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A straightforward and convenient pathway for the synthesis of functional bismacrocyclic ligands

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ABSTRACT

The synthesis of a novel DO3A-based bismacrocyclic ligand is reported. The synthetic pathway involved a series of simple and convenient steps, which can easily provide the desired product in larger quantities than produced in current synthetic procedures. This method enables the facile preparation of binuclear macrocyclic complexes which can be used in MRI, as well as other molecular imaging modalities.

A number of imaging techniques in medicine, such as X-ray computed tomography (CT), positron emission tomography (PET) and magnetic resonance imaging (MRI), allow the non-invasive diagnosis of disease or damage in living systems by providing three-dimensional images of tissues. Among these, MRI remains the most widely employed technique in the clinical field. Unlike CT and PET, MRI does not require ionizing radiation, and exploits the difference in water relaxation rates in tissues to generate spatially resolved images. The contrast of such images may be considerably enhanced by using paramagnetic contrast agents. Ever since it was demonstrated that Gd-DTPA can increase the relaxation rate (R_1) of water protons,[1] several Gd-based contrast agents (CAs) (e.g., Gd-DOTA, Gd-HPDO3A) have been developed to improve image quality.[2]

Over the last decade, however, contrast agents have been also developed for detecting and reporting specific biological events.[3] Such bioactivated, smart contrast agents (SCAs) are capable of responding to enzymatic activity, pH or biologically relevant metal ions such as Zn^{2+}, K^+ or Ca^{2+}, by changing the MRI signal induced by a change in their microenvironment.[4–8]

Efforts to develop new Ca^{2+}-responsive, MRI-detectable CAs, have been particularly strong, as they can potentially revolutionize neuroimaging by providing noninvasive functional markers.[9] We have recently synthesised several bismacrocyclic molecules that contained various Ca^{2+}-chelators.[8,10,11] The physicochemical characterization of the Gd^{3+} complexes indicated their considerable potential to operate as SCAs. Interestingly, the use of responsive bismacrocylic complexes proved to be significantly more effective than their monomacrocyclic analogs. Specifically the binding of a metal ion in a 1:1 stoichiometry, was actually found to affect two Gd^{3+} in the SCA altering both their r_1. Therefore the resulting MR signal was twice as strong as the signal change produced from an SCA that contains only one Gd^{3+} center. Unfortunately, however, the synthesis of the bismacrocyclic SCAs is more challenging when compared to their monomacrocyclic equivalents.

There are several possible strategies to couple the Ca^{2+}-chelating moiety of the SCA to its MR reporting unit (Gd-DO3A). The linking of BAPTA-, DTPA- or EDTA-modified Ca^{2+} chelators was achieved by the reaction of a corresponding bis-anhydride with a DO3A-alkylamine.[10,11] The reaction yields were up to 55% and purification by HPLC was required due to the high polarity of the two resulting carboxylic acids. Alternatively, EGTA-derivatized SCAs were made by coupling a DO3A-alkylamine with 2-bromoacetic acid, followed by an *N*-alkylation reaction to attach these two DO3A-derivatives to the Ca^{2+} chelator. In this case, the isolated yields of the bismacrocyclic derivatives were high (up to 81%), however purification by size exclusion chromatography was required. Most significantly the purification methods needed to isolate the bismacrocyclic ligand precursors, or the final products, are not always readily available in all laboratories.

Here we report a simple and convenient synthesis of a new DO3A-based bismacrocyclic ligand. The practical advantage of this synthetic methodology involves the facile purification of all the

