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Digest Paper

Synthetic strategies for preparation of cyclen-based MRI contrast agents

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ABSTRACT

Cyclen-based macrocyclic ligands have an essential role in the development of contrast agents for magnetic resonance imaging (MRI). A prevailing need for preparation of multifunctional probes triggered a number of attempts to synthesize and derivatize ligands which efficiently chelate lanthanide ions and have advantageous MRI properties. This digest Letter summarizes the most common synthetic approaches for the preparation of macrocyclic ligands based on cyclen depending on the desired application.

Contents

Introduction

Magnetic resonance imaging (MRI) has become an important tool in biomedical research and is an essential diagnostic method in clinical radiology today. Moreover, the existence of different types of MRI contrast mechanisms in tissues provides for continuous development of this method.[1] MRI enables tracking of physiological changes noninvasively, allowing imaging of specific biological processes at the molecular or cellular level. To further improve the specificity of MRI, a number of contrast agents have been developed and employed to date. According to their mechanism of action, contrast agents suitable for ^{1}H MRI can be classified as T_1- and T_2-shortening agents or CEST agents.[2] The vast majority of them are based on lanthanide complexes with polyamino polycarboxylic ligands. Due to better thermodynamic and kinetic stability properties that reduce the potential toxicity of the MRI agents in vivo, multidentate macrocyclic chelators based on 1,4,7,10-tetra-azacyclododecane (cyclen) are the most commonly used chelating agents nowadays,[2] although their role in preparing contrast agents for positron emission tomography (PET), single photon emission computed tomography (SPECT), or optical imaging is also very important.[3,4]

The extensive use of cyclen-based contrast agents demands continuous improvements in derivatization and preparation of novel macrocyclic molecules with various chelating and functional groups. Synthetic changes aim to improve specific physicochemical or biological properties of the contrast agents thereby enabling their binding to particular macromolecules, localization to a specific organ or receptor and hence expanding the scope of their application.[5–7] To obtain various products with diverse desired structures and properties, an awareness of the synthetic chemistry

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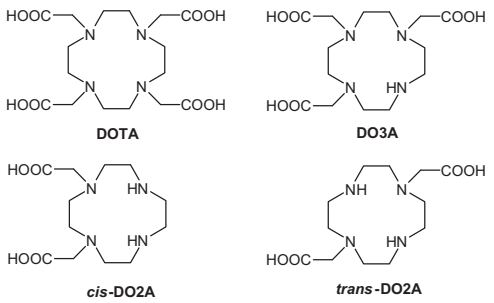


Figure 1. Structures of principal chelators described in this work.

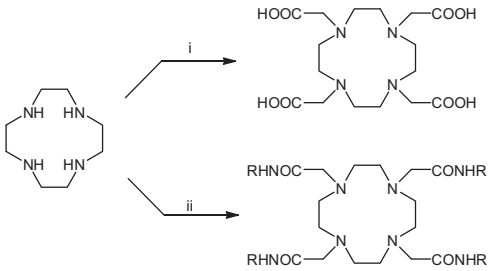
of these systems is required, especially of the numerous *C*- and *N*-functionalization procedures of cyclen.

To this end, a number of review articles and books focused on preparation, general chemical properties, or application of contrast agents have been already published.[1,2,8–13] In the present work, we concisely summarize existing methodologies for the synthesis of the most common macrocyclic chelators (cyclen-1,4,7,10-tetraacetic acid – DOTA, cyclen-1,4,7-triacetic acid – DO3A and cyclen-1,4- or 1,7-diacetic acid – *cis*- or *trans*-DO2A, respectively, Fig. 1) and their derivatives, emphasizing the most straightforward synthetic pathways for their preparation. Furthermore, we discuss different methodologies that lead to functionalization of the cyclen pendant arms. Finally, we briefly list some recent examples of contrast agents that were prepared according to such procedures, finding useful applications.

Ligands based on DOTA

DOTA is an octadentate ligand with four carboxylate and four amino groups which coordinate with lanthanide ions. Consequently, their complexes with DOTA possess high thermodynamic stability and kinetic inertness, making these compounds useful in MRI as contrast agents. DOTA can be easily prepared by tetra N-alkylation of cyclen with chloroacetic acid. This synthetic procedure was reported almost four decades ago and is still an acceptable method for DOTA preparation (Scheme 1).[14] Other haloacetic acid derivatives (bromo- or iodo-) can also be used as alkylating agents, and such a procedure is especially useful for the preparation of the tetraamide (DOTAM) chelators. Here the common precursor is chloroacetyl chloride which is first converted to the desired chloroacetamide; subsequently the conversion of chloro- to iodoacetamide is done prior to alkylation (Scheme 1),[15] resulting in numerous compounds that are suitable for use as contrast agents for CEST MRI.[16,17]

