Synthesis of Templated Polymeric Materials for Plasma LPA Enrichment

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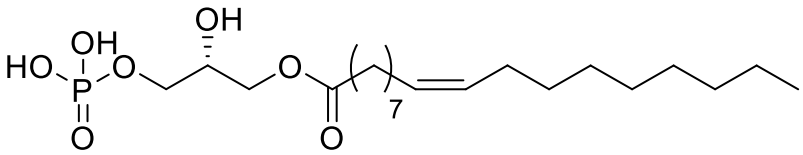
Introduction

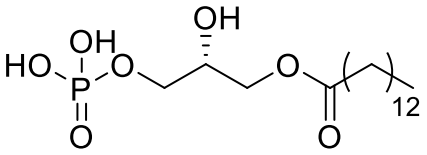
- Ovarian cancer is a deadly disease that around 10 million women in the United States are at risk for, with 21,000 new cases each year

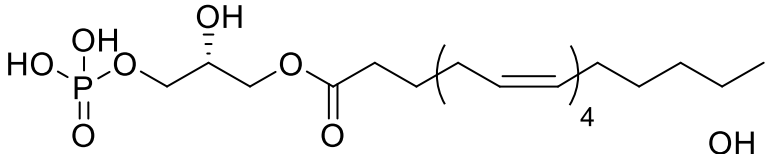
- CA125 (cancer-antigen) is the main biomarker used now, but it has a very low detection rate so it is usually discovered only during the late stages and when that occurs, the survival rate is 30%

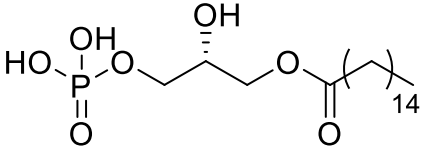
- Previous studies have shown that LPA could be a biomarker that can help identify ovarian cancer in its earliest stage, which would raise the survival rate to 90%

