ligands

The ligands DTPA, DOTA, and their derivatives form high stability ML complexes with trivalent metal ions in which generally all the donor atoms of the hepta- or octadentate ligands are coordinated. The stability constants (log $K_{ML}$) of the lanthanide$^{III}$ and some divalent metal complexes formed with DTPA and DOTA derivative ligands are listed in Tables 5.2.3 and 5.2.4.

The stability constants of complexes of the ligands DTPA, EOB-DTPA, BOPTA and MS-325, which contain three amine nitrogen and five carboxylate oxygen donor atoms, are very similar, showing that the side chain only slightly affects the basicity of donor atoms and the coordination environment of the metal ions. Similarly to DTPA, the ligands listed in Table 5.2.3 are all octadentate and according to NMR studies, all the donor atoms are coordinated to the $Ln^{3+}$ ions.[157] The stability constants of complexes strongly depend on the charge of the ligand. The replacement of one or two carboxylate group/s with non-ionic –CO–NHR amide group/s leads to the decrease of the stability constants of $Gd^{3+}$ complexes by about 2 – 3 and 5 – 6 log $K$ units, respectively. The decrease in the log $K_{ML}$ values is smaller when the amide group does not contain amide hydrogen (*e.g.* DTPA-B(BBuA)) since in this case the H-bond cannot form and thus the coordinated amide oxygen is more basic. (The electron withdrawing effect of the amide groups increases in the following

**Table 5.2.3** Stability constants of the $Ca^{2+}$, $Eu^{3+}$, $Gd^{3+}$, $Yb^{3+}$, $Zn^{2+}$ and $Cu^{2+}$ complexes formed with DTPA and their derivates at 25 °C.

| *Ligands* | *Electrolyte* | *log$K_{CaL}$* | *log$K_{EuL}$* | *log$K_{GdL}$* | *log$K_{YbL}$* | *log$K_{ZnL}$* | *log$K_{CuL}$* | *Ref.* |
|---|---|---|---|---|---|---|---|---|
| DTPA | 0.1 M KCl | 10.75 | 22.39 | 22.46 | 22.62 | 18.6 | 21.5 | 206 |
| BOPTA | 0.1 M KCl | – | – | 22.59 | – | 17.04 | 21.94 | 43,218 |
| EOB-DTPA | 0.1 M KCl | 11.82 | 23.1 | 23.6 | 23.0 | 18.78 | 20.2 | 208 |
| MS-325 | 0.1M $NaClO_4$ | 10.45 | 22.21 | 22.06 | – | 17.82 | 21.3 | 209 |
| DTPA-N-MA | 0.1 M KCl | – | 18.70 | 19.40 | 19.5 | 16.00 | 18.71 | 48 |
| DTPA-N'-MA | 0.1 M KCl | – | 19.90 | 19.0 | 20.4 | 16.82 | 18.50 | 48 |
| DTPA-BMA | 0.1 M NaCl | 7.17 | – | 16.85 | – | 12.04 | 13.05 | 140b |
| DTPA-BMEA | 0.1 M $NaClO_4$ | – | – | 16.84 | – | – | – | 99 |
| DTPA-B(BbuA) | 0.1 M KCl | – | – | 19.0 | – | 16.38 | 17.85 | 158 |
| DTPA-BBzA | 0.15 M NaCl | 7.13 | – | 16.48 | – | 11.98 | 12.28 | 101 |
| DTPA-BAMA | 0.1 M KCl | 7.49 | – | 16.85 | – | 11.9 | 12.86 | 211 |
| DTPA-TrA | 0.1 M KCl | – | – | 17.93 | – | 13.36 | 13.75 | 158 |
| 15-DTPA-EAM | 0.1M KCl | 5.65 | 11.7 | 11.4 | 10.6 | 12.08 | 15.1 | 45, 212 |
| EPTPA | 0.1 M $Me_4NNO_3$ 14.45 | – | 22.77 | – | 18.59 | 19.31 | | 159 |
| *p*-$NO_2$-Bn-EPTPA | 0.1 M $Me_4NCl$ | 9.38 | – | 19.20 | – | 16.01 | 18.47 | 160 |

Some other log $K_{ML}$ values of M(DTPA) complexes: ScL: 23.9, InL:29.5, GaL: 23.32 (0.1 M KCl and $KNO_3$; 25 °C)[219,220]; Y(p-$NO_2$-Bn-DTPA): 21.5, Y(CHX-DTPA): 24.2, Y(*p*-$NO_2$-Bn-CHX-DTPA): 24.4 (0.1 M $NaClO_4$; 25 °C)[105].

order: –CO–$NR_2$ < –CO–NHR < –CO–$NH_2$.) The coordination number of $Cu^{2+}$ and $Zn^{2+}$ is lower than the denticity of DTPA, so the free donor atoms can coordinate to another $Cu^{2+}$ or $Zn^{2+}$ ion, giving rise to dinuclear complexes. The formation of similar dinuclear complexes was not detected for the DTPA–bis-amide derivatives.[140] However, more recently the formation of binuclear $Cu_2L$ and $Zn_2L$ has been reported with DTPA–bis(butyl) amide and DTPA–bis(bis-butyl) amide.[158]

The log $K_{ML}$ values of the EPTPA complexes are higher than expected, because the presence of a six-membered chelate ring should normally result in the decrease of the stability.[159] However, the complexes of *p*-$NO_2$-Bn-EPTPA are less stable than those of DTPA, showing the expected loss in the stability.[160]

The stability constants of the complexes of DOTA derivatives strongly depend on the number of pendant arms attached to the cyclen ring and on the charge of the ligand. The log $K_{ML}$ values decrease in the order DOTA > DO3A > DO2A. The stability constants of the Ln(DOTA) complexes increase

**Table 5.2.4** Stability constants of the $Ca^{2+}$, $Eu^{3+}$, $Gd^{3+}$, $Yb^{3+}$, $Zn^{2+}$ and $Cu^{2+}$ complexes formed with DOTA and their derivates at 25 °C.