A number of procedures for preparation of DOTA derivatives exist to date, resulting in products that can be divided into two general groups: (a) *N*-functionalized and (b) *C*-functionalized

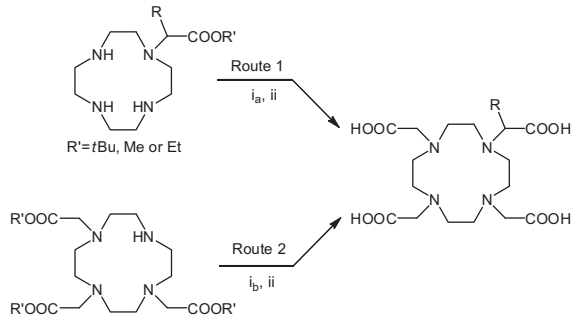


Scheme 1. General synthetic scheme for the preparation of DOTA (top) and DOTAM-type chelators (bottom) from cyclen. The most common conditions are: (i) XCH_2COOH or (ii) XCH_2CONHR (X = Cl, Br, I), DIPEA or K_2CO_3, MeCN or DMF. R stands for hydrogen, an alkyl or an aryl group.

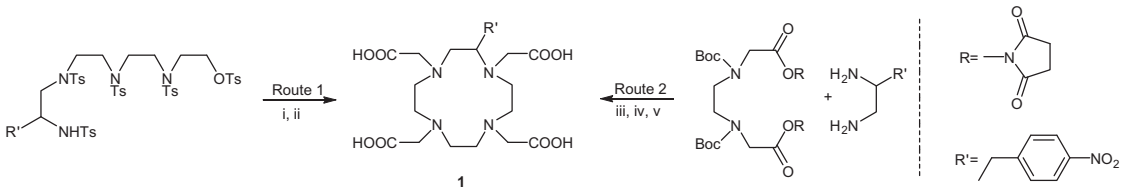
DOTA-type ligands. The former can be prepared starting from the monoalkylation of cyclen with one group, followed by the alkylation of the remaining three secondary amine positions with another group (Scheme 2, route 1). The second approach for *N*-functionalized DOTA ligand synthesis includes alkylation of the remaining amine on the ester-protected DO3A-type ligand (Scheme 2, route 2), which easily reacts with various alkyl halides in aprotic solvents. These two procedures will be described in more detail in the next section (see below). Older procedures to prepare *N*-functionalized DOTA derivatives involve the use of acyclic precursors and 2+2, 3+1 or 4+0 cyclization processes.[11] However, these procedures are no longer the most convenient, given the wide commercial availability of cyclen as the essential starting material in all procedures.

C-functionalized DOTA-type ligands can be prepared by intramolecular or intermolecular cyclization methods using appropriate scaffolds. For instance, the intramolecular cyclization of tosyl amide (prepared from tetrapeptide followed by borane reduction and tosylation) at high dilution and subsequent detosylation with a concentrated strong acid results in *C*-functionalized cyclen.[18] Its alkylation with *tert*-butyl bromoacetate, followed by hydrolysis yields a *C*-functionalized DOTA-type ligand **1** (Scheme 3, route 1). This synthesis has been improved by initiating the cyclization of diamine with carbamate-protected disuccinimido ester at high-dilution at a higher temperature (90 °C) (Scheme 3, route 2).[19] Using these synthetic approaches, a number of different *C*-functionalized DOTA can be prepared by a simple variation of side arm (R′).

In general, because preparation of *N*-functionalized DOTA derivatives is much more convenient, they are the more frequently used ligands. One of the first DOTA bifunctional chelators **2** was developed for protein and antibody labeling (Fig. 2). The synthetic approach to obtain **2** includes side arm transformation following the strategy from Scheme 2 (route 1). The initial precursor contains a *p*-nitrophenyl group, which is then transformed to isothiocyanate, allowing further synthetic transformations and coupling reactions with primary amines.[20] Following a similar synthetic strategy, *N*-functionalized DOTA derivatives with self-immolative arms as potential enzyme-responsive MRI contrast agents were reported (**3**, Fig. 2).[21] Recently, a range of building blocks for the preparation of DOTA-like chelating agents was also prepared. The procedure starts from DOTAGA-anhydride (GA = glutaric acid) which can be selectively opened with different nucleophiles, resulting in a variety of bifunctionalized DOTA derivatives of type **4** (Fig. 2).[22] This kind of ligand is useful for both in vitro and in vivo applications. For instance, the reactive moiety of these DOTA-type chelators allows further coupling procedures and



Scheme 2. Synthetic routes for the preparation of *N*-functionalized DOTA ligands. The most common reagents: (i_a) $BrCH_2COOR'$, K_2CO_3/MeCN; (i_b) $RCH(X)COOR'$, K_2CO_3 or Et_3N/MeCN or DMF; (ii) HCl/MeOH, HCOOH or TFA (for R′ = *t*Bu), or LiOH, NaOH or KOH, EtOH/H_2O (for R′ = Me or Et). R stands for a number of diverse substituents, R′ = *t*Bu, Me or Et and X = Cl or Br.