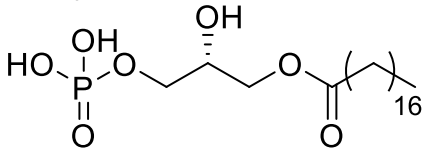
LPA background

LPA 18:1 

LPA 14:0 

LPA 20:4 

LPA 16:0 

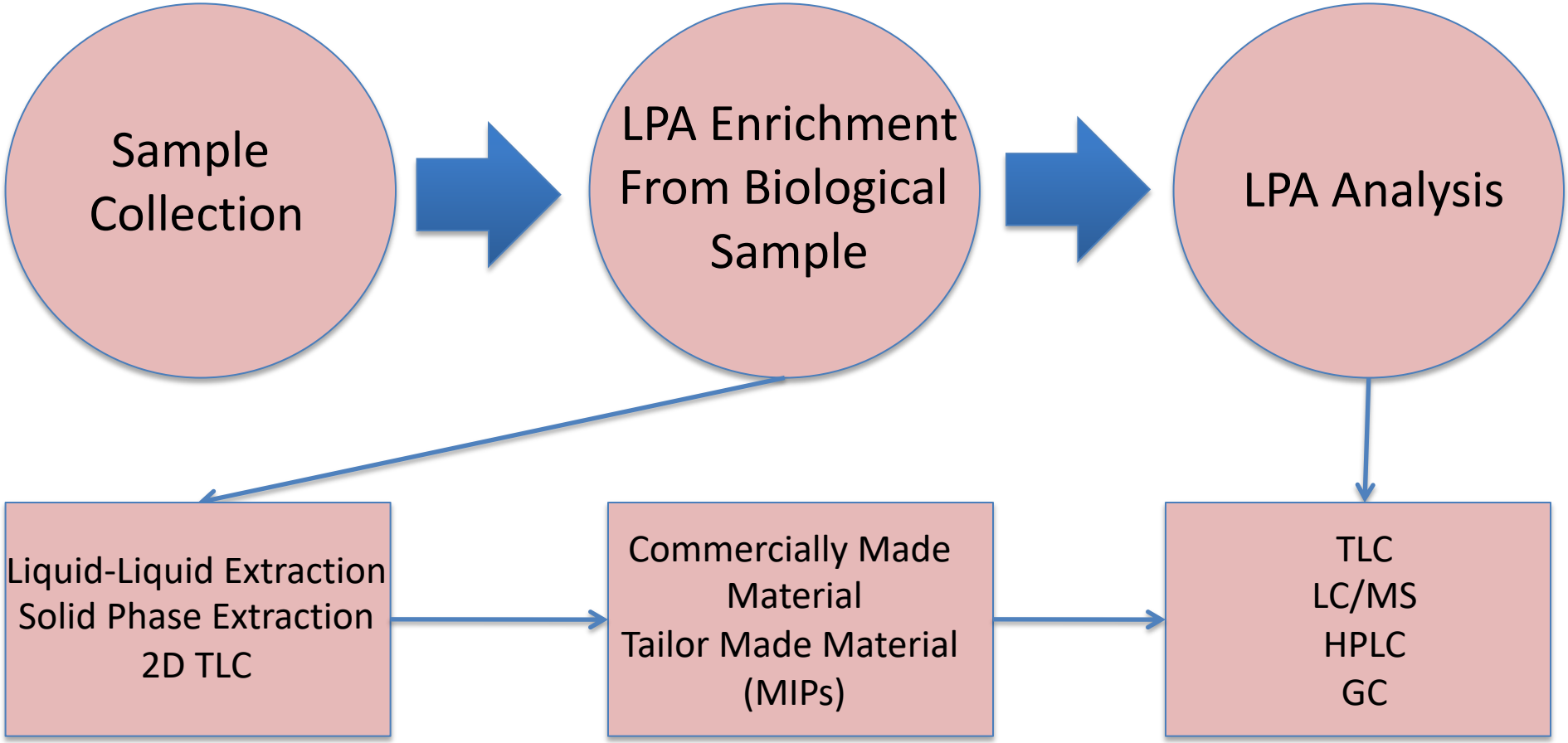
LPA 18:0 

- Lysophosphatidic acid (LPA) is a phospholipid that have been show to stimulate the reproduction of cancer cells and it appears to be in larger numbers in the blood of people with ovarian cancer.

Total LPA Concentration (µmol/L)	
No Ovarian Cancer	Ovarian Cancer
1.86	11.53

Sedláková, I, J Vávrová, J Tošner, and L Hanousek. "Lysophosphatidic acid (LPA)—a perspective marker in ovarian cancer." *Tumour Biology*. 32.2 (2011): 311-316. Web.

Objective
LPA Analysis Process



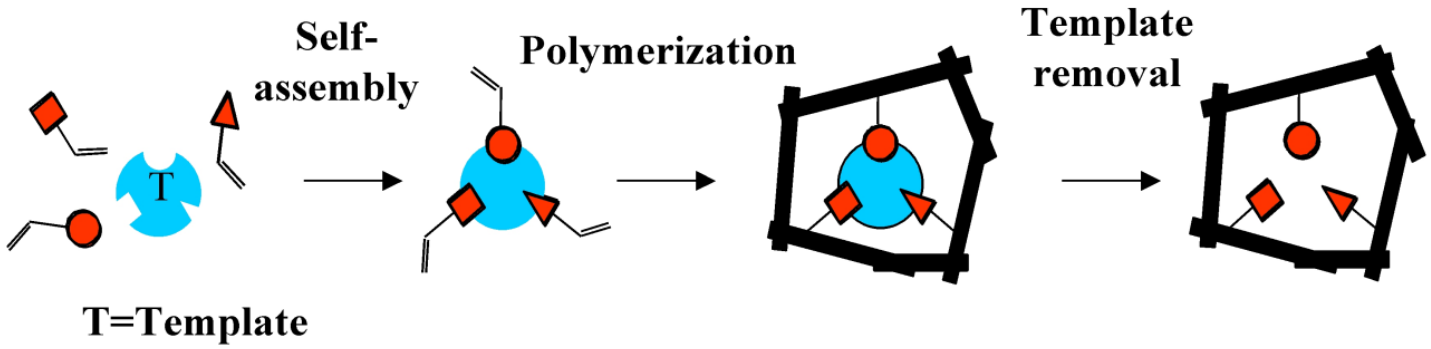
Method
Proposed Approach

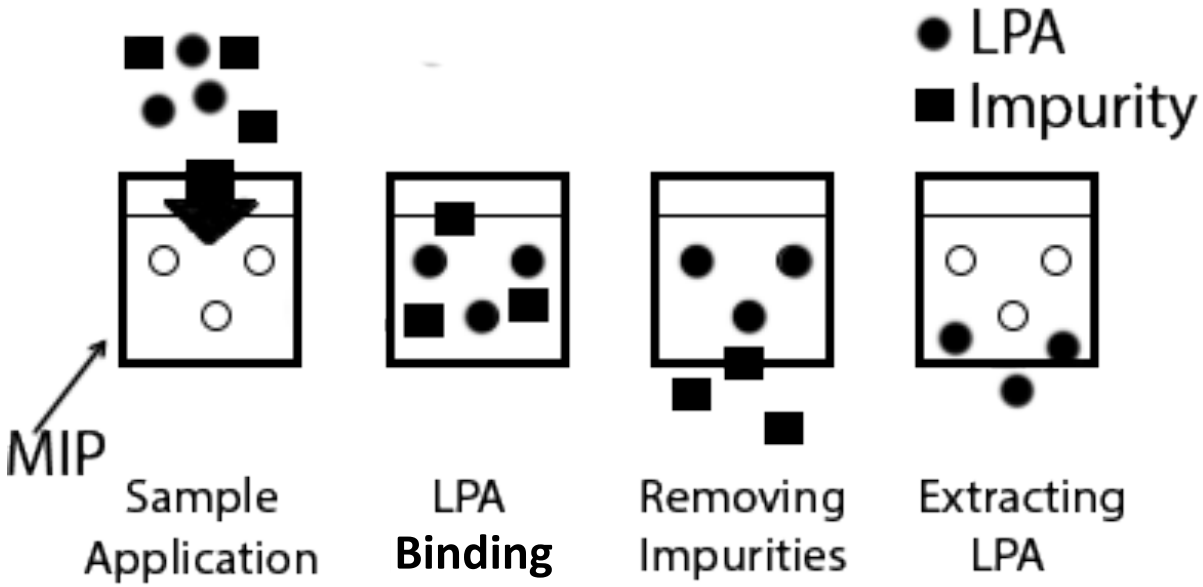
- Strongin's group has developed a method that uses a reversed phase solid support for the solid phase extraction (SPE) of LPA, but a liquid-liquid extraction is needed beforehand in order to remove impurities