| *Ligands* | *Electrolyte* | *log* $K_{CaL}$ | *log* $K_{EuL}$ | *log* $K_{GdL}$ | *log* $K_{YbL}$ | *log* $K_{ZnL}$ | *log* $K_{CuL}$ | *Ref.* |
|---|---|---|---|---|---|---|---|---|
| DOTA | 0.1M NaCl | 16.37 | 23.5 | 24.7 | 25.0 | 18.7 | 22.72 | 145,217,221 |
| *p*-$NO_2$-Bn-DOTA | 1.0M $Me_4NCl$ | – | – | 24.2 | – | – | – | 198 |
| DO3A | 0.1M $Me_4NCl$ | – | 20.69 | 21.0 | – | – | – | 197, 213 |
| HP-DO3A | 0.1M $Me_4NCl$ | – | – | 23.8 | – | 17.32[218] | 20.55[218] | 197, 213 |
| BT-DO3A | 0.1M $Me_4NCl$ | 14.3 | 21.2 | 20.8 | – | 19.0 | 21.1 | 79 |
| P-730 | 0.1M $Me_4NCl$ | – | 24.01 | 24.03 | – | – | – | 214 |
| DO2A | 0.1M $Me_4NCl$ | 7.16 | 12.99 | 13.06 | 13.26 | – | – | 215 |
| DO3AP | 0.1M $Me_4NCl$ | – | 27.8 | 27.5 | – | – | – | 155 |
| DO2A2P | 1.0 M KCl | 15.1 | 25.6 | 25.7 | – | 22.5 | 24.9 | 81 |
| DOA3P | 1.0 M KCl | 14.5 | 27.5 | 27.3 | – | 22.9 | 27.3 | 83 |
| DOTP | 0.1 M $Me_4NNO_3$ | 11.12 | 28.1 | 28.8 | 29.5 | 24.8 | 25.4 | 132,152,153 |
| $DO3AP^{ABn}$ | 0.1M $Me_4NCl$ | – | 25.0 | 24.04 | – | – | – | 154 |
| DOTEP | 0.1 M $KNO_3$ | 9.39 | – | 16.5 | – | 15.8 | 19.57 | 216 |
| PCTA | 1.0 M KCl | 12.72 | 20.26 | 20.39 | 20.63 | 20.48 | 18.79 | 87 |
| *p*-$NO_2$-Bn PCTA | 1.0 M KCl | 12.72 | 19.02 | 19.42 | – | 21.36 | 19.11 | 131 |
| DOTAM | 1.0 M KCl | 10.32 | 13.80 | 13.12 | – | 13.77 | 14.50 | 113 |
| DTMA | 1.0 M KCl | 10.11 | 13.67 | 13.54 | – | 13.66 | 14.61 | 113 |
| TRITA | 0.1 M KCl | 11.99 | – | 19.17 | – | 18.04 | 22.49 | 145,217 |

Some other log $K_{ML}$ values of M(DOTA) complexes: ScL: 24.2, InL:23.9, GaL:21.33 (0.1 M KCl, 25 °C).[217,222]

from the $La^{3+}$ to the $Sm^{3+} - Eu^{3+}$, indicating that the best fit of the $Ln^{3+}$ ions in the coordination cage of DOTA is realised for the ions $Sm^{3+} - Eu^{3+}$. The replacement of one carboxylate with an alcoholic OH results in the decrease of the log $K_{ML}$ values by 1 – 3 log $K$ unit (HP-DO3A and BT-DO3A).

The substitution of phosphonate groups for the acetate arms results in the increase of the stability constants of complexes in the following order: DO2A2P < DOA3P < DOTP (the log $K_{ML}$ values of the DOA3P complexes are larger than expected in comparison with those of the DOTP complexes). The replacement of the carboxylate groups of DOTA with amide groups results in a significant drop in the log $K_{ML}$ values. The amide groups decrease the basicity of the ring nitrogens and the stability constants of the complexes are very low. However, the trend of the stability constants of the $Ln(DOTAM)^{3+}$ complexes is similar to that of the $Ln(DOTA)^-$ complexes. The stability constants increase from $La^{3+}$ to $Sm^{3+} - Eu^{3+}$ and for the heavier elements the log $K_{ML}$ values are similar.[113,161] In these $Ln(DOTAM)^{3+}$ complexes the $Ln^{3+}$ ion is in the coordination cage defined by the 4 macrocyclic N atoms and the 4 O atoms of the amide groups. The ninth coordination site is occupied by an inner sphere water molecule. Generally, the water exchange rate of Ln-DOTA tetraamide complexes is significantly slower than that of the corresponding DOTA complexes and in particular, the Eu-complexes have the lowest water exchange rate. The favorable paramagnetic properties of $Eu^{3+}$ (relatively large Ln induced shift and negligible relaxation enhancement effects) combined with the extremely slow water exchange rate makes Eu-DOTA tetraamides ideal candidates for paramagnetic chemical exchange (PARACEST) imaging agents.[162–164]

### 5.2.3.4 Equilibria of the Transmetallation Reactions of the DTPA and DOTA Derivative Complexes

The metal complexes of DTPA and DOTA derivatives used in biomedicine may react *in vivo* with endogenous metals and ligands. The body fluids, where these reactions occur, are very complicated systems, which contain several metal ions and a great number of complex forming ligands. The new species formed in these reactions cannot be exactly predicted, but some assumptions can be made on the basis of our experiences and knowledge in chemistry. The concentration of various complexes, the "species distribution", can be calculated using the known stability constants. Such calculations give some information about the *in vivo* fate of the complexes used as imaging agents. These species distribution calculations are particularly interesting for $Gd^{3+}$-based MRI contrast agents, because the amount of $Gd^{3+}$ administered into the body is high (0.1 – 0.3 mmol $Gdkg^{-1}$ body weight), so decomplexation as low as 1–2% of the $Gd^{3+}$ complex would result in a relatively significant amount of residual $Gd^{3+}$ in the body.

The study of the complexation equilibria in the blood plasma has attracted renewed interest lately due to a new disease, Nephrogenic Systemic Fibrosis (NSF), which is assumed to be related to the release of $Gd^{3+}$ from CAs.[165–167] The elimination of the CAs from the body is generally fast (the half-time of excretion, $t_{1/2} = 1.5$ h) and so the extent of de-chelation in the body due to the slow dissociation of $Gd^{3+}$ complexes is negligible. However, in patients with severe renal insufficiency, the half-time of elimination (with dialysis) is 30–40 h,[165–167] and consequently the $Gd^{3+}$ complex spends longer in the body and the extent of dissociation can be observable. Practically all known cases of NSF are associated with the use of the $Gd^{3+}$ complexes of DTPA derivatives, first of all Gd(DTPA-BMA),[165–168] which has the lowest stability constant among commercial CAs. The $Gd^{3+}$ complexes formed with the macrocyclic DOTA derivatives are extremely inert, so their de-chelation in the body does not take place.

