Interuniversity Master in Drug Discovery

2019 - 2020

OPTIMIZATION OF SYNTHETIC ROUTES TOWARD GADOXETATE DISODIUM: A CONTRAST AGENT EMPLOYED IN NUCLEAR MAGNETIC RESONANCE IMAGING (MRI)

OPTIMIZACIÓN DE RUTAS SINTÉTICAS PARA LA OBTENCIÓN DEL GADOXETATO DISÓDICO: UN AGENTE DE CONTRASTE EMPLEADO EN RESONANCIA MAGNÉTICA NUCLEAR DE IMAGEN (MRI)

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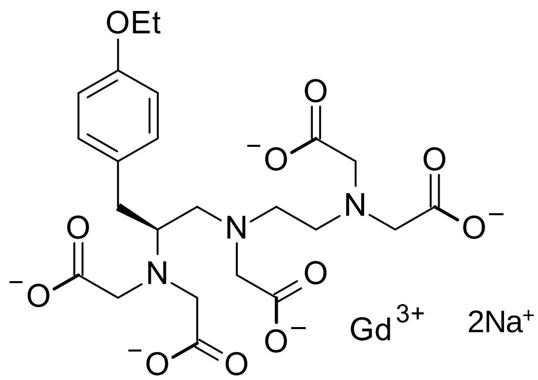

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que ha realizado **D./Dña. Esmael Ahbiti Laarab** como Trabajo Fin de Máster, para el Máster Interuniversitario en Descubrimiento de Fármacos, ha sido realizado en los laboratorios de este Departamento bajo su dirección y autorizan su presentación.

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Sincerely,

Thank you all!

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ABSTRACT

Gadolinium-based contrast agents (GBCAs) enhance the tissue visualization allowing to the doctor differentiate between abnormal tissues and normal tissues in a patient by using magnetic resonance imaging (MRI). Among all commercialised agents, the mixed contrast agent Primovist® or, chemically known as Gadoxetate Disodium, has a great interest in application in MRI studies. This fact can be explained due to its ability to have dual excretion pathway which allow the detection of the vascular and hepatic injuries in one single injection. For this reason, this review will be focused on the synthetic route toward Primovist® to find out a better pathway for this pharmacological product in terms of yield, purity and suitability to operate at industrial scale.

RESUMEN

Los agentes de contraste basados en gadolinio (GBCA) mejoran la visualización de los tejidos, facilitando a los médicos llevar a cabo una buena diferenciación entre los tejidos patológicos y los tejidos normales de un paciente mediante resonancia magnética nuclear de imagen (RMI). Entre todos, los agentes de contraste mixtos como Primovist®, conocido como Gadoxetato Disódico, han tenido un gran interés de aplicación en los estudios de resonancia magnética nuclear por imagen. Este hecho se puede explicar debido a que presentan como característica principal la de poseer una vía de excreción dual, permitiendo la detección de lesiones vasculares y hepáticas en una sola inyección. Por esta razón, en esta revisión bibliográfica se prestará mucha atención en la única ruta sintética descrita para la obtención de Primovist®, con el fin de encontrar una mejor ruta para este producto farmacológico en términos de rendimiento, pureza e idoneidad para operar a nivel industrial.

KEYWORDS

Gadolinium-Based Contrast Agent, Primovist®, Synthetic Route

PALABRAS CLAVE

Agentes de contraste basados en gadolinio, Primovist®, Ruta sintética

ABBREVIATIONS

CAs: Contrast Agents

Cbz-Cl: Benzyl chloroformate

CT: Computed Tomography

DTPA: Diethylenetriaminepentaacetato

EOB: Ethoxybenzyl

GBCAs: Gadolinium Based Contrast Agents

HPLC: High Performance Liquid Chromatography

MRA: Magnetic Resonance Angiography

MRI: Magnetic Resonance Imaging

MTBE: Methyl *tert*-butyl ether

OATP1B1/B3: Organic anion transporting polypeptide

NMR: Nuclear Magnetic Resonance

NSF: Nephrogenic Systemic Fibrosis

PRE: Paramagnetic Relaxation Enhancement

r: relaxivity

T_1: Longitudinal Relaxation Time

T_2: Transverse Relaxation Time

Et_2SO_4: Diethyl sulphate

THF: Tetrahydrofuran

HCl: Chloridric acid

MsCl: Methane sulfonyl chloride

EtI: Ethyl iodide

$NaBH_4$: Sodium borohydride

ECAs: Extracellular Agents

MeI: Methyl iodide

AcOEt: Ethyl acetate

MRP2: Multi drug resistance

Boc_2O: Di-*tert*-butyl dicarbonate

Et_3N: Triethylamine

K_2CO_3: Potassium carbonate

L-Tyr: L-Tyrosine

ECF: Ethyl Chloroforornate

DMF: *N,N* - dimethylformamide

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1. INTRODUCTION

1.1. Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) was invented by **Paul C. Lauterbur** in 1971 and it is powerful technique for application in diagnostic clinical medicine and in biomedical research. This technique can transform spatial information of the water molecules present in tissues into an NMR signal using magnetic field gradients. After years gaining insight into this field, the first clinical full body MRI machine (Figure 1) was not installed until 1980 and since that time, essential improvements have been made, leading to its widespread use in medicine today.

Figure 1. First MRI scanner build in 1980.

Magnetic Resonance Imaging [1] (MRI), unlike conventional radiography and computed tomographic (CT) which employ potentially harmful (X-ray) radiation, is known as a harmless and non-invasive imaging technology that produces detailed three-dimensional anatomical images of biological tissues used in radiology. So, MRI is an essential technique which is useful for doctors in the early detection, diagnosis, and treatment monitoring of human disease. Therefore, it can be summarized as an elaborate proton nuclear magnetic resonance (NMR) that visualizes the magnetic properties from tissue water protons, whose concentration in our body is around 35 M. Before explaining the mainly applications and the evolution of MRI, it is crucial to understand a little bit about the physics phenomena behind MRI consists of, by explaining the experimental procedure of an MRI in a real situation.

During an MRI procedure, a patient is placed inside a cylindric magnet, called MRI scanner, and it must remain restful until the imaging process is finished so as not to get blurred images. MRI scanners [2] make use of powerful magnets which lead to a strong magnetic field that drive water protons inside the body to align with that field.

[1] De Leõn-Rodríguez, L. M.; Martins, A. F.; Pinho, M. C.; Rofsky, N. M.; Sherry, A. D. Basic MR relaxation mechanisms and contrast agent design. *J. Magn. Reson. Imaging* **2015**, *42* (3), 545-565.
[2] Chimie, L. D. E.; Et, I. Highly Efficient MRI Contrast Agents: from Monomers to Nanoparticles. Thesis, École Polytechnique Fédérale De Lausanne, December 2008.

Subsequently, an intense radiofrequency pulse, known as *Larmor frequency*, is then applied through the patient that tips the net magnetization vectors generated by the hydrogen nuclei in the direction of the receiver coil. Once the radiofrequency field is switched off, the MRI sensors are able to detect the energy released as the protons bring the net magnetization vectors back (only longitudinal component or $\boldsymbol{T_1}$ and without transverse component or $\boldsymbol{T_2}$) to its equilibrium position in alignment with the initial applied magnetic field. This magnitude signal in energetic terms detected by the receiver is used to form the MR image with the aid of computer software. Moreover, this signal is related to the time it takes for the protons to realign with the magnetic field, known as the *T_1 or T_2* relaxation *time* and changes depending on the environment and the chemical nature of the water molecules present in soft tissues. As I have just mentioned, water protons in tissue are characterised by these two relaxation times constants, T_1 and T_2, which both affect the images' signal intensity. By consensus, T_1 (or also called longitudinal relaxation time) is defined as the time it takes for 63% of its longitudinal magnetization component to recover, while T_2 of a tissue is defined as the time it takes to lose 63% of its total transverse magnetization signal. With this useful data in hand provided by MRI machine, doctors have been able to detect if there is a disease process or injury present inside the patient. So, the key of this technique for ensuring an appropriate image and being able to differentiate abnormal and normal tissues are both relaxation times T_1 and T_2, but as we are going to see later in case of gadolinium agents the effect is higher on T_1-weighted images. MRI in humans is extensively used today thanks to Raymon Damadian *et* al. [3] who discovered in the 1970's that the T_1 of tumours (Table 1)[3] in animals were significantly longer than T_1 values of the corresponding normal tissues. This outcome led to an extension of the study in humans to differentiate as much as possible abnormalities in our tissues.

Table 1. T_1 relaxation times in normal and malignant tissues.

Tissue	T_1 tumour (s)	T_1 normal (s)
Bone	1.027	0.554
Breast	1.080	0.367
Liver	0.832	0.570
Lung	1.110	0.788
Skin	1.047	0.616

[3] Damadian, R.; Zaner, K.; Hor, D.; DiMaio, T. Human tumors detected by nuclear magnetic resonance. *Proc. Natl. Acad. Sci. U. S. A.* **1974**, *71* (4), 1471-1473.

Due to the difference between the abnormal and normal tissues relaxation times together with the differences observed in their signal intensities, the medical doctors can determine or detect a possible case of an early tumour stage. Regarding the duration of an MRI examination, it can be said that it is quite long most of the cases and particularly the contrast between healthy and unhealthy tissues might be not enough to ensure the detection of a certain disease. For this reason, a class of pharmacological products, called MRI contrast agents [4,5,6] have been used years ago for improving the visualization of these tissues.

1.2. Contrast Agents

Despite the great advances in MRI, there are still some kind of pathologies that cannot be fully assessed by MRI. For this reason, the appealing for chemical agents, known as **contrast agents**, can be a useful tool to overcome the main weaknesses of MRI itself. These kinds of compounds provide detailed analysis and anatomic depiction of the vascular system as well as they help to improve the visibility in certain tissues making these pharmacological products widely used in diagnostic imaging for nearly 30 years. Just after the introduction of MRI technique, the first contrast agent developed in human study was reported in 1981[7], using oral ferric chloride for gastrointestinal (GI) tract. Since that time, many other contrast agents [8] have been launched to the market differing in their magnetic properties, chemical composition, biodistribution, imaging applications and its administration route.