- It is proposed that LPA enrichment from biological samples could be carried out by the use of tailor made materials called MIPs (Molecularly Imprinted Materials)

- It is anticipated that this will shorten sample process time and be more efficient than liquid-liquid extraction.

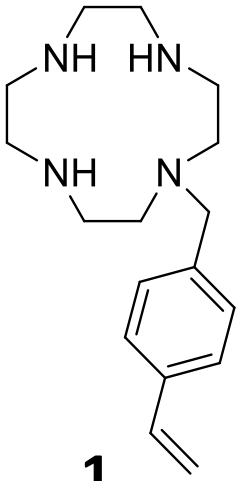
Molecularly Imprinted Materials



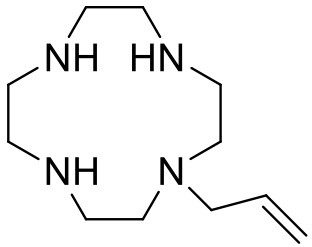


http://www.infu.tu-dortmund.de/images/big_090_Figure1.jpg

Functional Monomers Commonly Used for Molecular Imprinting of Phosphates

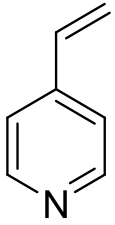


1



2

1 = 1-(4-vinylbenzyl)-1,4,7,10-tetraazacyclododecane
2 = 1-allyl-1,4,7,10-tetraazacyclododecane
3 = 1-vinyl imidazole
4 = 4-vinyl pyridine
5 = 2-vinyl pyridine
6 = LPA-Cyclen binding



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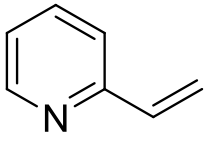

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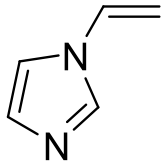

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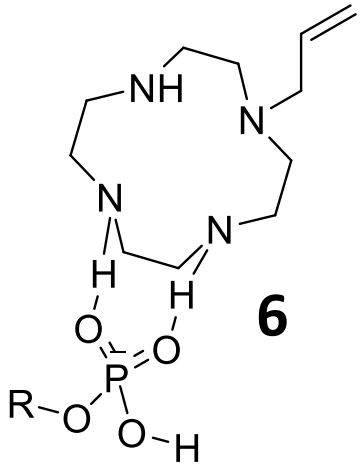
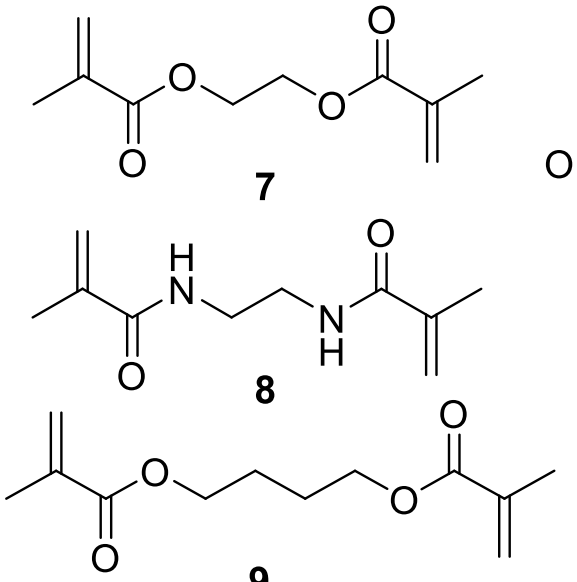


6

Crosslinking Monomers Used for Molecular Imprinting



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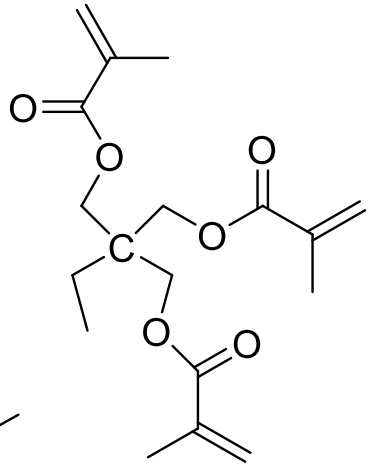

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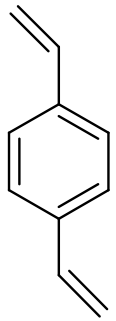

