

Review

## Synthetic Approaches to Heterocyclic Ligands for Gd-Based MRI Contrast Agents

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**Abstract:** Magnetic Resonance Imaging (MRI) methods are currently used in the clinic for the non invasive detection and characterization of a wide variety of pathologies. Increases in the diagnostic efficiency of MRI have been helped by both the design of dedicated MR sequences revealing specific aspects of the pathology and by the development of more sensitive and selective Contrast Agents (CAs), capable of more precisely delineating the borderline regions. In the present review we focus on the synthetic strategies used to obtain MRI CAs containing heterocyclic rings.

**Keywords:** Lanthanide Complexes, Contrast Agents, Heterocyclic Ligands, MR Imaging

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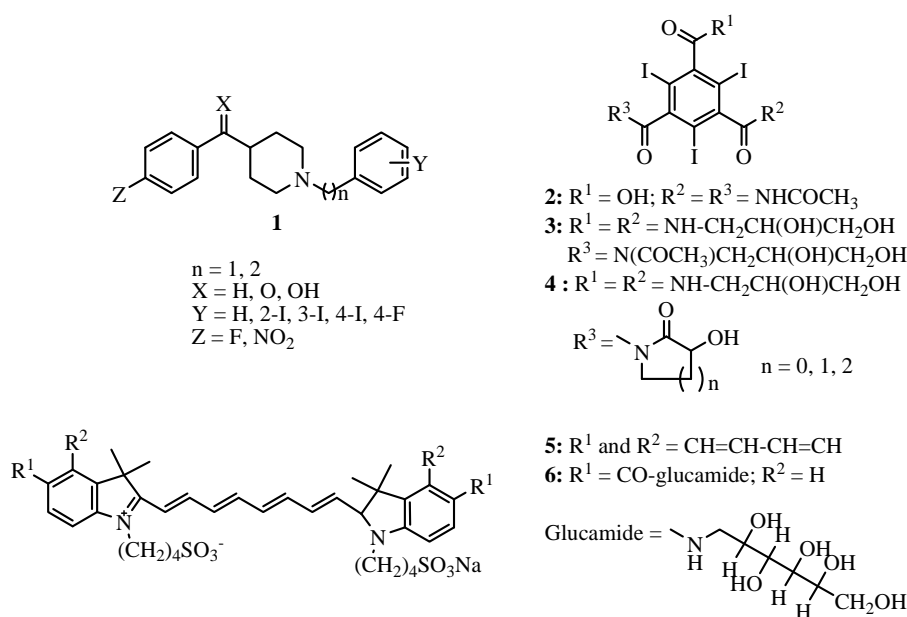
## Contents

1. Introduction	1772
2. Contrast Agents (CAs) for MRI: Gd-based complexes	1773
2.1. CAs derived from acyclic ligands	1774
2.2. CAs derived from macrocyclic ligands	1784
3. Concluding remarks and future perspectives	1790

## 1. Introduction

Cardiovascular and neurodegenerative diseases, as well as tumors, are often clinically diagnosed using non invasive methods such as PET (Positron Emission Tomography) [1,2], SPECT (Single Photon Emission Tomography) [2], MRI (Magnetic Resonance Imaging) [3], Optical Imaging [4], ultrasound methods [5] or their multimodal combinations [6]. In many cases all of these methods involve the additional use of specific probes (known as Contrast Agents, CAs) to increase image resolution and discrimination between healthy and pathological areas. Therefore, the development of more sensitive, selective and efficient CAs is an important task of strategic interest due to their potential applications in many biomedical imaging procedures. Classically the widespread presence of heterocyclic rings in Nature and their very favorable coordinating properties have prompted the use of a variety of nitrogen-based heterocycles in the manufacture of many CAs. As a more recent example, Fu *et al.* [7] have described a new family of benzoylpiperidines **1** (Figure 1) as serotonin 5-HT<sub>2A</sub> ligands for PET or SPECT Imaging of the brain. Notably, in spite of the considerable progress of MRI and PET protocols, X-Ray Imaging still accounts for 75-80% of all diagnostic imaging procedures. In this respect, non-ionic X-Ray CAs based on the attachment of heterocyclic moieties to the 5-position of diatrizoic acid (**2**) or iohexol (**3**) have been reported [8]. Most of these new X-Ray contrast agents consist of sterically congested lactams **4**, derived from the 2,4,6-triiodoisophthalamide, which exhibit water solubility, stability and osmolality, depending on the heterocycle included. Approaches based on optical methods are fast emerging as alternatives to conventional X-Ray Imaging. Near Infra-Red light (NIR) is increasingly being considered nowadays as a powerful non-invasive biomedical imaging tool. It is specially recommended as a complementary method to X-ray mammography for examinations of young women with dense breast tissues or patients with scars and implants, often employing NIR absorbing dyes. Indocyanine green (ICG, **5**) is a clinically approved NIR dye used for testing of hepatic function and fluorescence angiography in ophthalmology and even for detection of breast tumors [9]. NIR dye **6**, a modification of ICG, overcomes many of its limitations with regards to spatial resolution and sensitivity [10].

In the following sections we address the various synthetic strategies developed to produce heterocyclic ligands useful for preparing Gadolinium based CAs for MRI [11]. The present work complements these contributions by addressing in more detail the heterocyclic chemistry involved in these processes.

**Figure 1.** Some useful probes for PET, SPECT, X-Ray and Optical Imaging.

## 2. Contrast Agents (CAs) for MRI: Gd-based complexes

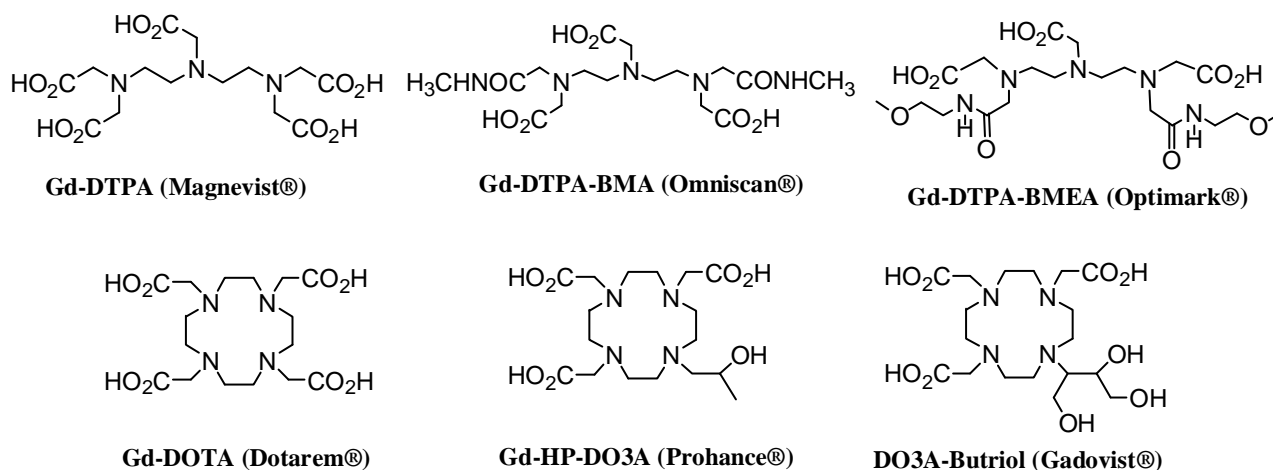
As mentioned above, the diagnostic efficiency by many MRI methods frequently relies on the use of a new type of drugs, referred to as contrast agents (CAs), able to discriminate between normal and pathological tissues due to their different MR properties [11-13]. The role of the CAs is to enhance the MRI signal by shortening the relaxation times of water protons in those tissues in which they distribute. Generally, the most investigated paramagnetic CAs are lanthanide complexes, with particular emphasis on the corresponding Gd(III)-chelates. Mn(II) and Fe(III) salts have also been investigated as paramagnetic metals, but they often become weakly chelated and dissociate spontaneously under *in vivo* conditions [14].

As mentioned, the paramagnetic metal of choice in clinical practice is generally Gd(III), but free Gd(III) is toxic *in vitro* as well as *in vivo*, and the use of Gd(III) chelates becomes mandatory in biomedical applications to reduce its toxicity. Gd(III) remains the optimal paramagnetic ion because of its high electronic spin ( $S=7/2$ ), relatively long electronic relaxation time, high magnetic moment and relatively labile hydration sphere for water exchange. The first generation of Gd(III) chelates was derived from linear or macrocyclic polyaminopolycarboxylates such as diethylenetriaminepentaacetic acid ( $[\text{dtpaGd(H}_2\text{O)}]^{2-}$ ) or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid ( $[\text{dotaGd(H}_2\text{O)}]^-$ ), respectively (Figure 2) [11]. Gd-Dtpa, patented as Magnevist<sup>®</sup> by Schering (Germany), was the first CA approved for clinical use. Gd-Dota (Dotarem<sup>®</sup>, Guerbet, France), Gd-Dtpa-BMA (Omniscan<sup>®</sup>, Amersham, Great Britain), Gd-HP-DO3A (Prohance<sup>®</sup>, Bracco, Italy), Gd-Dtpa-BMEA (Optimark<sup>®</sup>, Mallinkrodt, USA) and Gd-DO3A-Butriol (Gadovist<sup>®</sup>, Schering, Germany) followed as other Gd-based CAs commonly used in clinical practice. All of them present similar pharmacokinetic properties and renal elimination rates.

The modification of both linear and macrocyclic parental structures, Gd-Dtpa and Gd-Dota, is currently found to be an essential part of the investigations generating new CAs with improved magnetic properties. In this respect, new Gd-based CAs must exhibit sufficiently high thermodynamic

and kinetic stabilities as important determinants for their use in MRI diagnosis. In addition, the CA's must have improved molecular relaxivity properties,  $r_1$  or  $r_2$  ( $s^{-1} mM^{-1}$ ), defined as the longitudinal or transversal relaxation rates of the water protons in a 1 mM aqueous solution of the Gd(III)-chelate.