Scheme 3. Synthetic routes for the preparation of *C*-functionalized DOTA ligand. The most common reagents: (i) Cs_2CO_3/DMF; (ii) H_2SO_4, then $BrCH_2COOH$; (iii) Et_3N/dioxane; (iv) HCl/dioxane, then BF_3·THF; (v) $BrCH_2COOH$.

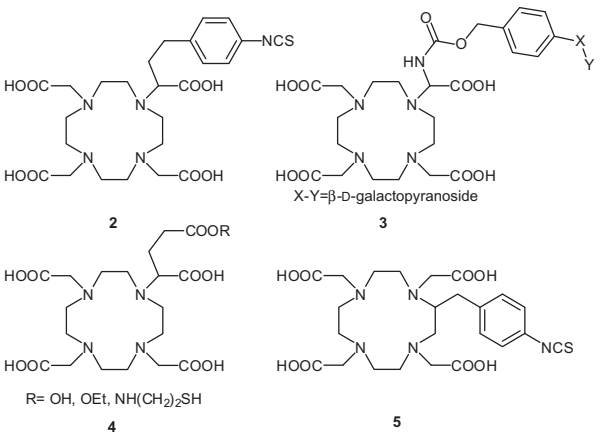


Figure 2. Some examples of *N*- and *C*-functionalized DOTA derivatives.

preparation of high molecular weight MRI contrast agents. Such agents have high relaxivity and long half-lives in the circulation, which is very advantageous for MRI applications.[23] For example, polyamidoamine (PAMAM) or polylysine (Gadomer) dendrimeric contrast agents can be prepared in this manner, carrying large numbers of monomeric DOTA-type units, and hence amplifying the MRI signal.[24,25] *C*-functionalized DOTA derivatives with isothiocyanate pendant arms (**5**, Fig. 2) can be used for the same purpose, to react with the terminal amino groups of the dendrimer, providing high molecular weight contrast agents of different dendrimer generations (G4-6).[26]

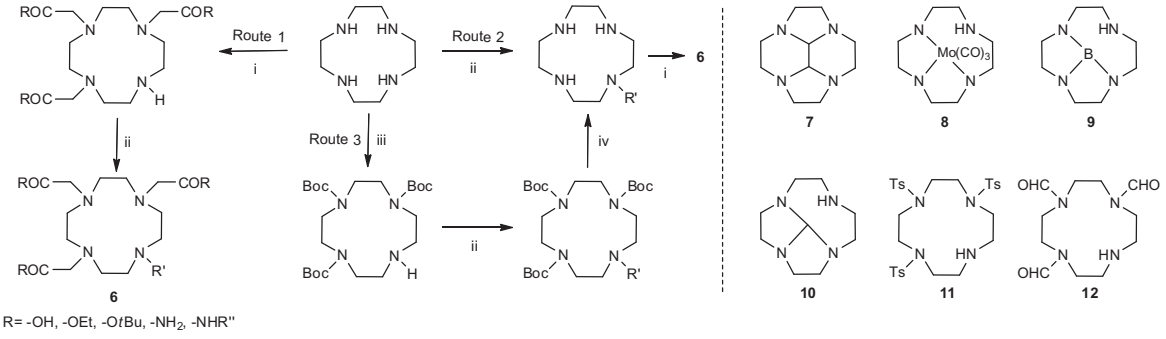
Ligands based on DO3A

As well as DOTA, DO3A is also a compound of great importance for MR imaging. This heptadentate ligand possesses one fewer carboxylic group for chelating lanthanide ions, but has a secondary amine in the cyclen ring available for a number of synthetic transformations. It therefore represents a suitable scaffold and one of the most commonly used precursors for the preparation of a variety of macrocyclic chelates. DO3A can be easily transformed to an octadentate ligand by different derivatization reactions to yield various bifunctional, targeted, or responsive (smart) contrast agents.[2,9] In this way, these molecules can also be converted to DOTA derivatives with one functionalized arm (see above), employing similar synthetic pathways for obtaining typical DO3A derivatives, which we describe in this section.