Up to date, MRI contrast agents may be divided into different two groups. The first group comprises paramagnetic compounds, like lanthanides elements such as gadolinium, whereas the second group is composed by transition elements such as manganese and

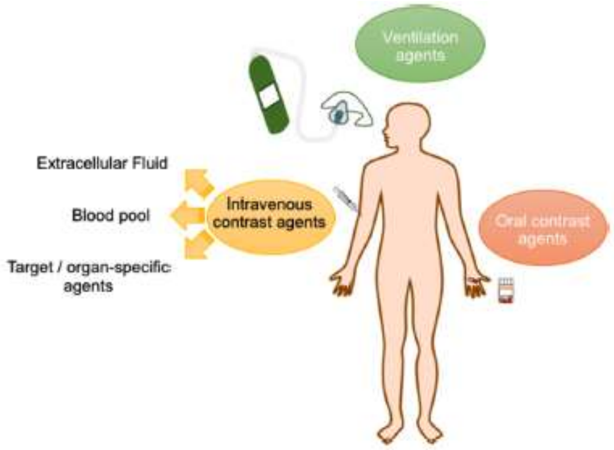
[4] Carrasco Muñoz, S.; Calles Blanco, C.; Marcin, J.; Fernández Álvarez, C.; Lafuente Martínez, J. Contrastes basados en gadolinio utilizados en resonancia magnética. *Radiologia*. **2014**, *56* (S1), 21-28.
[5] Choi, Y.; Huh, J.; Woo, D. C.; Kim, K. W. Use of gadoxetate disodium for functional MRI based on its unique molecular mechanism. *Br. J. Radiol.* **2016**, *89* (1058), 1-9.
[6] Le Fur, M.; Caravan, P. The biological fate of gadolinium-based MRI contrast agents: a call to action for bioinorganic chemists. *Metallomics* **2019**, *11* (2), 240-254.
[7] Young, I. R.; Clarke, G. J.; Baffles, D. R.; Pennock, J. M.; Doyle, F. H.; Bydder, G. M. Enhancement of relaxation rate with paramagnetic contrast agents in NMR imaging. *J. Comput. Tomogr.* **1981**, *5* (6), 543-547.
[8] Lohrke, J.; Frenzel, T.; Endrikat, J.; Alves, F. C.; Grist, T. M.; Law, M.; Lee, J. M.; Leiner, T.; Li, K. C.; Nikolaou, K.; *et* al. 25 Years of Contrast-Enhanced MRI: Developments, Current Challenges and Future Perspectives. *Adv. Ther.* **2016**, *33* (1), 1-28.

iron (also known as superparamagnetic contrast agents). The available contrast agents possess a similar mechanism of action, consisting in accelerate the relaxation rates of the surrounding protons (longitudinal (T_1) and transverse (T_2) relaxation times), allowing a reduction in the examination time and improving the accuracy of imaging contrast. The gadolinium-based chelates have represented the mainstay of intravenous contrast-enhanced MR imaging since their introduction into clinical use of Gadopentetate Dimeglumine (Gd-DTPA) in 1998[9]. Since that time, several Gd chelates have been developed to improve clinical efficacy and patient safety.

Gadolinium Based Contrast Agents

Gadolinium Based Contrast Agents (GBCA) can be divided in function of their administration route. Thus, they can be found as intravenous, oral or ventilation contrast agents (Figure 2), being all of them using for MRI. For example, oral MRI agents are primarily used to produce detailed images of the small intestine, enterography. However, the ventilation contrast agents are suitable for lung examination: the intravenous contract agents are useful to enhance the visibility of the vascular system and structures and some specific tissues. So, their use can be tailored to a specific disease.

Figure 2. Route of administration of MRI contrast agents.



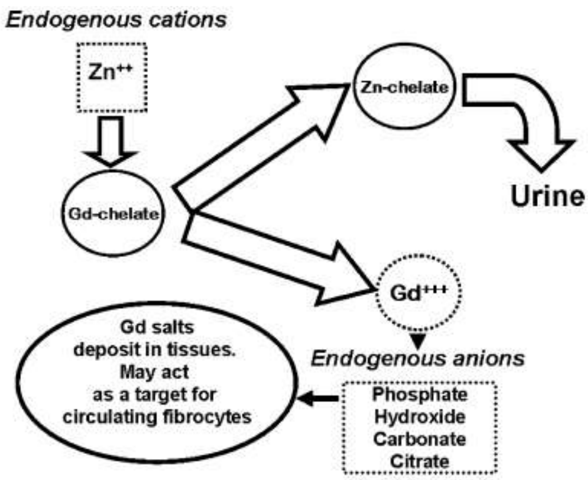
But now, intravenous contrast agents are by far the most predominant in clinical practice since their huge scope of application. Regarding the mechanism of action, intravenous gadolinium contrast agents modify the T_1 relaxation time in most cases, whereas iron contrast agents work through T_2 effect. Another fact, that explain the huge use of gadolinium complexes is about the adverse effects which tend to be mild in comparison to other metal complexes.

[9] Pierre, V. C.; Allen, M. J.; Caravan, P. Contrast agents for MRI: 30+ years and where are we going? Topical issue on metal-based MRI contrast agents. Guest editor: Valérie C. Pierre. *J. Biol. Inorg. Chem.* **2014**, *19* (2), 127-131.

Therefore, the most commonly used contrasts currently are those based on Gadolinium (Gd), although there are also contrast media for other elements, such as Mn (Mangafodipir trisodium marketed under the name Teslascan®) and Fe (Super Paramagnetic Iron Oxide nanoparticles, Sinerem®), but they are currently in disuse since that none have presented at large scale as well as their poor clinical performance and concerns over toxicity issues.

Based on their chemical structure, GBCA are molecules which contain two different moieties. The first one is composed by lanthanide rare metal (in this case gadolinium) and the second one tends to be an organic ligand such as polyamino-polycarboxylic derivatives which are able to reduce the toxicity generate by the presence of the free ionic form.

Figure 3. A diagram of the process of transmetallation between Gd^{3+} and endogenous cations such as zinc (Zn^{2+}).



Considering that the stability of these kind of compounds is important since body fluids contain a large number of ligands or endogenous metal ions. So, various competitive reactions can occur *in vivo*. For instance, endogenous cations like Fe^{3+}, Ca^{2+}, Zn^{2+}, Cu^{2+} can react with gadolinium chelates by displacing Gd^{+3} in a process known as transmetallation exchange. Between the endogenous ions located in blood pool, only zinc (Figure 3) can displace significant amount of Gd^{3+} due to its high concentration in the blood (55–125 mol/L). Due to the low copper concentration (1–10 mol/L) and the low calcium affinity to organic ligands, these two metals have not influence on the transmetallation exchange. Moreover, iron ions are tightly bound to the storage proteins ferritin and haemosiderin and are not available for transmetallation with Gd^{3+}. As a result, zinc ions are the best competitors for gadolinium species. On the other hand, free Gd^{+3} can be complexed by binding proteins, endogenous ions (citrate) or endogenous anionic precipitants (CO_3^{2-}, OH^-, PO_4^{3-}), so the chelation is essential to minimize the risk of deposition of free gadolinium in certain organs to prevent the onset of some diseases like nephrogenic systemic fibrosis (NSF).[10] Focused on their molecular structure, the main GBCA used may be divided into macrocyclic and linear agents (see Table 2 and Figure 4).

Table 2. List of approved Gd MRI contrast agents.

Trade Name	Name	Structure	Log K_{therm}	Biodistribution	Elimination Pathway
Omniscan®	Gadodiamide	Linear nonionic	16.8	Extracellular	Kidney
OptiMARK®	Gadoversetamide	Linear nonionic	16.6	Extracellular	Kidney
Magnevist®	Gadopentetate dimeglumine	Linear ionic	22.1	Extracelullar	Kidney
MultiHance®	Gadobenate dimeglumine	Linear ionic	22.6	Extracellular / Liver	93% Kidney, 3% bile
Primovist®	Gadoxetic acid disodium	Linear ionic	23.5	Extracellular / Liver	50% Kidney, 50% bile
Ablavar®	Gadofosveset trisodium	Linear ionic	22.0	Blood pool	91% Kidney, 9% bile
Gadovist®	Gadobutrol	Macrocyclic nonionic	21.8	Extracellular	Kidney
ProHance®	Gadoteridol	Macrocyclic nonionic	22.8	Extracellular	Kidney
Dotarem®	Gadoterate meglumine	Macrocyclic nonionic	25.4	Extracellular	Kidney

***In November 2017, the European Commission [11] adopted the decision of the European Medicines Agency to suspend the marketing authorizations for intravenous use like Omniscan®, Optimark®, Ablavar® and Magnevist® and to restrict the use of MultiHance® to liver scans only.

In macrocyclic compounds, the gadolinium ion is captured in a molecular cavity within the chelating agent being more stable than linear compounds, allowing a lower rate of dissociation of free gadolinium ion and much less adverse effects for these complexes (see values in Table 2). For instance, Dotarem® possesses a higher value of LogK_{term} in comparison with other contrast agents. By contrast, linear agents can be subdivided into ionic and nonionic compounds. It is essential for the use of these compounds their stability parameters since the availability of free gadolinium has been related with the pathogenesis of NFS among other diseases, for example using Omniscan® as a nonionic linear GBCA.

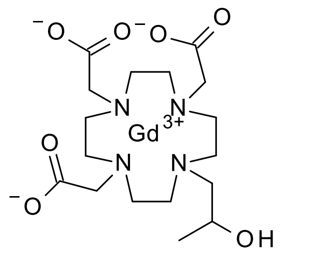
[10] Weller, A.; Barber, J. L.; Olsen, Ø. E. Gadolinium and nephrogenic systemic fibrosis: an update. *Pediatr. Nephrol.* **2014**, *29* (10), 1927-1937.
[11] Medicines Agency, E. https://www.ema.europa.eu/en/news/emas-final-opinion-confirms-restrictions-use-linear-gadolinium-agents-body-scans (accessed April 23, 2020).

The less adverse effects on these agents are due to the dosage. While in radiographic and computed tomography (CT) what you see and analyse is directly the iodine molecule, by contraposition in MRI what is analysed after its administration is the effect of the paramagnetic metal on the adjacent water protons. This mean that a single Gd atom modifies the relaxation times of many water protons. This fact can be seen reflected in dosage, which is an important feature in general medication, since the lowest possible dose of any drug prevent from suffering the onset of certain diseases. A great advantage of GBCAs in front of iodine-based contrast agents used in CT is that a single molecule of GBCA can affect many surrounding waters protons. The standard administration dose is 0.1 mmol/Kg of weight, equivalent to 0.2 ml/Kg in case of gadolinium agents. However, with iodine contrast agents though have a different mechanism of action it is needed more amount of CA to observe a certain contrast. It is said "The more iodine, the denser the X-ray effect", so we need much amount of iodine CA to have a similar contrast like GBCA. As a result, a much lower dose of GBCA is required compared to iodine CAs to achieve a similar degree of contrast enhancement.