**Figure 2.** Gd(III) complexes commonly used in clinical practice.

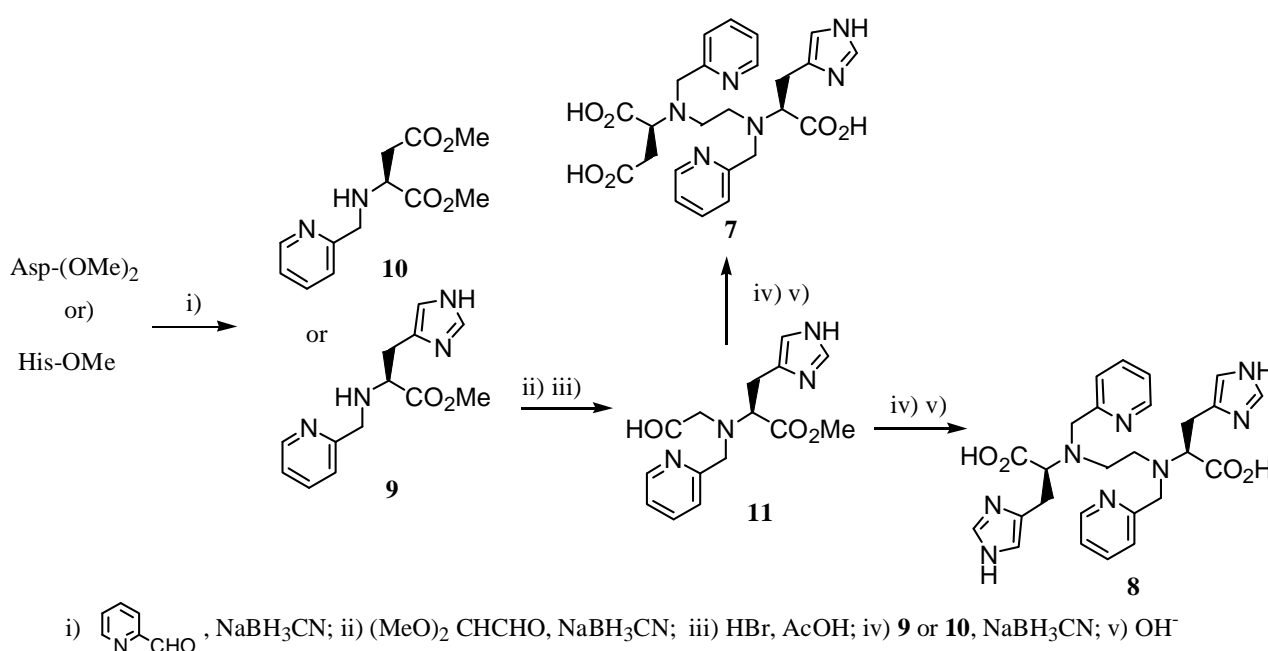
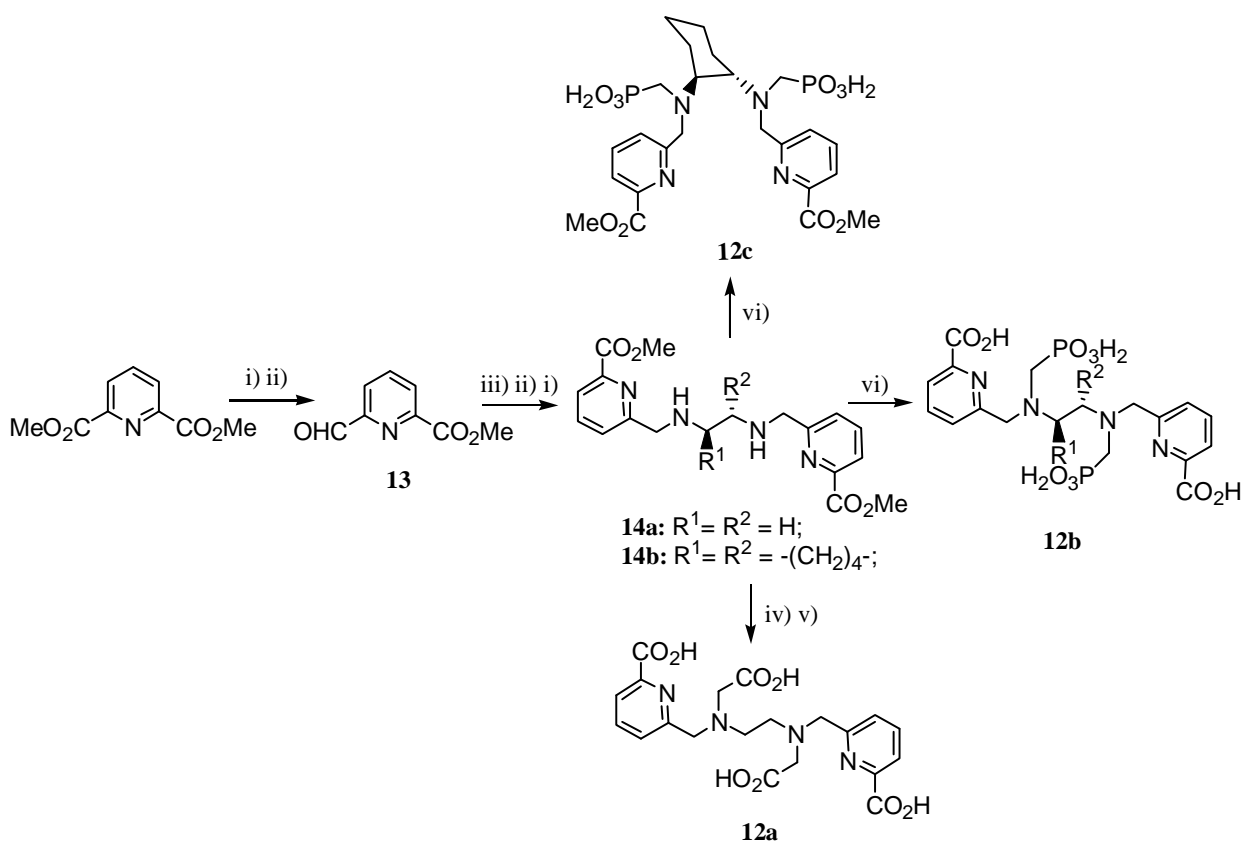


The following sections cover the synthetic approaches used to prepare these ligands, emphasizing the synthetic methodologies implemented to obtain heterocyclic chelators resulting progressively in more stable Gd complexes.

## 2.1. CAs derived from acyclic ligands

Miyake *et al.* [15] have reported a new type of optically active acyclic ligands **7** and **8** derived from (*S*)-aspartic acid and (*S*)-histidine (Scheme 1). Their Gd(III)-complexes exhibited a high relaxivity ( $r_1 = 9.4$  and  $9.9 s^{-1} mM^{-1}$ , respectively, at 300 MHz and 295 K), but their thermodynamic stability was not sufficient to be clinically relevant. Inclusion of pyridine ring was carried out by reductive amination between the corresponding amino acid and pyridine 2-carbaldehyde, affording compounds **9** and **10**. Reaction of **9** with 2,2-dimethoxyacetaldehyde in the presence of  $NaBH_3CN$ , followed by treatment in acidic medium yielded compound **11**, which by condensation with **9** or **10** under the mentioned conditions and subsequent basic hydrolysis, afforded the chelating ligands **7** and **8**.

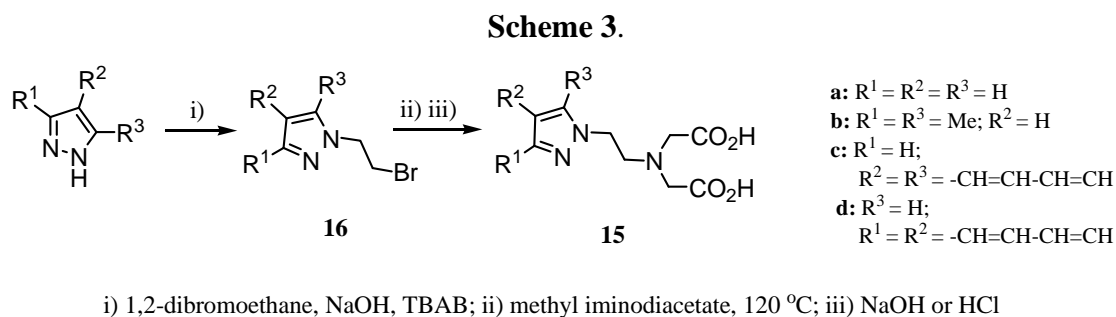
Pyridine derivatives **12a-c**, also based on ethylenediamine backbone, were recently reported by Platas-Iglesias *et al.* (Scheme 2) [16]. Compounds **12a-c** were synthesized from 2,6-pyridinedicarboxylic acid dimethyl ester by reduction of one of the ester groups using  $NaBH_4$  in MeOH, followed by oxidation of the corresponding alcohol yielding compound **13**. Condensation of **13** with ethylenediamine and subsequent reduction of the imine derivative gave compounds **14a-b**. Alkylation of **14a** with *tert*-butyl bromoacetate and deprotection of the ester moieties in acidic medium afforded **12a**. Compound **12b** was synthesized from precursor **14b** by Mannich-type reaction with paraformaldehyde and phosphorous acid in 6 M HCl [17]. Finally, chelate **12c**, containing the same structural skeleton with the ethyl bridge substituted by a more rigid cyclohexyl moiety, was prepared using the same synthetic sequence used to prepare **12b** (Scheme 2) [18]. **Gd-12a** induced an  $r_1$  value  $5.0 s^{-1} mM^{-1}$  measured at 20 MHz and 25 °C.

**Scheme 1.** Optically active acyclic ligands derived from (*S*)-aspartic acid and (*S*)-histidine.**Scheme 2.** Pyridine derivatives **12a-c** based on an ethyldiamine chelate.

i) NaBH<sub>4</sub>, MeOH; ii) SeO<sub>2</sub>, dioxane; iii) ethyldiamine or 1,2-cyclohexyldiamine, MeOH; iv) *tert*-butyl bromoacetate, Na<sub>2</sub>CO<sub>3</sub>, acetonitrile; v) HCl; vi) paraformaldehyde, phosphorous acid, HCl (6M)

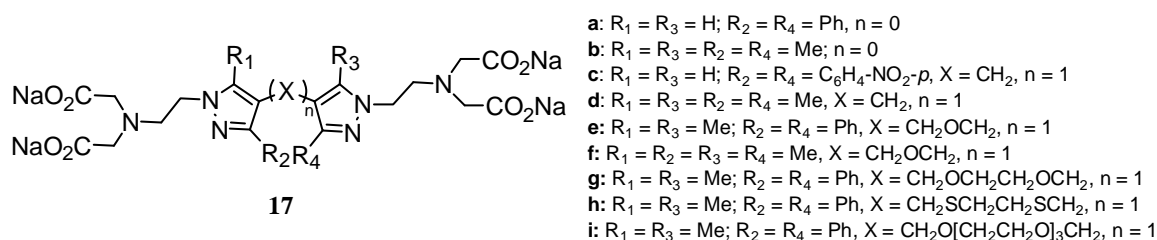
López *et al.* [19] have reported the synthesis of a novel series of chelating ligands **15** containing nitrogen heterocycles, such as pyrazole and indazole, which form tetradentate complexes with Gd(III).

These Gd(III)-complexes were considered as  $T_2$  relaxation agents for MRI ( $r_1$  4.6-5.9  $s^{-1}mM^{-1}$ ;  $r_2$  7.4-13.9  $s^{-1}mM^{-1}$  at 360 MHz and 25 °C). Their synthesis was carried out starting from the corresponding heterocyclic rings by alkylation, using phase transfer conditions, to give compounds **16**. Reaction of **16** with methyl iminodiacetate and subsequent acid or basic hydrolysis of the corresponding methyl ester afforded the chelating ligands **15** (Scheme 3).



