CHALMERS
UNIVERSITY OF TECHNOLOGY

Synthesis of Macrocyclic Diether Lipids Using Coordination Cages

Stability of a Nanobarrel in the Absence and Presence of a Guest

Master's thesis in Materials Chemistry

JOHN LEO GARMA BARING

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING

CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2023
www.chalmers.se

MASTER'S THESIS 2023

Synthesis of Macrocyclic Diether Lipids Using Coordination Cages

Stability of a Nanobarrel in the Absence and Presence of a Guest

JOHN LEO GARMA BARING

CHALMERS
UNIVERSITY OF TECHNOLOGY

Department of Chemistry and Chemical Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2023

Synthesis of Macrocyclic Diether Lipids Using Coordination Cages
Stability of a Nanobarrel in the Absence and Presence of a Guest
JOHN LEO GARMA BARING

Supervisor: Angela Beth Grommet, Division of Chemistry and Biochemistry, Chalmers University of Technology

Examiner: Nina Kann, Division of Chemistry and Biochemistry, Chalmers University of Technology

Master's Thesis 2023
Department of Chemistry and Chemical Engineering
Chalmers University of Technology
SE-412 96 Gothenburg
Sweden
Telephone + 46 31 772 1000

Synthesis of Macrocyclic Diether Lipids Using Coordination Cages
Stability of a Nanobarrel in the Absence and Presence of a Guest
JOHN LEO GARMA BARING
Department of Chemistry and Chemical Engineering
Chalmers University of Technology

Abstract

Archaea that thrive in extreme conditions have cell membranes that are composed of diether and tetraether lipids. Those archaeal lipids have high chemical resistance, rigidity, and impermeability. Thus, they can be incorporated into drug delivery platforms such as lipid nanoparticles to improve their properties. In this thesis, a novel strategy using coordination cages for synthesizing archaea-inspired macrocyclic diether lipids was investigated. We propose that linear precursors would fold into a hairpin conformation when confined in a barrel-shaped coordination cage that would render macrocyclization more favourable than polymerization. **MB1**, a symmetrical nanobarrel that has open windows at the top and bottom, was successfully synthesized following the procedure by Howlader *et al*.[14] Also, dotriaconta-1,31-diene, a linear terminal diene, was successfully synthesized following the procedure by Drescher *et al*.[19] Behaviour of **MB1** in the presence of the diene was checked, but **MB1** was observed to break down during these studies. We therefore decided to study the effect of heating, stirring and presence of diene on the stability of **MB1**. We found that **MB1** is not stable when stirred or heated. Thus, it will no longer be used by the group. Coordination cages with better stability will be explored in future work for synthesizing macrocyclic diether lipids.

Keywords: archaea, coordination cage, host-guest, lipid nanoparticle, macrocyclization, synthesis

Acknowledgement

Una sa lahat, maraming salamat Panginoong Diyos sa lahat ng mga biyaya at pagpapala na ipinagkakaloob Niyo po sa akin araw-araw.

Maraming salamat Mama Lolita at Papa Leonardo sa inyong pagmamahal sa akin, at sa lahat ng inyong pagpupursigi at pagsasakripisyo para sa aking pag-aaral.

Thank you very much Swedish Institute for the Swedish Institute Scholarship for Global Professionals (SISGP) that enabled me to study Master of Science in Materials Chemistry at Chalmers University of Technology.

Thank you very much Angela for being a very good supervisor, and for all your support and advices.

Tack så mycket Nina för att du är en mycket bra examinator och för att du hjälper oss att utföra organiska reaktioner i laboratoriet.

Tack så mycket Lars för att du är mitt hem i Sverige, och för allt ditt känslomässiga stöd till mig.

Maraming salamat Sir Carlo, Sir Dennis, Kuya Nes Daniel, at Ate Theresa sa inyong suporta at tulong sa aking SISGP application.

Maraming salamat Gothenburgers sa pagiging pamilya ko dito sa Sweden, at sa lahat ng mga pagkaing ating pinagsasaluhan.

Maraming salamat PH SI Scholars 2021 sa inyong gabay at tulong na makarating ako rito sa Sweden.

Thank you very much friends at Floor 9 and in Grommet research group for all your support, and for all the fun fikas we shared.

Thank you very much to all my family and friends who have been helping and supporting me all my life. MARAMING SALAMAT SA INYONG LAHAT! ❤️ ❤️ ❤️

John Leo Garma Baring, Gothenburg, June 2023

Table of Contents

List of Abbreviations 7
1. Introduction 8
1.1 Aim 9
1.2 Specification of Issue Under Investigation 9
1.3 Limitations 9
2. Theory 10
2.1 Coordination Cages 10
2.2 Host-Guest Chemistry 11
3. Results and Discussion 12
3.1 Synthesis of Nanobarrel 12
3.1.1 Synthesis of tetrapyridyl ligand **L'** 12
3.1.2 Synthesis of nanobarrel **MB1** 14
3.2 Synthesis of Linear Precursor 15
3.2.1 Synthesis of diene **5** 15
3.2.2 Synthesis of diol **6** 16
3.3 Stability Tests of the Nanobarrel 17
4. Conclusion and Future Work 21
References 22
Appendix 24
A.1 General Experimental 24
A.2 Synthesis of Benzene-1,4-di(4-terpyridine) 24
A.3 Synthesis of Nanobarrel **MB1** 25
A.4 Synthesis of Dotriaconta-1,31-diene 25

List of Abbreviations

$B(OH)_3$	boric acid
BH_3	borane
CD_3CN	trideuteroacetonitrile
$CDCl_3$	deuterochloroform
$CHCl_3$	chloroform
D_2O	deuterium oxide (heavy water)
DLS	dynamic light scattering
DMSO	dimethyl sulfoxide
DMSO-d_6	hexadeuterodimethyl sulfoxide
DODAP	1,2-dioleoyl-3-dimethylammonium propane
DOTAP	1,2-dioleoyl-3-trimethylammonium propane
Et_2O	diethyl ether
EtOH	ethanol
^1H NMR	proton nuclear magnetic resonance
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
K_2CO_3	potassium carbonate
Li_2CuCl_4	dilithium tetrachlorocuprate(II)
LNP	lipid nanoparticle
mRNA	messenger RNA
Na_2SO_4	sodium sulphate
NaOH	sodium hydroxide
NH_3	ammonia
NMR	nuclear magnetic resonance
Pd(II)	palladium(II)
$Pd(NO_3)_2 \cdot 2H_2O$	palladium(II) nitrate dihydrate
ppm	parts per million
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	tetramethylethylenediamine

1. Introduction

Archaea are extremophilic microorganisms that thrive in extreme conditions such as very high temperature, very high salt concentration, and extreme acidic (pH 0) or extreme basic (pH 12.8) environments.[1] They have been found in hot springs, hydrothermal vents, volcanic steam vents, soda lakes, and salt lakes.[2] Cell membranes of archaea are fundamentally composed of diether and tetraether lipids [3]—lipids made up of isoprenoid in ether-linkage to glycerol with unique stereochemistry [4]. The ether-linkage of archaeal membrane lipids is more chemically resistant than the ester-linkage of bacterial membrane lipids, and allows tight compaction.[5] Diether and tetraether lipids can be present as macrocycles. The macrocyclic diether lipids form a bilayer, while the tetraether lipids form a monolayer. Compared to bacterial bilayer membranes, monolayer membranes formed by archaeal tetraether lipids are more rigid and impermeable.[6] Structures of saturated isoprenoid-based archaeal lipids is provided in Figure 1.

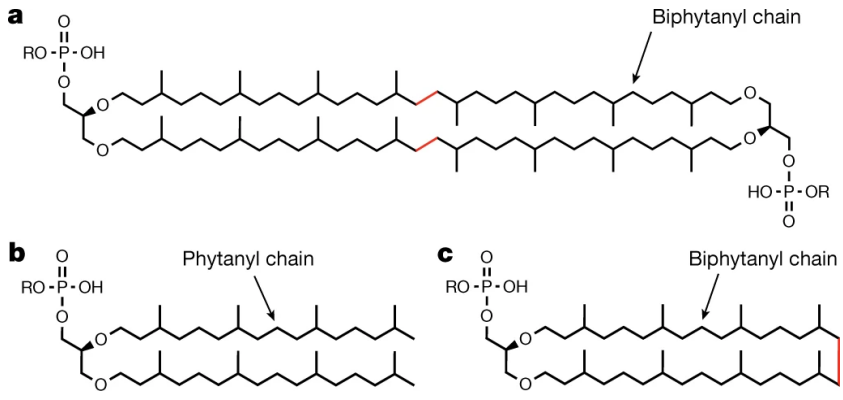


*Figure 1. Structures of a macrocyclic glycerol dibiphytanyl glycerol tetraether (GDGT) (**a**), diether archaeal lipid (**b**) and macrocyclic archaeol (**c**).*
From [7], used under Creative Commons CC-BY license.