It is generally assumed that the *in vivo* dissociation of the $Gd^{3+}$ complexes occurs through transmetallation reactions, first of all with $Zn^{2+}$.[169] However, the equilibrium of:

$$Gd(DTPA)^{2-} + Zn^{2+} \rightleftharpoons Zn(DTPA)^{3-} + Gd^{3+} \qquad (5.2.12)$$

is shifted to the left hand side, because log $K_{GdL} >>$ log $K_{ZnL}$. Transmetallation reactions with $Zn^{2+}$ occur only in the presence of a second ligand (*e.g.* citrate), which forms more stable complexes with $Gd^{3+}$ than with $Zn^{2+}$:

$$Gd(DTPA)^{2-} + Zn(cit)^{-} \rightleftharpoons Zn(DTPA)^{3-} + Gd(cit) \qquad (5.2.13)$$

This is well illustrated by the following example: if $[Gd(DTPA)^{2-}] = 0.3$ mM and $[Zn^{2+}] = 0.01$ mM, pH = 7.4, then in the equilibrium (Equation 5.2.11) only 4% of the $Zn^{2+}$ is in the form of $Zn(DTPA)^{3-}$. If citrate is also present at a concentration of [Cit] = 0.11 mM, then 75% of the $Zn^{2+}$ is in the form of $Zn(DTPA)^{3-}$ and 2.5% of the $Gd^{3+}$ forms Gd(cit). These data clearly show that the transmetallation reactions and the release of $Gd^{3+}$ from CAs can take place in the blood plasma, but for the calculations of the species distribution, some plasma models should be used.

The blood plasma model, developed by *May et al.*,[170] takes into account seven metal ions and 40 endogenous ligands, which can form more than 5000 different complexes. The program ECCLES calculates the concentration of all species formed in the equilibrium, using the stability constants of complexes and the concentration of the components.[170] This blood plasma model was applied for the $Gd(DTPA)^{2-}$ as CA, but only the free $Gd^{3+}$ concentration ($10^{-13}$ M, if $[Gd(DTPA)^{2-}] = 1$mM) was reported; the formation of other $Gd^{3+}$ containing species was not mentioned.[171] A simplified blood plasma model does not predict significant de-chelation of Gd(DTPA-BMA).[140] However, the experimental data clearly contradict this result.[172] Another simplified model, where the more probable reactions were taken into account, indicated significant de-chelation of $Gd(DTPA)^{2-}$.[173] The results of the species distribution calculations performed with the stability constants are valid only for equilibrium systems. Since the de-chelation rates of $Gd^{3+}$-based CAs are much lower than the rates of their elimination from the body, the residual $Gd^{3+}$ in the body due to de-chelation is generally very low. The situation is different for patients with severe renal impairment, when the elimination of the CAs from the body is 20–30 times slower. The longer residence time of the complexes formed with DTPA derivatives can lead to partial dissociation, which should be considered when these complexes are used as CAs.

## 5.2.4 Kinetic Properties of the Complexes

The trivalent lanthanides, $Sc^{III}$, $Y^{III}$, $Ga^{III}$ and $In^{III}$ have closed, symmetric outer electronic shells so the kinetic behaviour of their complexes is determined mainly by the nature of the ligands and only slightly influenced by the size of the metal ions. The formation reactions of the complexes are fast with flexible multidentate ligands, but the complexation with rigid ligands is relatively slow.[174] The rapid complex formation is particularly important for the synthesis of radiopharmaceuticals, when the complex forming BFC ligand is bound to a temperature sensitive protein (*e.g.* to monoclonal antibody) and the radioisotope has a short half-life. In such cases the slow formation reaction and the need for high temperature processing is very unfavorable. The formation rates of the complexes of DTPA and its derivatives are high, but the kinetic inertness of these complexes is not always sufficient for nuclear medicine applications. The complexes formed with the macrocyclic DOTA derivatives are kinetically extremely inert, which makes them suitable for use in medicine. In order to delineate the problems of complexation, we shall discuss briefly the kinetics of formation of the DOTA based complexes. Since the kinetic inertness is a general requirement for imaging agents and in this respect the behaviour of DTPA and DOTA complexes differ considerably, the kinetics of their decomplexation will be discussed separately.

### 5.2.4.1 Formation Kinetics of Complexes of DOTA Derivatives

In the formation reactions of the complexes of DOTA, the rigid "spider like" structure of the ligand and the large difference between the first two and the remaining protonation constants (Table 5.2.2) play a crucial role. During the complex formation, the metal ion has to enter the coordination cage formed by the four nitrogen atoms of the 12-membered macrocycle and the four carboxylate oxygens of the four acetate groups. The formation of the DOTA complexes with $Ln^{3+}$, $In^{3+}$, $Sc^{3+}$ $In^{3+}$ and $Ga^{3+}$ ions is usually slow in the pH range 3–6, where DOTA is present in the form of protonated species $H_4DOTA$, $H_3DOTA$ and $H_2DOTA$. The kinetic data indicated the fast formation of a reaction intermediate which was detected by spectrophotometry,[175] $^1$H-NMR[176] and luminescence spectroscopy,[177,178] and EXAFS.[179] Due to the slow rearrangement at pH < 4, pH-potentiometric studies could be used to show that the intermediate was a diprotonated complex, $Ln(H_2DOTA)^{+*}$ and its stability constants could be determined.[175,178] Similarly, the stability constants of the intermediates $Ln(H_2L)^{*}$, formed with several other DOTA derivatives, have been determined.[155,180,181] Regarding the structure of the intermediate, it has been assumed that the $Ln^{3+}$ ion is positioned outside of the coordination cage and only the four carboxylates are coordinated to the $Ln^{3+}$, while the two protons are attached to two diagonal nitrogens.[175,178] With luminescence decay studies it was found that in the intermediate $Eu(H_2DOTA)^{+*}$ four or five water molecules remained coordinated to the $Eu^{3+}$ ion beside the four carboxylate oxygens.[177,178] At higher pH (pH > 7) monoprotonated intermediates, $Ln(HDOTA)^{*}$ are also formed.[182]

The mechanism of the formation of the $Ln(DOTA)^{-}$ complexes through the deprotonation of the di- and mono-protonated intermediates has been disputed.[24,176,183] However, all the authors agree that the rate determining step of the formation of the $Ln(DOTA)^{-}$ complexes is the loss of the proton from the monoprotonated $Ln(HDOTA)^{*}$ intermediate, which is followed by the rearrangement of the deprotonated intermediate to the product. Similar assumptions were made for the formation of complexes with other DOTA based ligands.[155,177,179,181] The deprotonation presumably occurs *via* the transfer of

proton from the nitrogen to a surrounding water molecule (or $OH^-$ ion) in which a carboxylate oxygen may play an important role. For the formation of the $Ln(DOTA)^-$ complexes the general base catalysis is valid.[182]

The formation of complexes of DOTA and DOTA derivatives occurs in a first-order reaction because the rate determining step is the deprotonation of the monoprotonated intermediate. The experimentally measured first-order rate constants, $k_{obs}$ values, are generally directly proportional to the $OH^-$ concentration, so $k_{obs} = k_{OH} \cdot [OH^-]$, where the $k_{OH}$ ($M^{-1}\ s^{-1}$) rate constant characterizes the formation rate. The $k_{OH}$ rate constants have been determined for the formation of a number of DOTA derivatives and are presented in Table 5.2.5.