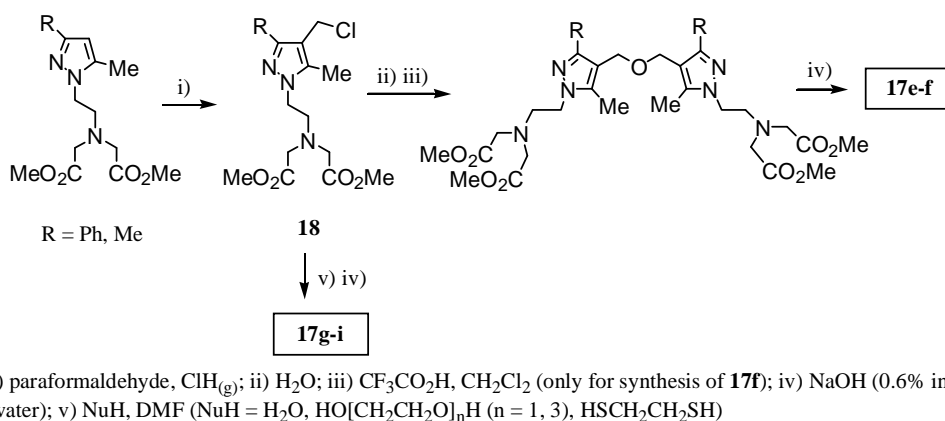
In a similar fashion, Mayoral *et al.* [20] described a novel family of chelating agents **17** containing bi- and bis-pyrazole structure, which form double tetradentate complexes with Gd(III). They exhibited a larger relaxivity in a range of 13.8-37.0  $s^{-1}mM^{-1}$  (60 MHz and 37 °C), even compared to dendrimers with numerous metallic centers (Figure 3). Ligands **17a-d** were obtained by alkylation of the corresponding bi or bispyrazole, reaction with methyl iminodiacetate and followed by the basic hydrolysis as mentioned above for the preparation of **15** (Scheme 3).

**Figure 3.** Chelating ligands for Gd(III) with Bi and bis-pyrazole skeleton.



Chelators **17e-i** were synthesized from the chloromethyl pyrazoles **18** prepared by condensation of the appropriate pyrazole with paraformaldehyde (Scheme 4) [21].

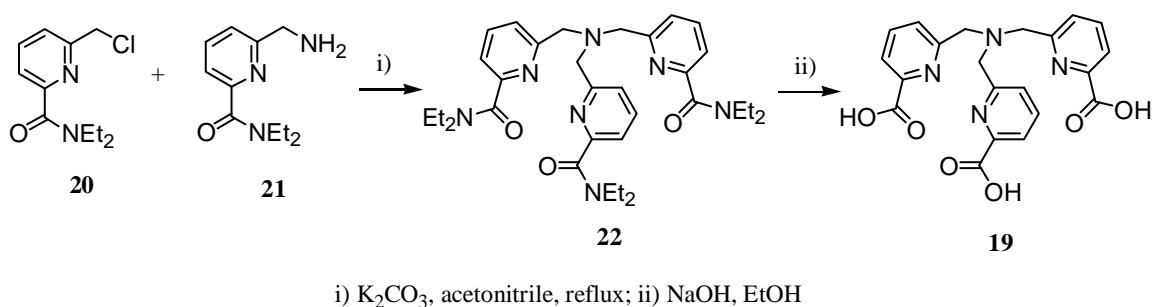
**Scheme 4.** Gd(III) Chelators from 4-chloromethylpyrazoles.



Compounds **18** were extremely reactive species, reacting with the corresponding nucleophiles to give different products, depending on the starting substrate. When compounds **18** reacted with water, complexes **17e-f** were isolated after basic hydrolysis of the corresponding ester, while compounds **17g-i** were prepared by reaction of **18** with the nucleophile shown in Scheme 4.

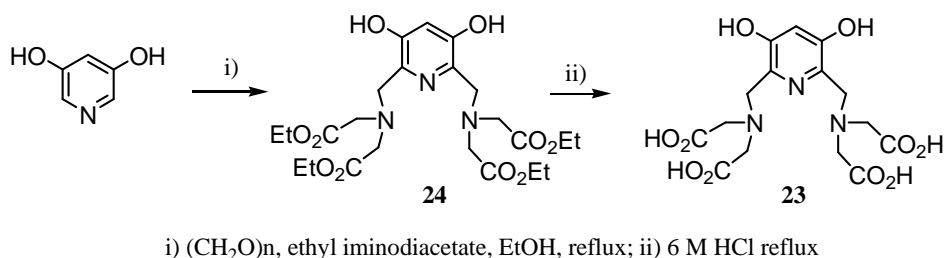
Heptadentate ligand **19** containing three pyridine rings was synthesized in two steps according to Scheme 5. Alkylation of **21** with the pyridylmethyl chloride **20** lead to compound **22**, which when treated with NaOH/EtOH afforded the ligand **19**. **Gd(III)-19** showed an unusual and higher relaxivity with respect to the other heptadentate complexes ( $13.3 \text{ s}^{-1}\text{mM}^{-1}$  measured at 60 MHz and 298 K) [22].

Scheme 5.

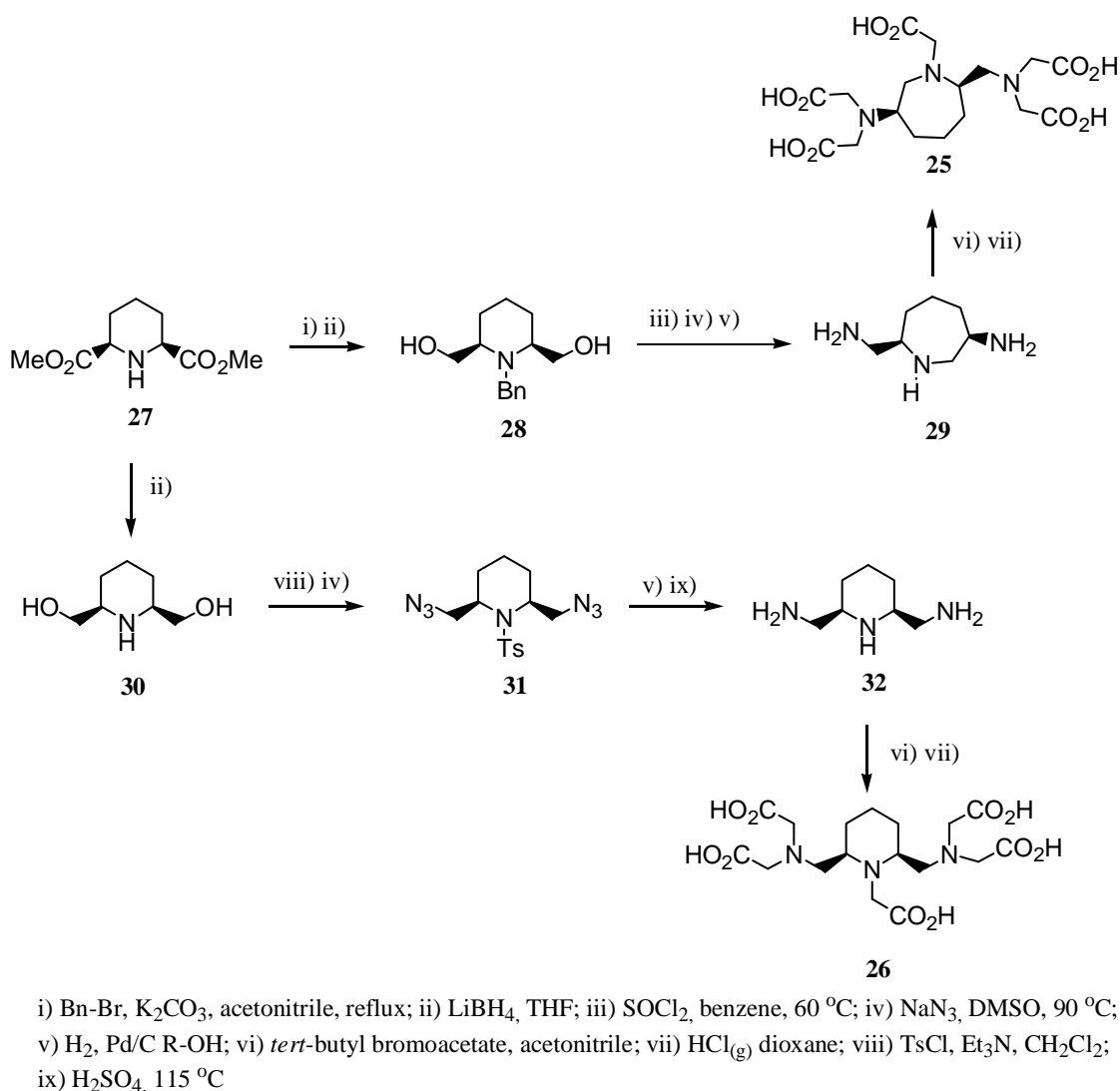


The Gd(III)-complex of ligand **23** with a pyridine ring as part of the triamine skeleton was reported by Aime *et al.* [23]. This complex showed a high relaxivity ( $9.1 \text{ s}^{-1}\text{mM}^{-1}$  at 20 MHz and 25 °C) in comparison with other heptacoordinating complexes, probably due to the presence of the hydroxyl groups that contribute to an increase of the second coordination sphere of the complex. Compound **23** was synthesized in two steps through a double-Mannich reaction, as shown in Scheme 6. Thus, reaction of 3,5-dihydroxypyridine with paraformaldehyde and ethyl iminodiacetate gave compound **24**, whose treatment with 6M HCl afforded **23** in good overall yield (56%).

Scheme 6.



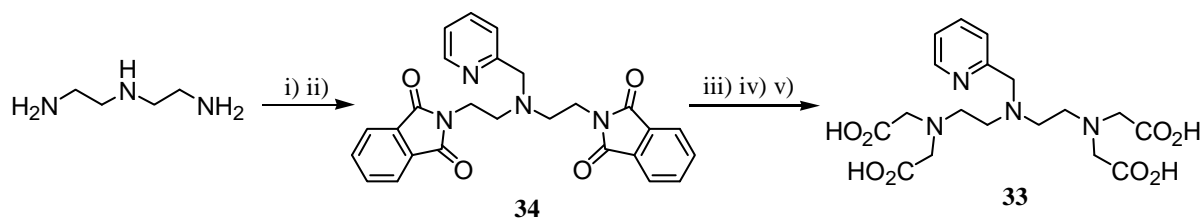
High stability constants and high relaxivity values are the essential requirements of a Gd-complex to be a potential CA for MRI. Consequently, design of octadentate chelating ligands is pursued in most investigations of new CAs with high stability constants. Dtpa analogues derived from piperidine and azepane **25** and **26** have been reported as potential CAs for MRI [24]. The synthetic routes used to prepare them firstly involved the functionalised heterocycles synthesis and subsequent alkylation (Scheme 7). The synthesis of **25** and **26** started from the piperidine **27**, which after benzylation of amine group and subsequent ester reduction afforded the compound **28**.

**Scheme 7.** Dtpa analogues derived from piperidine and azepane.

Treatment of **28** with thionyl chloride followed by reaction with sodium azide induces the ring expansion giving the corresponding azide, which was reduced to give the diamine **29**. On the other hand, reduction of **27** using LiBH<sub>4</sub> lead to compound **30**, which by reaction with *p*-toluensulfonyl chloride followed by treatment with sodium azide gave compound **31**. Reduction of the azide groups in compound **31** and subsequent treatment with concentrated H<sub>2</sub>SO<sub>4</sub> afforded diamine **32**. Finally, alkylation of **29** and **32** using *tert*-butyl bromoacetate and subsequent treatment with an acidic medium lead to **25** and **26**, respectively.