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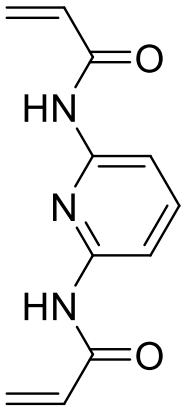

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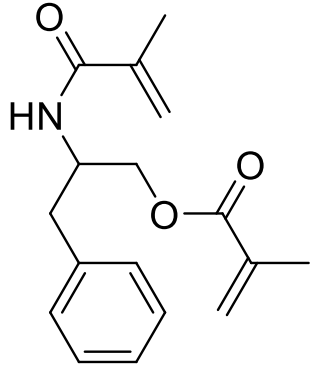


13

7 = EGDMA

8 = Ethylendimethacrylamide

9 = Butanediolmethacrylate (BDMA)

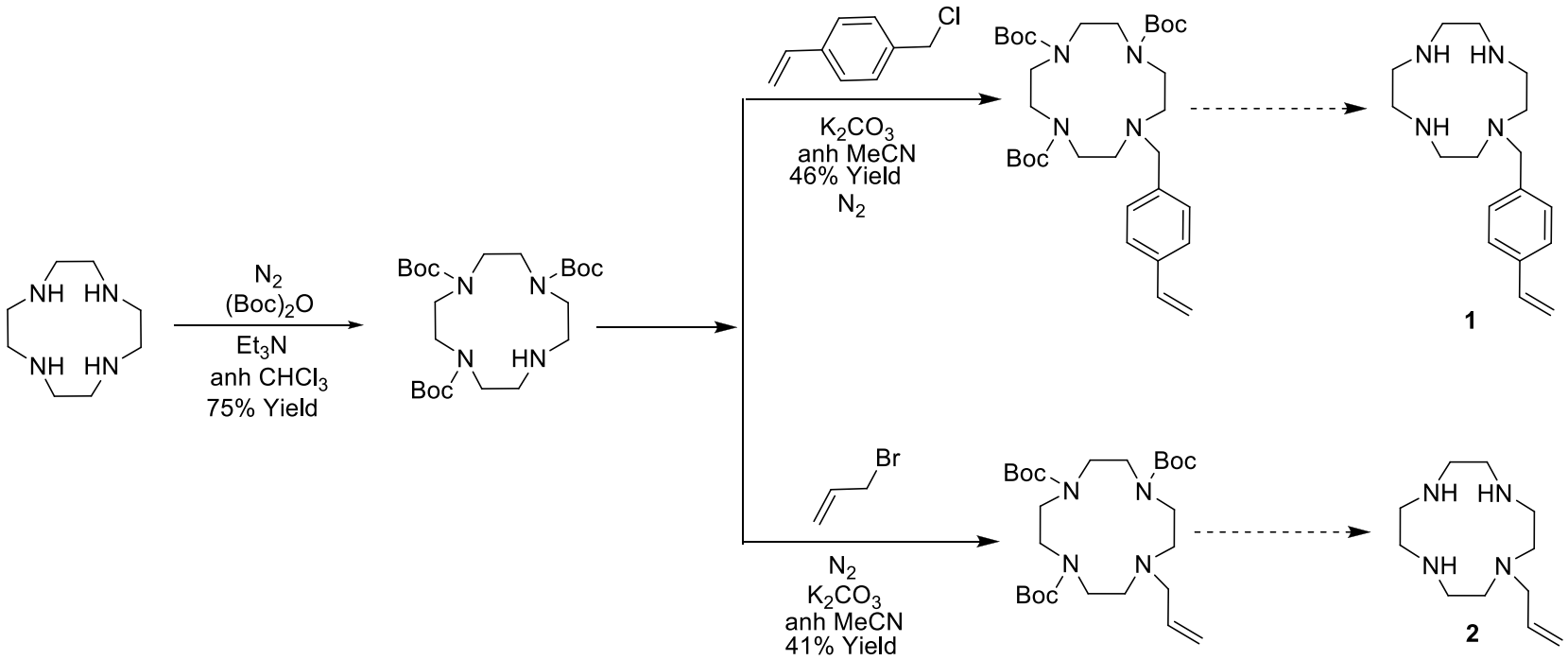
10 = TRIM