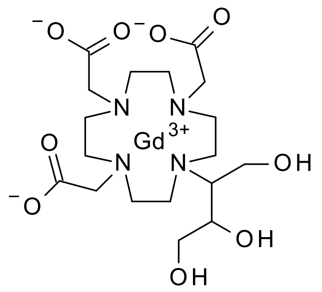
Regarding to the mechanism of action of GBCAs, it is said that gadolinium agents possess a pronounced effect through the T_1 relaxation times. Previous studies [12] confirmed that water T_2 is generally 5-20 times shorter than T_1. As a result, the effect of a Gd (III) contrast agent will be much more pronounced on T_1 but it should not be ruled out in some pathologies T_2 can be predominant as well.

[12] Wahsner, J.; Gale, E. M.; Rodríguez-Rodríguez, A.; Caravan, P. Chemistry of MRI contrast agents: Current challenges and new frontiers. *Chem. Rev.* **2019**, *119* (2), 957-1057.

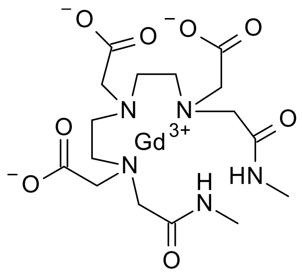
Figure 4. Structure of marketed Gadolinium MRI contrast agents.



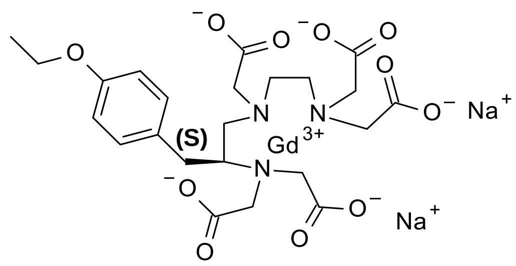
Gd-HP-DO3A, ProHance®



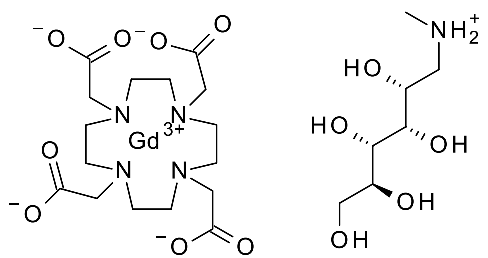
Gd-BT-DO3A, Gadovist®



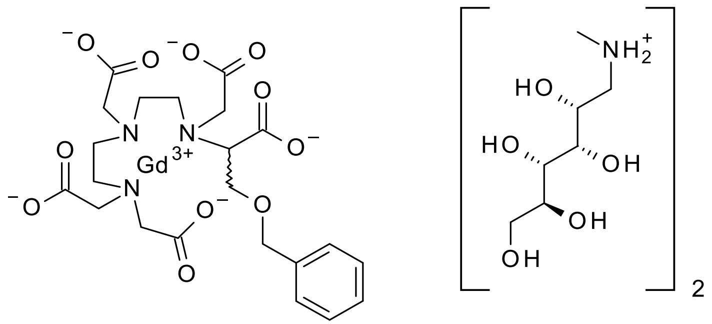
Gd-DTPA-BMA, Omniscan®



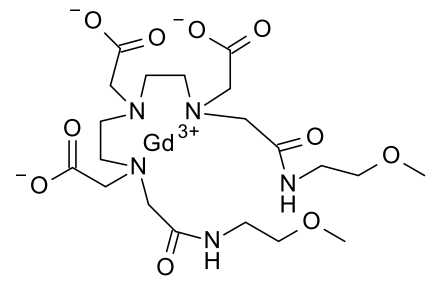
Gd-EOB-DTPA, Primovist®



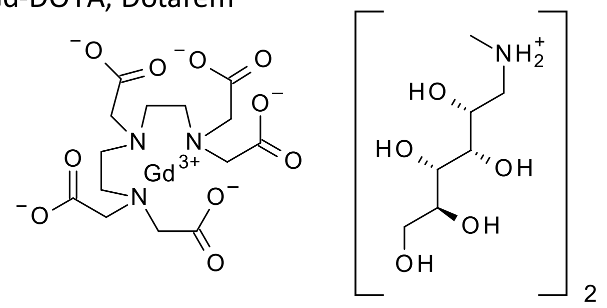
Gd-DOTA, Dotarem®



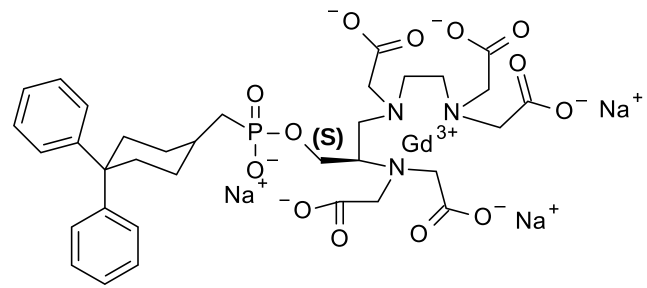
BOPTA, MultiHance®



Gd-DTPA-BMEA, Optimark®



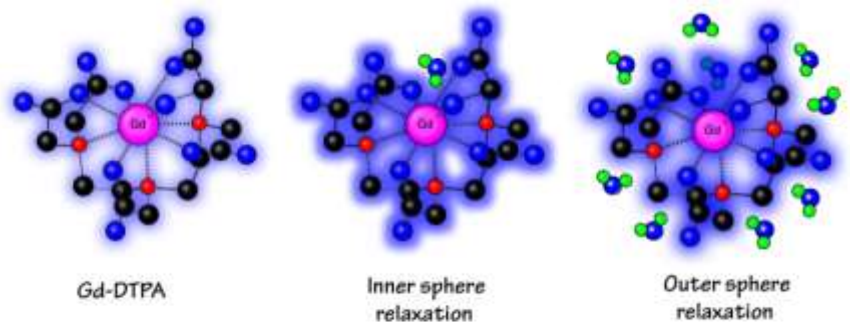
Gd-DTPA, Magnevist®



MS325, Vasovist®

Going into a brief detail in his mechanism, gadolinium complexes preferentially induce T_1 relaxation in tissues where they accumulate. Focusing on Gd features, this metal exhibits powerful paramagnetic behaviour since possess 7 unpaired electrons in its 4f shell. Previous experiments confirmed that these inner electrons are not involved with bonding and this evidence concluded that Gd complexes possessed the same paramagnetic whether is free or attached to organic ligands. Moreover, most Gd contrast agents commercially available have nine coordination sites for bonding and chemical interactions. In most cases, the ligand group complexes to Gd^{3+}, which occupies eight of these sites while the last site is available for interacting with water as one can see depicted in Figure 5.[13]

Figure 5. Ball and stick model of the contrast agent Gd-DTPA (Magnevist®). *



*The Gd^{+3} ion is coordinated with 5 oxygen atoms (blue) and 3 nitrogen atoms (red). Structural carbon atoms are black. The crevice in the molecule leaves room for a single water molecule (blue and green) to interact directly with the Gd^{+3} ion (inner sphere relaxation). Beyond this a second shell of other water molecules experience outer sphere relaxation.

Figure 6. Enhanced contrast imaging after Gadolinium injection.

In a nutshell, dipolar interactions between water nuclei and unpaired electrons of the metallic center are taken placed and known as *paramagnetic relaxation enhancement (PRE).* As a result, this led to improve the contrast in some tumours (Figure 6*).* After briefly explaining how T_1 is induced by contrast agents we should know that in speaking terms MRI contrast agents are characterized by its relaxivity value (*r*), which is defined as the paramagnetic enhancement of the

[13] Paramagnetic relaxation - Questions and Answers in MRI http://mriquestions.com/paramagnetic-relaxation.html (accessed abr 23, 2020).

longitudinal relaxation rate of water protons (T_1 and T_2) in presence of 1 mM paramagnetic ion concentration.

This value reflects how the relaxation rates of T_1 and T_2 changes as a function of concentration as shown in equations below.

Equation 1. Relaxivity expression.

$$\mathbf{1/\Delta T_1 = \mathit{r_1} \bullet [C]} \qquad \textit{and} \qquad \mathbf{1/\Delta T_2 = \mathit{r_2} \bullet [C]}$$

Since ΔT_1 and ΔT_2 are given in seconds and [C] in milimoles per liter, $\mathbf{r_1}$ and $\mathbf{r_2}$ have units of L/mmol-s. Considering this parameter, some contrast agents clinically approved have certain variability among r_1 and r_2 values. Agents with larger relaxivities (r_1 and r_2) are those with higher molecular weights, higher protein binding like Eovist®, which provide a brighter image on T_1-weighted (*see* Table 3). Eovist® is the CA that possess a greater relaxivity value (without taking into consideration Ablavar® which is a blood pool agent and possess the high protein binding) which determines how bright the contrast agent appears on T_1-weighted MRI. This fact makes Eovist® optimal for detecting enhancement on T_1 and T_2 weighted images at standard doses.

Table 3. r_1 and r_2 relaxivities for marketed GBCA obtained in plasma at 37ºC at 1.5T.[14]

Brand Name	T_1 relaxivity (r_1)	T_2 relaxivity (r_2)
Magnevist®	3.9 – 4.3	3.8 – 5.4
MultiHance®	6.0 – 6.6	7.8 – 9.6
Primovist/Eovist®	6.5 – 7.3	7.8 – 9.6
Vasovist/Ablavar®	18.0 – 20.0	32.0 – 36.0
Omniscan®	4.0 – 4.6	4.2 – 6.2
OpmiMARK®	4.4 – 5.0	4.3 – 6.1
Dotarem®	3.4 – 3.8	3.4 – 5.2
ProHance®	3.9 – 4.3	4.2 – 5.8
Gadovist®	4.9 – 5.5	5.2 – 7.0

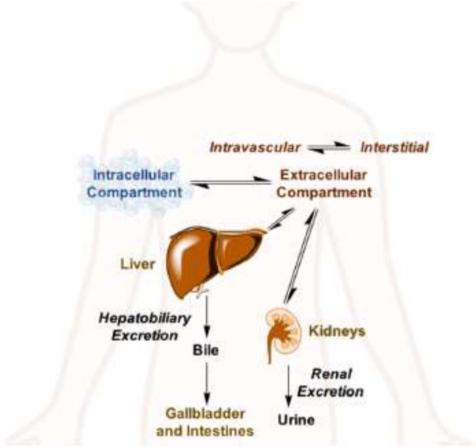
According to their pharmacokinetics properties, Figure 7 shows the distribution and excretion pathways for GBCAs. An intravenous agent rapidly equilibrates between intravascular and interstitial fluid compartments (known as extracellular compartment).

After that depending on its structure, the complex might also be distributed into intracellular environments (such as liver) by passive diffusion or specific uptake processes or excreted directly through the kidney.

[14] Hao, D.; Ai, T.; Goerner, F.; Hu, X.; Runge, V. M.; Tweedle, M. MRI contrast agents: Basic chemistry and safety. *J. Magn. Reson. Imaging.* **2012**, *36* (5), 1060-1071.