In the same way, Cheng T.-H. *et al.* [25] reported the Gd(III)-complex of *N'*-2-pyridylmethyl derivative **33** showing similar relaxivity values (4.2 s<sup>-1</sup>mM<sup>-1</sup> at 20 MHz and 310 K) and stability constants to Gd(III)-dtpa. This ligand was synthesized from diethylenetriamine with protection of the terminal amine groups according to the route shown in Scheme 8. Alkylation of the central nitrogen of the amine backbone gave compound **34**, which was sequentially treated with acid, alkylated with *tert*-butyl bromoacetate and finally hydrolysed in acidic medium yielding ligand **33**.

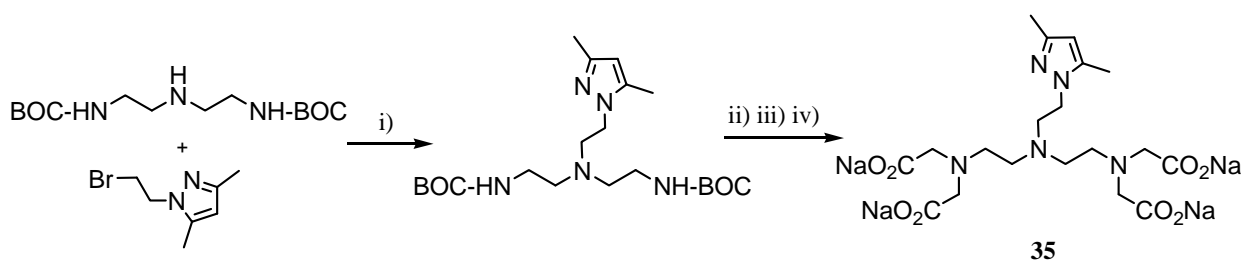
Scheme 8.



i) Phthalic anhydride, MeOH; ii) 2-picolyl chloride, NaOH; iii) HCl, reflux; iv) *tert*-butyl bromoacetate, K<sub>2</sub>CO<sub>3</sub>, acetonitrile; iv) HCl, rt

Recently, we reported an experimental and theoretical study of lanthanide complexes of the modified dtpa derivative **35** that includes a 3,5-dimethylpyrazolyethyl arm, corroborating the effective azole coordination with the metal center [26]. This compound was synthesized starting from *N,N'*-Boc-diethylenetriamine using a similar synthetic approach as for **33**, which gave **35** in 83% overall chemical yield (Scheme 9). The corresponding Gd-complex of **35** exhibited an  $r_1$  value of 5.1 s<sup>-1</sup>mM<sup>-1</sup> measured at 60 MHz and 310 K.

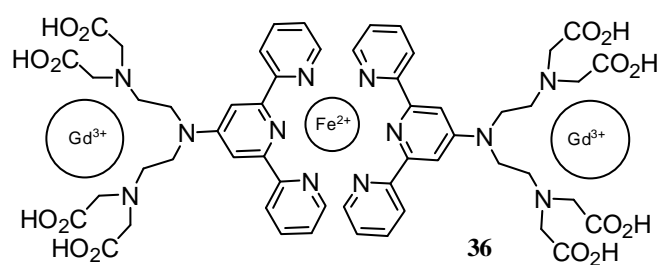
Scheme 9.



i) Na<sub>2</sub>CO<sub>3</sub>, acetonitrile; ii) HCl/MeOH (6N); iii) methyl bromoacetate, Na<sub>2</sub>CO<sub>3</sub>, acetonitrile; iv) NaOH

Relaxivity properties of Gd-based CAs can be increased restricting the motion of the complexes by linking to macromolecules through covalent or non-covalent bonds [11,12b]. This approach was used by Ruloff *et al.* [27] to prepare the complex [Fe{Gd **36**(H<sub>2</sub>O)<sub>2</sub>}<sub>3</sub>] from ligand **36**, obtained by a similar synthetic pathway as mentioned for compound **35** (Scheme 9 and Figure 4). The  $r_1$  for this complex was 22.9 s<sup>-1</sup>mM<sup>-1</sup> (60 MHz, 298 K).

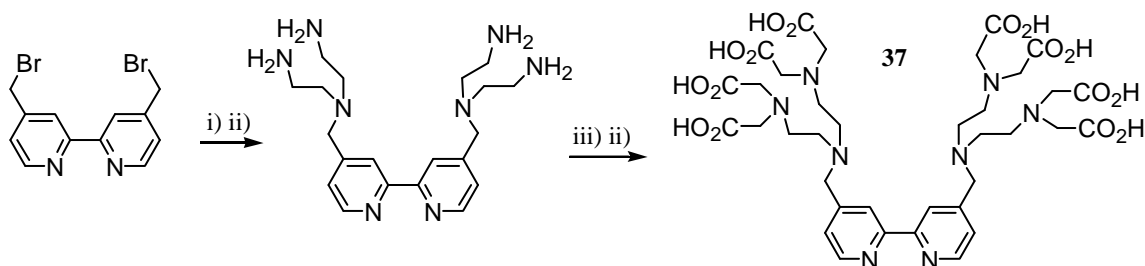
Figure 4.



On the other hand, a heterometallic self-assembled metallostar was prepared from ligand **37** synthesized using the same route mentioned above (Scheme 10) [28]. The multinuclear complex [Fe{Gd<sub>2</sub>**37**(H<sub>2</sub>O)<sub>4</sub>}<sub>3</sub>]<sup>4-</sup> is a rigid supramolecular structure containing two water molecules per Gd(III)

ion in the inner-sphere. Its high relaxivity ( $27 \text{ s}^{-1}\text{mM}^{-1}$  at 20 MHz and 25 °C) is comparable to a Gd(III)-Dota-loaded generation 10 dendrimer.

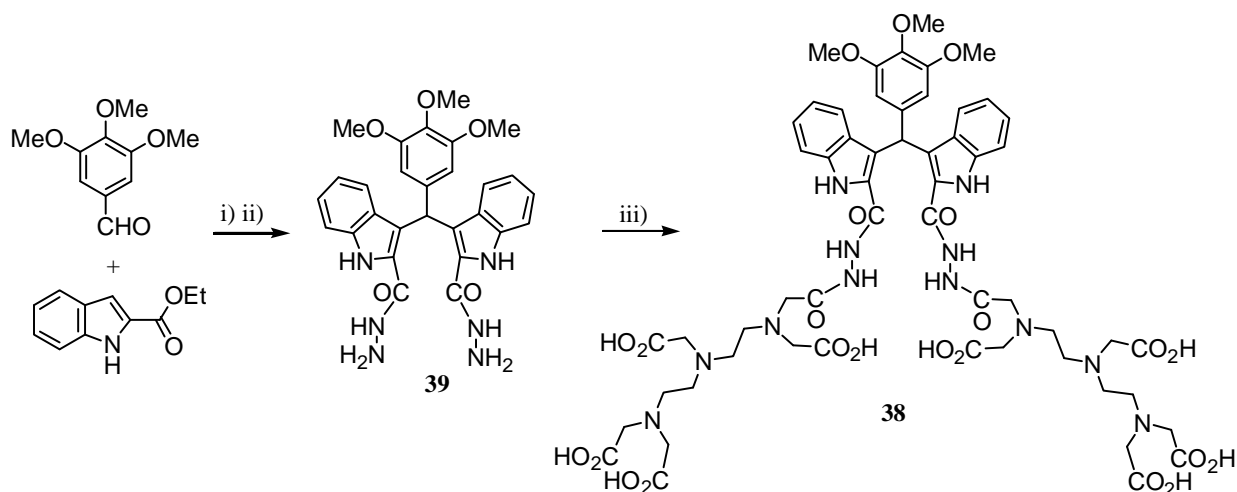
Scheme 10.



i) protected triamine,  $\text{K}_2\text{CO}_3$ , acetonitrile; ii) 6M HCl; iii) *tert*-butyl bromoacetate, DIEA, KI, DMF

Parac-Vogt *et al.* [29] reported a stable dinuclear Gd(III)-complex of compound **38** exhibiting a  $r_1$  value of  $13.6 \text{ s}^{-1}\text{mM}^{-1}$  (20 MHz, 25 °C). Compound **38** was prepared from the bis-indole derivative **39**, which was attached to two units of dtpa using TBTU/TEA in DMSO, according to Scheme 11.

Scheme 11. Dtpa bis-indole derivative.



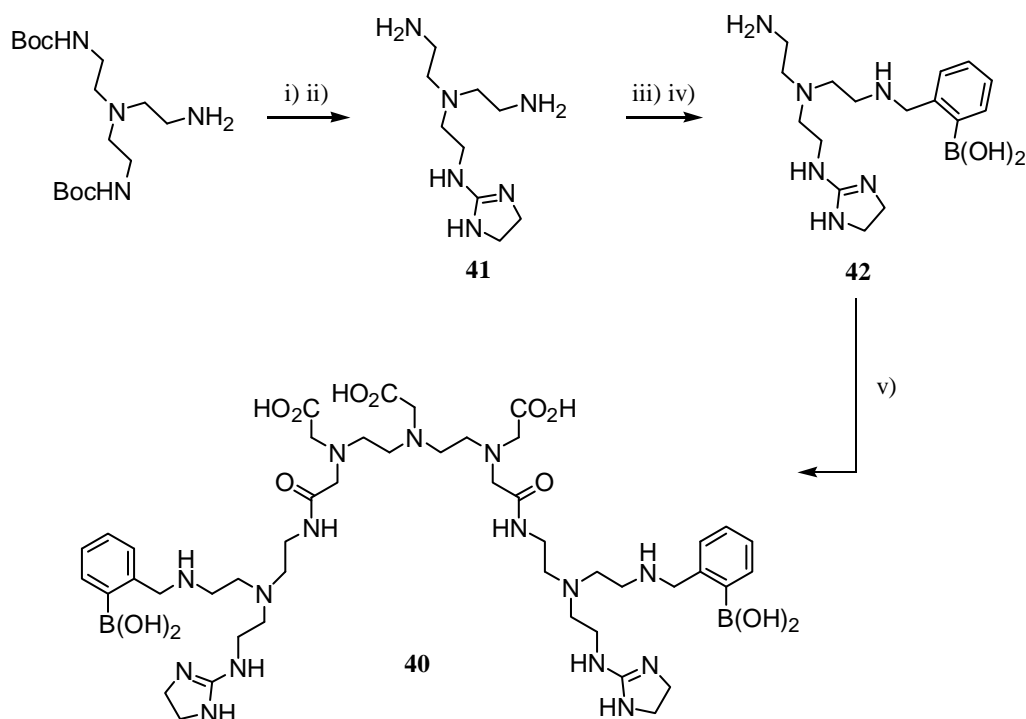
i) EtOH, HCl, reflux; ii) MeOH,  $\text{NH}_2\text{NH}_2$ , Py, 100 °C; iii) ethylentriaminepentaacetic acid, TBTU/TEA, DMSO

Gd-complexes have been also produced to recognize molecules such as sialic acids, a generic term for the *N*- or *O*-substituted derivatives of neuraminic acid, a nine-carbon monosaccharide containing a carboxyl group at the anomeric carbon. Glycoproteins or glycolipids containing sialic acids as terminal residues are often present in the cell surface or intracellular membranes.