11 = DVB

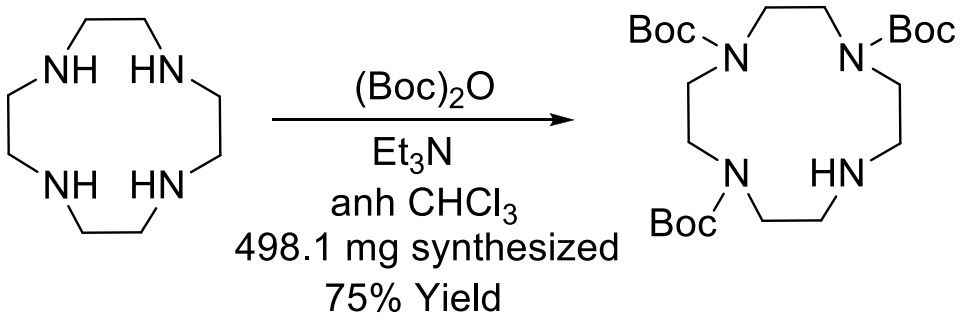
12 = bis(acrylamide)pyridine

13 = N,O-bismethacryloyl phenylalanine

Results
Functional Monomer Synthesis

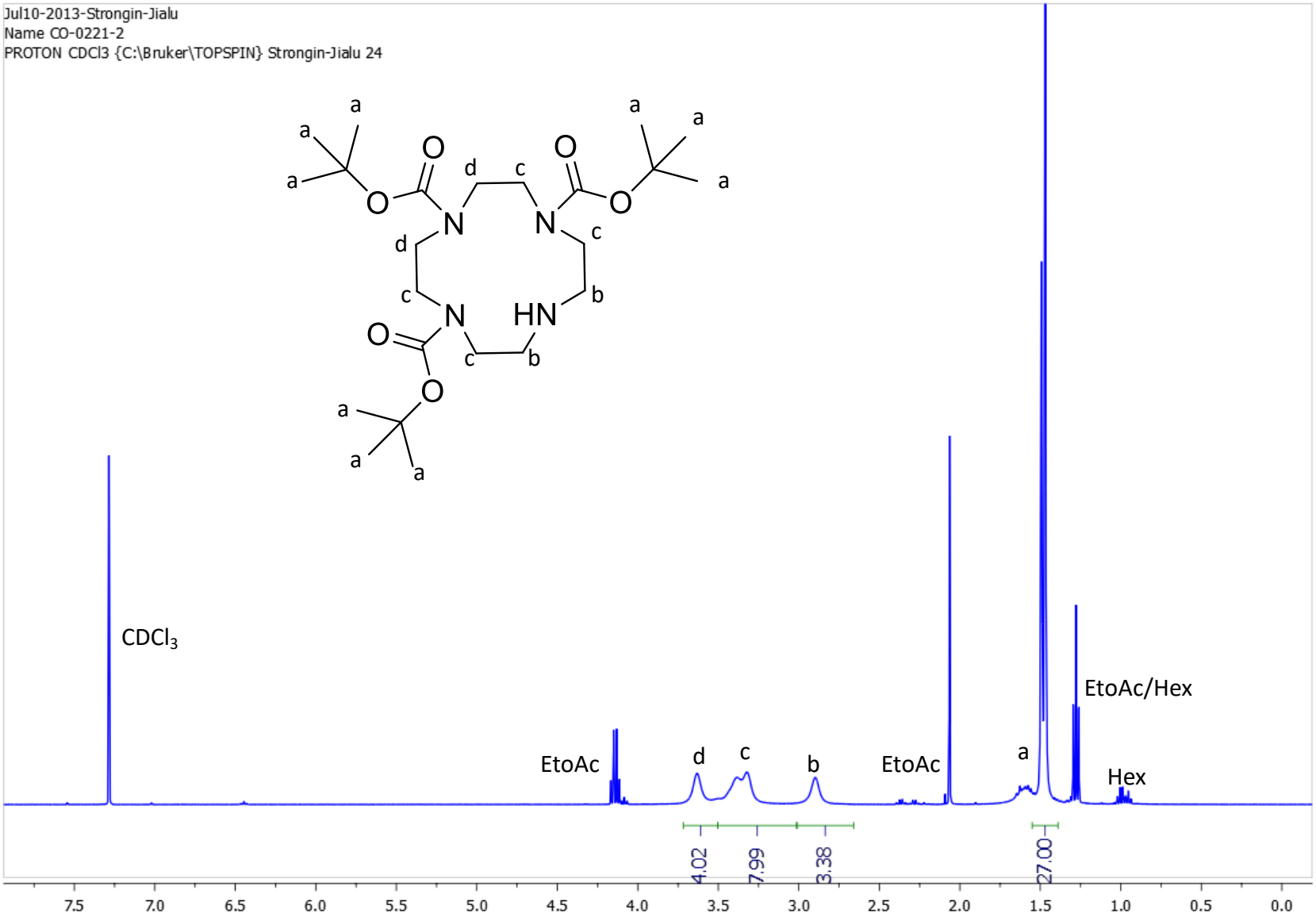


Synthesis of tri-tert-butyl 1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (Boc-protected Cyclen)
Step 1