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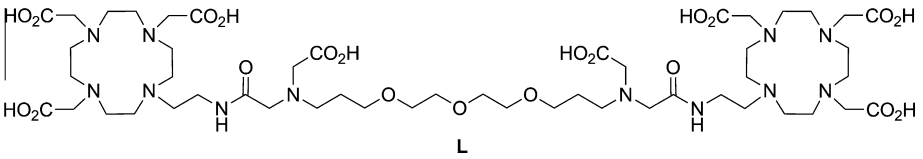
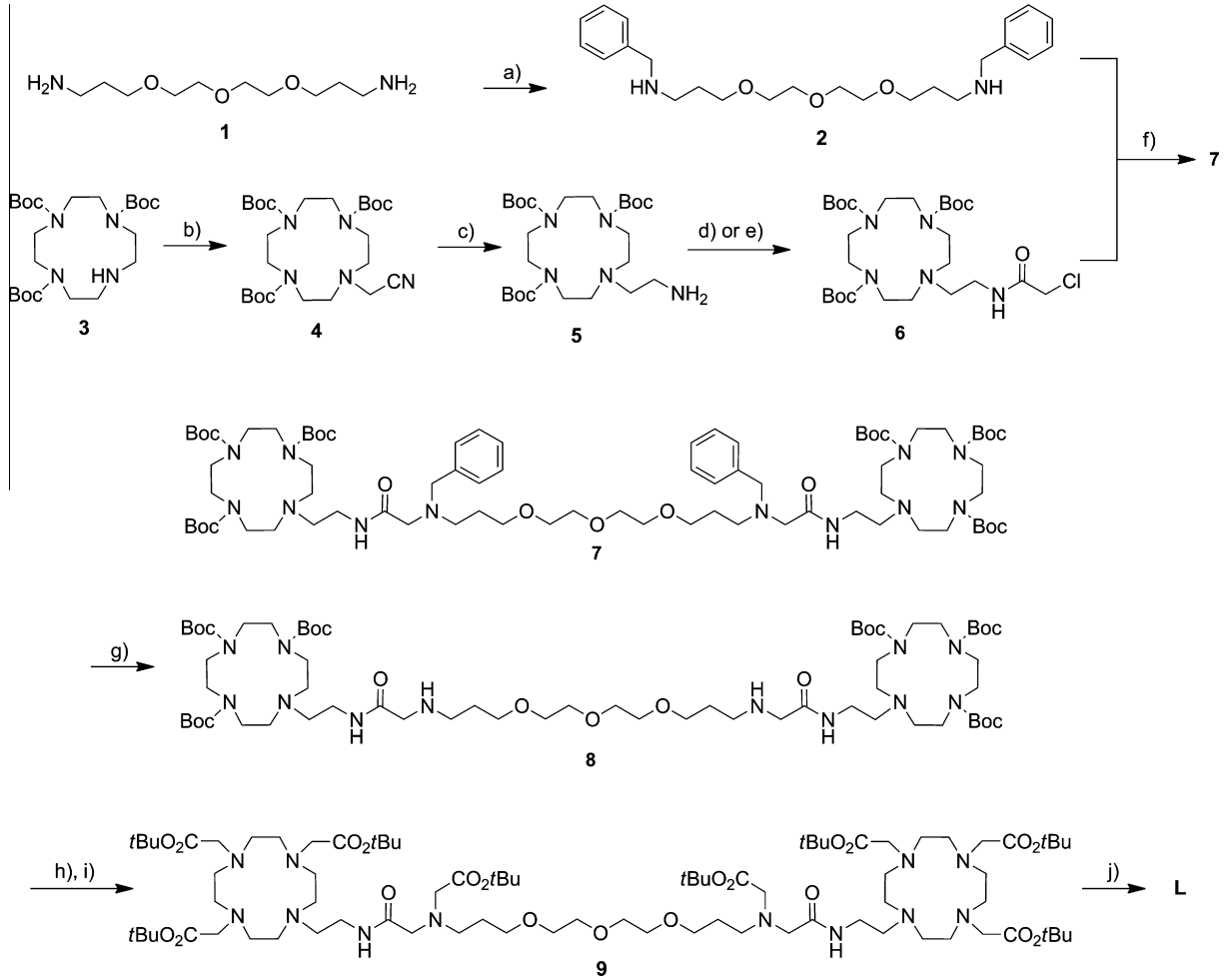