Frullano *et al.* [30] synthesized a Gd-complex of **40** able to recognize and reversibly bind to sialic acid residues (Scheme 12), often proposed as diagnostic indicators in several diseases. Ligand **40** was synthesized from tri(ethylamine)amine (TREN) with two amine groups protected as *tert*-butoxycarbonyl (Boc) moieties and subsequent treatment with 2-methyl-2-thioimidazoline hydroiodide in refluxing ethanol. Removal of the Boc protecting groups in acidic medium afforded compound **41**, which reacted with 2-formylphenylboronic acid giving the corresponding imine. Reduction of the

latter and subsequent separation led to the monoamine **42**. Finally, **40** was prepared by condensation between compound **42** and the dtpa-bis-anhydride (Scheme 12).

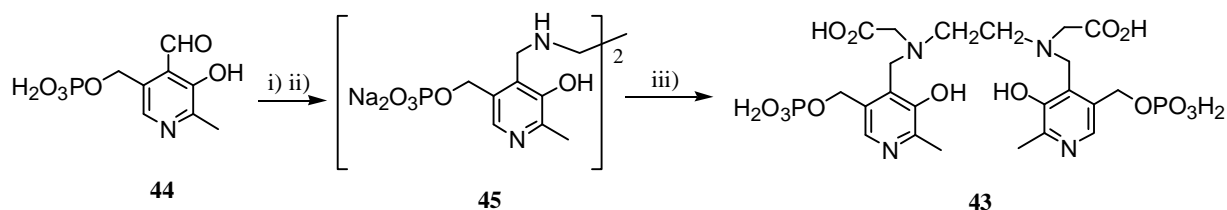
**Scheme 12.** Gd(III)-**40** recognizing and reversibly binding to the sialic acid residues.



i) 2-methylthio-2-imidazoline hydroiodide, EtOH, reflux; ii) HCl 37% EtOH; iii) 2-formylphenylboronic acid, MeOH, TEA; iv) NaBH<sub>4</sub>, MeOH; v) Dtpa-bisanhydride, zeolite KA, EtOH

One of the first CAs that included pyrimidine rings in its structure was the Mn(II) complex of *N,N'*-dipyridoxylethylenediamine-*N,N'*-diacetate 5,5'-bis(phosphonate) (DPDP, **43**), considered the active component of Teslascan<sup>®</sup>, a contrast medium for hepatic MRI [31]. This complex was synthesized in 1989 from pyridine **44** by condensation with ethylenediamine and subsequent reduction of the corresponding imine affording compound **45**. Alkylation of amine groups in **45** with bromoacetic acid in basic medium gave **43** (Scheme 13) [32].

**Scheme 13.** Synthesis of DPDP as chelating ligand for Mn(II).

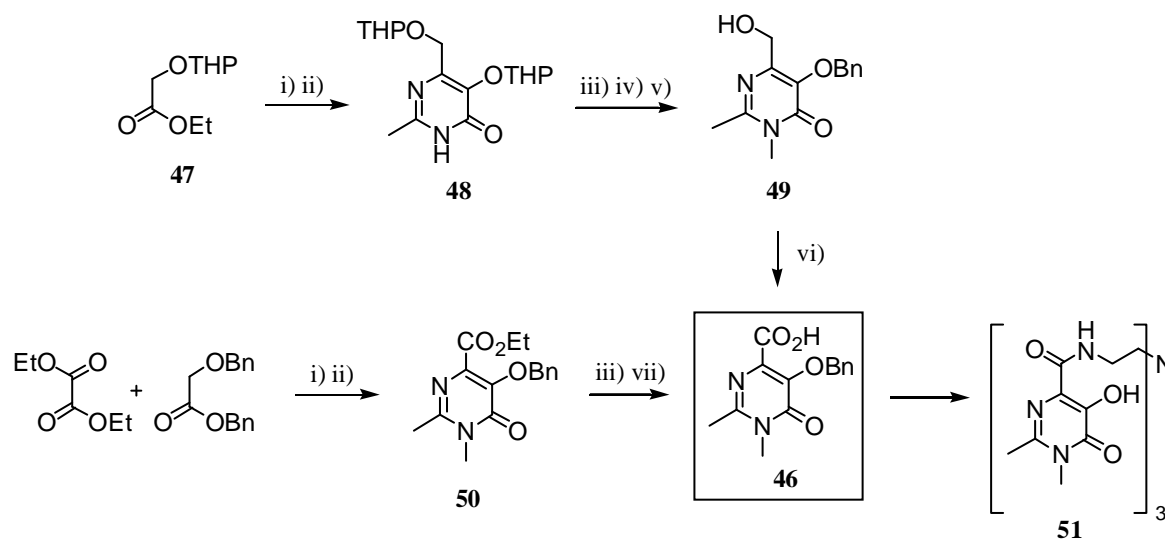


i) ethylenediamine, NaOH, MeOH; ii) H<sub>2</sub>, Pt/C; iii) bromoacetic acid, NaOH

Recently, 4-benzyloxy-5-pyrimidinone carboxylic acid **46** was reported as a building block to prepare several ligands through coupling with the corresponding amine. Two synthetic routes leading

to **46** were reported (Scheme 14) [33]. One of them starts with self-condensation of **47** and subsequent *in situ* reaction with acetamidine, giving compound **48**. Nitrogen methylation followed by hydroxyl group deprotection in an acidic medium and benzylation of the freed hydroxyl moiety at the 5-position yielded compound **49**. Finally, oxidation of **49**, using phase transfer conditions, afforded the acid **46**. The second approach was a shorter one starting from diethyl oxalate and benzyl benzyloxyacetate. Condensation of both, in the same conditions mentioned above, led to ester **50**, which by nitrogen methylation and basic hydrolysis of the ester group yielded the desired acid **46**. Coupling of **46** with amines may produce a large series of hydroxypyrimidinones. Particularly, the Gd-complex of **51** exhibited a relaxivity value of  $9.0 \text{ s}^{-1}\text{mM}^{-1}$  measured at 20 MHz and 25 °C.

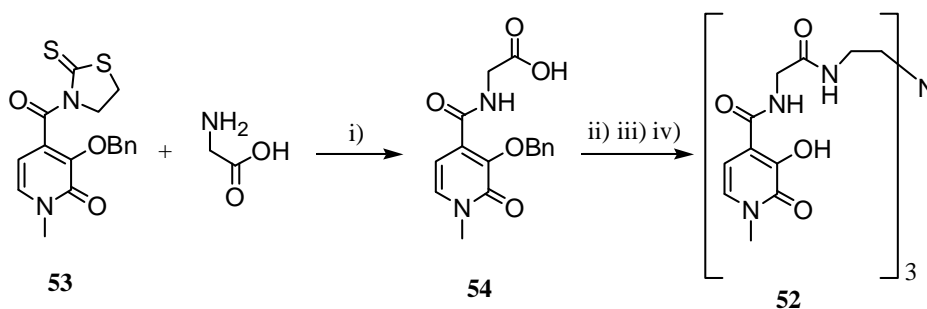
**Scheme 14.** Two synthetic strategies to prepare **51**.



i) Na or NaH, Et<sub>2</sub>O or THF; ii) acetamidine; iii) NaH, DMF, MeI; iv) H<sup>+</sup>, ROH; v) K<sub>2</sub>CO<sub>3</sub>, DMF, BnCl; vi) aq NaOCl, PT; vii) a) KOH, MeOH, b) H<sub>3</sub>O<sup>+</sup>

In 2003, a new tripodal hydroxypyridonate Gd-complex of **52** was prepared (Scheme 15) and its stability constant and relaxometric studies were described [34]. Ligand **52** was obtained from thiazolidine **53** by coupling with glycine to give compound **54**.

**Scheme 15**

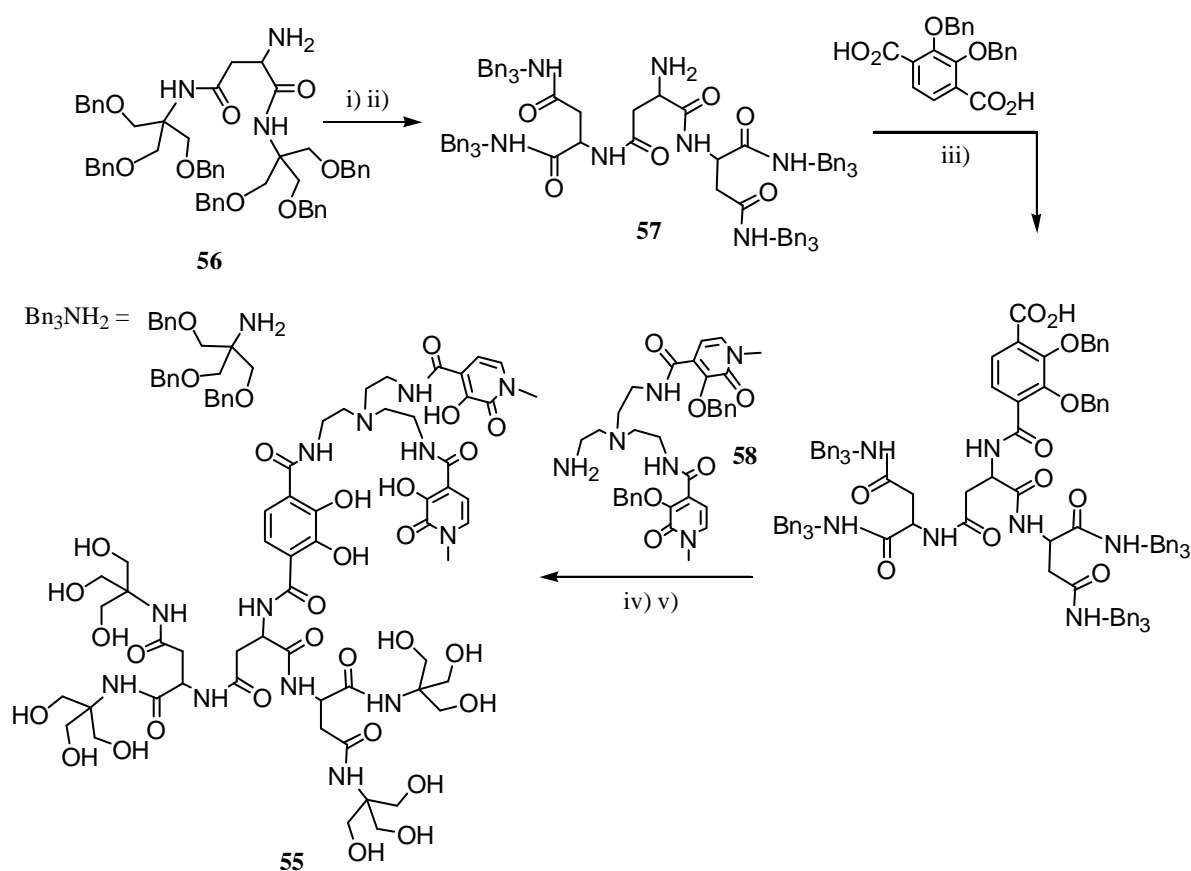


i) NaOH; ii) NHS, DCC; iii) TREN; 5% Pd/C, H<sub>2</sub>

Activation of **54** using *N*-hydroxy-succinimide (NHS), followed by amidation with TREN and subsequent benzyloxy group deprotection afforded compound **52**. The corresponding Gd(III)-complex showed improved relaxivity ( $6.6 \text{ s}^{-1} \text{ mM}^{-1}$  at 20 MHz and 298 K), compared to the CAs normally used in clinical diagnosis. However, its stability constant decreased slightly with respect to the analogous **51** without the glycine unit. Bis-hydroxypyridonate chelates were investigated, the MR imaging investigations and biodistribution being reported [35].