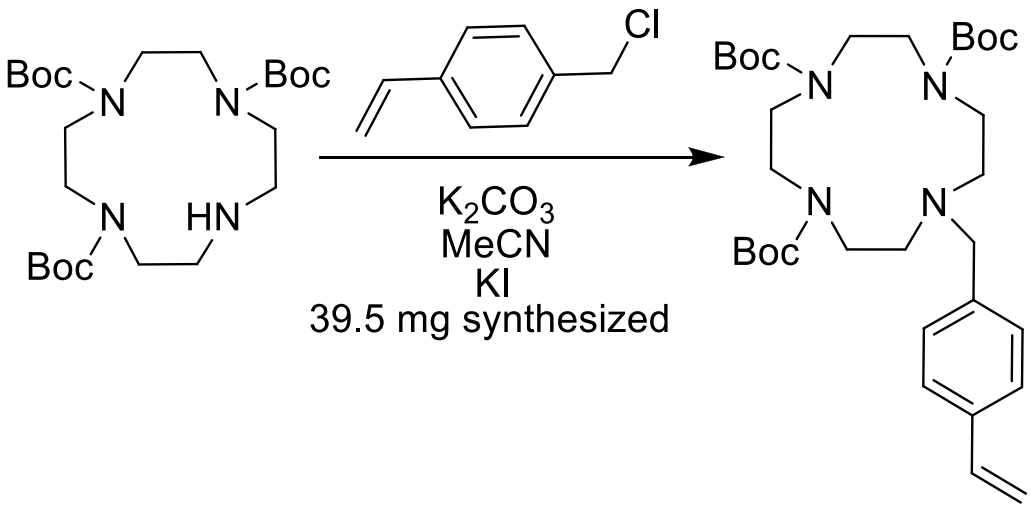
- Hwang S.; Cha W.; Meyerhoff M. Polymethacrylates with Covalently Linked Copper(II)-Cyclen Complex for in situGeneration of Nitric Oxide from Nitrosothiols in Blood. *Chem. Int. Ed.* **2006**, 45, 2745-2748

^{1}H NMR Boc-Protected Cyclen



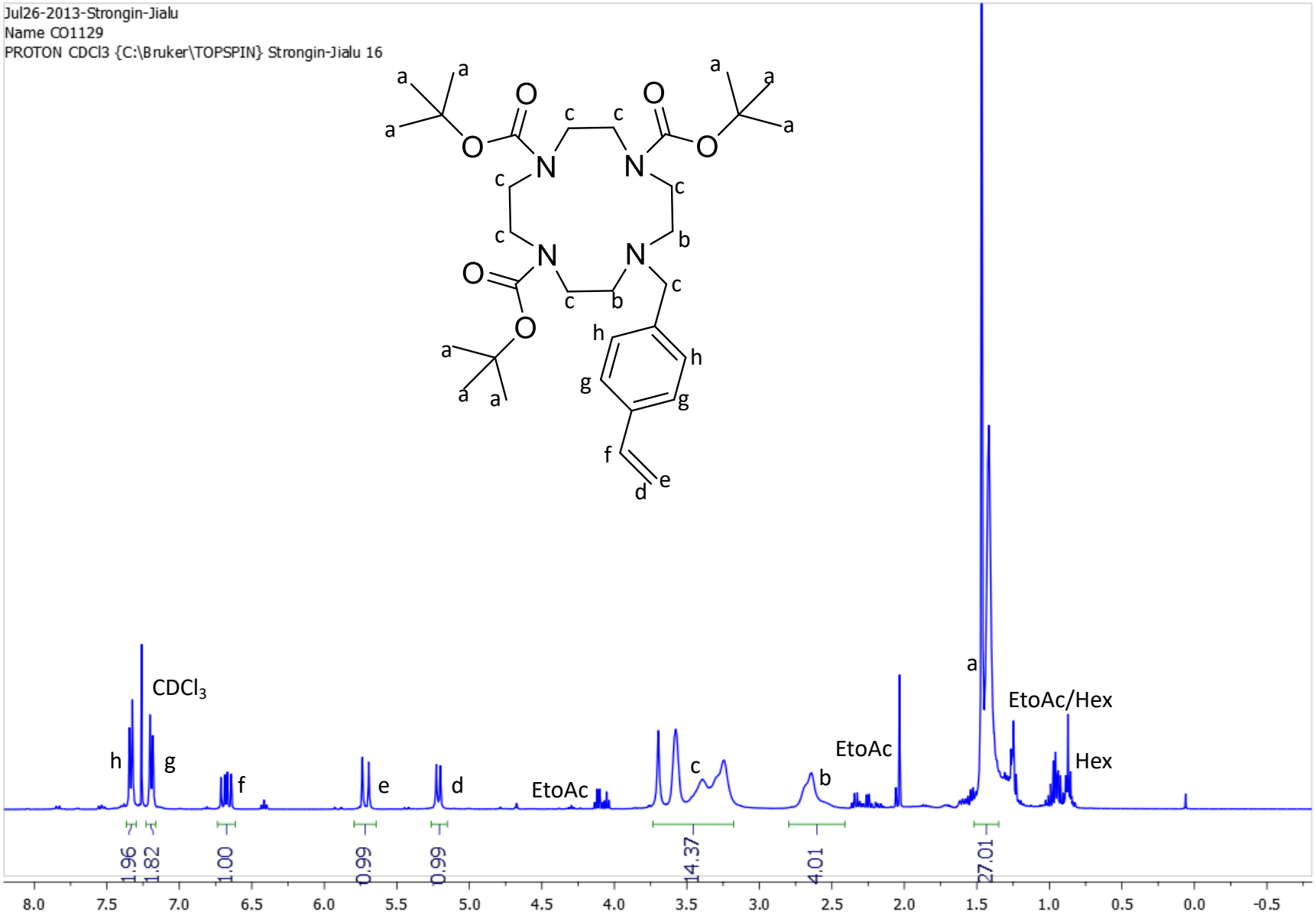
Synthesis of tri-tert-butyl 1-(4-vinylbenzyl)-1,4,7,10-tetraazacyclododecane-4,7,10-tricarboxylate