A few general correlations can be established between the ligand structures and formation rates. The data presented in Table 5.2.5 reveal that for the complexes of ligands containing carboxylate and alcoholic OH oxygen donor atoms, the formation rates increase with the decrease in the size of the $Ln^{3+}$ ions. The replacement of the carboxylate group(s) of DOTA with alcoholic OH group(s) leads to a decrease in the formation rates of $Ln^{3+}$ complexes. The formation rates decrease progressively with the gradual replacement of the carboxylate groups with phosphonates, probably because of an increase in the stability of the intermediates (DO3AP > DO2A2P > DOA3P > DOTP). The substitution of a propionate group for an acetate in DOTA results in the ligand DO3A-Nprop, which forms complexes faster than DOTA.[184] The ligand TRITA obtained by the enlargement of the 12-membered ring of DOTA to a 13-membered macrocycle also forms complexes somewhat faster than DOTA.[180] The results are very promising with the ligand PCTA, which forms $Ln^{3+}$ complexes significantly faster than DOTA. The kinetic behaviour of the bifunctional *p*-$NO_2$-Bz-PCTA is similar; that is, the attachment of the side arm to the PCTA has practically no effect on the reactivity of the ligand.[87,131]

The mechanism of formation of the complexes of the DOTA-*tetra*amide ligands differs considerably from that of the DOTA complexes. In the reactions of DOTAM and DTMA the formation of a diprotonated intermediate cannot be detected in solution. The reactions occur with the direct encounter of the $Ln^{3+}$ ion and the fully deprotonated ligand (L), so the rate is directly proportional to $[Ln^{3+}]$ and [L].[113,185] DOTAM and DTMA contain four amide groups and the basicity of the amide oxygens (which coordinate to the $Ln^{3+}$ ions) is much lower than that of the carboxylate oxygens of DOTA. So the proton loss from the protonated intermediate with the proton transfer from a ring nitrogen to an amide oxygen is not possible. In this reaction the diprotonated intermediate is a "dead end" complex.[176] However, the diprotonated $[Gd(H_2DOTAM)(H_2O_4)](ClO_4)_5$ has been prepared in the solid state. The X-ray crystal structure has shown that the $Gd^{3+}$ is coordinated by four amide oxygens and four $H_2O$ molecules but the metal ion is located outside the coordination cage of the diprotonated $H_2DOTAM^{2+}$.[186] The structure of this complex is similar to that of the intermediates assumed in the formation reactions of the $Ln(DOTA)^-$ complexes.

**Table 5.2.5** Formation rate constants $k_{OH}$ ($M^{-1}\ s^{-1}$) of the DOTA derivative complexes.

| *Ligand* | $Ce^{3+}$ | $Eu^{3+}$ | $Gd^{3+}$ | $Yb^{3+}$ |
|---|---|---|---|---|
| DOTA[175] | $3.5 \times 10^6$ | $1.1 \times 10^7$<br>$7.2 \times 10^{6}$[178] | $5.9 \times 10^{6}$, [183] | $4.1 \times 10^7$<br>$9.3 \times 10^{7}$[183] |
| DO3A[183] | – | – | $2.1 \times 10^7$ | |
| DO2A[181] | $2.8 \times 10^5$ | – | – | $2.5 \times 10^5$ |
| HP-DO3A[183] | – | – | $1.2 \times 10^7$ | – |
| BT-DO3A[181] | $2.1 \times 10^6$ | $4.8 \times 10^6$ | – | $1.6 \times 10^7$ |
| DO3A-Nprop[184] | $1.7 \times 10^7$ | – | $2.9 \times 10^7$ | $3.9 \times 10^7$ |
| PCTA[87] | $9.7 \times 10^7$ | $1.7 \times 10^8$ | – | $1.1 \times 10^9$ |
| *p*-$NO_2$-Bz-PCTA[131] | $1.0 \times 10^7$ | $1.4 \times 10^8$ | – | $5.6 \times 10^8$ |
| TRITA[180] | $6.9 \times 10^6$ | | $2.6 \times 10^7$ | $5.0 \times 10^7$ |
| DO3AP[155] | $9.6 \times 10^6$ | $2.7 \times 10^{6}$[177] | $9.0 \times 10^4$ | – |
| DO2A2P[81] | $1.7 \times 10^5$ | – | $6.6 \times 10^4$ | – |
| DOA3P[83] | – | – | $2.2 \times 10^4$ | – |
| DOTP[223] | – | – | $7.2 \times 10^3$ | – |
| DOTPMB[223] | – | – | $1.3 \times 10^3$ | – |
| DOTAM[185] | $7.7 \times 10^3$ | $2.7 \times 10^4$ | – | $6.6 \times 10^3$ (Lu) |
| DTMA[113] | $3.0 \times 10^4$ | $4.8 \times 10^4$ | – | $6.5 \times 10^4$ (Lu) |

### 5.2.4.2 Kinetics of Dissociation of Complexes

The metal chelates used in medical diagnosis and therapy must be very inert, which means they essentially should not dissociate into the free metal ion and ligand once administered into the body. The $Gd^{3+}$ used for increasing the relaxation rate of protons in MRI must be complexed to ensure its elimination from the body. The complexes of radiometals bound to proteins or monoclonal antibodies, used for diagnosis or therapy, must be also very inert. The delivery of the radiopharmaceuticals to the target site is relatively slow and in the case of dissociation of the complex, the free radioactive isotope would damage the healthy organs and the efficiency of the treatment would also decrease.