The properties of archaeal lipids will be helpful in improving drug delivery platforms such as lipid nanoparticles (LNP's). For example, archaeal lipids can be incorporated into LNP mRNA therapeutics to increase their required storage and shipping temperature and to improve mRNA cargo stability.[8] However, harvesting them from archaea is arduous and unsustainable, and they are currently difficult to synthesize in the laboratory.[9] In the Grommet research group, we endeavour to synthesize hybrid lipids that are inspired by archaeal lipids. In this thesis, a novel supramolecular strategy that would involve folding a linear precursor under confinement was investigated for synthesis of macrocyclic diether lipids.[8]

1.1 Aim

Investigate a novel strategy using coordination cages for synthesizing macrocyclic diether lipids that can be incorporated into LNP mRNA therapeutics to increase their required storage and shipping temperature and to improve mRNA cargo stability.

1.2 Specification of Issue Under Investigation

Coordination cages could be used for synthesizing macrocyclic diether lipids inspired by archaeal lipids. Hydrophobic molecules such as linear precursors could be encapsulated within the cavity of a nanobarrel, pre-organizing them into conformations that would render macrocyclization more favourable than polymerization. In this thesis, the following objectives were pursued:

1. Synthesize a linear precursor and a nanobarrel.

2. Determine the stability of the nanobarrel in the absence and presence of the linear precursor.

1.3 Limitations

Synthesis of novel LNP's, their permeability, and stability of cargo under storage conditions were not examined.

2. Theory

2.1 Coordination Cages

Coordination cages are three-dimensional supramolecular structures resulting from coordination-driven self-assembly of metal ions and organic ligands in solution. The metal-ligand coordination bond is weaker than the organic covalent bond but is stronger than dispersive interactions.[10] Generally, it is reversible, and enables error-checking and self-repair that ensures formation of thermodynamically most stable structures.[11] However, in cases when the ligand is insoluble in solvent (e.g., water), the coordination bond is no longer reversible; there is no self-repair in the system and the cage simply breaks down. Stoichiometric amounts of metal ions with well-defined coordination geometries, and symmetrical rigid organic ligands can be combined to create highly symmetrical coordination cages of varied geometries.[10],[11]

Palladium(II) (Pd(II)) is a metal cation with square planar coordination geometry.[12] Following the molecular panelling approach to coordination cage self-assembly developed by Fujita *et al* [13], Pd(II) coordinates with two organic ligands at 90°-angle to each other when two of the adjacent coordination sites of Pd(II) are blocked with a *cis*-coordinating ligand (e.g., tetramethylethylenediamine (TMEDA)) [11]. In this thesis, a barrel-shaped coordination cage (i.e., a nanobarrel) developed by Howlader *et al* was used.[14] Tetrapyridyl ligand **L'** and cis-(TMEDA)Pd(NO_3)$_2$ **M** self-assemble in 1:2 molar ratio into nanobarrel with four closed sides and two open parallel windows that may facilitate encapsulation or release of guest molecules. However, Howlader *et al* determined that the nanobarrel exists in two isomers in dimethyl sulfoxide (DMSO)—**MB1** is highly symmetrical and has two large open windows, and **MB3** is asymmetrical and has four small open windows. Nevertheless, they reported that heating the mixture of **MB1** and **MB3** in water will result in conversion of **MB3** to **MB1** (see Figure 2).

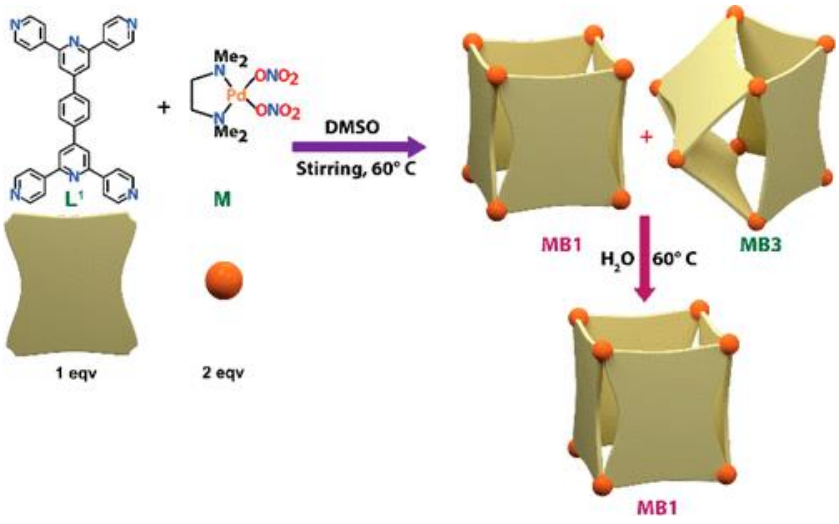


*Figure 2. Self-assembly of the molecular nanobarrel **MB1** from tetrapyridyl ligand **L¹** and **M** in 1:2 molar ratio. Reprinted with permission from [14]. Copyright 2018 American Chemical Society.*

2.2 Host-Guest Chemistry

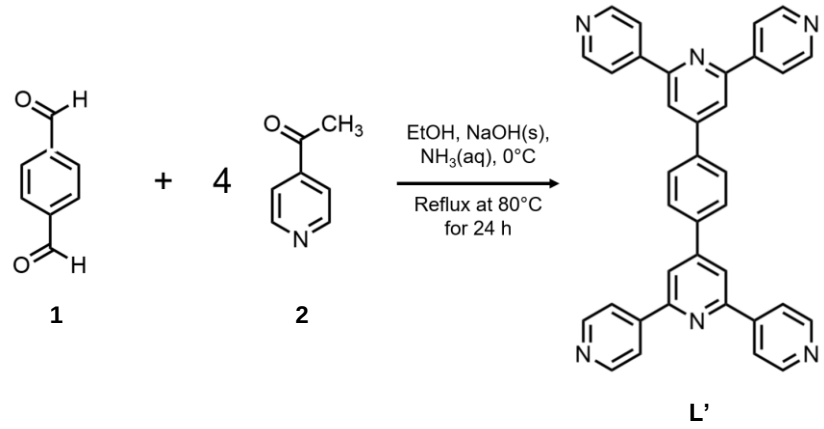
Coordination cages have well-defined three-dimensional nanocavities and can selectively bind guest molecules.[11] They can be used in selective molecular transport [15] and catalytic chemical transformations [16]. Since most organic ligands that are used to create coordination cages have extended rigid aromatic regions, the enclosed nanocavities are hydrophobic. Also, inside the nanocavities of "empty" coordination cages in aqueous solution are "high-energy" water molecules that are ordered and that cannot form the optimal number of hydrogen bonds. Thus, releasing the "high-energy" water molecules to the bulk solution is both entropically and enthalpically favourable, an effect which drives binding of hydrophobic guest molecules.[10]

In this thesis, the guest molecule is a linear precursor which has a hydrophobic chain and polar ends. It will be reacted with a headgroup via Williamson ether synthesis to synthesize a macrocyclic diether lipid. However, when left to react just by themselves in solution, the headgroup may collide and react with two different molecules of the precursor resulting to polymerization instead of macrocyclization. That is especially true when the two ends of the precursor are far apart. And, the precursor in solution will be in a stretched-out conformation as driven by entropy. Thus, there is an energy barrier that needs to be overcome for the precursor to be folded into a hairpin conformation. To overcome that, we propose to confine the precursor in **MB1**—the nanobarrel that is closed on four sides by **L'** and has wide open windows at the top and bottom, allowing rapid guest exchange kinetics (i.e., the precursor can bind inside **MB1** and the product can be readily released after reaction). Upon addition of the precursor to an aqueous solution of **MB1**, we anticipate that the hydrophobic chain will get into the hydrophobic nanocavity as driven by the hydrophobic effect. Whereas, the polar ends will avoid the hydrophobic nanocavity and will protrude by the open windows.[8] The internal diameter of **MB1** should not be too wide to ensure that the polar ends will be in proximity in the same window and to avoid complex coiling or knotting within the nanocavity. It should not be too narrow either and should allow the desired hairpin fold to form.