The most straightforward and commonly used synthetic route for the preparation of DO3A-type ligands is direct and selective alkylation of the three secondary amine positions in the cyclen (Scheme 4, route 1). Depending on the need for further DO3A modifications, electrophiles with acid-labile (the most commonly *tert*-butyl),[27] or base-labile (methyl or ethyl) esters can be used.[28]

The products thus obtained (*tert*-butyl, ethyl or methyl esters of DO3A) easily react further with a range of alkylation agents resulting in a range of functionalized ligands of type **6** (Scheme 4, route 1). Moreover, the hydrolysis of esters is usually mild and fairly clean, resulting in convenient preparation of the desired DO3A derivative. It is of note that tris-*t*Bu-DO3A is probably the most widely used precursor among these derivatives today; it is commercially available, and its preparation is also quite convenient and easy.[27]

The same route 1 (Scheme 4) is also very suitable for the synthesis of amide derivatives of DO3A using previously prepared amide-containing electrophiles.[29] Different reaction conditions and influences of the chosen electrophiles on three- or tetra-substituted cyclen with arginine pendant groups were recently studied.[30] For instance, the usage of iodo-derived electrophiles favored the formation of DO3A-type, while the DOTA-type products predominated when chloro-derived electrophiles were used. This study indicates that the choice of the electrophiles is an important step for the controlled synthesis of functionalized DO3A/DOTA-type ligands.



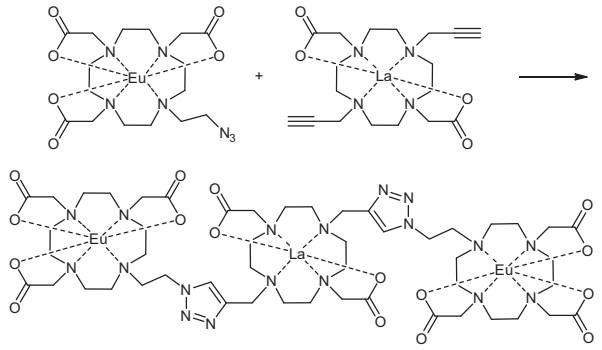
Scheme 4. The most common routes (1–3) for the synthesis of DO3A derivatives (left) and examples of protected cyclen intermediates **7**–**12** (right). The most common reagents: (i) $BrCH_2COR$, K_2CO_3/MeCN; (ii) R′X, K_2CO_3 or Et_3N/MeCN or DMF; (iii) Boc_2O, $Et_3N/CHCl_3$; (iv) HCl/MeOH or TFA. R′ and R″ stand for a number of diverse substituents. Cleavage reagents for deprotection: NaOH or KOH in H_2O (for **7**, **9** and **12**); HCl (for **8**); H_2O, EtOH (for **10**); (a) Na/NH_3, urea or (b) sodium amalgam, Na_2HPO_4, MeCN (for **11**).

Another convenient and easy method for the synthesis of DO3A functionalized molecules is the direct cyclen monoalkylation (Scheme 4, route 2), followed by substitution of the remaining three amine positions with *tert*-butyl bromoacetate or other electrophiles. A number of mono *N*-alkylated products with a range of alkylating agents were prepared using this strategy and mild conditions.[31] More recently, a monoalkylation procedure using a four-fold excess of cyclen was described where the unreacted cyclen can be successfully recovered. Depending on the alkyl halides used, moderate to good yields were obtained; the final products were isolated without purification by column chromatography.[32] Various further reaction conditions exist to obtain different *N*-monofunctionalized cyclens. These include further alkylation, acylation, sulfonylalkylation or sulfonylarylation procedures.[11] Mono-amide cyclen derivatives can be also prepared in such a manner.[33] The drawback of these procedures is the possibility of side product formation, thus requiring column chromatography purification of the obtained product.[34]

Although this strategy appears to be easy and straightforward for the synthesis of various functionalized cyclen-type contrast agents, it is actually not widely used. Reasons for this could be the bis- and tris-substituted byproduct formation which complicates isolation of the desired product. In such cases, when the synthetic route is more demanding or direct alkylation methods are not suitable for the preparation of the targeted molecule, a slightly modified strategy can be followed. It involves protection of three cyclen amines with Boc groups, followed by alkylation of the fourth position with terminally functionalized electrophile. After Boc deprotection under mild acidic conditions, various carboxylic acid derivatives can be introduced into the molecule with this strategy (Scheme 4, route 3).[35] The disadvantage of this route is a need to prepare tris-Boc-cyclen,[36] and a limited choice of electrophiles. Namely, in the majority of cases, tris-Boc-cyclen alkylation with halides is not optimal most likely due to sterical constraints and therefore a better option for alkylation is reductive amination.[37]