More recently, Pierre *et al.* [36] described a new dendrimeric Gd(III) chelator **55**, including 12 hydroxyl groups to ensure the overall solubility in water, and exhibiting a  $r_1$  values of 1.6 and 1.8 times greater than its corresponding monomer at 20 and 90 MHz, respectively ( $14.3$  and  $18.0 \text{ s}^{-1} \text{ mM}^{-1}$  25 °C, respectively). Ligand **55** derived from hydroxypyridonate was synthesized according to Scheme 16.

**Scheme 16.** Dendrimeric chelating ligand derived from hydroxypyridonate.



i) HATU, DIPEA, DMA, 20 °C; ii) TFA,  $\text{CH}_2\text{Cl}_2$ , 20 °C; iii) (a)  $\text{C}_2\text{O}_2\text{Cl}_2$ , toluene, DMF, 20 °C, (b) DIPEA, THF, 20 °C; iv) HATU, DIPEA,  $\text{CH}_2\text{Cl}_2$ , 20 °C; v)  $\text{H}_2$ , Pd/C, acetic acid

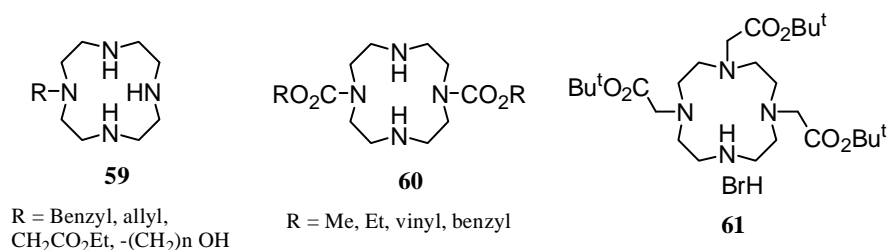
Reaction of tris-benzyloxyethanolamine and *N*-*tert*-butoxycarbonylaminoaspartic acid in the presence of the coupling reagent, HATU (*N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide), and subsequent deprotection of the amino group in an acidic medium led to compound **56**. Coupling of **56** with an additional aspartic acid unit and subsequent deprotection of the amino group afforded **57**. Amidation reaction between 2,3-benzyloxyterephthalic chloride and **57**, followed by coupling with compound **58**, and

hydrogenolysis of benzyl moieties yielded ligand **55**. This chelating ligand coordinated one Gd(III) ion showing two water molecules in the inner-sphere.

## 2.2 CAs derived from macrocyclic ligands

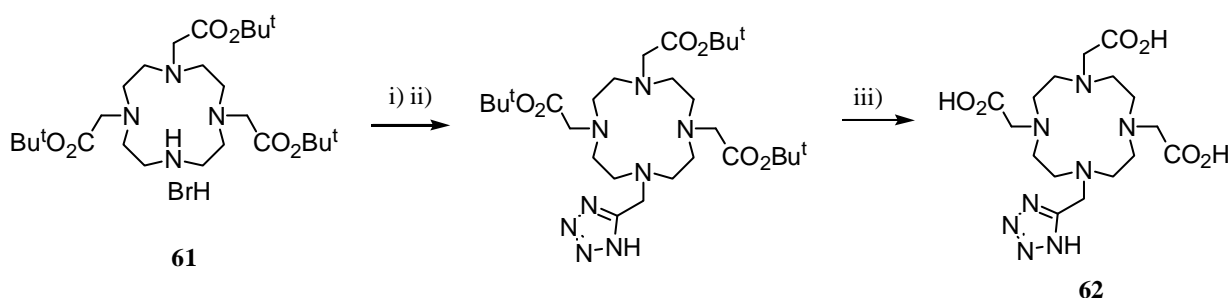
The development of novel generations of macrocyclic CAs for MRI, such as DOTA derivatives, is an important area of research because of the higher thermodynamic and kinetic stability of these lanthanide complexes in comparison with the linear CAs [37]. Three important key intermediates are used to prepare macrocyclic ligands based on the cyclen skeleton: monoalkylated cyclen **59** [38], 1,7-disubstituted-1,4,7,10-tetraazacyclododecanes **60** [39] and the 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane hydrobromide salt **61** [40] (Figure 5).

**Figure 5.** Cyclen derivatives used to synthesize the most useful macrocyclic CAs.



Ligand **62** containing a tetrazolymethyl arm was prepared from compound **61** according to Scheme 17 [41]. Compound **61** was alkylated with freshly distilled chloroethylnitrile under heterogeneous conditions followed by treatment with TMS- $\text{N}_3$ , in the presence of  $\text{Bu}_2\text{-SnO}$ , yielding the corresponding tetrazole derivative, which by acid hydrolysis afforded **62** ( $r_1$  of Gd-**62** was  $4.8\text{ s}^{-1}\text{ mM}^{-1}$  measured at 60 MHz and  $37\text{ }^\circ\text{C}$ ).

### Scheme 17

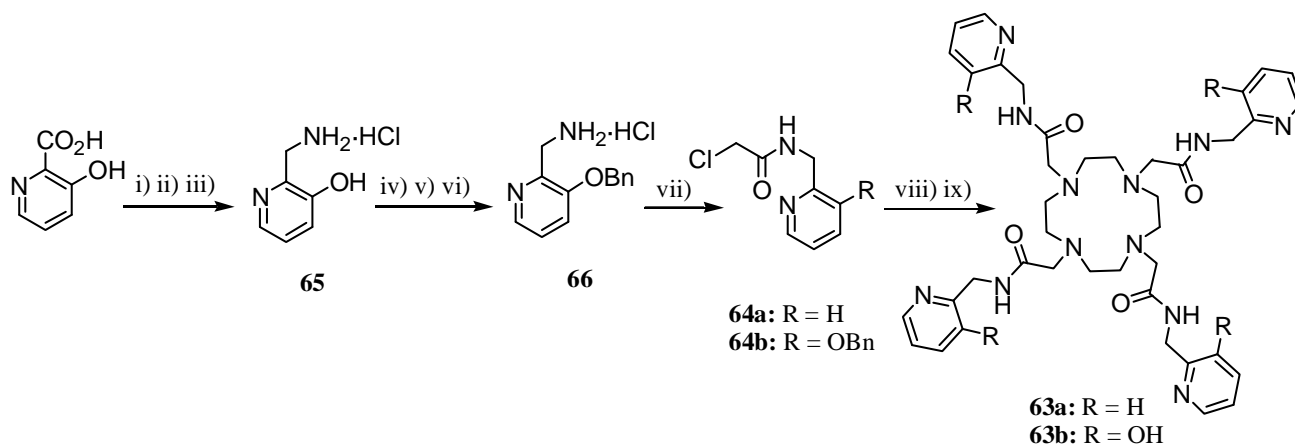


i)  $\text{ClCH}_2\text{CN}$ , KI,  $\text{K}_2\text{CO}_3$  acetonitrile, reflux; ii) a) TMS- $\text{N}_3$ ,  $\text{Bu}_2\text{-SnO}$ , toluene, reflux. b) 5% aq. HCl; iii) 12 N HCl reflux.

The pH environment of a complex can modify some of the determinant factors that contribute to the relaxivity of CAs, such as the number of water molecules ( $q$ ) in the inner-sphere of the complex, or even the second coordinating sphere. Pyridylmethyltetraamides **63** derived from DOTA were studied as pH dependent CAs [42]. Gd-**63b** showed a  $r_1$  values of 5.6 and  $4.1\text{ s}^{-1}\text{ mM}^{-1}$  at pH of 8.5 and 3.3, respectively (20 MHz). Compounds **63** were prepared by alkylation of cyclen with the corresponding chloroacetamide **64** (Scheme 18). Ligands **64a-b** were obtained by reaction of chloroacetyl chloride

and the corresponding amine. Compound **64b** was synthesized from 2-hydroxypicolinic acid by esterification with methanol, transformation of the ester in its amide by reaction with ammonia and subsequent reduction with borane giving amine **65**. The protection of the hydroxy group as benzyloxy moiety in **65** was carried out in three steps: i) protection of the amine as *tert*-butylcarbamate, ii) benzylation of hydroxyl group and, iii) deprotection of amine group in acidic medium affording compound **66**.

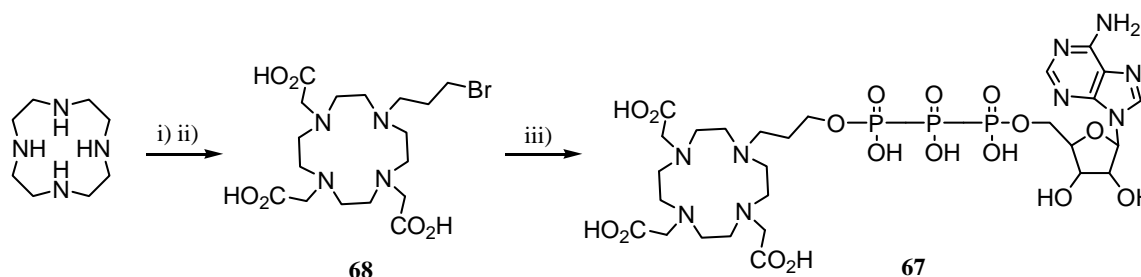
**Scheme 18.** Pyridylmethyltetraamides as pH responsive CAs.



i) MeOH, H<sub>2</sub>SO<sub>4</sub>; ii) NH<sub>3</sub> (aq); iii) a) BH<sub>3</sub>, THF; b) 2 M HCl; iv) (Boc)<sub>2</sub>O, MeOH, NaHCO<sub>3</sub>, H<sub>2</sub>O; v) BnBr, K<sub>2</sub>CO<sub>3</sub>, acetonitrile; vi) HCl, EtOH; vii) chloroacetyl chloride, THF, NaHCO<sub>3</sub>, H<sub>2</sub>O; viii) cyclen, K<sub>2</sub>CO<sub>3</sub>, acetonitrile; ix) H<sub>2</sub>, Pd/C, EtOH (only to synthesize **63b**)

Another example of a CA displaying pH dependent relaxivity is the Gd(III)-complex of ATP-conjugated DO3A **67**, reported by Ratnakar and Alexander (6.5 s<sup>-1</sup> mM<sup>-1</sup> measured at 24 MHz, 308 K and pH of 8.5) [43]. Compound **67** was prepared from cyclen by trialkylation using bromoacetic acid and subsequent reaction with 1,3-bromopropane in basic medium leading to compound **68**. Treatment of **68** with ATP disodium salt in water afforded **67** (Scheme 19).