3. Results and Discussion

3.1 Synthesis of Nanobarrel

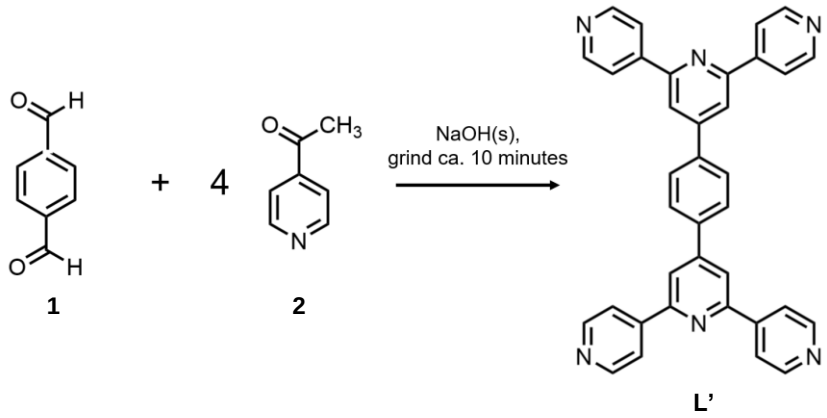
3.1.1 Synthesis of tetrapyridyl ligand **L'**



*Scheme 1. Synthesis of tetrapyridyl ligand **L'** [benzene-1,4-di(4-terpyridine)] in ethanol.[14]*

Tetrapyridyl ligand **L'** [benzene-1,4,-di(4-terpyridine)] was synthesized from two commercially available starting reactants terephthalaldehyde (**1**) and 4-acetylpyridine (**2**) following the procedure from literature (see Scheme 1).[14] The reaction was carried out in ethanol. Vacuum filtration of the precipitate using a Hirsch funnel took about two hours because the amount of precipitate obtained was much more than anticipated. The subsequent washing of the precipitate with ethanol and water became difficult and inefficient due to lumping of the product slurry at the bottom of the Hirsch funnel.

The product was checked using thin layer chromatography (TLC). Aliquots of reactants were dissolved in ethanol, while an aliquot of the product was dissolved in chloroform; the eluent solvent used was ethanol. The product was free of reactants but contained ethanol-soluble side-products. Thus, further washing of the product with ethanol was conducted. The product was further washed by dissolving and stirring in ethanol (about 150 mL) for one to two hours. The suspension formed did not settle and was difficult to filter (i.e., the fine powder passed through the filter paper). As an alternative, the powder was separated from the washing solvent by centrifugation followed by decantation. Subsequent washings of the solid product were done by mixing in ethanol/water, followed by centrifugation then decantation. The ^{1}H NMR peaks of the product obtained corresponded to those reported in the literature, but there were extra peaks corresponding to impurities. The product was not very soluble in $CDCl_3$, and phase separation of the NMR sample was observed upon standing for some time.



1 **2** **L'**

*Scheme 2. Synthesis of tetrapyridyl ligand **L'** [benzene-1,4-di(4-terpyridine)] by grinding.[17]*

Synthesis of **L'** via a sequential solventless aldol condensation and Michael addition[17] (see Scheme 2) was tried because **L'** obtained using the first method was difficult to purify. Compounds **1** and **2** and NaOH were combined using mortar and pestle. Early on, the mixture got stuck on the surface of the mortar as a hard yellow-orange paste, which made further mixing difficult. No powder product was being formed after ten minutes of grinding. Few drops of ethanol were added to get the paste off the mortar surface and to facilitate mixing. Once the ethanol evaporated, a pinkish white substance began to form. More ethanol was added to get more of the paste stuck on the mortar. The mixture turned red after grinding for about 30 minutes. A red sticky solid formed as the ethanol evaporated. It redissolved in ethanol making a red solution. When water was added, pink powder precipitated which was then filtered. An attempt was made to wash the product with ethanol, but it redissolved in ethanol, which should have not been the case. The ^{1}H NMR peaks of the product obtained did not correspond to those reported in the literature. Grinding may had been too prolonged than necessary (more than 30 minutes versus 10 minutes in literature procedure).

L' was synthesized again following the solution-based literature procedure.[14] An oil bath was used to heat the reaction to reflux temperature instead of metal heating mantle that was used in the first attempt. Also, a Büchner funnel was used during vacuum filtration instead of a Hirsch funnel. Batches of ethanol and water were poured onto the precipitate before it started to dry up and lump together. The precipitate was washed solely with ethanol until filtrate became colorless; then, the precipitate was washed with water until filtrate became colourless. However, the ^{1}H NMR spectrum of the product still showed impurities which was about the same as the first time. Column chromatography could not be used for purification because the impurities had fairly the same hydrophobicity as the product due to the aromatic rings. Soxhlet extraction or recrystallization of the product may be employed in the future to improve product purity. Also, the reaction may be carried out using microwave radiation to shorten the required reaction time since the literature procedure requires refluxing for 24 hours. We decided to proceed with the impure ligand because the impurities were anticipated to be not as soluble as **L'** in DMSO, where synthesis of **MB1** would be carried out.

3.1.2 Synthesis of nanobarrel **MB1**

Nanobarrel **MB1** was synthesized following the procedure by Howlader *et al* (see Figure 2).[14] However, palladium(II) nitrate dihydrate ($Pd(NO_3)_2$•$2H_2O$) and TMEDA were mixed in DMSO, prior adding **L'**, instead of using *cis*-(TMEDA)$Pd(NO_3)_2$ as reported in literature to save several steps of synthesis. Also, one-pot synthesis of complexes from Pd(II), TMEDA and pyridine ligands has been reported by Bandi *et al*.[18] **MB1** was synthesized in two occasions. In the first occasion, $Pd(NO_3)_2$•$2H_2O$ was added first to DMSO making a brown solution. The solution turned yellow upon addition of TMEDA. It turned dark green after adding **L'** and being heated at 60°C for 10 minutes. Then, it turned brown after 20 minutes more had passed. The product obtained was not very soluble in D_2O. It appeared to dissolve in D_2O with aid of mechanical stirring. The ^1H NMR peaks of the product obtained were very small compared to the solvent peak, but they corresponded to those reported in the literature. Thus, adding $Pd(NO_3)_2$•$2H_2O$ and TMEDA to **L'** in one pot was successful.

In the second occasion, amounts of reactants used were twice of the literature scale, including DMSO. This time, TMEDA was added first to DMSO making a yellow solution, and the solution remained yellow after addition of $Pd(NO_3)_2$•$2H_2O$ and **L'**, and heating at 60°C for six hours. When ethyl acetate was added for the work-up, the product that precipitated dissolved in DMSO making filtration of the product difficult. DMSO and the ethyl acetate that was added were evaporated in a rotary evaporator (80°C, 8 mbar) leaving a yellow grease. When ethyl acetate was added again after, an insoluble precipitate was obtained which was then filtered and washed. The double amount of DMSO may have been too much relative to the product formed resulting to formation of a solution instead of a suspension. In future occasions, the amount of DMSO may be kept the same as in the literature, when all other reactants are scaled up.

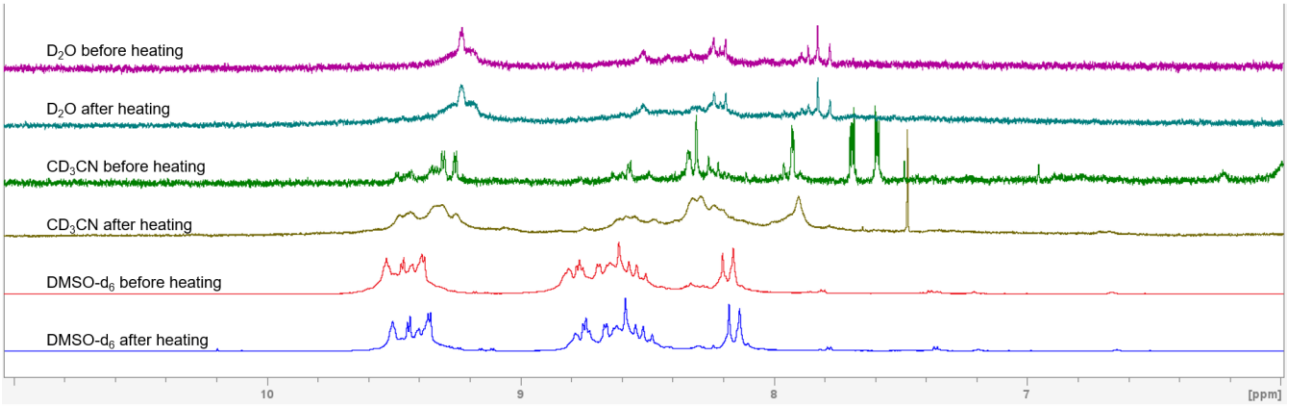
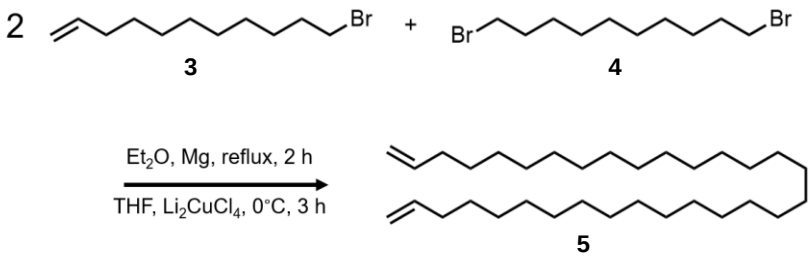


Figure 3. ^1H NMR (600 MHz) spectra of the nanobarrel before and after heating in solvents of different polarities at 60°C for ten minutes.