Along with Boc, different protected cyclens can be used as intermediates (Scheme 4): cyclen–glyoxal bis-aminal (**7**),[38] cyclen–tricarbonylmolybdenum (**8**),[39] triheterocycles borane derivative (**9**),[40] orthoamide (tricycloderivative—**10**),[41] tritosylated cyclen (**11**),[41] triformylcyclen (**12**),[42] or phosphoryl species.[43] The procedures involving these protected cyclens usually include multistep reactions and were summarized in a recent review article.[11]

Once DO3A derivatives or DO3A precursors with appropriate protecting groups have been prepared, further synthetic transformations can be performed to yield the desired so-called bifunctional agents, these products may be used for lanthanides complexation and MRI contrast agent preparation. In general, there are two distinct methods for the synthesis of bifunctional contrast agents (Scheme 5). These include either further functionalization of one of the DOTA carboxylic groups (usually accomplished by conversion into various amide derivatives), or a direct monoalkylation of cyclen or tris-protected DO3A with an α-halogenated molecule. For instance, a terminally positioned amino group can be

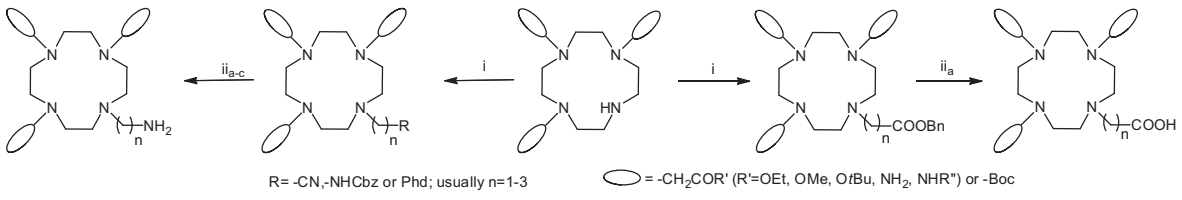


Scheme 6. CuI-catalyzed 'click' 1,3-dipolar azide–alkyne cycloadditions. Reagents: CuI, piperidine, MeCN.

introduced to DO3A, protected as Cbz carbamate or phthalimide. A primary amine suitable for further synthetic modifications is subsequently obtained using mild deprotection conditions.[44,45] Alternatively, a pendant arm with a terminal nitrile group can be introduced into the molecule. Upon nitrile reduction in a hydrogen atmosphere using Raney-nickel as a catalyst, an amine-containing DO3A precursor can be obtained to serve as a precursor for the synthesis of the bismacrocyclic chelators.[35] On the other hand, a carboxylic group on the pendant arm that is suitable for further functionalization can be introduced in a similar fashion as Bn-ester. Subsequently, the free acid will be obtained upon the Pd/C-catalyzed hydrogenation.[46] Overall, the reductive reactions discussed in this paragraph are good solutions for performing the necessary synthetic transformations on DO3A derivatives. Namely, the presence of multiple acid- or base-labile protecting groups on the DO3A (see above) limits the possibilities to conduct further transformations. Therefore, reactions under neutral conditions (i.e., reductions) appear to be a good solution in such cases.

Azide-functionalised DO3A derivatives can also serve as useful compounds with activated side arms. Recently, these kinds of compounds were reported to link two kinetically stable metal complexes together,[34] or to link a chromophore to lanthanide complexes via CuI-catalyzed 'click' 1,3-dipolar azide–alkyne cycloadditions (Scheme 6).[47] It should be noted that unmetallated cyclen-based scaffolds should not undergo 'click' cyclizations due to Cu^{+}-sequestration by the macrocycle. It is therefore necessary to first prepare the desired lanthanide complex with azide- or alkyne-appended DO3A or DOTA before performing the 'click' reaction.[47]

It is worth to note that isolation and purification of majority of these compounds appears to be one of the main challenges throughout their synthesis. Most commonly, the protected ligands are purified by column chromatography on silica gel using polar eluent systems combined from CH_2Cl_2 or $CHCl_3$ with MeOH or EtOH, in occasional cases with addition of NH_4OH or Et_3N (few%).[20,36–38,48] Less polar intermediates can sometimes be eluted with combination of ethyl acetate with hexane or dichloromethane.



