Synthesis of a Bifunctional Macrocycle

Hasina Noory

University at Albany, State University of New York

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Synthesis of a Bifunctional Macrocycle

An honors thesis presented to the
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Hasina Noory

Research Mentor: Leah Seebald
Research Advisor: Maksim Royzen, Ph.D.

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Abstract

Macrocycles are important organic ligands for encapsulating metal ions. This work describes the first step of a synthesis to create a bifunctional macrocyclic ligand suitable for nuclear magnetic resonance spectroscopy (NMR) studies. Cyclen is an organic macrocycle that contains twelve atoms total, with four nitrogen atoms incorporated into the cyclic backbone. Cyclen can be modified to coordinate a variety of metal ions by adding additional chelating arms. This synthesis is focused on functionalizing three of the donor nitrogens with carboxylate arms, while strategically leaving the fourth nitrogen available for further modification.
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Introduction

Macrocycles are organic molecules which contain at least nine atoms with at least three donor atoms incorporated into their cyclic backbone. They are known to coordinate metal ions whose ionic crystal radius matches best with both the cavity formed by the ring and the types of additional arms. Due to both their size and choice of chelating arm, macrocycles are able to target multiple types of metal, increasing both binding affinity and selectivity. For example, synthetic macrocycles such as crown ethers are used to quench metal ions such as sodium or potassium in a reaction. Because of the macrocyclic effect, these molecules are ideal for biological studies where metals might otherwise be prone to exchange or displacement. This selective process enables them to be used for many applications including ion storage and transport in vivo, solvent extraction of metals, development of metal-ion selective reagents for analytical applications, metal ion protection in biomedical imaging and drug delivery.

Magnetic resonance imaging (MRI) is an essential noninvasive diagnostic method in clinical radiology. Small quantities of macrocyclic ligands chelated with metal ions are used as contrast-enhancing agents to exhibit the different between diseased and normal tissue. For example, gadolinium, which has seven unpaired electrons, is considered an effective contrast agent. However, due to its toxicity as a lone metal ion, it is administered with a chelating compound, such as DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), a cyclen based macrocycle. MRI is an identical technique to NMR, where typically MRI is the term used in a clinical setting, and NMR is used in an academic setting. From herein, the terms will be used interchangeably.

The overall goal of this project is the development of an alternative macrocyclic probe for studying RNA by NMR. This project is broken down into the synthesis of the macrocycle and
the choice of the metal. Specifically, this work focuses on cyclen, an azamacrocycle, which contains four donor nitrogens. However, it is important to briefly explain why the choice of the metal is significant. There are two types of metals: paramagnetic and diamagnetic metals. Paramagnetic metals have unpaired electrons which cause proximal nuclei to shift and broaden on an NMR spectrum whereas diamagnetic metals do not affect a spectrum at all.\textsuperscript{13,14} Lanthanide ions, such as dysprosium and europium, are our metals of interest because of the useful paramagnetic effects on the NMR spectra.\textsuperscript{15,16} Thus, the macrocycle that will be synthesized needs to be suitable for coordinating the aforementioned lanthanide ions. This honors thesis will discuss in depth the first synthetic step towards the formation of a bifunctional azamacrocycle and its potential application towards magnetic resonance imaging.
Design and Synthesis

The goal of this project is to prepare a DOTA-type probe with a chelated lanthanide metal. This molecule will have three chelatable arms and a fourth arm with an N-hydroxy succinimide ester capable of covalent coupling to a modified RNA strand. Looking at Figure 1, there is a cyclen macrocycle with four arms. However it is evident that three of the arms of the cyclen are identical and one arm is different. This means the synthesis must first start with the addition of three identical arms and then, after workup and purification, the addition of the fourth. The following described procedure was used to make the three armed cyclen.

![Figure 1: Target molecule, a DOTA-like macrocycle](image)

The synthetic procedure is as follows. Cyclen (1 g, 5.80 mmol) and sodium acetate (1.57 g, 19.16 mmol) were dissolved in 20.0 mL dry N,N-dimethylformamide (DMF). The flask was then cooled to -20°C in an acetone bath with a cooling unit (also known as a “cold finger”). After stirring for approximately 45 minutes, three equivalents of methyl bromoacetate (1.92 mL, 17.41 mmol...
mmol) in DMF was added dropwise over a six hour period. Direct Analysis in Real Time (DART) mass spectrometry was used to monitor the molecular weight of the desired product (389.2981 g/mol) over this six hour time period. Upon completion the reaction was diluted with dichloromethane (DCM). After filtering the insoluble salts, the solvent was evaporated under reduced pressure. The compound was purified using flash column chromatography on silica with a gradient of dichloromethane to 5% methanol. The overall synthetic scheme of the cyclen macrocycle can be seen in Figure 2.