In order to check what isomer of the nanobarrel was predominant, ^{1}H NMR spectra were obtained before and after heating the nanobarrel in solvents of different polarities at 60°C for ten minutes. Contrary to that reported in the literature, nanobarrel in D_2O that was not heated showed ^{1}H NMR spectrum that was already similar to that reported for **MB1**. In addition, the nanobarrel in less polar solvents such CD_3CN and DMSO-d_6 showed ^{1}H NMR spectra that were similar to that reported for a mixture of **MB1** and **MB3**. Thus, solvent polarity, and not heating, affects what isomer or isomers the nanobarrel adapts.

3.2 Synthesis of Linear Precursor

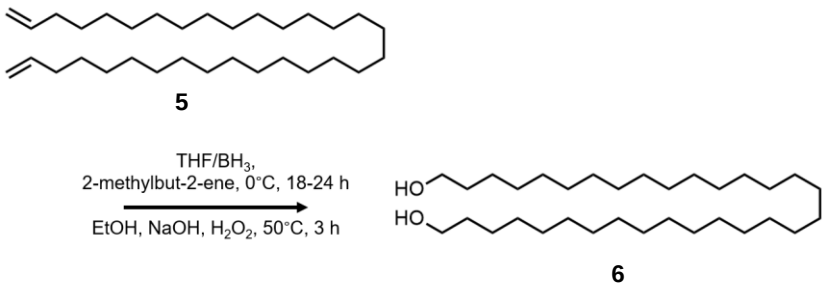
3.2.1 Synthesis of diene **5**



Scheme 3. Synthesis of dotriaconta-1,31-diene.[19]

Dotriaconta-1,31-diene (**5**), a 32-carbon linear terminal diene, was synthesized from two commercially available starting reactants 11-bromoundec-1-ene (**3**) and 1,10-dibromodecane (**4**) by Grignard bis-coupling following the procedure by Drescher *et al*.[19] Compound **3** was reacted with magnesium turnings to make a Grignard reagent, which was then cross-coupled with compound **4** in the presence of dilithium tetrachlorocuprate(II) (Li_2CuCl_4).[20] The reactions were carried out under nitrogen instead of argon atmosphere. The synthesis was done three times. In the first attempt, amounts of reactants used were one-sixth of the literature scale (i.e., 1 g compound **4**). The ^{1}H NMR peaks of the product obtained corresponded to those reported in the literature. Product yield was 13 wt%, and product purity estimated from the ^{1}H NMR spectrum was 96 wt%. In the second attempt, amounts of reactants used were increased to one-half of the literature scale (i.e., 3 g compound **4** basis). The Grignard bis-coupling reaction was carried out for two hours only that time, instead of three hours, resulting to low product yield of 3 wt%. However, product purity increased to 99 wt% following use of a silica gel column taller than previous. The scale for the third attempt was the same as the second attempt. The Grignard bis-coupling reaction was carried out for full three hours, resulting to relatively high product yield of 12 wt%; product purity was 98 wt%.

3.2.2 Synthesis of diol **6**



Scheme 4. Synthesis of dotriacontane-1,32-diol.[19]

Diene **5** was to be converted into dotriacontane-1,32-diol (**6**) via a hydroboration-oxidation reaction. Three attempts were made to synthesize diol **6**. In the first attempt, amounts of reactants used were 1% of the literature scale (i.e., 50 mg diene **5** basis). Hydroboration reaction was carried out under argon atmosphere until the diene **5**/tetrahydrofuran (THF) solution was added; argon was then switched to nitrogen for the overnight hydroboration. During recrystallization of the product using heptane, and methanol, powder instead of crystals precipitated even when the solution was brought to boiling then cooled to room temperature very slowly. In addition, the product obtained was very little making recovery from solvent difficult. The product was separated from solvent by centrifuging aliquots in an Eppendorf tube, followed by decantation and evaporation of solvent. The ^{1}H NMR peaks of the product obtained did not correspond to those reported in the literature; most of the peaks corresponded to solvents used.

In the second attempt to synthesize diol **6**, amounts of reactants used were increased to 4% of the literature (i.e., 200 mg diene **5** basis) to obtain more product than in the first attempt. Also, hydroboration reaction was carried under nitrogen atmosphere throughout. Completion of the hydroboration was checked with TLC, but the result was inconclusive. THF used to make the diene **5** solution was not dried with molecular sieves; borane (BH_3)may have reacted with water in THF, instead of diene **5**, to form boric acid ($B(OH)_3$). Nevertheless, oxidation reaction was proceeded since hydroboration had been going on for 24 hours already. After oxidation, white insoluble precipitate formed and accumulated at the interface of the organic and aqueous phases. During work-up, the combined organic phases were not dried with sodium sulphate (Na_2SO_4) due to foreseen difficulty in separating the insoluble precipitate with Na_2SO_4. White powder was obtained by recrystallization using methanol. The ^{1}H NMR peaks of the product obtained still did not correspond to those reported in the literature.

In the third attempt to synthesize diol **6**, amounts of reactants used were 2% of the literature (i.e., 100 mg diene **5** basis). THF used to make the diene **5** solution was dried with molecular sieves. Also, complete hydroboration of diene **5** was confirmed by a conclusive TLC test. After oxidation, white insoluble precipitate formed and accumulated at the interface of the organic and aqueous phases again. The pH of the reaction mixture was verified to be 14 (i.e., basic). Thus, the white precipitate was thought to be deprotonated diol **6** with its charged oxygen ends attracted to water molecules in the aqueous phase, and its hydrophobic hydrocarbon chain sticking out to the organic

phase. Addition of potassium carbonate (K_2CO_3) did not change the pH of the reaction mixture since it was a basic salt. Hydrochloric acid (HCl) was added to an aliquot of the reaction mixture, but the precipitate did not dissolve at pH 7, nor at pH 1. The precipitate still did not dissolve when 100 mL diethyl ether was added to 10 mL aliquot of the reaction mixture. Thus, albeit using different starting materials and reactions for synthesis of diol **6**, product work-up protocol by McKiernan *et al* was tried.[21] HCl was added to the reaction mixture up to pH 2, then the white insoluble precipitate was filtered. Fine white crystals were finally obtained by recrystallization using methanol/chloroform ($CHCl_3$) (1/1 vol/vol) as observed using an optical microscope. However, the ^{1}H NMR peaks of the product obtained still did not correspond to those reported in the literature. The old stock of hydrogen peroxide (H_2O_2) used in the oxidation step may no longer have been effective. In the future, fresh stock of H_2O_2 should be used.

3.3 Stability Tests of the Nanobarrel

While working on the synthesis of the linear precursor, we were interested to see guest binding in **MB1** and to try to determine how the guest would fold in **MB1**. Since diene **5** had already been synthesized, it was used as the guest molecule. However, we failed to observe conclusive evidence of guest binding, and we also noticed that **MB1** was breaking down. That led us to investigate the stability of **MB1**. We decided to study the effect of heating, stirring and presence of diene **5** to the stability of **MB1**. A stock solution was made by mixing 50 mg **MB1** with 10 mL D_2O (5 mg **MB1**/mL D_2O, or 1.3 millimolar **MB1** in D_2O), from which 5 mL aliquots were used for each sample in Figure 4. For samples that were heated, the heating temperature was set at 65°C because it was the intended temperature for the Williamson ether synthesis for the macrocyclization of the linear precursor. For samples that were stirred, a magnetic stir bar was added and stirring was set at 200 rpm. For samples that had diene **5**, 10 mg diene **5** was added (3 mol diene **5**/mol **MB1**). Samples were run in parallel for 22 hours, then their ^{1}H NMR spectra were taken. Figure 5 shows that there are observable visual differences between samples subjected to different conditions. Samples that were not stirred had solid **MB1** settled at the bottom of the NMR tubes indicating difficulty in dissolution.