Scheme 5. The most convenient strategies for preparation of the bifunctional DO3A derivatives precursors. Common reagents: (i) $Br(CH_2)_nR$ (R = COOBn, CN, NHCbz or Phd), K_2CO_3/MeCN; (ii_a) H_2, Pd/C, EtOH (for NHCbz or COOBn); (ii_b) H_2, Raney-nickel, NaOH, EtOH (for CN); (ii_c) aq NH_2NH_2 or ethylenediamine (for Phd). R″ stands for a number of diverse substituents.

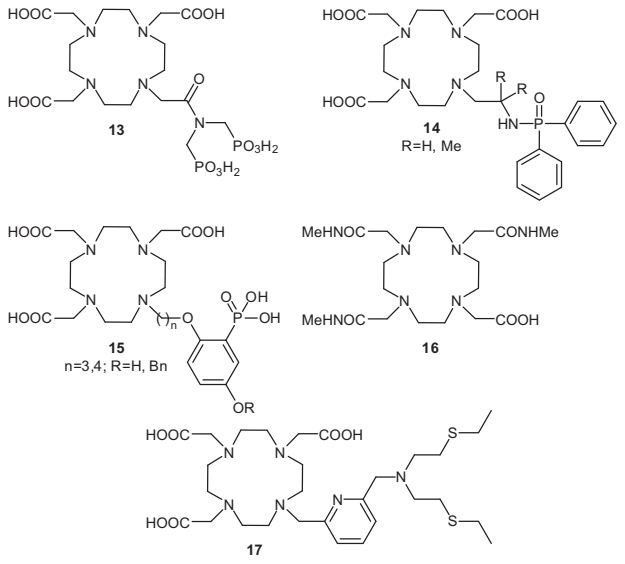


Figure 3. Some examples of DO3A-functionalized derivatives.

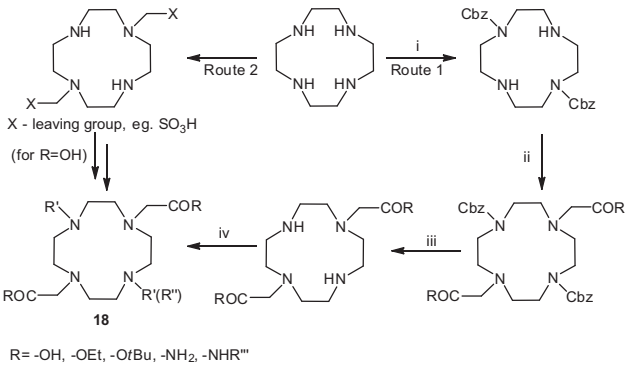
Once ester protecting groups are hydrolyzed and free acids on the chelators are formed, an HPLC on reverse phase C_{18} columns is performed, using water and acetonitrile or methanol as eluents.[22,38,45] Occasionally after the reaction work-up is done in acid or based medium, the purification can be performed using strong or weak cation or anion exchange resins.[49,50] Finally, different crystallization techniques using appropriate combination of solvents can be used for compound isolation in very specific cases.[37,46]

A methodology which can dramatically reduce number of tedious purification steps mentioned above is the synthesis on solid support. This synthetic strategy was indeed used to obtain bifunctional DO3A-peptide conjugates. It mainly involves coupling tris-*t*Bu-DO3A to the N-terminus of peptides bound on the polymer resin. In the same manner, tris-allyl-, tris-methyl- or tris-benzyl-DO3A esters can be employed. It is important to note that protecting groups on the DO3A derivatives used have to be compatible with the solid phase synthesis conditions. A range of DOTA-peptide conjugates have been synthesized and investigated up to now.[51]

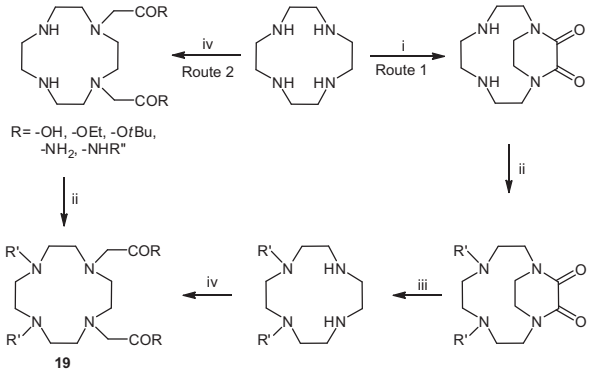
The abovementioned methods described for preparation of various DO3A functionalized compounds list just the most commonly used strategies for preparation of potentially useful MRI contrast agents. A number of further bifunctional DO3A derivatives obtained via the introduction of different pendant arms have also been reported (Fig. 3). Mono- and bis(phosphonate)-containing ligand **13** showed potential as a positive MRI contrast agent for bone and other calcified tissues.[52] The molecule **14** bearing a pendant diphenylphosphinamide moiety enables non-covalent binding to human serum albumin,[49] while molecules of type **15** with an aryl phosphonate group can serve as bimodal (optical and MR imaging) agents.[53] The ligand **16** represents a PARACEST agent investigated for its potential for labeling adenovirus particles,[50] while compound **17** has been developed as a responsive MR contrast agent for selective copper sensing.[48] Listing all the procedures and applications of contrast agents thus obtained is beyond the scope of this work; however, a number of recent review articles that deal with these topics can be found for further reading.[8,9,54,55]