Step 2

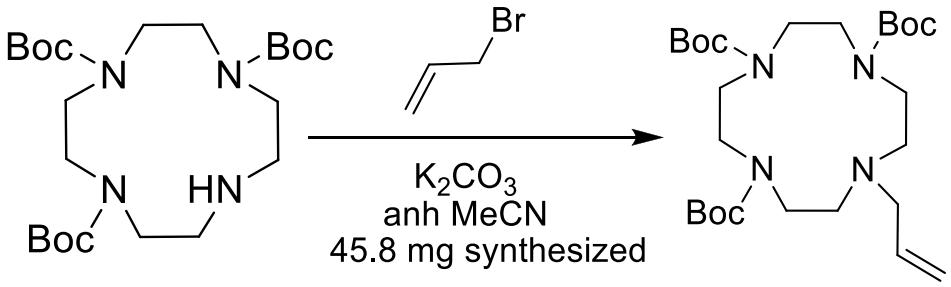


Exp	Reactants ratio Cylen:RX	Temp (°C)	Reaction time (h)	Yield (%)
1	1:4	90	70	0
2	1:4	90	96	TBA
3	1:1.5	70	3	43

^{1}H NMR Boc-Protected-Benzyl Cyclen

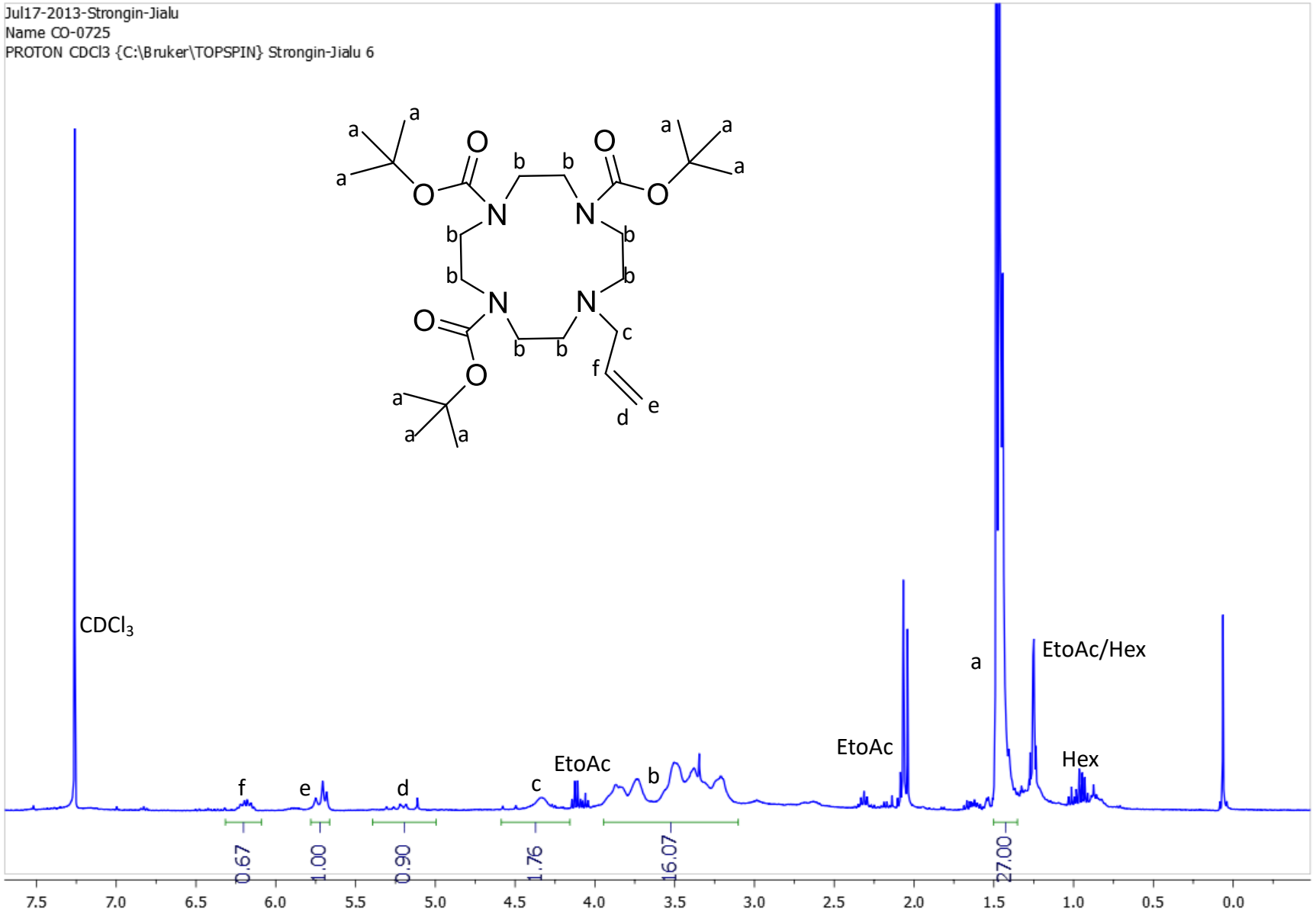


Synthesis of tri-tert-butyl 1-allyl-1,4,7,10-tetraazacyclododecane-4,7,10-tricarboxylate Step 2