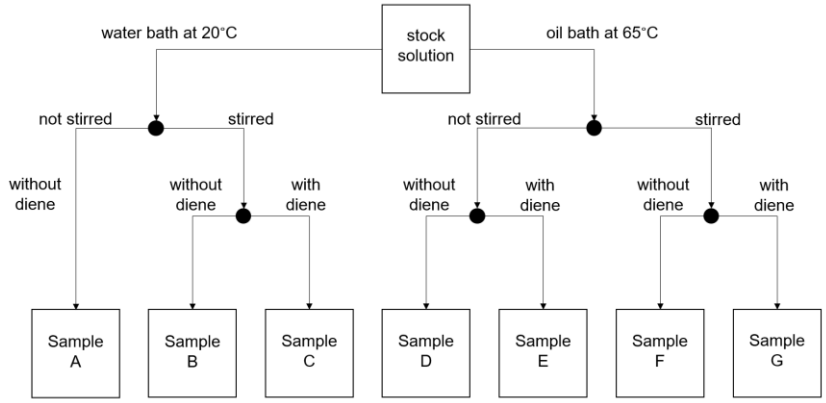


Figure 4. Experiment tree for stability tests of the nanobarrel.

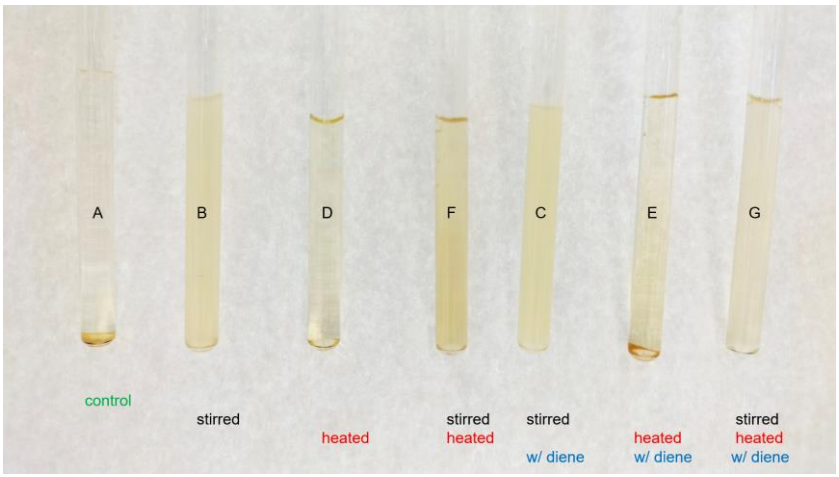


Figure 5. Visual differences between nanobarrel samples subjected to different conditions.

The nanobarrel dissolved in D_2O better with stirring than without. However, prolonged stirring broke down the nanobarrel whether diene **5** was present or not. Also, prolonged heating broke down the nanobarrel whether diene **5** was present or not. Finally, prolonged stirring with heating broke down the nanobarrel. We could not see diene **5** binding into the nanobarrel, but its presence had some impact.

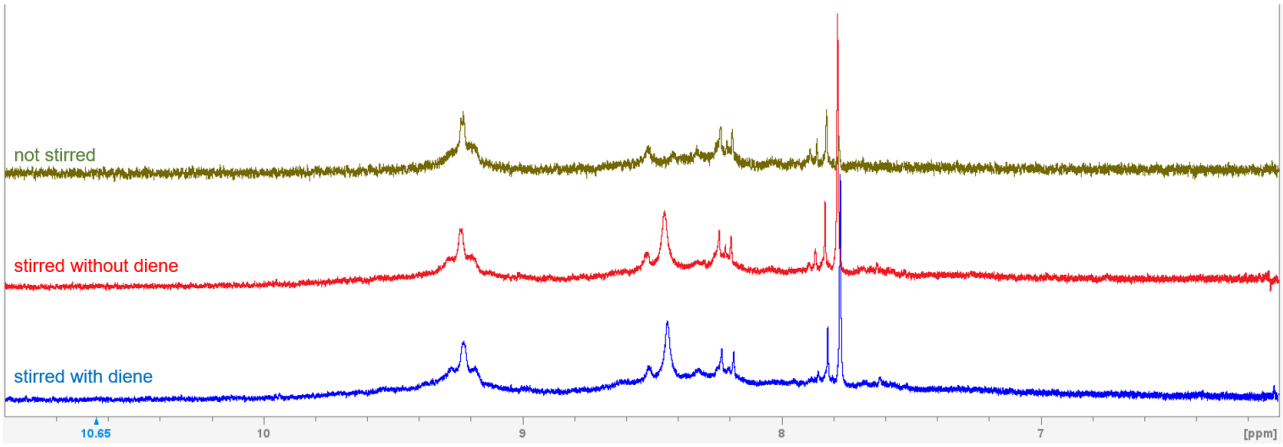


Figure 6. ^1H NMR (600 MHz, D_2O) of nanobarrel in water bath at 20°C for 22 hours that was not stirred, stirred without diene, and stirred with diene.

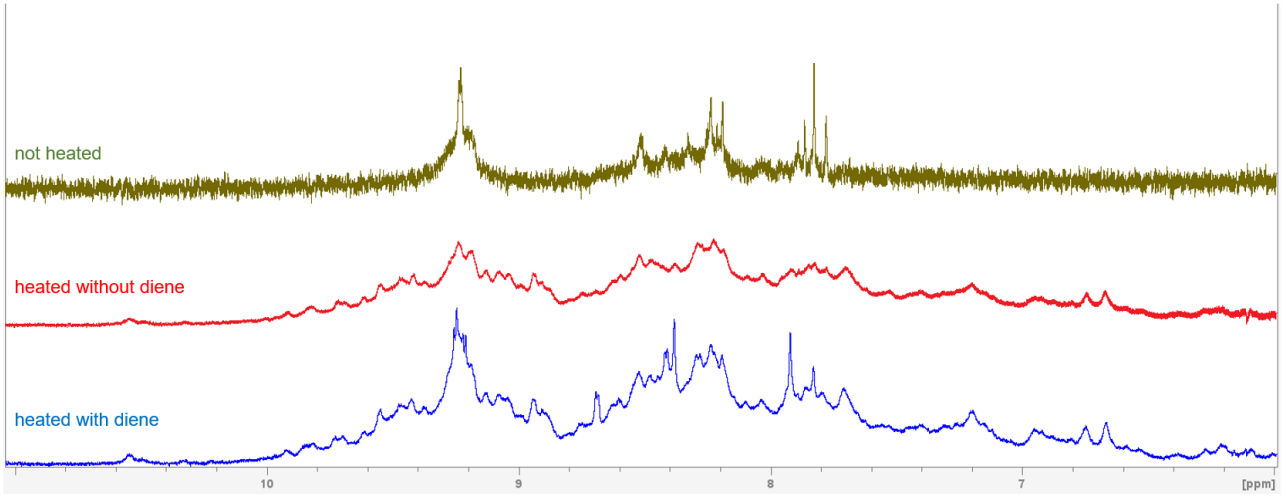


Figure 7. ^{1}H NMR (600 MHz, D_2O) of nanobarrel in water bath at 20°C for 22 hours and nanobarrel in oil bath at 65°C for 22 hours without diene, and with diene.