The importance of the kinetic inertness of the $Gd^{3+}$ complexes was recognized quite early in the research into MRI contrast agents.[187] Animal experiments performed with different $Gd^{3+}$ containing CAs indicated that the elimination of $Gd^{3+}$ from the body of mice was not complete. The long term (14 days) whole body deposition was about 0.01 – 1.0% depending on the properties of the $Gd^{3+}$ complex.[187] The amount of the residual Gd was always lower when $Gd^{3+}$ complexes of macrocyclic ligands were used. For characterizing the rates of decomplexation, first order-rate constants ($k_d$) were determined in 0.1 M HCl, where the complexes are not thermodynamically stable. The half-time of dissociation ($t_{1/2} = 0.693/k_d$) for the complexes are given in parenthesis: $Gd(DOTA)^-$ (338 h); Gd(BT-DO3A) (43 h); Gd(HP-DO3A) (3.9 h); complexes of DTPA derivatives (<5 s).[169] These data clearly show that the complexes formed with the DOTA derivatives are extremely inert compared to the complexes of DTPA derivatives.

It is generally assumed that the de-chelation of complexes in biological fluids can take place *via* transmetallation reactions with $Zn^{2+}$ and $Cu^{2+}$. Another possibility is a ligand exchange reaction, when an endogenous ligand displaces the chelating agent in the $Gd^{3+}$ complex. Phosphate ions were found to compete with the open chain ligands, DTPA, and DTPA-BMA for the $Gd^{3+}$, because of the formation of insoluble $GdPO_4$ in the presence of $ZnCl_2$ or $CuCl_2$. Under similar conditions the formation of $GdPO_4$ was not observed from the $Gd^{3+}$ complexes of the macrocyclic DOTA and HP-DO3A.[188] Based on the formation of $GdPO_4$ precipitate, a simple relaxometric method was proposed by *Laurent et al.*,[189] for the comparison of the rates of de-chelation of $Gd^{3+}$ complexes. In phosphate buffer at pH = 7 the free $Gd^{3+}$, released from the $Gd^{3+}$ chelate in the presence of $Zn^{2+}$, forms $GdPO_4$, when the relaxivity of the solution decreases with time. In the case of $Gd(DOTA)^-$ and Gd(HP-DO3A) the dissociation of the complexes could not be detected.[189] This method is suitable for a fast comparison, but it cannot be used for kinetic studies, because $Zn_3(PO_4)_3$ precipitate is also formed in the reaction, and so the concentration of $Zn^{2+}$ decreases with time. Besides, the large phosphate excess significantly increases the rate of dissociation of the $Gd^{3+}$ complexes. The kinetic properties of the complexes formed with other lanthanide$^{III}$ and $Y^{3+}$ ions are very similar to those observed for the $Gd^{3+}$ complexes.[190]

### 5.2.4.3 Kinetics of Decomplexation of Complexes of DTPA Derivatives

The kinetics of metal exchange reactions of the aminopolycarboxyate complexes of transition metals, lanthanides and $Y^{3+}$ have been studied in detail for 40–50 years.[174] The isotopic exchange reactions of $Ln(EDTA)^-$ and $Ln(DTPA)^{2-}$ occur predominantly through proton assisted dissociation. The rates of transmetallation reactions were found to be inversely proportional to the stability constants of the complexes.[191] The kinetics of metal exchange reactions between the $Gd^{3+}$ complex of DTPA and DTPA derivatives and $Cu^{2+}$, $Zn^{2+}$ or $Eu^{3+}$ have been studied more recently.[158,173,192] These studies were carried out in the presence of $Cu^{2+}$, $Zn^{2+}$ or $Eu^{3+}$ excess. The rates of the exchange reactions can be expressed by Equation 5.2.14 ($k_d$ is the pseudo-first-order rate constant and $[LnL]_t$ is the total concentration of the complex.):

$$-\frac{d[LnL]_t}{dt} = k_d[LnL]_t \qquad (5.2.14)$$

The rates of metal exchange reactions have been studied by varying the $H^+$, $Zn^{2+}$, $Cu^{2+}$ or $Eu^{3+}$ concentration and following the reaction by spectrophotometry ($Cu^{2+}$, $Eu^{3+}$) or relaxometry ($Zn^{2+}$). The $k_d$ values increase with increasing $H^+$ and metal concentration, but at pH values higher than about 4.5–5 the $Cu^{2+}$ and $Zn^{2+}$ assisted reactions predominate. The increase of the $k_d$ values with increasing $[H^+]$ indicates that the transmetallation can take place



with the proton-assisted dissociation of the $Ln^{3+}$ complexes, which is followed by a fast reaction between the free ligand L and the $Zn^{2+}$ or $Cu^{2+}$. The effect of $H^+$ can be interpreted by the formation of a protonated complex, which dissociates faster than the non-protonated species because one or more functional group(s) are decoordinated by protonation:

$$LnL + H^+ \overset{K_{LnHL}}{\rightleftharpoons} LnHL \overset{k_{LnHL}}{\longrightarrow} Ln^3 + HL \qquad (5.2.15)$$

At pH < about 4, the $k_d$ values show a second order dependence on the $[H^+]$, which can be explained with the proton assisted dissociation of the monoprotonated complexes, but this pathway is not important at pH around 7. The increase in the $k_d$ values with increasing $Zn^{2+}$ or $Cu^{2+}$ concentration demonstrates the contribution of the reactions taking place with the direct attack of the exchanging metal $M^{2+}$ ($Zn^{2+}$ or $Cu^{2+}$) on the complex:

$$LnL + M^{2+} \overset{K_{LnLm}}{\rightleftharpoons} LnLM \overset{k_{LnLM}}{\longrightarrow} Ln^3 + ML \qquad (5.2.16)$$