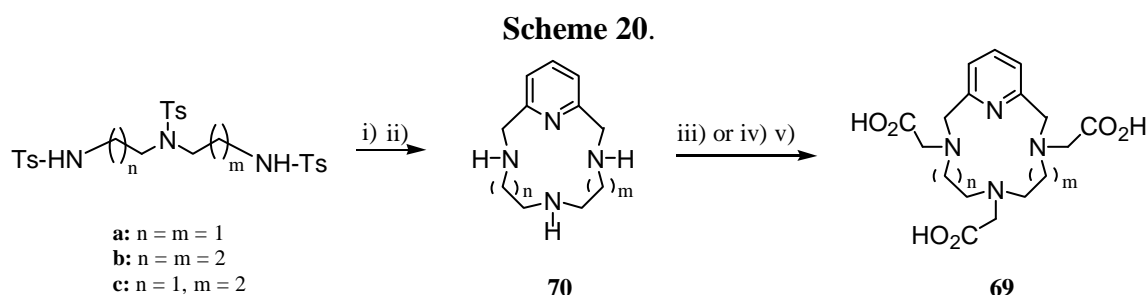
**Scheme 19.** ATP-conjugated DO3A **67** which its Gd(III)-complex showing pH dependence relaxivity.



i) chloroacetic acid, H<sub>2</sub>O, NaOH (pH 10), -4 °C; ii) 1,3-dibromopropane, DMF, H<sub>2</sub>O, triethylamine; iii) adenosine 5'-triphosphate disodium salt, H<sub>2</sub>O, rt

Several contributions have reported novel series of tetraazamacrocycles containing one pyridine ring as part of the macrocycle. Their lanthanide complexes generally showed high stability and improved relaxivity. These heptadentate ligands form stable complexes with lanthanide ions, allowing the coordination of two water molecules in the inner-sphere and consequently providing higher

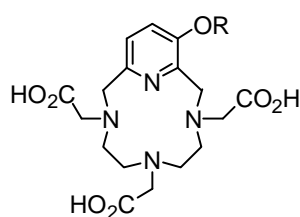
relaxivity than DOTA chelates. In general these complexes are characterized by a relatively fast water exchange rate as compared to the octacoordinated ligands. Scheme 20 shows the synthetic approach to prepare **69** [44]. Ligand **69a** was synthesized by reaction of 1,4,7-tritosyl-1,4,7-triazaheptane with bis(2,6-chloromethyl)pyridine, followed by deprotection of the amine groups in an acidic medium yielding compound **70a**. Alkylation of **70a** with chloroacetic acid in the presence of sodium carbonate, and subsequent treatment in acidic medium yielded **69a** (Gd-**69** showed a  $r_1$  of  $6.9 \text{ s}^{-1} \text{ mM}^{-1}$  at 20 MHz and 25 °C). Compounds **69b-c** were synthesized from the corresponding triprotected amine via the macrocycles **70b-c**. The latter reacted with methyl chloroacetate as alkylating agent yielding the corresponding esters, which were hydrolyzed using KOH in methanol. Complexes Gd-**69b** and Gd-**69c** exhibited a  $r_1$  values of 5.9 and  $6.3 \text{ s}^{-1} \text{ mM}^{-1}$ , respectively (20 MHz and 25 °C).



i) bis-(2,6-chloromethyl)pyridine,  $\text{Na}_2\text{CO}_3$ , acetonitrile, reflux; ii) HBr, AcOH, phenol, reflux; iii) a) KOH, b) KOH, chloroacetic acid,  $\text{H}_2\text{O}$  80 °C, c) 2N HCl, ( $n = m = 1$ ); iv) a) methyl chloroacetate,  $\text{Ag}_2\text{CO}_3$ , THF, b)  $\text{H}_3\text{O}^+$ ,  $\text{H}_2\text{S}$ ; v) KOH, MeOH ( $n = m = 1$  and  $n = 1, m = 2$ )

Analogous systems **71** with substituents on a pyridine ring were prepared using a similar synthetic approach to that employed to obtain **69**, starting from the corresponding bis(2,6-chloromethyl)pyridine derivative (Figure 6) [45].

**Figure 6.**

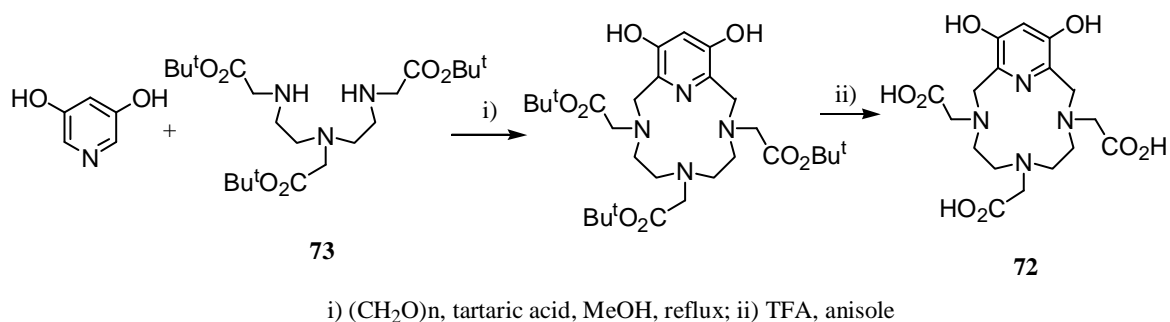


**71a:**  $\text{R} = \text{CH}_2-\text{C}_6\text{H}_4-\text{Br-}p$   
**71b:**  $\text{R} = \text{C}_{12}\text{H}_{25}$

Their corresponding Gd(III)-complexes depicted improved relaxivity with respect to **69** ( $r_1 = 8.25 \text{ s}^{-1} \text{ mM}^{-1}$  measured at 20 MHz and 25 °C, for Gd-**71a**). Studies on the binding of the Gd(III)-**71a** to biomacromolecules such as the human serum albumin (HSA), were described and even the formation of the inclusion compounds using  $\beta$ -cyclodextrins and poly- $\beta$ -cyclodextrins were reported. These non covalent adducts showed higher relaxivity due to the reduced motion of the corresponding Gd(III)-complexes, becoming well tolerated in animal tests. Gd(III)-**71b** was considered as micellar CAs, its relaxivity depending on concentration (maximum value of  $r_1$  was  $29.2 \text{ s}^{-1} \text{ mM}^{-1}$  at 20 MHz, 25 °C and 1.5 mM).

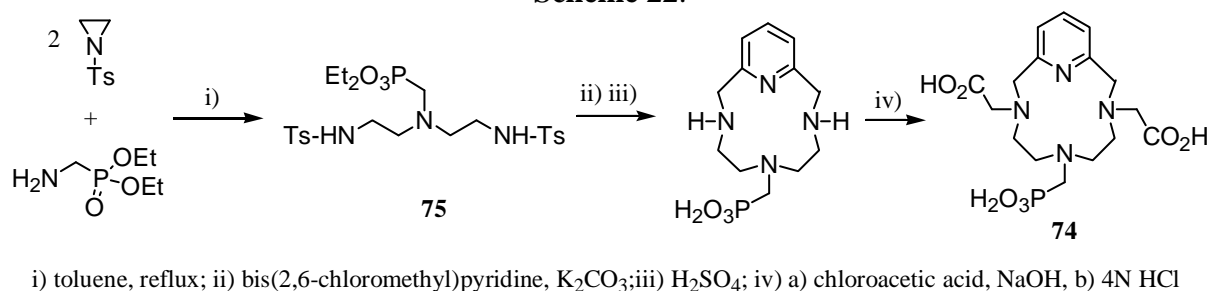
The relaxivity of pyridine-based complexes can be improved through the introduction of polar moieties supported over the heterocyclic ring, a circumstance probably improving the effects of the second coordination sphere. An example of that is the Gd(III)-complex of macrocycle **72**, containing two free hydroxyl groups, with  $r_1$  of  $8.5 \text{ s}^{-1} \text{ mM}^{-1}$  at 20 MHz and 25 °C [23]. Ligand **72** was prepared by double Mannich reaction between 3,5-dihydroxypyridine, paraformaldehyde and amine **73** followed by treatment with neat TFA-anisole (Scheme 21).

Scheme 21.



On the other hand, inclusion of the methylenephosphonic arm on triamine backbone of these macrocycles induced higher relaxivity of the corresponding Gd(III)-complexes. Thus, the complex of **74** showed two water molecules in its inner-sphere and another water molecule bound to the phosphate group providing an important contribution to the higher relaxivity ( $r_1 = 8.3 \text{ s}^{-1} \text{ mM}^{-1}$  at 20 MHz and 25 °C) [46]. Compound **74** was prepared from *N*-tosylaziridine and diethyl aminomethylphosphonate leading to the amine **75** (Scheme 22). Cyclization of **75** with bis(2,6-chloromethyl)pyridine in basic medium, deprotection of amine groups and subsequent alkylation of them afforded compound **74** in high overall chemical yield.

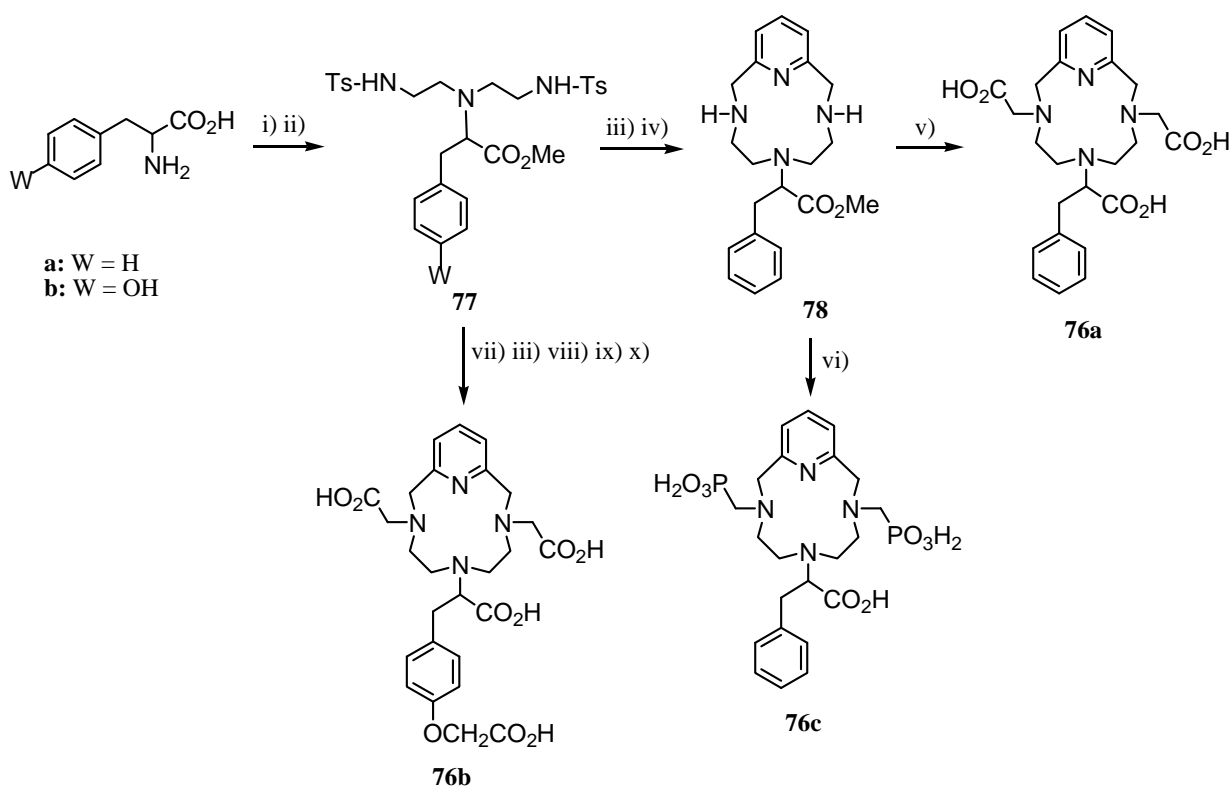
Scheme 22.