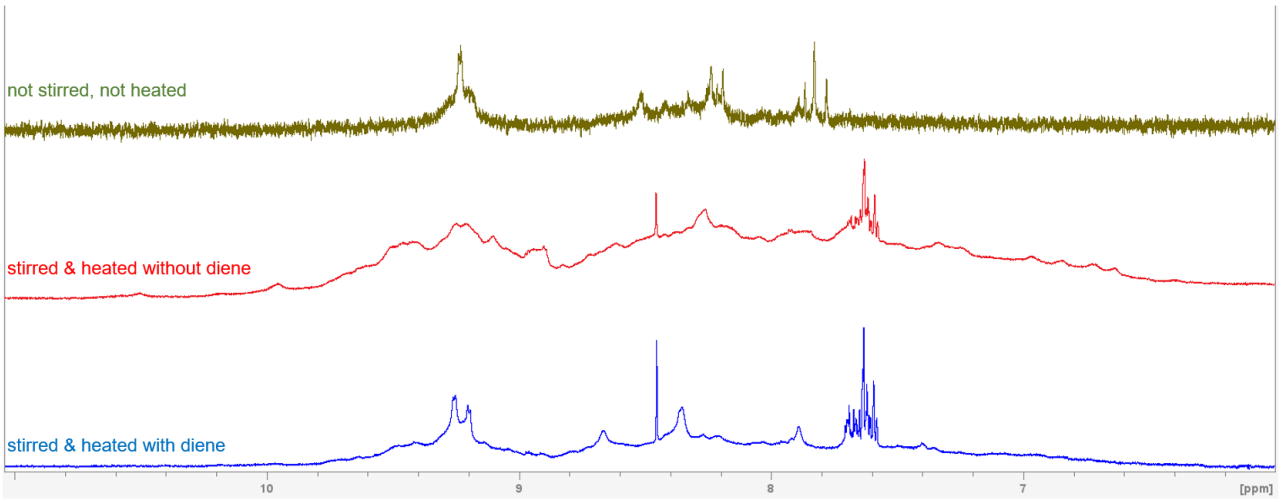


Figure 8. ^{1}H NMR (600 MHz, D_2O) of nanobarrel in water bath at 20°C for 22 hours, and nanobarrel in oil bath at 65°C for 22 hours that was stirred without diene, and stirred with diene.

The nanobarrel was not expected to break down due to stirring because it was expected to exist as discrete particles in aqueous solution. Also, despite the insolubility of the **L'** subcomponent of the cationic nanobarrel in water, the counterions (i.e., NO_3^-) were expected to increase the solubility and stability of the nanobarrel complex in solution.[22-24] The particle size of the nanobarrel in aqueous solution was measured employing dynamic light scattering (DLS). Particle size ranged between 150 nm and 420 nm versus the nanobarrel diagonal which was just 2 nm (see Figure 9). Clearly, the nanobarrel existed as aggregates in aqueous solution. The effect of the NO_3^- counterions on the solubility of the nanobarrel complex was not sufficient to counteract the effect of the big hydrophobic aromatic panels **L'** of the nanobarrel. Instead, the nanobarrel aggregated such that less of the hydrophobic panels **L'** was in contact with water molecules resulting to stability of the complex. However, the nanobarrel aggregates collapsed upon stirring, and more of the hydrophobic panels **L'** got into contact with water molecules disrupting the stability of the complex resulting to breaking down of the nanobarrel. And in this case, the nanobarrel could not self-repair after breaking down.

In addition, the poor dissolution of the nanobarrel resulted to low concentration in the solution and small NMR peaks, and the observed sediments at the bottom of the NMR tubes (see Figure 5).

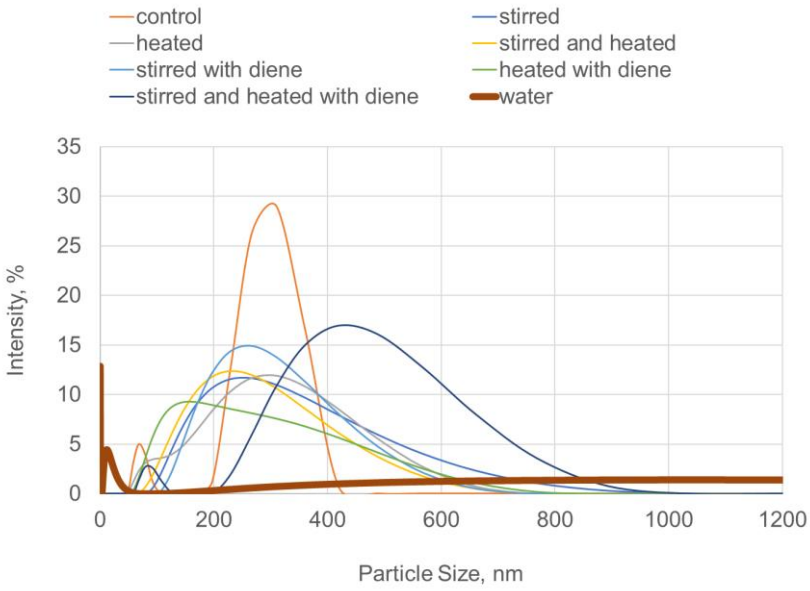


Figure 9. Particle size distribution of nanobarrel in samples subjected to different conditions.

4. Conclusion and Future Work

MB1 was successfully synthesized from $Pd(NO_3)_2 \cdot 2H_2O$, TMEDA, and tetrapyridyl ligand **L'** in one pot, instead of in several steps as reported in the literature[14]. **L'**, successfully obtained via solution-based synthesis[14] albeit impure, was effective in making nanobarrel **MB1**. Contrary to the literature[14], solvent polarity, and not heating, affected what isomer or isomers the nanobarrel adapted in solution. The nanobarrel aggregated in aqueous solution and dissolved poorly even with mechanical stirring. The nanobarrel was not stable when stirred or heated. The instability of the nanobarrel upon stirring was attributed to nanobarrel aggregates collapsing and the hydrophobic panels **L'** getting into contact with water molecules resulting to insolubility that was not sufficiently counteracted by the NO_3^- counterions. The nanobarrel will no longer be used by the group.

Dotriaconta-1,31-diene (diene **5**), a linear terminal diene, was successfully synthesized following the procedure by Drescher *et al*.[19] Fresh stock of H_2O_2 should be used in future synthesis dotriacontane-1,32-diol (diol **6**).

Synthesis of macrocyclic diether lipids was temporarily paused so that we could investigate the stability of the nanobarrel, but we will return to this when the linear precursor and a suitable coordination cage have been successfully synthesized.

References

1. Rampelotto, P. H. (2013). Extremophiles and extreme environments. *Life*, *3*(3), 482-485.

2. Chaban, B., Ng, S. Y., & Jarrell, K. F. (2006). Archaeal habitats—from the extreme to the ordinary. *Canadian Journal of Microbiology*, *52*(2), 73-116.

3. Boyd, E. S., Hamilton, T. L., Wang, J., He, L., & Zhang, C. L. (2013). The role of tetraether lipid composition in the adaptation of thermophilic archaea to acidity. *Frontiers in Microbiology*, *4*, 62.

4. Eme, L., & Doolittle, W. F. (2015). Archaea. *Current Biology*, *25*(19), R851-R855.

5. Baba, T., Toshima, Y., Minamikawa, H., Hato, M., Suzuki, K., & Kamo, N. (1999). Formation and characterization of planar lipid bilayer membranes from synthetic phytanyl-chained glycolipids. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1421*(1), 91-102.

6. Chong, P. L. G. (2010). Archaebacterial bipolar tetraether lipids: Physico-chemical and membrane properties. *Chemistry and Physics of Lipids*, *163*(3), 253-265.

7. Lloyd, C. T., Iwig, D. F., Wang, B., Cossu, M., Metcalf, W. W., Boal, A. K., & Booker, S. J. (2022). Discovery, structure and mechanism of a tetraether lipid synthase. *Nature*, *609*(7925), 197-203.

8. Grommet, A.B. (2021). Research proposal.

9. Eguchi, T., Ibaragi, K., & Kakinuma, K. (1998). Total synthesis of archaeal 72-membered macrocyclic tetraether lipids. *The Journal of Organic Chemistry*, *63*(8), 2689-2698.

10. Percástegui, E. G., Ronson, T. K., & Nitschke, J. R. (2020). Design and applications of water-soluble coordination cages. *Chemical Reviews*, *120*(24), 13480-13544.

11. McConnell, A. J. (2022). Metallosupramolecular cages: From design principles and characterisation techniques to applications. *Chemical Society Reviews*, *51*(8), 2957-2971.

12. Fujita, M., Tominaga, M., Hori, A., & Therrien, B. (2005). Coordination assemblies from a Pd (II)-cornered square complex. *Accounts of Chemical Research*, *38*(4), 369-378.

13. Fujita, M., Umemoto, K., Yoshizawa, M., Fujita, N., Kusukawa, T., & Biradha, K. (2001). Molecular paneling via coordination. *Chemical Communications*, (6), 509-518.