Ligands based on DO2A

This cyclen derivative allows immediate synthetic transformations on two secondary amines on the macrocyclic rim. DO2A possesses two acetic arms which chelate lanthanide ions and can be either at position 1,4 (*N-cis*) or 1,7 (*N-trans*). Nevertheless, the



Scheme 7. Different methods for synthesis of *trans*-DO2A. The most common reagents: (i) Cbz-Cl/1,4-dioxane and H_2O; (ii) K_2CO_3, $BrCH_2COOtBu$/MeCN; (iii) Pd(C), H_2; (iv) R′(R″)Br, K_2CO_3/MeCN. R′, R″ and R‴ stand for a number of diverse substituents.



Scheme 8. Different methods for *cis*-DO2A synthesis. The most common reagents: (i) diethyl oxalate/EtOH; (ii) R′Br, (*i*-Pr)$_2$NEt/MeCN; (iii) NaOH/EtOH; (iv) $BrCH_2COR$, DIPEA/MeCN. R′ and R″ stand for a number of diverse substituents.

thermodynamic and kinetic stability of lanthanide complexes with DO2A is too low for further in vivo studies due to an insufficient number of coordination bonds between the DO2A chelator and the lanthanide ion (coordination number 6: four amines in the cyclen ring and two carboxylic acids). Therefore, DO2A is mainly used as a precursor for preparation of multifunctional contrast agents, and not as a final chelator for preparation of the lanthanide complexes and their use in MRI.

Up to now several different approaches for the double functionalization of cyclen were reported. Although direct derivatization is convenient for preparation of tri- or tetra-substituted cyclen (see above), this approach exhibits lack of regioselectivity in the case of di-substituted cyclen derivatives, resulting in a mixture of mono-, di-, tri- and tetra-functionalized products. The most commonly accepted methodology for DO2A synthesis thus includes a series of protection-functionalization-deprotection steps (Schemes 7 and 8, route 1). Principally, the initial steps involve reaction of selective amine protection at *trans*- or *cis*-positions (1,4 or 1,7), followed by alkylation of remaining two amines with suitable acetic esters or amides. The final stage involves the amine deprotection and their further derivatization (usually alkylation). It is also possible to switch these two steps of alkylation by preparing 1,4- or 1,7-disupstituted alkyl- or aryl-cyclens that can be further transformed into DO2A derivative in the second alkylation stage.[11] Either of these synthetic strategies results finally in symmetrical or unsymmetrical *N-trans* or *N-cis* DO2A derivatives (compounds of type **18**, Scheme 7 and type **19**, Scheme 8).

Following these strategies, a selective *N*1,*N*7-difunctionalization of cyclen can be achieved at high yield under acidic condition

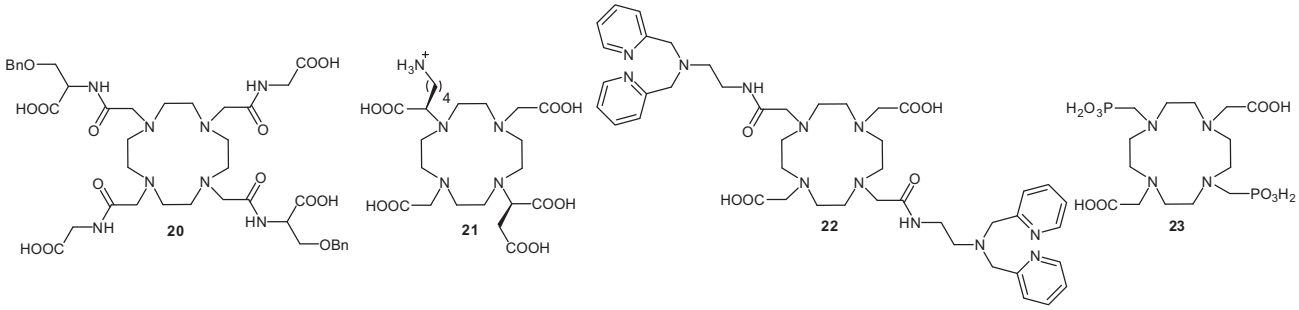


Figure 4. Structures of some symmetrical and unsymmetrical *trans*-DO2A based derivatives.