According to its biodistribution GBCA may be categorized in 3 types: (a) extracellular agents (ECAs), (b) blood pool agents and (c) target/organ-specific agents.

Figure 7. Main distribution sites and excretion pathways for intravenously administered soluble metal complexes.



Extracellular agents (ECAs) were the first to be introduced into clinical practice. [15] They are hydrophilic and typically small molecular weight (around 500 daltons) compounds with nonspecific distribution. They are consisting of a chelating agent that tightly encapsulates the gadolinium ligand, preventing its cellular uptake. After intravenous administration, GBCA are quickly and freely equilibrate between the extracellular compartments (interstitial and intravascular spaces), and then they are excreted exclusively through the kidneys with a plasma half-life of 1.5 hours in patients with normal renal function. By contrast, in patients with renal impairment an increase in plasma half-life of gadolinium compounds is noticed and depending on renal function it can varies from 7-8 hours to days.

Blood Pool Agents are compounds used mostly in magnetic resonance angiography (MRA). After their injection they are distributed exclusively in the intravascular space due to their reversible binding to albumin, providing a high contrast for imaging in arteries and veins. Due to the high capacity to bind (80-90%) to albumin protein, blood pool agents have longer intravascular half-live than ECA. The most important of the low-molecular-weight agents is Gadofosveset trisodium (Ablavar®), a monomer which noncovalently binds to albumin (80-90%) in human plasma, making it a blood pool agent. The remain fraction unbound is then eliminated through the kidneys, but now is no longer in use since the main drawback of this agent is the risk of gadolinium dissociation related to their prolonged retention in the body.

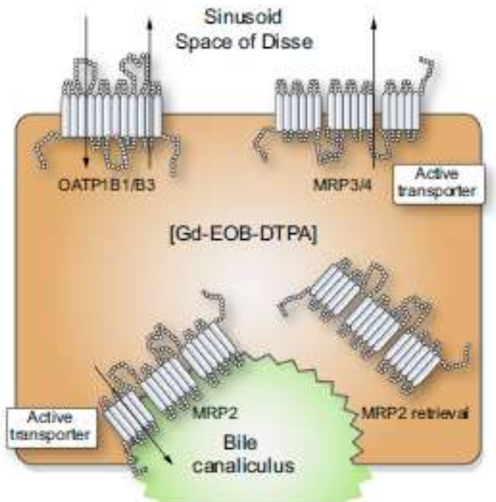
[15] Aime, S.; Caravan, P. Biodistribution of gadolinium-based contrast agents, including gadolinium deposition. *J. Magn. Reson. Imaging* **2009**, *30* (6), 1259-1267.

Hepatobiliary Agents or also known as mixed contrast agents were developed to detect the diagnosis of focal hepatic lesions, and include Gadobenate Dimeglumine (Gd-BOPTA, MultiHance®) and Gadoxetic acid (Gd-EOB-DTPA, Eovist®, Primovist®). These agents are taken up by hepatocytes through an active transport mechanism and are cleared intact via the hepatobiliary system. Thanks to their cellular liver uptake, these agents can therefore be used to image the liver and biliary system. Both agents in the first few minutes after their administration act as a conventional ECAs and after 20 min or more (60-120 min in case of MultiHance®), an enhancement of liver parenchyma is taken place in order to stand out hepatic lesions as dark spots. The degree of hepatic clearance varies from 2% (Gadobenate dimeglumine [Multihance®]) to 50% (Gadoxetate disodium [Eovist®]) depending on the agent, making Eovist® the most useful liver contrast enhancement.

1.3. Gadoxetate disodium, Primovist®

Gadolinium ethoxybenzyl dimetilglumine (Gd-EOB-DTPA, sold as Primovist® in Europe and Evoist® in USA) is a liver-specific MRI contrast agent manufactured by Bayer HealthCare designed for liver imaging. The chemical structure of the complex closely seems to Gd-DTPA, but with one arm of the ligand containing a highly lipophilic ethoxybenzyl (EOB) group responsible for the liver uptake. [16]

Figure 8. Cellular pharmacology of Gd-EOB-DTPA.



This agent is useful since it has up to 50% hepatobiliary excretion in the normal liver followed by 50% renal excretion. After intravenous injection, Primovist® initially distributes into the vascular and extracellular spaces in a similar way to non-specific gadolinium agents. Afterwards, a half of the administered dose progressively accumulates into hepatocytes cells and then is excreted into the biliary tract. Subsequently, the remainder dose is excreted renally by the kidneys through glomerular filtration. Overall, unlike other agents, Eovist® has

[16] Van Beers, B. E.; Pastor, C. M.; Hussain, H. K. Primovist, eovist: What to expect? *J. Hepatol.* **2012**, *57* (2), 421-429.

double distribution and elimination pathways allowing it to be used to assess both vascularity and function of liver lesions. The resulting hepatic enhancement in the liver is achieved by the transport through **OATP1B1/B3** (Organic Anion Transporting Polypeptide) and **MRP2** (Multi Drug Resistance Protein) transporters located in the sinusoidal and canalicular membrane (Figure 8) respectively. Several human and animal studies [17] have shown that the detection of nonhepatocellular tumors can be improved much more by the administration of Gadoxetate Disodium or Gadobenate Dimeglumine. This fact can be explained by the selective uptake of hepatobiliary contrast agents in functional hepatocytes and the lack of uptake in tumors generating a substantial contrast. For these benefits, this review will pay much attention on the synthesis of Gadoxetate Disodium in order to seek a new synthetic route suitable and practical to be transferred at commercial scale.

1.4. Synthetic Route Towards Gadoxetate Disodium

The design of octadentate ligands derivatives such as diethylenetriaminepentaacetato (DTPA) is still a current challenge at large scale for the synthesis of contrast agents. Its importance is due to the need for a high thermodynamic stability complex in order to prevent the release of free Gd$^{(III)}$ into the body's patient. Many approaches have been done in literature [18] to achieve the synthesis of a large variety of chelators used to the formation of desired complex.

Years before, around 1996-98 two patents (**US5798092** and **US5482700**)[19, 20] were published regarding the synthesis of Primovist®. Unfortunately, both presented some drawbacks at the time to transferring at large scale owing to the use of expensive and complex reagents such as diborane compounds. Apart from that, some procedures were undesired at industrial scale such as chromatographic purification which is usually avoided. But not until 2017, a patent was filed to the synthesis of Gadoxetate at level

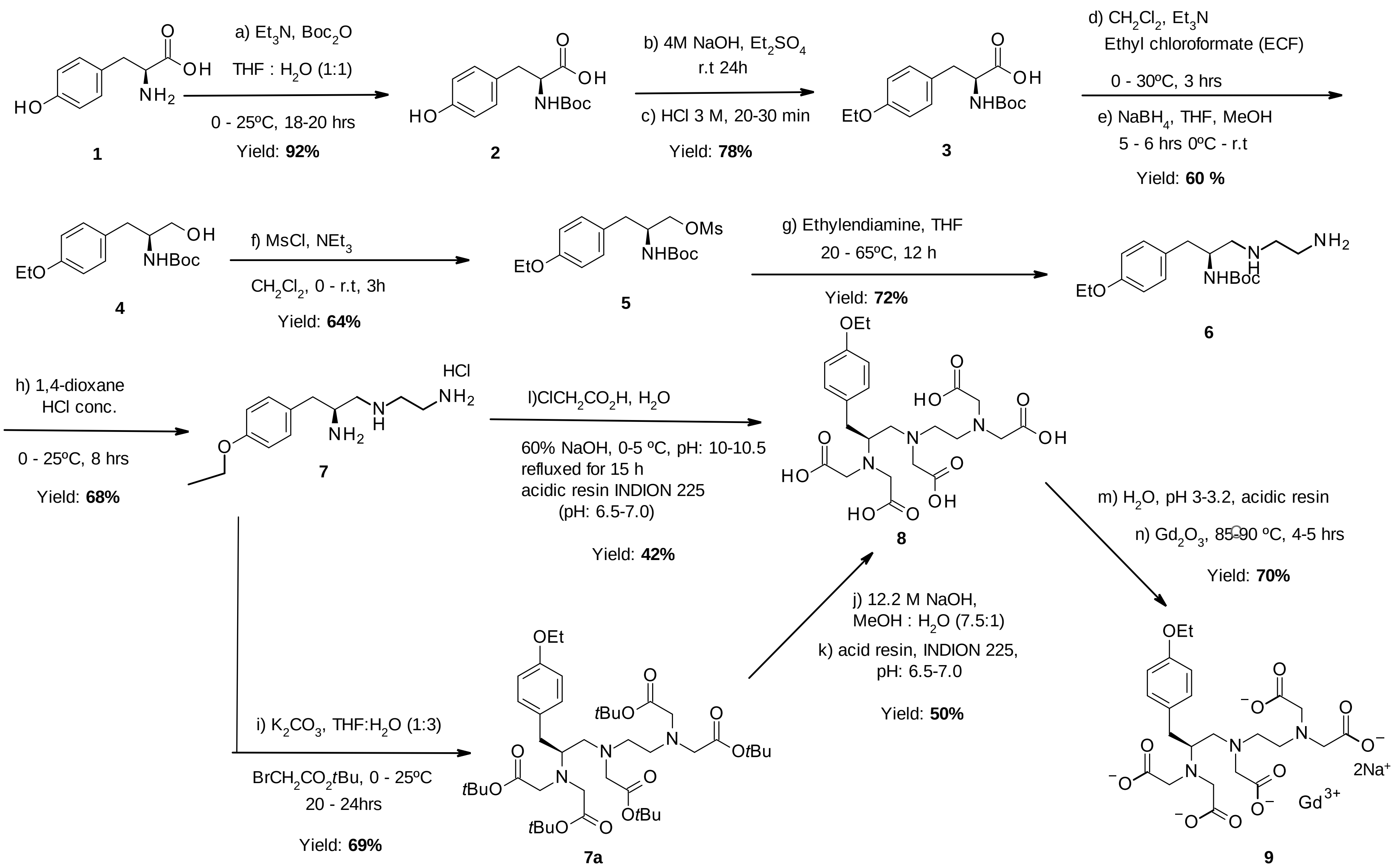
[17] Müller, A.; Clément, O.; Vexler, V. Hepatobiliary Enhancement with Gd-EOB-DTPA: Comparison of Spin-Echo and STIR Imaging for Detection of Experimental Liver Metastases. *Radiology*.**1998**, *184*, 207-213.
[18] Frullano, L.; Caravan, P. Strategies for the Preparation of Bifunctional Gadolinium(III) Chelators. *Curr. Org. Synth*. **2011**, 8 (4), 535-565
[19] Deutsch, J.; Gries, H. *Substituted polyamino, polycarboxy complexing agent dimers for MRI and X-Ray contrast.* U.S Patent 5,482,700, Jan 9, 1996.
[20] Schmitt-Willich, H.; Platzek, J. *Derivatized DTPA complexes pharmaceutical agents containing these compounds, their use, and processes for their production.* U.S Patent 5,798,092, Aug 25, 1998.