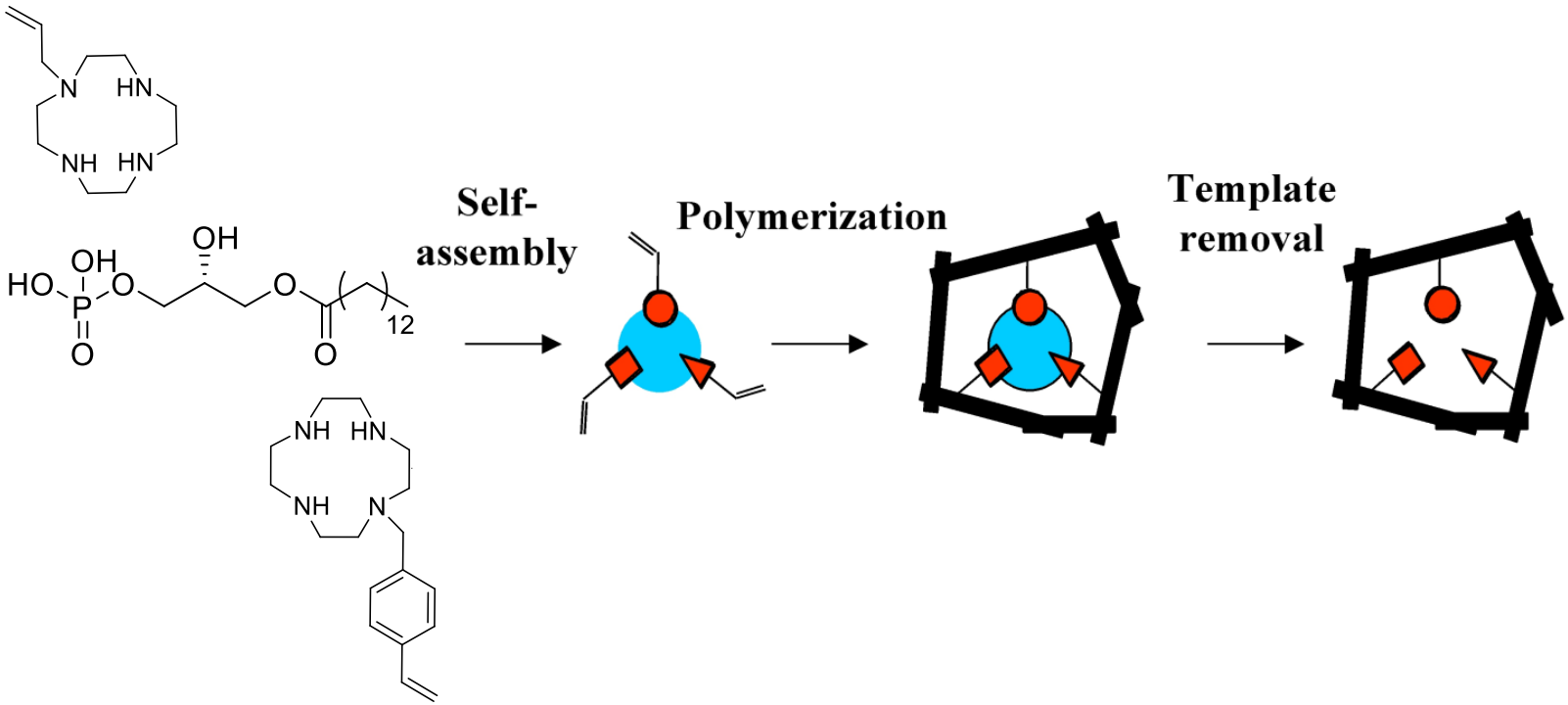


Exp	Reactant Ratio Cyclen:RX	Temp (°C)	Reaction time (h)	Yield (%)
1	1:8	90	70	43.16
2	1:8	90	96	35.34

^{1}H NMR Boc-Protected-Allyl Cyclen



Conclusions
Creation of the MIP



Future Research

- Synthesize deprotected benzyl and allyl monomers.
- LPA-Monomer Binding Studies by H NMR.
- Synthesis of MIPs.
- LPA Screening of MIPs using control samples.
- LPA-MIP enrichment from human plasma.

Acknowledgements

- National Science Foundation
- Woman and Infants Hospital of Rhode Island
- Brown University
- Dr. Robert Strongin, Dr. Martha Sibrian-Vazquez, Jialu Wang, and everyone else in the Strongin lab

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UNIVERSITY

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Questions?

- If so, ask away!