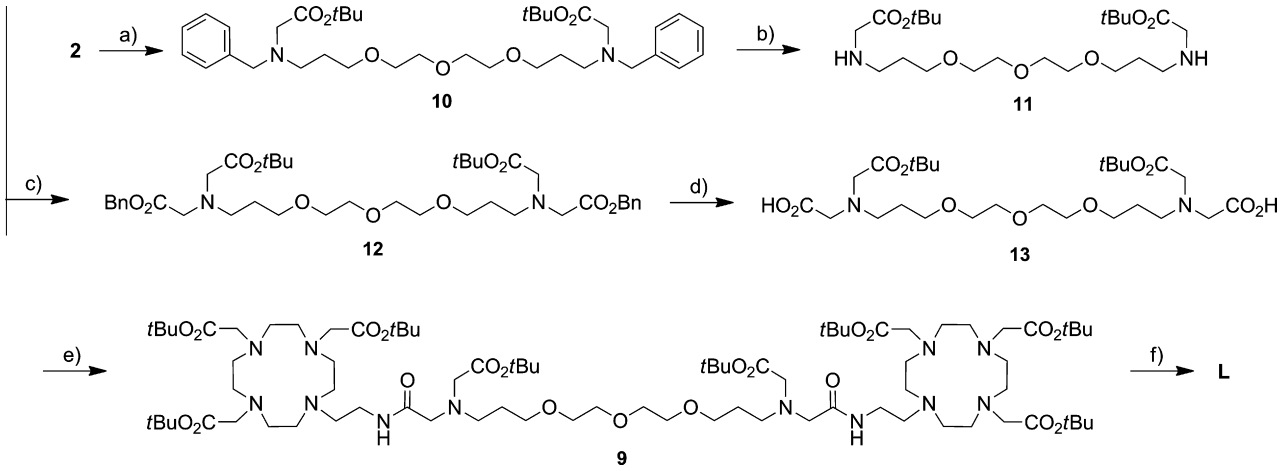
L

Chart 1. The structure of the synthesized bismacrocycle **L**.

intermediates throughout the synthetic route. The bismacrocyclic ligand **L** (Chart 1) was synthesized in eight steps starting from the commercially available 1,13-diamino-4,7,10-trioxatridecan **1** (Scheme 1). The primary amines of **1** were easily converted to secondary *N*-benzyl amines by reductive amination using $NaBH_4$, leading to **2** in a 63% yield. In parallel, the tris-Boc-protected cyclen **3** was prepared as previously described[12] and then reacted with bromoacetonitrile in CH_3CN in the presence of K_2CO_3 to provide **4** in a high yield (93%). Thereafter the nitrile functionality was readily reduced to the amine **5** by hydrogenation at room temperature, employing the Raney-nickel catalyst and a solution of 2 N sodium hydroxide. This was transformed into the corresponding amide **6** by the use of chloroacetic anhydride in CH_2Cl_2. Note that **6** can also be obtained using chloroacetyl chloride under the same conditions yielding the same amount of product (81%). The condensation of 2.4 equiv of **6** with 1.0 equiv of the bis-amine **2** in the presence of KI resulted in the formation of the bismacrocyclic compound **7**. The presence of the Boc protecting groups on the cyclen nitrogens apparently reduces the polarity of this whole molecule when compared to the analogous *tert*-butyl ester derivative of DO3A. Thus **7** is easily purified by column chromatography over silica gel, resulting in a 75% yield. The removal of the benzyl groups to give **8** was achieved by hydrogenolysis in EtOH using Pd/C as a catalyst at 60 °C. It is important to note that no trace of product could be observed when the hydrogenolysis was attempted at room temperature, possibly because of the bulkiness of the Boc protective groups. Following the successful removal of the benzyl groups, **8** was treated with a mixture of TFA/CH_2Cl_2 to cleave the Boc protecting groups. The excess TFA was removed by evaporation under reduced pressure and the resulting crude compound was alkylated with *tert*-butyl bromoacetate in basic conditions producing the ligand precursor **9**. Finally, the complete deprotection of the *tert*-butyl esters to obtain the desired ligand **L**, was accomplished with formic acid at 60 °C.[13]



Scheme 1. Synthesis of **L**. Reagents and conditions: (a) benzaldehyde, $NaBH_4$, CH_3OH, 63%; (b) bromoacetonitrile, K_2CO_3, CH_3CN, 70 °C, 93%; (c) Raney-nickel, 2 N NaOH, EtOH, rt; (d) chloroacetic anhydride, Et_3N, CH_2Cl_2, 0 °C, 81%; (e) chloroacetyl chloride, Et_3N, CH_2Cl_2, 0 °C, 81%; (f) KI, K_2CO_3, CH_3CN, 60 °C, 75%; (g) H_2, Pd/C, EtOH, 60 °C; (h) TFA/CH_2Cl_2, rt; (i) *tert*-butyl bromoacetate, K_2CO_3, CH_3CN, 70 °C, 59% over three steps; (j) formic acid, 60 °C, 97%.



Scheme 2. Reagents and conditions: (a) *tert*-butyl bromoacetate, K_2CO_3, 80 °C, 59%; (b) H_2, Pd/C, CH_3OH, rt; (c) benzyl bromoacetate, K_2CO_3, CH_3CN, 80 °C, 50%; d) H_2, Pd/C, CH_3OH, rt; e) tris-*t*-Bu-DO3A-EA, EDCI/NMM/HOBt or HBTU or carbonyldiimidazole, DMF, 60 °C; f) formic acid, 60 °C.