A similar synthetic route (Scheme 23) was used to obtain the macrocycles **76** starting from the corresponding amino acids [47]. Relaxivity of their Gd(III)-**76a-c** was 8.3, 10.5, and  $8.1 \text{ s}^{-1} \text{ mM}^{-1}$  at 20 MHz and 25 °C, respectively, and in the range of the major of the heptadentate complexes. However, the inclusion of two phosphonate groups in **76c** caused a decrease of the number of water molecules in the inner-sphere ( $q = 1$ ) remaining two of those in second coordinating sphere ( $q^{2nd} = 2$ ). Esterification of the corresponding amino acid followed by reaction with two units of the *N*-tosylaziridine yielded compounds **77**, which gave the corresponding macrocycles under the conditions mentioned above; deprotection of amine groups in **77a** using acidic medium led to compound **78**. While alkylation of the free amine groups in compound **78** using chloroacetic acid gave **76a**, treatment of **78** with phosphoric acid and paraformaldehyde yielded **76c**. Ligand **76b** was synthesized from **77b** in five

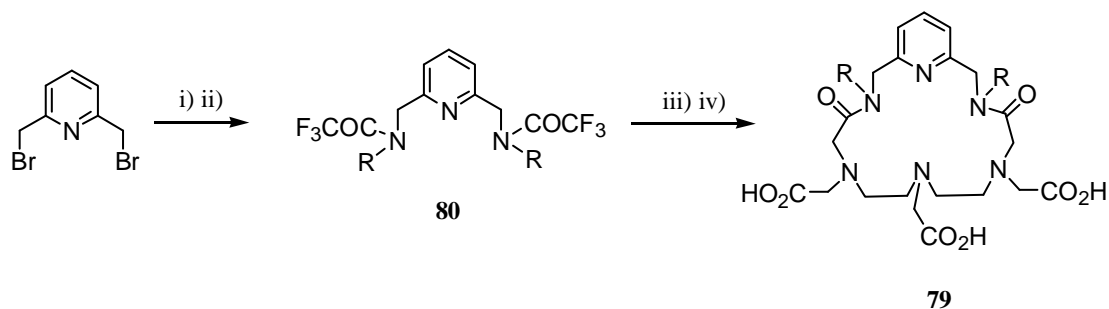
steps as follow: i) protection of hydroxyl group, ii) formation of macrocycle, iii) deprotection of amine groups, iv) alkylation of those and hydroxyl using methyl chloroacetate and, v) basic hydrolysis.

Scheme 23.



i)  $\text{SOCl}_2$ , 0 °C, MeOH; ii) 1-tosylaziridine, toluene, reflux; iii) 2,6-bis(chloromethyl)pyridine,  $\text{K}_2\text{CO}_3$  acetonitrile reflux; iv) 48 % HBr, PhOH, AcOH, reflux; v) chloroacetic acid, KOH, 80 °C; vi)  $\text{H}_3\text{PO}_3$ ,  $(\text{CH}_2\text{O})_n \text{HCl}$ , reflux; vii)  ${}^t\text{BuMe}_2\text{SiCl}$ ,  ${}^i\text{Pr}_2\text{EtN}$ , acetonitrile, 0 °C; viii) 30 % HBr, AcOH, 80 °C; ix) methyl bromoacetate,  $\text{Ag}_2\text{CO}_3$ , acetonitrile, rt; x) KOH, MeOH 80 °C

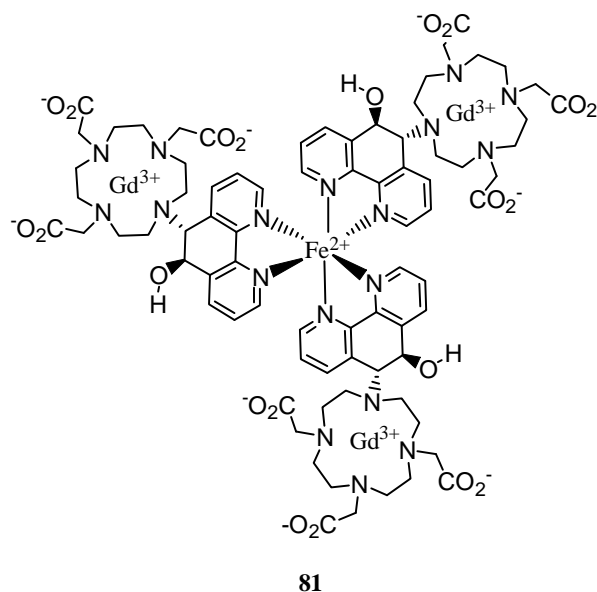
Zheng *et al.* [48] have developed new lipophilic macromolecular chelators of lanthanide ions **79** for *in vivo* applications. Some types of cells can be labeled with these complexes in order to visualize cell migration in different biological systems. Compounds **79** were prepared by reaction of bis(2,6-bromomethyl)pyridine and the corresponding amines and subsequent reaction with  $(\text{CF}_3\text{CO})_2\text{O}$  to give compound **80**, this last additional step being necessary to purify the corresponding amines (Scheme 24). Finally, treatment of **80** in basic medium and reaction of those with DTPA-bisanhydride afforded compounds **79**.

Scheme 24. Lipophilic macromolecular chelators of lanthanide ions **79**.

i)  $\text{R-NH}_2$ , toluene ( $\text{R} = \text{C}_4\text{H}_9$ ,  $\text{C}_{10}\text{H}_{21}$  or  $\text{C}_{12}\text{H}_{25}$ ); ii)  $(\text{CF}_3\text{CO})_2\text{O}$ , acetonitrile,  $\text{K}_2\text{CO}_3$ ; iii)  $\text{NaOH}_{(\text{aq})}$ , THF; iv) DTPA-bisanhydride, DMF

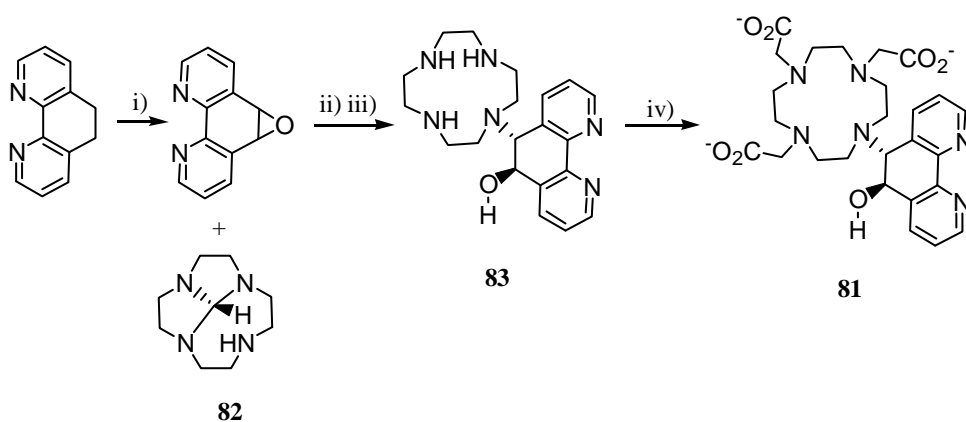
Multinuclear complexes based on 5,6-dihydro-1,10-phenanthrolin-5-yl-DO3A **81** were reported as complexes with higher molecular weights and consequently reduced motion of the complex and increased relaxivity [49] (Figure 7). Complex **Gd(III)-81** spontaneously forms highly stable tris-complexes with Fe(II) and Ni(II) characterized because of their relaxivity is not dependent on the temperature in a 5-30 °C range. Relaxivity values reported for Gd-**81** and Fe[Gd-**81**]<sub>3</sub> were 3.7 and 36.6 s<sup>-1</sup> mM<sup>-1</sup> at 20 MHz and 37 °C, respectively.

**Figure 7.** Multinuclear complex based on 5,6-dihydro-1,10-phenanthrolin-5-yl-DO3A.



Ligand **81** was prepared from phenanthroline by epoxidation, subsequent reaction with cyclen derivative **82**, followed by refluxing in HCl in MeOH affording compound **83**. Finally, alkylation of **83** yielded the desired compound **81** (Scheme 25).

**Scheme 25.**



i) NaOCl, Na<sub>2</sub>HPO<sub>4</sub>, (*n*-butyl)<sub>4</sub>N<sup>+</sup>HSO<sub>4</sub><sup>-</sup>; ii) X, LiClO<sub>4</sub>, acetonitrile; iii) ClH in MeOH, b) K<sub>2</sub>CO<sub>3</sub>, acetonitrile; iv) BrCH<sub>2</sub>CO<sub>2</sub>K, MeOH, K<sub>2</sub>CO<sub>3</sub>

### 3. Concluding Remarks and Future Perspectives

We have described above the main synthetic strategies used to produce heterocyclic CAs, a family of ligands depicting very appropriate stability and relaxivity properties for MRI. Basically, most of them are produced through two general organic reactions, namely amine alkylations or amidation. In general heterocycles provide good electron donor ligands suitable for improving the chelating capacity of the earlier complexones. The present review provides an adequate frame to analyze the importance of the heterocyclic ring in determining the coordination chemistry, the relaxivity and the stability properties of the resulting complexes.

On these grounds, the use of heterocyclic CA's is expected to increase in the future. The possibilities to obtain physiologically responsive agents, reflecting tissue properties beyond anatomy has already started [50]. Further improvements are expected from the combination of novel synthetic approaches and updated MR imaging techniques, as Magnetization Transfer [51]. The combination of both, synthetic and MRI approaches will certainly exceed the capabilities of their independent use. As a complementary field, the development of heterocyclic contrast agents useful for in vivo spectroscopy and spectroscopic imaging of pH and pO<sub>2</sub> in healthy and pathological tissues, constitutes an area of growing interest [52-55]. The combination of spectroscopic and imaging approaches in multiparametric studies will further enhance the diagnostic potential of these new methods [56]. Finally, the development of multimodal heterocyclic probes, active in different imaging modalities (MRI, MRS, PET), is currently envisioned as one of the most attractive goals for the immediate future.

### Acknowledgements

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*Sample Availability:* Contact the authors.