The dinuclear complexes LnLM are formed in an equilibrium reaction but during the intramolecular rearrangement of the complex LnL, the functional groups of the ligand can be slowly transferred to the attacking $M^{2+}$ metal ion, step-by-step. Considering all the reaction pathways, the rate of transmetallation can be expressed as:

$$-\frac{d[LnL]_t}{dt} = k_0[LnL] + k_{LnHL}[LnHL] + k_{LnLM}[LnLM] \qquad (5.2.17)$$

where the term $k_0[LnL]$ is characteristic for the spontaneous dissociation of the complex. By comparing equations 5.2.14 and 5.2.17, considering the total concentration of LnL ($[LnL]_t = [LnL] + [LnHL] + [LnLM]$) and the equations which define the $K_{LnHL}$ and $K_{LnLM}$ stability constants, the $k_d$ value can be expressed as follows:[173]

$$k_d = \frac{k_0 + k_1[H^+] + k_3^M[M^{2+}]}{1 + K_{LnHL}[H^+] + K_{LnLM}[M^{2+}]} \qquad (5.2.18)$$

where $k_1 = k_{LnHL} \cdot K_{LnHL}$ and $k_3^M = k_{LnLM} \cdot K_{LnLM}$. The rate constants $k_0$, $k_1$, $k_3^M$ and the stability constant $K_{LnLM}$ can be calculated by fitting the $k_d$ values to Equation 5.2.18 ($K_{LnHL}$ is often known from equilibrium studies, but if $K_{LnHL}$ is too low, the term $K_{LnHL} \cdot [H^+]$ in the denominator can be neglected at pH > 4). The rate constants $k_1$ and $k_3^M$ ($M^{2+}$ is $Zn^{2+}$ or $Cu^{2+}$) determined for the different DTPA derivative complexes are presented in Table 5.2.6. The $k_0$ values are not shown because their calculated values are generally very low and often have a negative sign and high error limits.

Comparing the rate constants obtained for different $Gd^{3+}$ complexes (Table 5.2.6) reveals that the attachment of a hydrophobic group to an acetate group

**Table 5.2.6** Rate constants, characterizing the decomplexation of the DTPA derivative complexes.

| *Complex* | $k_1 (M^{-1} s^{-1})$ | $k_3^{Zn} (M^{-1} s^{-1})$ | $k_3^{Cu} (M^{-1} s^{-1})$ |
|---|---|---|---|
| $Gd(DTPA)^{2-}$,[173] | 0.58 | 0.056 | 0.93 |
| $Gd(BOPTA)^{2-}$,[224] | 0.41 | 0.029 | 0.68 |
| $Gd(EOB\text{-}DTPA)^{2-}$,[208] | 0.16 | [a]N | [a]N |
| $Gd(DTTA\text{-}Nprop)^{2-}$,[184] | 48 | 0.64 | [a]N |
| $Gd(DTPA\text{-}N\text{-}MA)^{-}$,[192] | 1.5 | 0.032 | 1.9 |
| $Gd(DTPA\text{-}N'\text{-}MA)^{-}$,[192] | 1.6 | 0.08 | 0.62 |
| $Gd(DTPA\text{-}BMA)$[192] | 12.7 | 0.0078 | 0.63 |
| $Gd(DTPA\text{-}BMEA)$[225] | 8.6 | [a]N | [a]N |
| $Gd(DTPA\text{-}TrA)^{+}$,[158] | 0.40 | 0.0087 | 0.063 |
| $Gd(DTPA\text{-}N'\text{-}P)^{3-}$,[193] | 3380 | [a]N | 33 |
| $Gd(DTPA\text{-}N'\text{-}PhPi)^{2-}$,[193] | 1600 | [a]N | [a]N |
| $Gd(DTPA\text{-}EAM)$[226] | 0.12 | [a]N | 1.3 |

[a]not investigated.

or to the amine chain results in an increase in the kinetic inertness, which increases in the order $Gd(DTPA)^{2-} < Gd(BOPTA)^{2-} < Gd(EOB\text{-}DTPA)^{2-}$. However, replacing an acetate of DTPA with a propionate group (DTTA-NProp) leads to a significant increase in the lability of the complex.

The replacement of a carboxylate with a phosphonate (DTPA-*N'*-P) or a phenylphosphinate (DTPA-*N'*-PhPi), results in a very significant increase in the rates of decomplexation, and both the proton and the $Cu^{2+}$ assisted dissociation of the $Gd^{3+}$ complexes becomes very fast. This significant change is probably caused by the high basicity of the phosphonate group (the complex is in protonated form at pH around 7) and the large steric requirement of the phenylphosphinate moiety.[193]

The replacement of one, two or three carboxylates of DTPA with amide groups leads to the mono-, bis- and tris-amides of DTPA. Although the protonation constants of the DTPA-amide complexes are very low (their formation cannot be detected by pH-potentiometry), the proton assisted dissociation of their $Gd^{3+}$ complexes is significantly faster than that of $Gd(DTPA)^{2-}$. However, the $k_3^{Cu}$ and $k_3^{Zn}$ rate constants for the DTPA-amide derivative complexes are lower than those of the $Gd(DTPA)^{2-}$, so the rates of de-chelation are very similar. This finding is surprising if we consider the results of the *in vivo* studies, when *e.g.* the residual $Gd^{3+}$ in mice was the largest for Gd(DTPA-BMA) of all CAs.[187] This contradiction is; however, only apparent. The rate constants presented in Table 5.2.6 have been determined generally in KCl solutions (at constant ionic strength), when the conditions are far from the biological ones. In body fluids both the equilibrium and kinetic properties of the $Gd^{3+}$-chelates are influenced by the presence of endogenous ligands. The rates of transmetallation reactions between the complexes $Gd(DTPA)^{2-}$ and $Gd(BOPTA)^{2-}$ and $Zn^{2+}$ or $Cu^{2+}$ in the presence of citrate and histidinate are significantly lower than in the absence of these ligands. However, the transmetallation reactions of Gd(DTPA-BMA) with $Zn^{2+}$ or $Cu^{2+}$ in the presence of citrate are approximately two orders of magnitude faster than in the absence of citrate.[194] These results clearly show that the endogenous ligands significantly accelerate the dissociation of the DTPA-amide derivative complexes.

In order to increase the kinetic inertness of the DTPA complexes several new, backbone substituted DTPA derivatives were synthesised. By replacing an ethylene group of DTPA with a cyclohexane ring (like in DCTA), the more rigid, cyclohexyl-DTPA derivatives were prepared. The acid assisted dissociation of the $Y^{3+}$ complexes of the more rigid ligands is slower than that of $Y(DTPA)^{2-}$.[2-105]