14. Howlader, P., Mondal, B., Purba, P. C., Zangrando, E., & Mukherjee, P. S. (2018). Self-assembled Pd (II) barrels as containers for transient merocyanine form and reverse thermochromism of spiropyran. *Journal of the American Chemical Society*, *140*(25), 7952-7960.

15. Nguyen, B. N. T., Thoburn, J. D., Grommet, A. B., Howe, D. J., Ronson, T. K., Ryan, H. P., Bolliger, J. L., & Nitschke, J. R. (2021). Coordination cages selectively transport molecular cargoes across liquid membranes. *Journal of the American Chemical Society*, *143*(31), 12175-12180.

16. Xue, Y., Hang, X., Ding, J., Li, B., Zhu, R., Pang, H., & Xu, Q. (2021). Catalysis within coordination cages. *Coordination Chemistry Reviews*, *430*, 213656.

17. Cave, G. W., & Raston, C. L. (2001). Efficient synthesis of pyridines via a sequential solventless aldol condensation and Michael addition. *Journal of the Chemical Society, Perkin Transactions 1*, (24), 3258-3264.

18. Bandi, S., Debata, N. B., Ramkumar, V., & Chand, D. K. (2014). One-pot synthesis of self-assembled heteroleptic palladium (II) complexes with tmeda: An application of ligand exchange reactions. *Inorganic Chemistry Communications*, *39*, 75-78.

19. Drescher, S., Meister, A., Blume, A., Karlsson, G., Almgren, M., & Dobner, B. (2007). General synthesis and aggregation behaviour of a series of single-chain 1, ω-bis (phosphocholines). *Chemistry–A European Journal*, *13*(18), 5300-5307.

20. Ouali, A., & Taillefer, M. (2016). Grignard reagents and Copper. *Physical Sciences Reviews*, *1*(8), 20160037.

21. McKiernan, R. L., Gido, S. P., & Penelle, J. (2002). Synthesis and characterization of polyethylene-like polyurethanes derived from long-chain, aliphatic α, ω-diols. *Polymer*, *43*(10), 3007-3017.

22. Grommet, A. B., Hoffman, J. B., Percástegui, E. G., Mosquera, J., Howe, D. J., Bolliger, J. L., & Nitschke, J. R. (2018). Anion exchange drives reversible phase transfer of coordination cages and their cargoes. *Journal of the American Chemical Society*, *140*(44), 14770-14776.

23. Wang, W., Wang, Y. X., & Yang, H. B. (2016). Supramolecular transformations within discrete coordination-driven supramolecular architectures. *Chemical Society Reviews*, *45*(9), 2656-2693.

24. Sommer, F., Marcus, Y., & Kubik, S. (2017). Effects of solvent properties on the anion binding of neutral water-soluble bis (cyclopeptides) in water and aqueous solvent mixtures. *ACS Omega*, *2*(7), 3669-3680.

Appendix

A.1 General Experimental

Reagents and solvents were purchased from Sigma-Aldrich, Fisher Scientific, and VWR, and were used as supplied.

All ^{1}H NMR spectra were recorded at the Swedish NMR Center, University of Gothenburg using a 600 MHz Bruker Avance NEO spectrometer. Chemical shifts are expressed in parts per million (ppm), relative to solvent peaks used as internal standards: $CDCl_3$ at 7.26 ppm, and D_2O at 4.79 ppm. All spectra were measured at 298 K.

Particle size of the nanobarrel was measured by DLS using Zetasizer Ultra ZSU3305.

A.2 Synthesis of Benzene-1,4-di(4-terpyridine)

Benzene-1,4-di(4-terpyridine) was synthesized following the literature procedure.[14] In a 500-mL round bottom flask that was dried in an oven overnight, 50 mL ethanol and a magnetic stir bar were added. The solution was cooled to 0°C. Stirring was started, then terephthalaldehyde (670 mg, 5 mmol) and NaOH (400 mg, 10 mmol) were added. 4-acetylpyridine (2.2 mL, 20 mmol) in 10 mL ethanol was added dropwise to the cold solution. 60 mL NH_3 solution (30 wt%) was added, then the solution was stirred at 0°C for one hour. Next, the solution was heated to 85°C, then refluxed for 24 hours. After, the mixture was cooled to room temperature, then the precipitate was vacuum filtered using a Büchner funnel, washed with ethanol until filtrate was colorless, then washed with water until filtrate was colorless. The pinkish grey solid product was dried in a desiccator overnight. Yield 1.1 g (42 wt%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.83 (d, 8H), 8.13 (m, 12H), 7.96 (s, 4H).

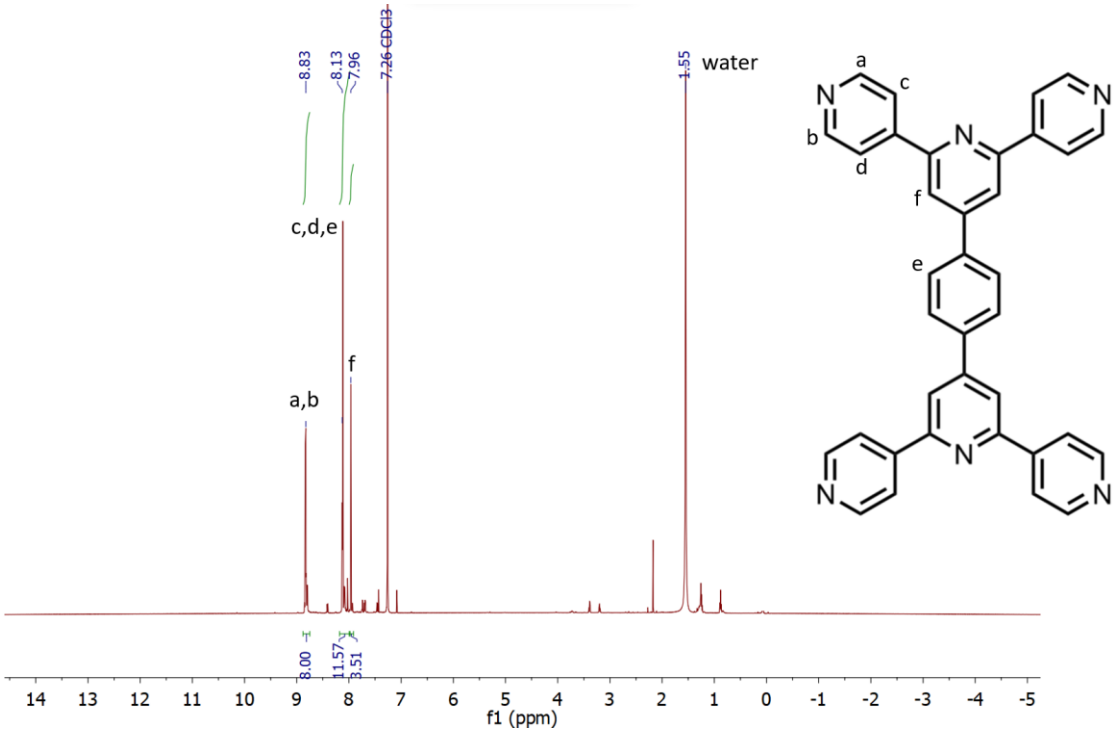


Figure A.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of benzene-1,4-di(4-terpyridine).

A.3 Synthesis of Nanobarrel **MB1**

The nanobarrel **MB1** was synthesized by modifying the procedure by Howlader *et al*.[14] Instead of having *cis*-(TMEDA)Pd(NO_3)$_2$ dissolved in DMSO per literature: in a 50-mL round bottom flask with a magnetic stir bar, TMEDA (30 µL, 0.2 mmol) was added to 3 mL DMSO; then, Pd(NO_3)$_2$•2H_2O (53.3 mg, 0.2 mmol) was added and the mixture was stirred for 30 minutes to make a clear yellow solution. Next, the ligand (54 mg, 0.1 mmol) was added. The solution was heated to 60°C and stirred for six hours. After, 20 mL ethyl acetate was added to the clear brownish solution. The peach precipitate was vacuum filtered and washed with acetone, then with ether. Finally, the brown solid product was dried under vacuum overnight. Yield 50 mg (51 wt%). ^{1}H NMR (600 MHz, D_2O): δ = 9.24 (broad s, 8H), 8.53 (broad s, 4H), 8.24 (m, 8H), 7.83 (s, 4H).