The effectiveness of this synthetic strategy was demonstrated when comparing it to those more frequently used. This includes the synthesis of the bismacrocyclic ligand **L** via the condensation of the biscarboxylic acid **13** with tris-*tert*-Bu-DO3A-ethylamine[11] by using common coupling reagents for peptide synthesis[14] to optimize the reaction yield (Scheme 2).

The biscarboxylic acid **13** was prepared initially from **2**, so that orthogonal protecting groups could be attached to both of the carboxylates of the desired product. The secondary amine **2** was alkylated with *tert*-butyl bromoacetate to yield **10**. The benzyl groups were removed by hydrogenation and subsequent alkylation of **11** with benzyl bromoacetate yielded the tetraester **12** which contains two different protecting groups that can be removed selectively under acidic (*tert*-Bu esters) or reductive (benzyl esters) conditions. The benzyl esters were converted to acids by hydrogenation using identical reductive conditions described previously for **13**.

The first condensation reactions utilized the conventional reagents EDCI, NMM and HOBt. However, only the trace amounts of the desired product **9** could be isolated (Table 1). The use of HBTU provided **9** in a moderate yield after column chromatography purification over silica gel. The *tert*-butyl esters were removed successfully using formic acid, however during the Gd^{3+}-complex formation and the subsequent relaxometric titration with Ca^{2+}, the formation of the precipitate was observed. This can be explained by the contamination of **9** with the hexafluorophosphate (PF_6^-) anions, which are then hydrolyzed to phosphoric acid during the acid mediated deprotection of the *tert*-butyl esters.[15] Ultimately, it is the presence of these phosphate anions in the solution that form insoluble salts in the presence of cations such as Gd^{3+} and Ca^{2+}.[16,17] Finally, the reaction involving the third coupling reagent, carbonyldiimidazole, gave **9** in a similar yield when compared to HBTU. However, in this case the complete cleavage of the *tert*-butyl esters using formic, trifluoroacetic or hydrochloric acid could not be achieved.

Finally Gd^{3+} and Eu^{3+} complexes of the novel ligand **L** were prepared and their physicochemical properties were investigated. The complexation was performed in H_2O at neutral pH from corresponding lanthanide chloride salt. The r_1 of the **Gd_2L** was 3.9 $mM^{-1}s^{-1}$ in HEPES buffer (pH 7.4), 25 °C and 300 MHz. The decay of the luminescent intensity of the **Eu_2L** was measured in buffered H_2O and D_2O, yielding with the lifetime values τ_{H2O} = 0.73 ms and τ_{D2O} = 1.43 ms. Using the empirical equation for the estimation of the hydration number,[18] q was determined to be 0.4. Both the r_1 and q are in the range typically observed for bismacrocyclic compounds of this type.[8] However, the relaxometric titration experiments, performed in the absence and presence of Ca^{2+} and Mg^{2+} exhibited only moderate r_1 changes upon the addition of up to 20 equiv. of either Ca^{2+} or Mg^{2+}. The maximum r_1 value reached 4.3 $mM^{-1}s^{-1}$, corresponding to a 10% increase from the initial r_1 value. The similar nature of the results obtained from these two titrations showed that the complex responds poorly to both of these ions, despite possessing a Ca^{2+}-chelator which exhibits a selective interaction towards alkaline earth metal ions when incorporated in a responsive fluorescence dye.[19]

Table 1
Conditions for the coupling reaction of **13** and tris-*t*-Bu-DO3A-ethylamine to yield **9**

Coupling agent	Solvent and temperature	Yield%
EDCI/NMM/HOBt	DMF, rt or 60 °C	—
HBTU	DMF, 60 °C	32
Carbonylimidazole	DMF, 60 °C	33

In summary, we have developed an easy and efficient procedure to obtain a new DO3A-based bismacrocyclic ligand. This simplified route involves a few facile synthetic steps requiring relatively easy purification procedures to obtain bismacrocyclic products. The effectiveness of this route was demonstrated by the synthesis of the molecule which can serve as potential SCA. The procedure can be applied to the future synthesis of a versatile range of bismacrocyclic products and can be used for preparation of various functional ligands that can find application in several molecular imaging methods.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.01.133.

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