scale with slightly improvements.[21] It is based on a multi-step synthesis with at least nine steps, from the commercially available L-tyrosine **1** as depicted in **Scheme 1.**

The first synthetic step involves the protection of amino group of L-tyrsoine **1** with di-*tert*-butyl dicarbonate (Boc_2O) in presence of base. The resulting compound **2** was alkylated in phenol position with diethyl sulphate (Et_2SO_4) in a basic medium affording the derivative **3**. The remain carboxylic acid present in **3** was activated by undergoing the reaction in presence of ethyl chloroformate (ECF) or methyl iodide in order to generate *in situ* the corresponding anhydride or methyl ester, which is then reduced in a slightly conditions by using sodium borohydride ($NaBH_4$) resulting the hydroxy derivative **4**. The obtained alcohol **4** was activated with methane sulfonyl chloride (MsCl) in presence of triethylamine (Et_3N), as a base, providing de mesyl derivative **5**. The subsequently condensation of this intermediate with ethylenediamine along with the deprotection of Boc group resulted in the chiral triamine intermediate **7**. Then, the alkylation reaction of chiral triamine to get the pentacarboxilic derivative **8** was conducted using two different strategies. The first one involves two steps, in which firstly took placed the alkylation of triamine using *tert*-butyl bromoacetate affording the intermediate **7a**. Then, it was hydrolysed in basic medium to provide pentaacetylated derivative **8**. The second strategy involves a direct alkylation process using chloroacetic acid, providing directly the pentaacetylated derivative **8** with a 42% yield after a purification process. At last, the complexation of EOB-DTPA with Gd^{+3} was conducted by adding gadolinium oxide (Gd_2O_3) to the ligand in water at 80ºC for 1h. The final compound **9** was isolated after a purification process.

[21] Pullagurla, M. R.; Pitta, B. R. *Novel process for the preparation of Gadolinium complex of (4S)-4-(4-ethoxybenzyl)-3,6,9-tris(carboxylatomethyl)-3,6,9 triazaundecanedioic acid disodium (Gadoxetate Disodium)*. WO Patent 2017/208258 A1, Dec 7, 2017.

Scheme 1. Synthetic pathway towards Gadoxetate Disodium. [21]



2. OBJECTIVES

As it was mentioned before, two hepatobiliary GBCA, Gadobenate Dimeglumine and Gadoxetate Disodium, which are still in use were discovered to this date. Unlike Gadobenate dimeglumine, Gadoxetate has the advantage of being faster offering abnormalities results in liver around 20 minutes in comparison to nearly 1 hour, in case of Gadobenate. For this reason, this review will be focused on a deeply way in the synthesis of Primovist®.

Currently, the production procedure belongs to Bayer which raise the price of 10 mL up to 182 Euros. For this reason, one of the current challenges into the industrialization of specific pharmacological products is to provide an economically efficient synthesis with high yields and purities to fulfil with the safety's specifications.

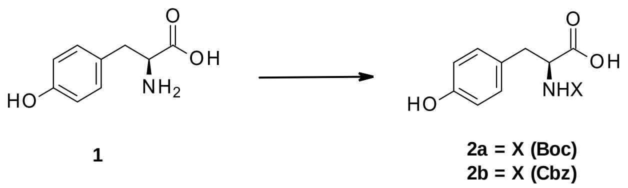
Thus, based on the recent synthetic pathway of Scheme 1 reported in the mentioned patent **WO2017208258A1**, the aim of this work is to make an exhaustive bibliographic research into the synthetic pathways of Primovist® and its intermediates, in order to seek new alternatives. These new synthetic procedures will result in an enhancement of the process at level scale, from its overall yield and purity to reduce the use of undesired reagents and improve its reproducibility.

3. RESULTS AND DISCUSSION

3.1. Synthesis of L-protected tyrosine

The first step in the synthesis involves the amino protection of L-tyrosine (**1**) as it was shown in the Scheme 1. According to bibliographic search, the protection of **1** can be carried out by using di-*tert*-butyl carbonate (Boc_2O) or benzyl chloroformate (Cbz-Cl) affording the *tert*-butyl (**2a**) or benzyl carbamate (**2b**) derivatives (Scheme 2).

Scheme 2. L-Tyrosine amine protection.



As it is shown in the Table 4 (entries 1&2*),* the formation of Boc protected L-tyrosine (**2a**) can be conducted under either an aqueous base (NaOH) or organic base (Et_3N) obtaining similar and good yields. By contrast, slightly better yields are obtained when Cbz is used as a protecting group (Table 4, *entries 3&4*). In this case, aqueous bases are the common conditions for taking place the subsequent formation. The key difference between these two protecting groups, is really in how they are removed as we will see in later steps, making Cbz a better protecting group for this synthetic process owing to its great endurance under acidic aqueous conditions.

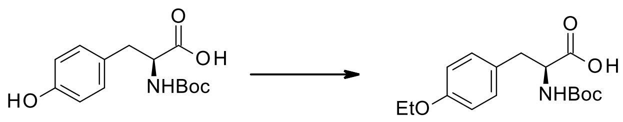
Table 4. Synthetic conditions for the synthesis of L-amino protected tyrosine.

Entry	Solvent	Reagents	Conditions	Yield (%)	Reference
1	THF:water (1:1)	Et_3N (1.7 eq) **Boc_2O** (1.1 eq)	20 – 25ºC 20hrs	92	Manik Reddi *et* al.[21]
2	THF	NaOH (2.2 eq) **Boc_2O** (1.1 eq)	20 – 25ºC 8-10 hrs	94	Yingjie Zhang *et* al. [22]
3	THF:water (1:2.5)	NaOH (4.5 M) **Cbz-Cl** (1.2 eq)	20 – 25ºC 12 hrs	95	Lixiong Dai *et* al. [23]
4	THF	NaOH (2.2 M) **Cbz-Cl** (1.2 eq)	20 – 25ºC Overnight	100	Arik A. Zur *et* al.[24]

3.2. O-Alkylation of protected L-tyrosine

The second step of the process consist on the O-alkylation of Boc or Cbz protected L-Tyrosine derivatives. In a first instance[21] (see Scheme 3), it was conducted under basic aqueous conditions (4M NaOH) in presence of diethyl sulphate (Et_2SO_4) as alkylating agent to get (S)-2-((*tert*-butoxy carbonyl) amino)-3-(4-hydroxyphenyl)propanoic acid (**3a**) in a 78% yield (see *entry 1*, Table 5).

Scheme 3. Phenol O-alkylation of Boc protected L-tyrosine.



2a **3a**

These conditions in terms of environmentally speaking are the most suitable for this process since neither solvents nor organic reagents are used in that methodology. O. Nyéki *et* al. [25] performed the same reaction with similar conditions obtaining a better yield, 94% in 24 hours. (see *entry 2*, Table 5). This was accomplished by changing the manipulation of the experimental procedure. In entry 1, once the reaction is finished,

[22] Zhang, Y.; Feng, J.; Liu, C.; Fang, H.; Xu, W. Design, synthesis and biological evaluation of tyrosine-based hydroxamic acid analogs as novel histone deacetylases (HDACs) inhibitors. *Bioorganic Med. Chem.* **2011**, *19* (15), 4437-4444.
[23] Dai, L.; Zhang, J.; Chen, Y.; Mackenzie, L. E.; Pal, R.; Law, G. L. Synthesis of Water-Soluble Chiral DOTA Lanthanide Complexes with Predominantly Twisted Square Antiprism Isomers and Circularly Polarized Luminescence (Supplementary material). *Inorg. Chem.* **2019**, *58* (19), 12506-12510.
[24] Zur, A. A.; Chien, H. C.; Augustyn, E.; Flint, A.; Heeren, N.; Finke, K.; Hernandez, C.; Hansen, L.; Miller, S.; Lin, L.; et al. LAT1 activity of carboxylic acid bioisosteres: Evaluation of hydroxamic acids as substrates (Supplementary material). *Bioorganic Med. Chem. Lett.* **2016**, *26* (20), 5000-5006.
[25] Taylor, P.; Nyéki, O.; Rill, A.; Klsfaludy, L. RACEMIZATION-FREE PREPARATION OF Boc-Tyr (Et) -OH ACTIVE ESTERS. *Organic Preparations and Procedures International : The New Journal for Organic Synthesis.* **2014**, *20* (1), 96-99.

the crude oil was dissolved in AcOEt and then acidified to reach a pH 1 with 1M aqueous solution of HCl. By doing the acidification process on this manner, the measurement of the pH of the solution is significantly difficult since the pH medium in organic solvent cannot be safely controlled. As a result, low yields are obtained in comparison to *entry 2*. For this reason, acidifying the crude oil before dissolved in AcOEt had a remarkable increasement in yielding process (*entry 2*).

Table 5. Phenol O-Alkylation conditions.

Entry	Solvent	Reagents	Conditions	Yield (%)	Reference
1	water	4M NaOH (3ml/g) Et_2SO_4 (2.0eq)	20 – 25ºC 2 – 3 hrs	78	Manik Reddi *et* al. [21]
2	water	4M NaOH(3.6ml/g) Et_2SO_4 (2.0eq)	20 – 25ºC 24 hrs	94	O. Nyéki *et* al. [25]
3	DMF	K_2CO_3(1.1 eq) EtI (1.1 eq)	20 – 25ºC Overnight	98	Schmitt-Willich *et* al. [26]

On the other hand, an alternative way for the alkylation process could be using ethyl iodide (EtI) instead of diethyl sulphate. We should take into consideration that this change in reagent conditions raise the price of the industrial process and the toxicity concerns as well. Schmitt-Willich *et* al[26] performed the resulting alkylation (*entry 3,* Table 5) using a similar subtrate (N_R-benzyloxycarbonyl-L-tyrosine methyl ester) at large scale, obtaining considerably high yields along with high purities without the need of purify the resulting product. Different conditions have been found to take place the corresponding phenol alkylation, but in this case the appeal for aqueous conditions as mentioned before in entry 2 are the best option in terms of green chemistry.