Figure 2: Overall Synthetic Scheme

DMF, a polar aprotic solvent, was utilized because it is polar enough for all the reagents to dissolve in and also reduced the likelihood of protonating the cyclen because of its aprotic characteristics. The reaction was conducted in low temperature conditions for steric control. Methyl bromoacetate is then added slowly in a dilute solution over a six hour time frame to optimize three armed cyclen formation. Addition of methyl bromoacetate occurs in an $S_N2$ fashion, as the bromine is a good leaving group and the amines of the cyclen are good nucleophiles. Both the starting material and the solvent conditions do no favor an $S_N1$ reaction pathway. Methyl bromoacetate also contains a methoxy protecting group which deprotects under strong basic conditions, yielding a carboxylic acid. This is important because carboxylic acids
are capable of coordinating metals, but are also susceptible to unwanted modification during later steps of the synthesis.\textsuperscript{18}

The challenge of the synthesis was the possible formation of undesirable byproducts. For instance, if methyl bromoacetate was added slowly in a dilute solution, one armed cyclen formation was encouraged (Figure 3).

\textbf{Figure 3:} Reaction scheme showing formation of one armed cyclen formation

If two equivalents of methyl bromoacetate were added, there are two armed cyclen formation (Figure 4). Due to steric hindrance, the second arm adds to the opposite amine, forming a symmetrical cyclen. This occurrence is well noted in literature and is thought to be the result of steric effects.\textsuperscript{18,19}

\textbf{Figure 4:} Reaction scheme showing formation of two armed cyclen
Finally, if methyl bromoacetate was added too quickly, there was an increase of four armed cyclen and lesser armed cyclen in the crude product, as shown in Figure 5.

![Reaction scheme showing formation of unwanted four armed cyclen](image1)

*Figure 5: Reaction scheme showing formation of unwanted four armed cyclen*

With the addition of three equivalents of methyl bromoacetate, the desired product is formed as shown Figure 6. By adding methyl bromoacetate slowly and very dilute over the six hour period, there was an optimization for three armed formation. However, the four armed cyclen and two armed cyclen were mixed with the desired product, as described in the results section.

![Reaction scheme showing formation of desired three armed cyclen](image2)

*Figure 6: Reaction scheme showing formation of desired three armed cyclen*
Results

The formation of the product was monitored by Direct Analysis in Real Time Mass Spectrometry (DART-MS), which gives the molecular weights of compounds by \([M+H]^+\).\textsuperscript{20} DART-MS was done in positive mode. The mass of the desired three armed cyclen is 389.2 g/mol. In figure 7, there is a peak at 389.281 m/z which confirms the desired product is present. The peak at 317.248 m/z is consistent with the mass of the two armed cyclen. Thus, more methyl bromoacetate was added to optimize the formation of the three armed cyclen.

![Figure 7: Positive ion DART-MS](image)

After the addition of more methyl bromoacetate, a byproduct of four armed cyclen is formed. DART-MS confirmed the mass at 461.317 m/z (Figure 8). Thus, the desired three armed...
cyclen was isolated and purified.

**Figure 8**: Positive ion DART-MS

In order to isolate the product, the solvent was removed and the compound was deposited on silica. The compound was purified using flash chromatography with dichloromethane (DCM) and 5% methanol (MeOH). The DART-MS of the desired product shows a single peak at 389.287 m/z, confirming the product was purified.
A Nuclear Magnetic Resonance (NMR) spectrum was also obtained for additional structure elucidation of the three armed cyclen. Looking at Figure 10, there are three different important proton environments present including the methyl ester, the methylene on the arm and the methylene in the cyclen. These different environment are confirmed by the NMR spectrum (Figure 11).
First, the methyl ester on the three armed cyclen has nine hydrogens. These hydrogens are expected to integrate to nine as a singlet. The NMR spectrum (Figure 11) confirms this peak at 3.7 ppm. The second proton environment, the methylene on the arm, contains six hydrogens. In this case, there would be two different singlets present because of the asymmetry of the cyclen. These two peaks are present from 3.4 ppm to 3.5 ppm. Finally, the methylene hydrogens are present from 2.5 ppm to 3.2 ppm because of the different environments each hydrogen is exposed to. There is a broad peak at 9.7 ppm which correlates with the hydrogen on the amine of the cyclen. The solvent used for the NMR spectrum is chloroform, which is present at 7.23 ppm. Thus, the NMR spectrum confirms the desired three armed cyclen produced.
Figure 11: NMR spectra of the three armed cyclen.
Conclusion

The bifunctionality of the target molecule comes with the addition of the fourth arm of a N-hydroxysuccinimide (NHS) ester. Initially, carboxylic acid is added to the cyclen with the three chelatable arms. Then, the NHS ester is attached, which is being coupled, making it a good leaving group (Figure 12). This NHS ester is also prone to nucleophilic attack at the carbonyl, which is important for further synthesis in the project.

*Figure 12: Future synthesis of the DOTA-like macrocycle*

Initially, the reaction contained a mixture of lesser armed cyclen with the target molecule. Therefore, the quantities of methyl bromoacetate were varied to encourage three armed formation. This modification was monitored by DART-MS. By increasing the quantity of methyl bromoacetate, DART-MS showed optimization of three armed cyclen formation. Overall, the goal was to produce a DOTA-like macrocycle with a cyclen backbone, 3 chelatable arms, and a fourth arm with an NHS ester to study RNA by NMR.
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