### 5.2.4.4 Kinetics of Decomplexation of DOTA Derivative Complexes

The complexes of tripositive metals formed with DOTA and DOTA derivative ligands are extremely inert, so the rates of their substitution reactions cannot be studied at pH around 7. The high inertness of the complexes originates from their rigid structure. The $Gd^{3+}$ or other metal ion in the coordination cage is inaccessible for another multidentate ligand and in addition, the coordinated functional groups of DOTA are not flexible enough to be transferred to an attacking metal ion. In contrast to metal ions, protons can successfully compete for the coordinated DOTA, when protonated complexes Ln(HL) are formed in the first step. The stability constants characterizing the formation of Ln(HL) complexes are relatively low ($K_{LnHL}$ values are about 10–400) because of the strong carboxylate oxygen–$Ln^{3+}$ interaction. The protons can be attached to a non-coordinated carboxylate oxygen atom as has been shown by $^1$H-NMR spectroscopy for Lu(HDOTA).[195] The protons from this oxygen can then be transferred to a ring nitrogen when the metal ion moves out of the coordination cage, and with the attachment of a second proton to a diagonally positioned nitrogen, a diprotonated intermediate is formed, in which only the four carboxylates are coordinated to the $Ln^{3+}$, which is now outside of the coordination cage. This diprotonated intermediate dissociates into $Ln^{3+}$ and $H_2DOTA^{2-}$, or the complex $Ln(DOTA)^{-}$ can be re-formed as it was discussed in Section 5.2.4.1. Since the complexes of DOTA and its derivatives are thermodynamically unstable at about $[H^+] > 0.05$ M, the kinetics of dissociation can be studied in the $H^+$ concentration range of 0.05–1.0 M by spectrophotometry, fluorescence spectroscopy, and relaxometry, or by separating the $Ln^{3+}$ ion from the complex by ion-exchange or HPLC.[175,176,189,196] In the presence of excess acid, first-order rate constants ($k_d$) are obtained which are directly proportional to the $[H^+]$ up to about 0.2 – 0.3 M, and the $k_d$ values can be given as follows:

$$k_d = k_0 + k_1[H^+] \qquad (5.2.19)$$

where $k_0$ and $k_1$ are the rate constants, characterizing the spontaneous and proton assisted dissociation of complexes. At higher $H^+$ concentration $k_d$ shows a saturation type dependence on $[H^+]$, which is interpreted by an

accumulation of the mono- and diprotonated complexes.[175,197] However, at pH values around 7 only the dissociation of the monoprotonated complexes can play a role, so for characterizing the kinetic inertness of complexes, we shall use only the $k_1$ rate constants. The $k_1$ values obtained in the studies of dissociation of different DOTA based complexes are presented in Table 5.2.7.

The rates of dissociation of complexes can also be studied at higher pH values, if the concentration of the $Ln^{3+}$ complex is relatively high (Equation 5.2.14). The rates of dissociation of Gd(HP-DO3A) and Gd(BT-DO3A) have been studied in the pH range of 3.2–5.3. The metal exchange between the complexes and $Eu^{3+}$ was followed by spectrophotometry at 0.1 M Gd(HP-DO3A) or Gd(BT-DO3A) and 0.01 M $Eu^{3+}$ concentration. The first-order rate constants were found to be independent of the $[Eu^{3+}]$ while the $k_d$ values linearly increased with increasing $[H^+]$.

There are several reported studies on the dissociation of $Sc^{3+}$, $Y^{3+}$, $Ga^{3+}$ and $In^{3+}$ complexes of DOTA derivatives. The rates of proton assisted de-chelation of Ga(DO3A) and In(DO3A) are even lower than those of the lanthanide complexes.[196] The kinetic behaviour of the $Y^{3+}$ complexes formed with DOTA, DO3A, PCTA, and several phosphonate and phosphinate derivatives of DOTA are very similar to that of the corresponding $Eu^{3+}$ and $Gd^{3+}$ complexes, and these chelates have satisfactory kinetic inertness for nuclear medicine applications.[87,154,190,196]

The comparision of the $k_1$ data presented in Table 5.2.7 shows that the complexes of DOTA derivatives are particularly inert if all four ring nitrogens possess pendant functional groups. The $k_1$ values of the complexes of DO3A, containing only three acetates, are 2 – 3 orders of magnitude higher than those

**Table 5.2.7** Rate constants $k_1$ ($M^{-1}$ $s^{-1}$) characterizing the proton assisted dissociation of the DOTA derivative complexes.

| *Ligand* | *$Ce^{3+}$* | *$Eu^{3+}$* | *$Gd^{3+}$* |
|---|---|---|---|
| DOTA | $8 \times 10^{-4,227}$ | $1.4 \times 10^{-5,177}$ | $8.4 \times 10^{-6,176}$ |
| | $3.4 \times 10^{-4,229}$ | | $2.0 \times 10^{-5}$ (37 °C)[175], $3.6 \times 10^{-5}$ (37 °C)[228] |
| DO3A | $1.1 \times 10^{-1,197}$ | – | $1.6 \times 10^{-3,197}$ |
| | | | $1.2 \times 10^{-2,196}$ |
| HP-DO3A | $2.0 \times 10^{-3,197}$ | – | $6.4 \times 10^{-4,197}$ |
| | | | $2.6 \times 10^{-4,79}$ |
| BT-DO3A[79] | – | – | $2.8 \times 10^{-5}$ |
| DO3A-Nprop[184] | $7.3 \times 10^{-3}$ | – | – |
| PCTA[87] | $9.6 \times 10^{-4}$ | $5.1 \times 10^{-4}$ | – |
| *p*-$NO_2$-Bz-PCTA[131] | $4.8 \times 10^{-5}$ | | $1.7 \times 10^{-4}$ |
| TRITA[180] | – | – | 0.35 |
| DO3AP | $1.2 \times 10^{-3,155}$ | $9.8 \times 10^{-5,177}$ | $2.8 \times 10^{-3,155}$ |
| DO2A2P[81] | – | – | $1.9 \times 10^{-4}$ |
| DOA3P[83] | – | – | $2.7 \times 10^{-4}$ |
| DOTP | $4.6 \times 10^{-2,155}$ | $1.3 \times 10^{-3,177}$ | $5.4 \times 10^{-4,223}$ |
| DOTPMB[223] | – | – | $2.1 \times 10^{-4}$ |
| DTMA[113] | $2.6 \times 10^{-5}$ | $5.6 \times 10^{-7}$ | – |



of the DOTA complexes. The secondary nitrogen of DO3A can be more easily protonated directly, which explains the faster dissociation of the DO3A complexes.[196,197]

The coordination of an alcoholic OH group is weaker than that of a carboxylate oxygen, so the dissociation of the complexes of HP-DO3A and BT-DO3A is faster than the de-chelation of the DOTA complexes. The $k_1$ value for Gd(BT-DO3A) is approximately ten times lower than that of Gd(HP-DO3A), although the log $K_{GdL}$ value of the latter is higher. Gd(BT-DO3A) is kinetically more inert than the Gd(HP-DO3A), probably because the size of the butrol group is larger than the size of the 2-hydroxy-propyl group, which makes Gd(BT-DO3A) more rigid.[79]