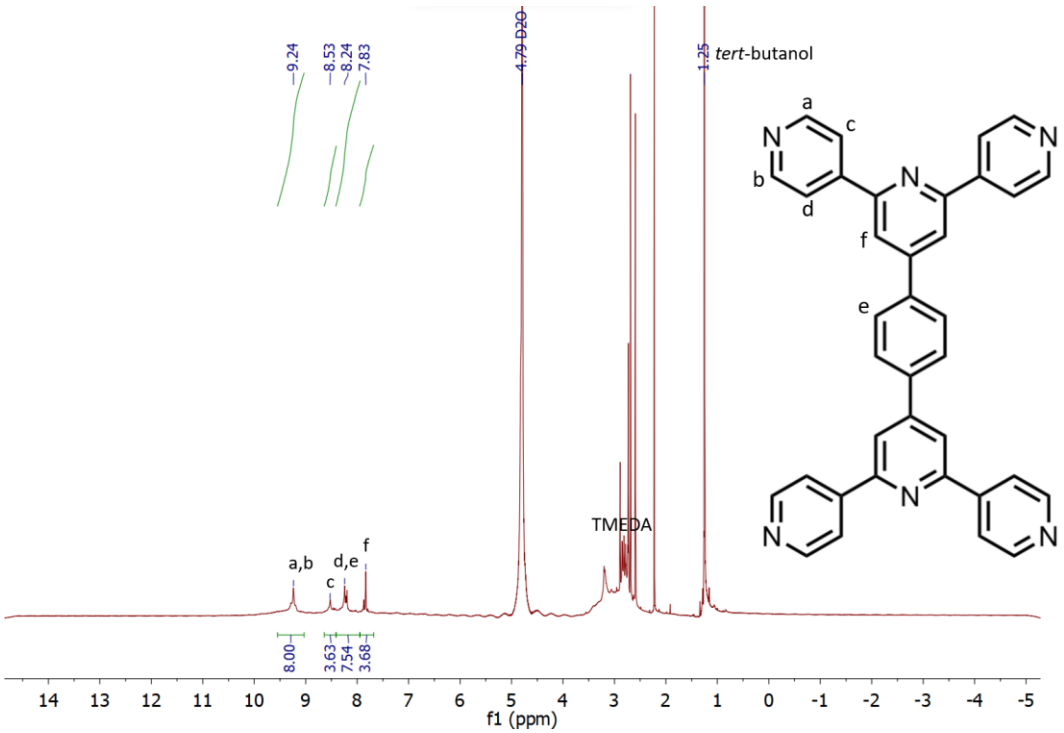


*Figure A.2 ^{1}H NMR (600 MHz, D_2O) spectrum of nanobarrel **MB1**.*

A.4 Synthesis of Dotriaconta-1,31-diene

Dotriaconta-1,31-diene was synthesized following one-half of the scale in the procedure by Drescher *et al*.[19] Glassware used during reaction were dried in an oven overnight. The organic solvents (i.e., diethyl ether, THF, and heptane) were dried using molecular sieves; acetone from newly opened container was used in lieu of dry acetone. Magnesium turnings (1.05 g, 42.5 mmol) were added to a 50-mL round bottom flask, and dried in an oven for an hour. After, magnetic stir bar was added, and the flask was connected to a condenser. The reflux set-up was evacuated and filled with nitrogen three times. Then, stirring was started to grind the surface of magnesium turnings to make them more reactive. 11-bromodec-1-ene (6.6 mL, 30 mmol) in 25 mL dry diethyl ether was added dropwise to the magnesium turnings. Before the exothermic reaction completely subsided, the mixture was heated to 45°C and refluxed for two hours. After, the excess magnesium turnings were removed by transferring the Grignard solution to a 250-mL round bottom flask with magnetic stir bar under nitrogen. The solution was cooled to 10°C, and the solvent was evaporated by evacuation in the Schlenk line. Then, the oily residue was cooled to 0°C, and 90 mL dry THF was added. Stirring was started, and dilithium tetrachlorocuprate (II) (0.1 M, 1.8 mL) and 1,10-dibromodecane (3 g, 10 mmol)

in 10 mL dry THF were added. After 20 minutes, the mixture was diluted with 10 mL dry THF, then stirring at 0°C was continued for three hours.

Next, 50 mL diethyl ether was added to the dark blue-green solution. Then, the mixture was mixed with 75 mL cold saturated solution of NH_4Cl in a 250-mL separatory funnel. The yellow organic phase was separated, and the blue aqueous phase was extracted twice with diethyl ether (30 mL x2). The combined organic phases were washed twice with water (75 mL x2). The organic phase was dried over Na_2SO_4 which was then removed using a glass filter. The organic phase was concentrated to dryness in a rotary evaporator. The milky peach residue was purified by filtering through a silica gel column with dry heptane. 250 mL dry heptane was passed through the column after loading the product on top of the column. [Dry hexane and/or pentane can also be used, instead of heptane.] Then, dry THF/diethyl ether (1/1 vol/vol) was passed through the column, while collecting the fraction in a new flask, until before the yellow front reached the bottom of the column. Acetone (about 80 mL) was added with stirring to the collected fraction until no further precipitation of diene occurred. [Acetone from not newly opened bottle may have more water than acetone, resulting to dilution and difficulty in precipitation of diene.] The waxy white product was filtered and dried under vacuum in Schlenk line. Yield 540 mg (12 wt%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.74-5.85 (m, 2H), 4.88-5.00 (m, 4H), 1.99-2.05 (q, 4H), 1.32-1.39 (m, 4H), 1.24-1.28 (m, 48H).

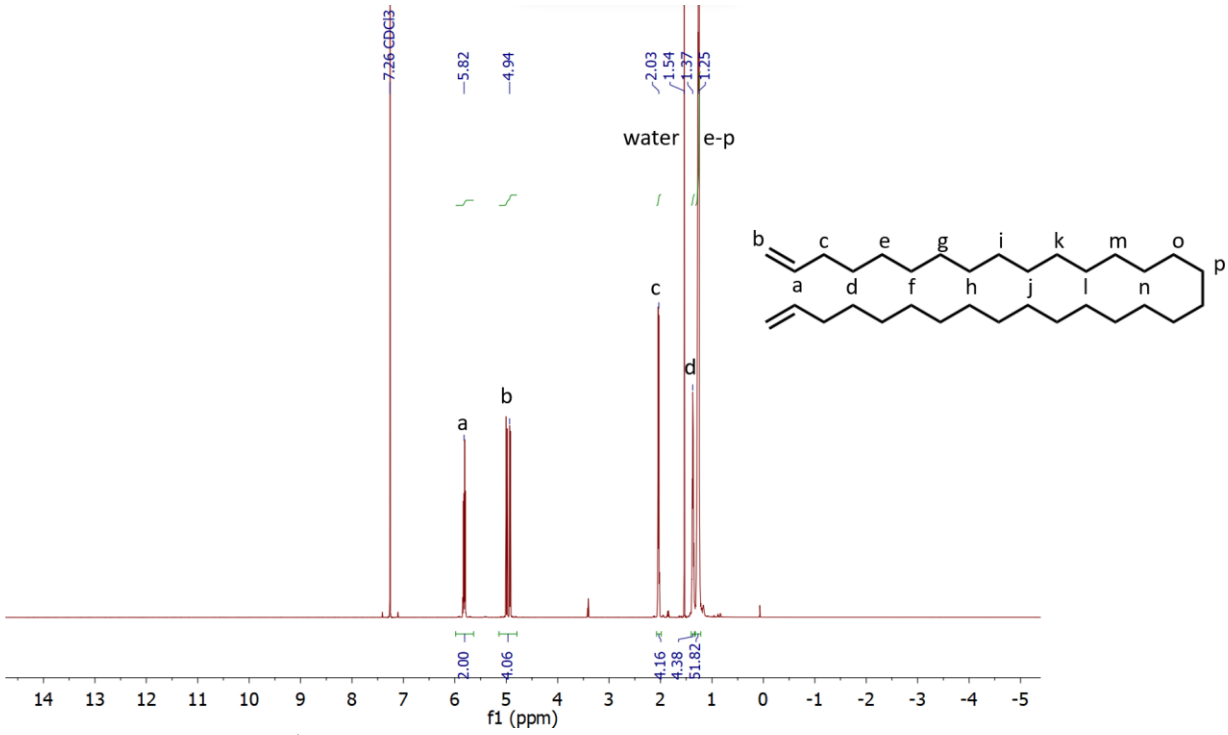


Figure A.3 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of dotriaconta-1,31-diene.

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2023
www.chalmers.se

CHALMERS
UNIVERSITY OF TECHNOLOGY