by slow addition of chloroformates.[56] Such a procedure avoids production of the undesired 1,4-substitution product, and allows the preparation of various carbamate-protected amines by using a range of chloroformates (methyl, ethyl, benzyl, or *tert*-butyl), depending on the choice of conditions required for their removal. The same regioselectivity can be achieved in nearly quantitative yields when the corresponding (oxycarbonyloxy)succinimide reagents are used as electrophiles.[57] Very recently, a *t*Bu-DO2A ester was also prepared from these reagents, however in a rapid manner using microwave-assisted hydrogenation.[58] Finally, using a completely different strategy, 1,7-cyclen derivatives can be prepared from protected cyclen **7** (Scheme 4) upon reaction with a number of alkyl-halogenides.[59]

On the other hand, a selective N1,N4-dialkylation of cyclen can be achieved using diethyl-oxalate as a protecting group to yield cyclenoxamides (Scheme 8, route 1). This intermediate can be further alkylated with *tert*-butyl bromoacetate or other electrophiles. Following the deprotection of the oxalate using a strong base, a *cis*-disubstituted cyclen product can be obtained, which is then available for second alkylation.[60]

In addition to these described methodologies, procedures for preparation of 1,4- or 1,7-DO2A that omit protection steps have also been reported. Thus, 1,7-DO2A can be prepared by *trans*-disulfomethylation of cyclen, followed by conversion of sulfonates to cyanides which upon hydrolysis finally result in acetate groups thereby forming the acetate moiety (Scheme 7, route 2).[61] On the other hand, the 1,4-isomer can be obtained upon alkylation of cyclen with *tert*-butyl bromoacetate in chloroform at room temperature in the presence of 10 equiv of triethylamine. After performing flash chromatography, the desired 1,4-disupstituted cyclen derivative can be isolated with a good yield (Scheme 8, route 2).[62]

Once the desired DO2A derivative has been prepared, it can undergo further synthetic transformations sequentially on the third and fourth secondary amine positions, or at both amines simultaneously. For instance, its mono alkylation with the alpha-halogenated protected carboxylic acid yields a functionalized DO3A derivative. This can be further modified by alkylating the fourth 'free' amine position on the cyclen ring to prepare biotinylated pH-responsive contrast agents that can interact with the protein avidin.[63] This strategy appears to be much more convenient as a similar bifunctionalized DO3A agent has also been reported (although using a different preparation strategy that did not start with a DO2A derivative), resulting in a much lower yield of the desired product.[64]

Further examples of DO2A derivatives (mainly *trans*-variants) that were prepared for use as MRI contrast agents have been summarized recently.[58] In most of cases, these include the symmetrical bifunctionalized DO2A chelators that have the same group in the *trans* position (in addition to two methylencarboxyl group). However, asymmetrical derivatives with two different pendant arms were also prepared (Fig. 4). For instance, although compound **20** is a DOTA derivative, it can also be classified as a symmetrical *trans*-DO2A agent according to the synthetic preparation pathway. Its Eu-complex exhibits a CEST effect and can be potentially useful as vascular MRI agent.[65] The same goes for compound **21**, belonging in this case to asymmetrical *trans*-DO2A derivatives. It is designed as an MRI agent with a potential to be a versatile tracer for multimodal imaging.[66] Additionally, responsive contrast agents can also be prepared from DO2A derivatives: compounds **22** and **23** were used to prepare MRI agents sensitive to zinc and pH, respectively.[67,68]

Conclusions

The recent expansion of different MRI methodologies has resulted in an increased demand for MRI contrast agents. Among these, a number of cyclen-based chelators such as DOTA, DO3A, DO2A and their derivatives have been prepared thus far. The most straightforward methodology for their synthesis is the direct alkylation of cyclen at the desired position. Nevertheless, due to lack of selectivity in most cases, strategies that involve diverse chemistries with protecting groups are required to obtain a product with the appropriate functional groups. The chosen protecting groups should be inert toward a wide range of reaction conditions and susceptible to easy cleavage under mild reaction conditions, leaving other functional groups in the chelator intact. Once these requirements are fulfilled, further modification of these chelators in terms of pendant arm functionalization is leading to diversification of contrast agents with a number of improved physicochemical and biological properties. The brief summary of well-established methods as well as also contemporary synthetic methods for the preparation of cyclen-based MRI contrast agents provided in this article should aid a better understanding of their chemistry, as well as assist in more convenient preparation of agents that are yet to be developed.

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