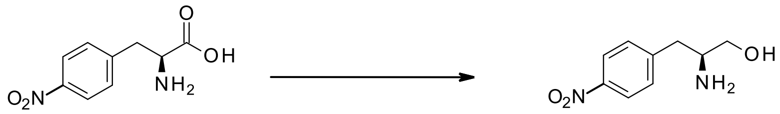
3.3. Reduction of Carboxylic Acid

The following step during the process is the activation of carboxylic acid, as an ester or anhydride, to make its reduction to alcohol under mild conditions. There are reported several conditions for taking the direct reduction to alcohol from the carboxylic acid starting material. For instance, Jan Willimiak Simek *et* al. [27] in 1997 described an alternative procedure, used more in academic labs rather than at level scale that

[26] Schmitt-Willich, H.; Brehm, M.; Ewers, C. L. J.; Michl, G.; Müller-Fahrnow, A.; Petrov, O.; Platzek, J.; Radüchel, B.; Sülzle, D. Synthesis and physicochemical characterization of a new gadolinium chelate: The liver-specific magnetic resonance imaging contrast agent Gd-EOB-DTPA. *Inorg. Chem.* **1999**, *38* (6), 1134-1144.
[27] Simek, J. W.; Tuck, T.; Bush, K. C. Reduction of Carboxylic Acids with Sodium Borohydride and an Electrophile. *Journal of Chemical Education*. **1997**, 74 (1), 107-108.

implicates the reduction of carboxylic acids with sodium borohydride and an electrophile, such as iodine in THF solution. A clear example in a 40 g scale was done by Lixiong Dai *et* al.[23] using the mentioned reaction with a similar substrate (**10**) obtaining the resulting alcohol (**11**) in 86% yield (*see* Table 6 *for the conditions*).

Table 6. Reaction conditions of carboxylic reductions in presence of an electrophile.

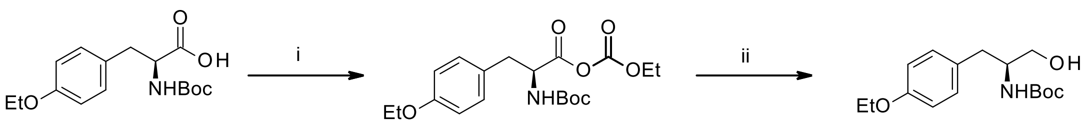


10 **11**

Entry	Solvent	Reagents	Conditions	Yield (%)	Reference
1	THF	$NaBH_4$ (3.5 eq) I_2 (1.2 eq)	Reflux at 70ºC over 16 hrs	86	Lixiong Dai *et* al.[23]

This synthetic methodology results in the reduction in one step, so if this strategy will consider in the synthesis of Gadoxetate it will shorten the required number of steps. However, in previous substrates some by-products were formed during the reaction and a rigorous workup is needed to obtain considerably purities making it unsuitable for its industrialization. For this reason, plus to the high cost of iodine this procedure is in disuse at industrial scale, but it could be tried in further trials using our substrate to verify the prior drawbacks. So as mentioned later, it seems that the best choice would be activating the carboxylic agent and then take place the subsequent reduction in presence of a reductant agent. Two different pathways have been carried out to achieve the desired alcohol. The first route reported in patent **WO2017208258**[21] (*see* Scheme 4) involves the formation of the anhydride intermediate **12**, by treating compound **3a** with ethyl chloroformate (ECF) under N_2 atmosphere and in basic medium and followed by a suitable reductant agent like sodium borohydride ($NaBH_4$) to obtain (S)-*tert*-butyl (1-(4-ethoxyphenyl)-3-hydroxypropan-2-yl) carbamate **(4a)**.

Scheme 4. Acid Carboxylic reduction via anhydride and subsequent reduction with $NaBH_4$.[a]

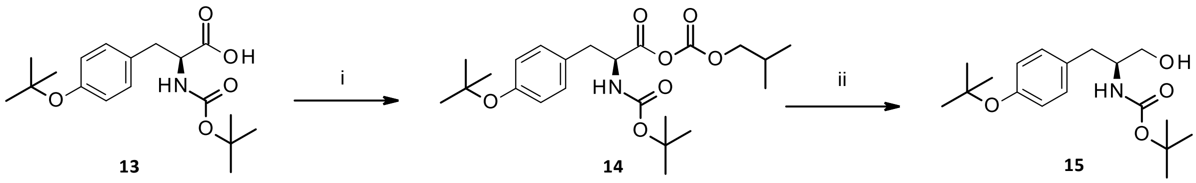


3a **12** **4a**

[a]*Reagents and conditions*: (i) CH_2Cl_2, NEt_3 (6 eq.), ECF (4 eq.), 0-30ºC, >3 hrs (ii) $NaBH_4$ (3.0 eq.), THF, 0ºC – r.t

The overall yield of the synthetic pathway illustrated in Scheme 4 was 60 % using the conditions mentioned above. Previous experiments using the same conditions and changing different variables, like reaction time and solvents, were carried out in CQAB facilities to isolate the compound **4a** in good yields. However, these changes in the experimental condition did not afford satisfactory results. These outcomes could be explained due to the high reactivity of the resulting intermediate **12** leading to its decomposition before its reduction. However, much more attempts can be done to improve the yielding process of this pathway by changing the base reagent or the chloroformate used. In patent **WO2019166937**, George Griesgraber *et* al.[28] reported the reduction of *(2S)-2-(tert-butoxycarbonylamino)-3-(4-tert-butoxyphenyl)propanoic acid* **(13)** (*see* Scheme 5) in presence of *N*-methylmorpholine as a base and isobutyl chloroformate as an electrophile to get the resulting anhydride intermediate **14**. Then, a subsequent reduction with sodium borohydride reached the desired alcohol **15**, as a syrup. Finally, the product was isolated by precipitation into vigorously stirred heptane to get the desired product as a white solid in a 95% yield.

Scheme 5. Acid Carboxylic reduction reported in patent **WO2019166937**.[a]

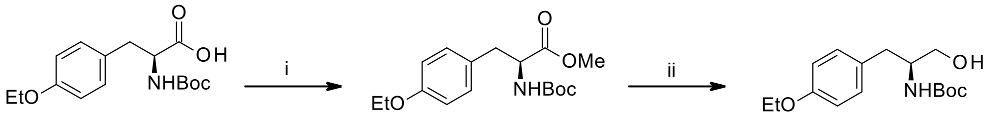


[a] *Reagents and conditions*: (i) THF anhydrous, N-methylmorpholine (1.0 eq.), Isobutylchloroformate (1.0 eq.), -15ºC – r.t (ii) $NaBH_4$ (2.0 eq.) in THF, 0ºC – r.t

This improvement, in yield terms, it could be explained by the less reactive anhydride intermediate **14** generated using a different chloroformate. For future experiments, these conditions might be used to test if it is possible to achieve compound **4a** in good yield and purity as well. On the other hand, a second approach to obtain the desired alcohol could be accomplished by the generation of an intermediate methyl ester **16**, followed by its reduction to alcohol as reported in patent **WO2017208258**[21] (*see* Scheme 6).

[28] Griesgraber, G. W.; *Substituted imidazo[4,5-c] quinoline compounds with an n-1 branched group*. WO 2019/166937 A1, Febr 28, 2019.

Scheme 6. Acid Carboxylic reduction via methyl ester and subsequent reduction with $NaBH_4$.[a]

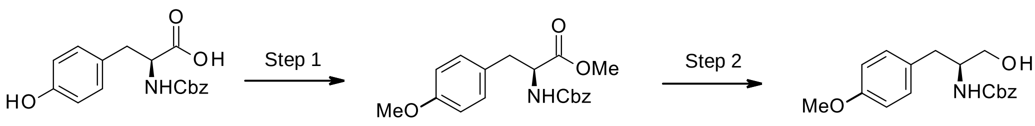


3a 16 4a

[a]*Reagents and conditions*: (i) DMF, K_2CO_3 (3.0 eq.), MeI (1.5 eq.) (ii) $NaBH_4$ (3.0 eq.), THF, MeOH, 18 hrs, 0ºC – r.t

This process seems to be easy handle for industrial manipulation since ester intermediate is less reactive than anhydride compound, obtaining the resulting alcohol between 70-75 % yield after precipitation in cyclohexane and with high purity (96%). Another interesting route for obtaining the alcohol can be carried out directly from L-protected tyrosine by shortening the number of synthetic steps as it is shown in Table 7. Lixiong Dai *et* al. [23] reported a two steps methodology pathway which involves in a single step the *O*-alkylation and the resulting esterification to get compound **17**. The second step involves a simple reduction of the resulting ester in presence of sodium borohydride to obtain **18** with 80 % overall yield. As we introduced in the synthetic pathway of Scheme 1, this was carried out in two steps at different conditions to get compound **4a** in a 60% yield. Even though in the conditions mentioned in Table 7 uses methyl iodide instead of ethyl iodide, it is of great interest to try these conditions with iodo ethane in order to find out if the synthetic pathway can shorten as well as increase the total yield of the process.

Table 7. Reaction conditions of the synthesis of alcohol 18 from L-protected tyrosine.

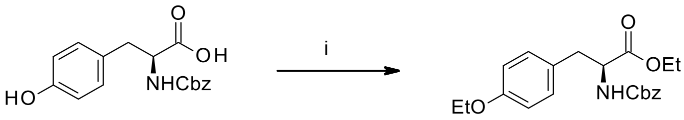


2b 17 18

Step	Solvent	Reagents	Conditions	Overall Yield (%)	Reference
1	Acetone	K_2CO_3 (3.9 eq) MeI (2.5 eq)	20 – 25ºC 16 hrs	80	Lixiong Dai *et* al[23]
2	Methanol	$NaBH_4$ (3.0 eq)	0 – 25ºC 16 hrs		

Moreover, in patent **WO03057722A2**[29], Shigeki Satoh and coworkers reported the direct phenol alkylation and ethyl esterification of Cbz-L-Tyrosine (**2b**) (see Scheme 7)

Scheme 7. Phenol alkylation and esterification of Cbz-L-Tyrosine.[a]



2b **19**

[a]*Reagents and conditions*: (i) DMF, K_2CO_3 (3.6 eq.), EtI (3.3 eq.), 0 – 25ºC, 48 hrs

At this point, the current challenge that we can face is if the reduction conditions with $NaBH_4$ mentioned before (Table 7*, entry 2*) are enough to take the resulting reduction of intermediate **19** in good yields or it would be needed different conditions which can provoke a change in the reductant agent leading a raise in the production cost. This doubt is yet to be determined in future experiments for our substrate, though in literature some examples have been described. Chuanxin Leng *et* al in-patent **CN 103896788**[30] described the exact reduction of compound **19** to get the resulting alcohol in presence of $NaBH_4$ as a reducing agent. They performed the reaction in THF solvent and 3.0 eq. of sodium borohydride at 25ºC for 3 hrs to get the resulting compound after the work-up procedure in a 95.6% yield and with purity of 99.3%.