In DOTA complexes the coordination of the nitrogen and oxygen donor groups result in the formation of five membered chelate rings, which contribute to the rigidity of complexes. The replacement of one acetate with a propionate or the enlargement of the 12-membered ring to a 13-membered macrocycle results in the formation of 6-membered chelate rings which decrease the rigidity and the kinetic inertness of the complexes of DO3A-Nprop and TRITA.[180,184]

The influence of the bifunctional linker on the kinetic inertness of the resulting complexes is an important issue in the design of the bifunctional ligands. Unfortunately, there are only very few systematic studies in this respect. The data known so far show *e.g.* that $k_1$ values of the complexes of DOTA and *p*-$NO_2$-Bn-DOTA or PCTA and *p*-$NO_2$-Bn-DOTA do not differ considerably.[131,198] Similar results were obtained in the study of the phosphonate and phosphinate derivatives of DOTA.[154,199]

The most inert complexes are formed with the DOTA-*tetra*amide derivative ligands. The half-times of dissociation of $Gd(DOTA)^-$, $Gd(DOTAM)^{3+}$ and $Gd(DTMA)^{3+}$ in 2.5 M $HNO_3$ at 25 °C were found to be 4.5 h, 68 h, and 155 h, respectively.[200] The $k_1$ values known for the DTMA complexes are also very low.[113] The slow proton assisted dissociation and the slow formation of the DTMA or DOTAM complexes can be interpreted in terms of low basicity of the ligands. The basicity of the amide oxygens is extremely low, so the protonation of the complex and the transfer of a proton to a ring nitrogen occurs with very low probability, which leads to the high kinetic inertness of the complexes. The inertness increases in the following order of substituents: $-CONH_2 < -CONHMe < -CONMe_2$.[113,200]

## 5.2.5 Summary

In recent years a number of metal complexes and metal complex bioconjugates have been developed for diagnostic imaging modalities such as MRI, SPECT, PET and optical imaging. Many of these agents contain a lanthanide ion; for example, in MRI the paramagnetic $Gd^{3+}$ is used as contrast agent, luminescent lanthanide complexes are involved in optical probes, and several radiolanthanides are applied in diagnosis and therapy. The chelating agents used for the complexation of these metal ions are generally the open-chain DTPA and the macrocyclic DOTA, and their derivatives. The metal ions released in

the body by de-chelation are very harmful ($Gd^{3+}$ is toxic, radiometals cause radiation damage), so high kinetic inertness of the complexes is very important for their safety. The de-chelation of $Gd^{3+}$ complexes (which are administered in large amounts) must be much slower than their elimination from the body ($t_{1/2} = 1.5$ h). This requirement is realized with the use of complexes of DOTA derivatives, since their proton-assisted dissociation is extremely slow at physiological pH ($t_{1/2}$ is about $10^5 - 10^7$ h).

The de-chelation of $Gd^{3+}$ complexes of DTPA derivatives pressumably occurs *via* transmetallation with $Zn^{2+}$ or $Cu^{2+}$. The dechelation of $Gd(DTPA)^{2-}$ and $Gd(BOPTA)^{2-}$ in the presence of citrate is slower, while that of Gd(DTPA-BMA) is much faster (about two orders of magnitude), than in the absence of citrate. The effect of citrate explains the larger residual $Gd^{3+}$ observed with the use of Gd(DTPA-BMA) in animal and human experiments.

The complexation equilibria in the blood plasma can be characterized by the species distribution calculations performed using the stability constants of all the complexes formed with the endogenous ligands and metal ions. However, the results of such calculations are not valid, because the real systems are far from the equilibrium, due to the fast elimination and slow de-chelation of contrast agents. For patients with severe renal impairment, the half-time of elimination is about 30 – 40 h and the amount of $Gd^{3+}$ released from complexes of DTPA derivatives, first of all from Gd(DTPA-BMA), is not negligible. It was assumed that in very few cases the residual $Gd^{3+}$ may give rise to the newly observed disease, Nephrogenic Systemic Fibrosis (NSF).

The permutations of DTPA and DOTA derived ligand structures made in the past two or three decades have resulted in a large number of bifunctional chelators designed for labeling proteins, protein fragments, monoclonal antibodies, dendrimers or fabricating multifunctional constructs and ligands for multimodal imaging. We believe that open chain bifunctional ligands are going to be replaced by suitable macrocyclic or bimodal bifunctional chelators that possess fast complexation kinetics under mild conditions and advanced kinetic inertness. On the other hand, conceptually different labeling reactions ("click chemistry") or labeling at specific amino acid moiety for instance need newly-designed reactive groups and coupling reactions besides the ones that are currently being used to conjugate bifunctional ligands to biological vectors. Finally, less common but potentially useful radionuclides such as $^{44,47}Sc$, $^{89}Zr$, $^{212}Pb$, $^{223}Ra$, $^{225}Ac$ *etc.* require the design and synthesis of more efficient bifunctional ligands capable of sequestering these metals in the form of stable chelates.

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CHAPTER 5.3

# *MRI Contrast Agents Based on Metallofullerenes*

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## 5.3.1 Introduction

Magnetic resonance imaging (MRI) has evolved into one of the most powerful techniques as a noninvasive diagnostic tool by providing high quality anatomical images of soft tissue;[1,2] and the rapid expansion of medical MRI has prompted the development of contrast agents (CAs). These agents can shorten the relaxation time of nearby water molecules, thereby enhancing the contrast between areas containing the contrast agent and the surrounding tissues, and so increasing diagnostic confidence.[2–6] Currently used MRI contrast agents include extracellular fluid (ECF) agents, intravascular blood pool agents and tissue-specific agents. The typical ECF agents are mainly gadolinium poly(aminocarboxylate) chelates with lower molecular weight such as Magnevist (gadolinium-diethylenetriaminepentaacetic acid, Gd-DTPA), Prohance (Gadoteridol), and Omniscan (Gd-diethylenetriaminepentaacetate-bismethylamide), which have a relatively short residence time in the vascular system; the developed intravascular contrast agents include Gd-DTPA labeled albumin, Gd-DTPA labeled dextran, and chromium-labeled red blood cells, which have longer residence times and allow extended imaging procedures as a result of having a molecular weight of approximately 70 000 and above; as for tissue-specific agents, superparamagnetic iron oxides (SPIOs) have been used as

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