3.4. Hydroxyl group activation

The next synthetic step implicates the activation of alcohol group of compound **4(a-c)** under N_2 atmosphere conditions at 0ºC as it is shown in Scheme 8 . Initially in patent **WO2017208258**[21], at large scale, it was carried out using the alcohol **4a** derivative in order to get the resulting mesylate compound **5a** in a 64% yield, by treating **4a** with methanesulfonyl chloride (1.5 eq.) in presence of basic medium such as trimethylamine (5 eq.) to provide the desired compound (*see*

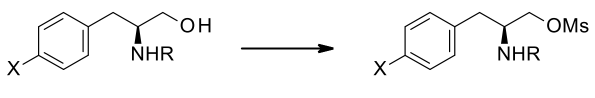
Table ***8***, *entry 1* for conditions). As we mentioned before, starting the synthesis by protecting the amine group in presence of benzyl chloroformate can be an alternative to our synthetic approach. One clear advantage in terms of stability is the endurance of

[29] Satoh, S.; Urano, Y. *Cyclic tetrapeptide compound and use thereof*. WO 03/057722 a2.2, Dec 27, 2002.

[30] CN103896788A - Preparation method of S-1-(4-ethyoxylbenzyl)-3-azapentane-1,5-diaminetrihydrochloride - Google Patents https://patents.google.com/patent/**CN103896788A**/en (accessed may 11, **2020**).

the resulting protecting group in acidic medium making it more suitable for all conditions throughout the synthetic pathway. Another advantage could be in that reaction specifically. Further literature [31] was found regarding the activation of hydroxyl groups in similar Cbz-protected tyrosine derivatives substrates which confirm us a better yielding process, as one can see in Table 9 (*Entries 2&3*).

Scheme 8. Alcohol activation via methane sulfonyl chloride.



4(a-c) **5(a-c)**

Table 8. Reaction conditions for the synthesis of mesylate derivatives.

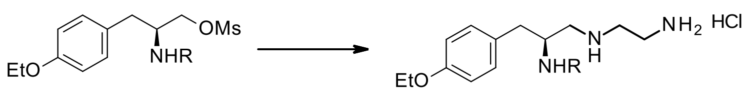
Entry	Comp.	R	X	Solvent	Reagents	Conditions	Yield (%)	Reference
1	5a	Boc	OEt	THF	1.5 eq. MsCl 5 eq. NMe_3	0 – 25ºC, N_2 3-4 hrs	64	Manik Reddi *et* al[21]
2	5b	Cbz	OEt	THF	1.1 Eq. MsCl 1.1 eq. Net_3	0 – 25ºC, N_2 30 min	90	Schmitt-Willich *et* al[26]
3	5c	Cbz	O*t*Bu	THF	1.1 Eq. MsCl 3 eq. Net_3	0 – 25ºC, N_2 20 min	94	Maite Sylla-Iyarreta Veitia *et* al [29]

At last, a crystallization process of compound **5(a-c)** can be taken place from a mixture water/tetrahydrofuran to get the desired compound with high purity.

3.5. Nucleophilic Substitution in presence of Ethylenediamine

The following synthetic step is based on a nucleophilic substitution reaction (SN_2 type) on mesylate derivative in presence of ethylenediamine (en) as a nucleophilic agent to get the resulting triamine-monoprotected derivative **6** (Scheme 9).

Scheme 9. Obtention of triamine monoprotected derivative **6(a-b)**.



5(a-b) **6(a-b)**

[31] Veitía, M. S. I.; Brun, P. L.; Jorda, P.; Falguières, A.; Ferroud, C. Synthesis of novel N-protected β3-amino nitriles: study of their hydrolysis involving a nitrilase-catalyzed step. *Tetrahedron Asymmetry* **2009**, *20* (18), 2077-2089.

The reaction conditions depend on the substrate **5(a-b),** which containing Boc or Cbz as a protecting group. Results shown below (Table 9), indicate that better results are achieved in yielding terms using Benzyl *N*-[S-1-(4-Ethoxybenzyl)-2-mesyloxyethyl]carbamate (**5b**), after its crystallization in methanol : MTBE. Then, the resulting solid was acidified in presence of HCl to form the salt **6b** which was isolated after washing with MTBE and drying at 50ºC.

Table 9. Reaction conditions for the amine condensation.

Entry	Compound	R	Solvent	Reagents	Conditions	Yield (%)	Reference
1	6a	Boc	THF	25 eq. en	20 – 65ºC 10 – 12hrs	72	Manik Reddi *et* al[21]
2	6b	Cbz	THF	25 eq. en	50 ºC 4 hrs	81	Schmitt-Willich *et* al[26]

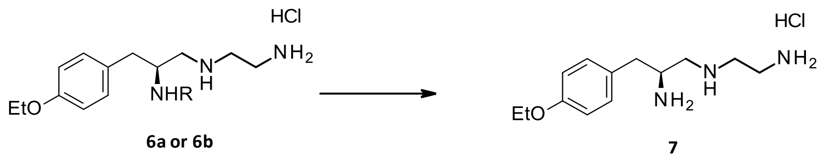
3.6. Deprotection groups conditions

At this point, two different conditions can be used for deprotecting the amine protecting groups depending on the substrate used. Firstly, as it is shown in the total pathway of Scheme 1, Boc's deprotection was carried out under acidic conditions (2M HCl) in dioxane to get the resulting triamine hydrochloride (**7**) in a 68% yield. Many conditions were found in literature to take this process with a suitable yield in a large variety of substrates. However, better yields in this step can be achieved by using different conditions as we mention below. Yeong Hun *et* al [32] in patent **WO2018084625** reported a better condition for the Boc's deprotection obtaining high yields in comparison to conditions mentioned before. They treated the compound **6a** with a mixture of MeOH and acetyl chloride at 50ºC for 1hour (*see* Table 10, *entry 2*) to get the resulting triamine trihydrochloride with a 99% yield, after its crystallization with MTBE. These outcomes might be useful for our interests to enhance the efficiency of the Boc's protection process. In case of preserving the same synthetic protecting group (Boc) it could be beneficial to test the deprotection conditions as *entry 2* to improve considerably the process´s yield

[32] Bureau, I.; Hun, Y.; Woo, H.; Jin, H.; Kyu, S.; Ki, S.; Jho, G.; States, D. *METHOD FOR PREPARATION OF (S)-NI-(2-AMINOETHYL)-3-(4-ALKOXYPHENYL)PROPANE-l,2 -DIAMINE TRIHYDROCHLORIDE.* WO Patent 2018/084625 A1, May 11, 2018.

By contrast, using Cbz as a protecting group provide us orthogonal conditions for its cleavage. The combination of hydrogen and a Pd/C catalyst has proved to be the most effective system. Two different conditions were found using Palladium catalyst which its conditions are shown in (Table 10, *entries 3 and 4*). First of all, Schmitt Willich *et* al.[26] reported exactly the Cbz deprotection using the same substrate **6b** for the synthesis of Gadoxetate by doing the hydrogenolysis over 10% Pd on charcoal at 15 bar for 1h at room temperature (*see entry 3*), obtaining the desired product **7** with 92.5 % yield after its crystallization from a mixture of methanol : MTBE (1:4). On the other hand, Lixiong *et* al. [23] reported the Cbz removal conditions in another substrate using the same amount of catalyst but differing in the pressure applied to the system. In this case, only 1 atmosphere of hydrogen was used obtaining the unprotected product after 16 hours with yield of 100%. Overall, similar results are achieved on the respective deprotection of Cbz group allowing us to choose the pressure value adequate to our needs.

Table 10. Reaction conditions for Boc and Cbz deprotection in **6a** or **6b**.



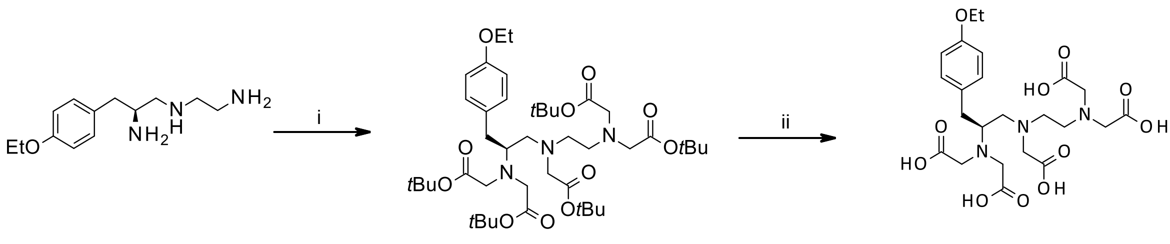
Entry	Substrate	Solvent	Reagents	Conditions	Yield (%)	Reference
1	**6a**	Dioxane	HCl 3M	20 – 25ºC 6 – 8 hrs	68	Manik Reddi *et* al[21]
2	**6a**	MeOH	AcCl (3.0 eq)	50 ºC 4 hrs	99	Yeong Hun *et* al[32]
3	**6b**	MeOH	10% Pd on charcoal	r.t, 15 bar H_2, 1 hr	92.5	Schmitt-Willich *et* al[26]
4	**6b**	EtOH	10% Pd on charcoal	r.t, 1 atm H_2, 16 hrs	100	Lixiong Dai *et* al[23]

3.7. Synthesis of Ethoxy Benzyl Diethylenetriamine Pentaacetic Acid

The penultimate step of this multi-lineal synthesis consists on the alkylation of triamine salt **7** in presence of a base along with an electrophilic agent to get the resulting triazaundecandioic acid **8**. Many synthetic approaches have been done to produce compound **8** as it is shown in Scheme 10, 11 & 12.

Schmitt-Willich *et* al[26] reported the synthesis of compound **8** from compound **7** in two steps as it is shown in Scheme 10 . The first step involves the alkylation of triamine salt **7** with *tert*-butyl bromoacetate in presence of potassium carbonate (K_2CO_3), as a base, to obtain the *tert*butyl ester intermediate **7a**, in a 73% yield after column chromatography. Whereas, the second step implicates the subsequent hydrolysis of intermediate **7a** in basic conditions, followed by acidification process using the cation exchange column chromatography resin Amberlite IR 120 (H^+ form). By using this strategy, the compound 8 was isolated in 94% yield.

Scheme 10. Synthetic pathway toward compound 8 by Schmitt-Willich and coworkers.[a]



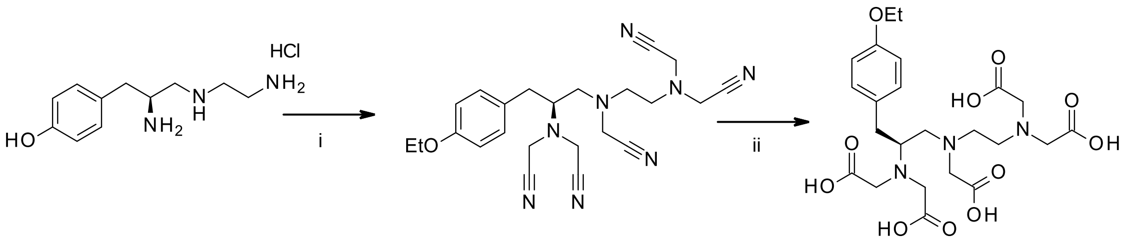
[a]*Reagents and conditions*: (i) $BrCH_2COO$*t*Bu, THF/H_2O, K_2CO_3, 20h, reflux; chromatography (ii) aqueous NaOH/methanol, 5h reflux, overnight r.t, IR 120 (H^+) to pH 3.

One drawback of this methodology is that after the alkylation process, an expensive chromatographic step is required to purify the intermediate **7a** from unreacted bromoacetic acid *tert*-butyl ester. On the other hand, after the saponification of *tert*-butyl ester, the residue must be passed through an ion exchange acidic column chromatography which becomes expensive for industrial sectors. As well, in patent of Scheme 1, the inventors also use the treatment of a strong acidic resin like INDION 225H.

For this reason, there is a need to avoid this expensive purification and try to use different conditions or different reagents to find out a better suitable and easy-handle procedure for the industrial production. In another patent **CN104130146A**[33] is reported an alternative method for the synthesis of ethoxy benzyl diethylenetriamine penta acetic acid part (EOB- DTPA) which compromised two steps as well, as it is shown in Scheme 11.

[33] CN104130146A - Preparation method of (4S)-3, 6, 9-triaza-3, 6, 9-tri(carboxymethyl)-4-(4-ethoxy benzyl)undecanedioic acid - Google Patents https://patents.google.com/patent/**CN104130146A**/en (accessed may 6, **2020**).

Scheme 11. Triamine salt alkylation via halo-acetonitrile and subsequent hydrolysis.[a][33]



7 7b 8

[a]*Reagents and conditions*: (i) Chloracetonitrile (7.5 eq.), Na_2CO_3 (8.0 eq.), THF : water, 80ºC, reflux, 5-8 hrs (60% yield) (ii) KOH (25.0 eq.), Methanol:water (30:1), reflux 20-30 hrs.

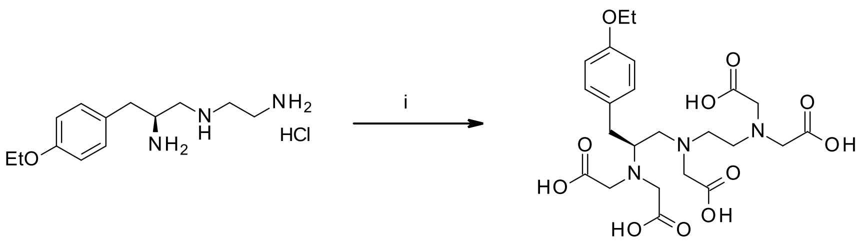
The first step consists on the amine alkylation using chloroacetonitrile as an electrophilic agent and sodium carbonate as a base in a mixture of THF:water, which react under reflux between 5-8 hrs to get compound **7b** in a 60 % yield after recrystallization with ethyl acetate. Next, the resulting penta nitrile compound is hydrolysed in basic solution of potassium hydroxide between 20-30 hrs at reflux. Then, the solution was concentrated, dissolved in water, and acidified with 2N aqueous sulfuric acid solution to reach a pH around 1.5. Under these conditions, the compound **8** is precipitated, filtered, washed, and recrystallized in hot water. After that, the compound **8** was isolated in a 66% yield. Compared with Schmitt[26] methodology, these conditions have the following advantages of avoiding chromatographic purification in the first step, owing to a changing in the electrophilic agent from halo-acetic acid *tert*-butyl to halo-acetonitrile, which then intermediate **7b** can adopt a recrystallization process that results in a simple way of purification. Finally, the compound **8** is obtained without the appealing of an ion exchange resin column chromatography where in some cases are beneficial in economic terms. In general, that synthetic route is brief, easy, and simple to handle and the most important thing as always is the easy cost. Unfortunately, this technique among the advantages that it has, it has been in disuse due to the toxicity concerns of halo-acetonitrile reagent as well as its cost.

However, in patent **DE102010023890A1**[34] is reported the obtention of crystalline form of ligand EOB-DTPA from intermediate **7a** *tert*-butyl ester. They used similar conditions in the saponification process like mentioned before (**CN104130146A**[33]) to obtain

[34] DE102010023890A1 - Preparing crystalline 3,6,9-triaza-3,6,9-tris(carboxymethyl)-4-(ethoxybenzyl)-undecanedioic acid comprises hydrolyzing 3,6,9-triaza-3,6,9-tris(tert-butoxy-carbonylmethyl)-4-(ethoxybenzyl)-undecanedioic acid-ditert-butylester and acidifying - Google Patents https://patents.google.com/patent/**DE102010023890A1**/en (accessed may 7, **2020**).

compound **8** in a high purity and in crystalline form saving at the same time the use of an ion exchange treatment. At last, there is an alternative way reported in patent **CN104761461A** [35] which shorten the reaction steps for the obtention of the ligand EOB-DTPA. As one can see illustrated in Scheme 12, it is based on the direct alkylation of chiral triamine salt (**7**) in presence of sodium hydroxide and a halo acetic compound, which can be sodium chloro- or bromoacetate.

Scheme 12. Synthesis of ligand EOB-DTPA in a single step.[a]



[a]*Reagents and conditions*: (i) $NaOCOCH_2X$ (X: Cl, Br), NaOH, Reflux 20-40 hrs.

In practical terms (*see* Table 12 *for the conditions*), compound **7** is solubilized in water, then between 5 – 12 eq. of sodium hydroxide is added along with sodium chloro- or bromo acetate (6 – 10 eq.) and the mixture is left rising reflux temperature around 20 – 30 hrs. After the reaction is completed, the residue is acidified with concentrated sulphuric or hydrochloric acid to pH 2-3. The resulting precipitated is filtered out and dried, obtaining better yields (73 – 77%) in comparison to Scheme 1 (42%) as well as with high purities (98 – 99%). The obtained solid might carried out further purification steps by water recrystallization or from a mixture of protic solvents like methanol and isopropyl alcohol.

This procedure presents better advantages from others. For example, the reaction conditions are environmental friendliness since does not adopt organic solvent and produce organic by-products. Moreover, the contaminants generated are few and the product purity is high. Finally, the raw material is easy to get which means in a lower production cost, making it a good procedure to be implemented at large scale.

[35] CN104761461A - Preparation method of novel gadoxetate disodium intermediate - Google Patents https://patents.google.com/patent/**CN104761461A**/en (accessed may 8, **2020**).

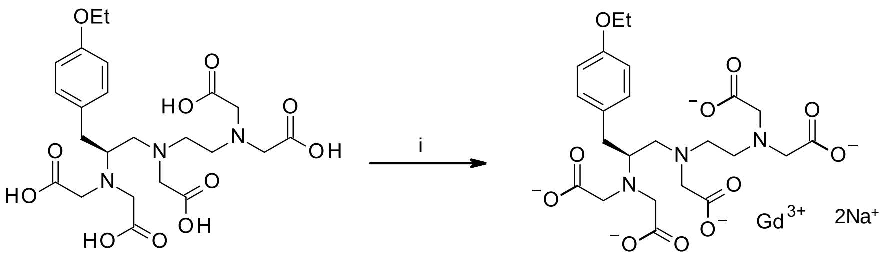
Table 12. Reaction conditions for the obtention of EOB-DTPA ligand.[35]

Entry	Solvent	Reagents	Conditions	Yield (%)	Purity
1	Water	NaOH (5.9 eq.) $NaOCOCH_2Cl$ (6 eq.)	Reflux 30 hrs	75.1	98.2 %
2	Water	NaOH (11.9 eq.) $NaOCOCH_2Br$ (7 eq.)	Reflux 20 hrs	73.8	98 %
3	Water	NaOH (11.9 eq.) $NaOCOCH_2Cl$ (10 eq.)	Reflux 40 hrs	77.4	98.7 %

3.8. Gadoxetate Disodium complex formation

The last step of the synthetic pathway involves the complexation of crystalline ligand EOB-DTPA obtained in previous step in presence of gadolinium oxide (Gd_2O_3) as it is shown in Scheme 13.

Scheme 13. Gadoxetate Disodium complex formation. [a][26]



8 **9**

[a]*Reagents and conditions*: (i) Gd_2O_3, 1-5 hrs, 80ºC, aqueous NaOH to pH 7, recrystallized from ethanol/H_2O

The complexation of compound **8** was performed with 0.5 eq. of gadolinium oxide (Gd_2O_3) in water at 80ºC to give the desired compound **9** between 1-5 hrs after its neutralization to pH 7 with 1M NaOH solution. Next, was treated activated carbon at 40-45ºC to remove undesired impurities and filtered through hyflo bed. The resulting solution can be recrystallized at 50ºC over 2 hours from a mixture of ethanol/water (95:5). After cooling at room temperature, the subsequent precipitation was observed to get Gadoxetate disodium **9** as a white powder after its drying under vacuum. Finally, the final compound was isolated with 85% yield and with high purity (>98%). Elemental analysis indicated that the gadolinium complex isolated at pH 7.0 has 1.75 sodium ions per molecule, obtaining the desired complex salt.

Anal. Calcd (**found**) for $C_{23}H_{28}GdN_3Na1.75O_{11}$: C, 38.36 (**38.27**); H, 3.95 (**3.70**); Gd, 21.83 (**21.08**); N, 5.83 (**5.78**); Na, 5.59 (**5.50**).

4. CONCLUSIONS

Based on the recent synthetic pathway of Scheme 1 at large scale reported in patent **WO2017208258A1** along with an exhaustive bibliographic search, it has been proposed an alternative route:

- Firstly, it was removed the Boc protecting group by replacing it to Cbz group, which provide a better endurance for the conditions required throughout the synthetic route.
- Secondly, in the second step which involves the *O*-alkylation of compound **2a**, it was found that in presence of ethyl iodide and K_2CO_3 a direct *O*-alkylation along with the resulting ester formation could be carried out allowing us to shorten the synthetic steps of the process. Furthermore, further trials should be carried out to test if by increasing the equivalents of Et_2SO_4 similar outcomes could be achieved.
- On the other hand, a better improvement in the reproducibility and cost of the process was achieved by obtaining the resulting ligand EOB-DTPA after its acidification using an inorganic acid aqueous solution, instead of the appealing of an ion exchange resin column chromatography which increase the cost as well as the system design of the process.
- Finally, the Scheme 14 provide a viable synthetic route towards Gadoxetate, which possess some special and appealing features like low cost, easy and simple to handle and the most essential thing, the great suitability for its industrialization obtaining the resulting compound with theorical better yield and considerably higher purities than the previous one described by Manik Reddi *et* al.[21]

Scheme 14. Synthetic route proposal for Gadoxetate Disodium based on bibliographic search.