The Design and Synthesis of Pnictogen Cages for Pnictogen Bond Driven Self-Assembly of Reversed Bilayer Vesicles for Aqueous Environments

Serena Yeh
BASIS Independent Silicon Valley
corresponding author: serenayeh10@gmail.com

Lina Delgado
Department of Chemistry and Biochemistry
Texas Tech University

Anthony Cozzolino, PhD
Department of Chemistry and Biochemistry
Texas Tech University

INTRODUCTION

Bilayer vesicles support biological functions, most importantly the transportation of materials.[1] Synthesizing them would allow access to manipulating such functions and aid in numerous biomedical applications (e.g. targeted drug delivery, disease monitoring).[1,2] Currently, anthropogenic methods mainly branch off liposomes (lipid building blocks naturally found in the body) or polymersomes (adjustable copolymers)[3] (**Fig. 1**).

Some have also looked towards non-covalent bonding solutions, including pnictogen bonding (PnB), which this study takes advantage of to synthesize water dispersible molecules capable of self-assembling into reversed bilayer vesicles. With three electronegative species bonded to the pnictogen, three intermolecular sites are introduced,[4,5] allowing molecules to self-assemble into reversed bilayer vesicles, with a mutable 'tail' and a hydrophobic 'head' consisting of the PnB site (**Fig. 1**).

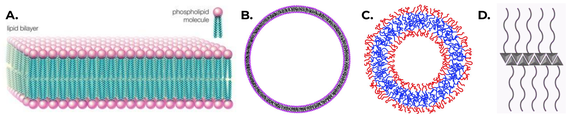


Figure 1. Phospholipid bilayer (**A.**), compared to liposomes (**B.**), polymersomes (**C.**) and PnB for vesicle formation (**D.**). (Source: Rideau et. al, Moaven et. al)

Previous studies utilized hydrophobic tails and could therefore only form vesicles in organic solvents. To allow for water solubility (cheaper, more environmentally friendly, necessary for applications in the body), [2-(2-methoxyethoxy)ethoxy]acetic acid was chosen as a hydrophilic and biocompatible polyethylene glycol (PEG) chain.[6]

Antimony (a well-studied heavy pnictogen) was used to make PnB possible and was configured into a cage formation, creating sufficient space for PnB.[6,7,8] The antimony is bonded to two sulfur atoms and one oxygen atom, benefitting from the stability and water insensitivity of the Sb–S bonds, and from the large electronegativity difference of the Sb–O bond. This antimony-dithiothreitol (Sb-DTT) cage structure represents the heads of the bilayer vesicle, and the PEG chains represents the tails (**Fig. 2**).

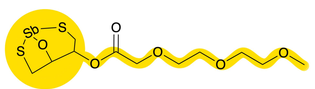


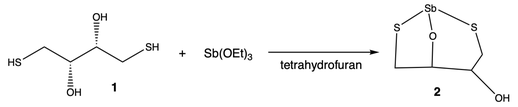
Figure 2. Synthetic reversed bilayer 'lipid' for this study, depicting the head (Sb-DTT cage) and the tail (PEG chain).

Steglich esterification is used to esterify the Sb-DTT cages and the PEG chains, with dicyclohexylcarbodiimide (DCC) as the carbodiimide coupling reagent and dimethylaminopyridine (DMAP) as an acylation catalyst.[9,10] The solvent, dichloromethane (DCM), is dried to prevent water from reacting with the DCC.

ACKNOWLEDGEMENTS

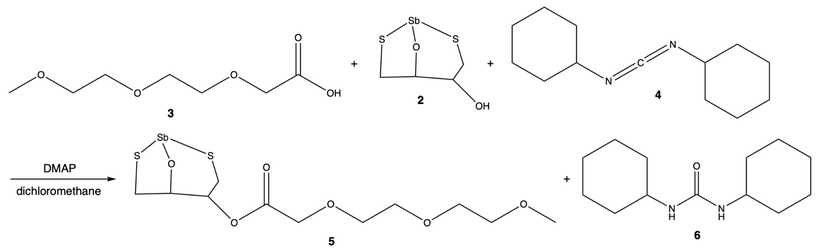
The first author would like to acknowledge the second and third authors for all their help and feedback on the project. Thanks also to the Anson L. Clark Scholars 2022 cohort and program directors, J. Carr and W. Flores.

SYNTHESIS OF Sb-DTT CAGES



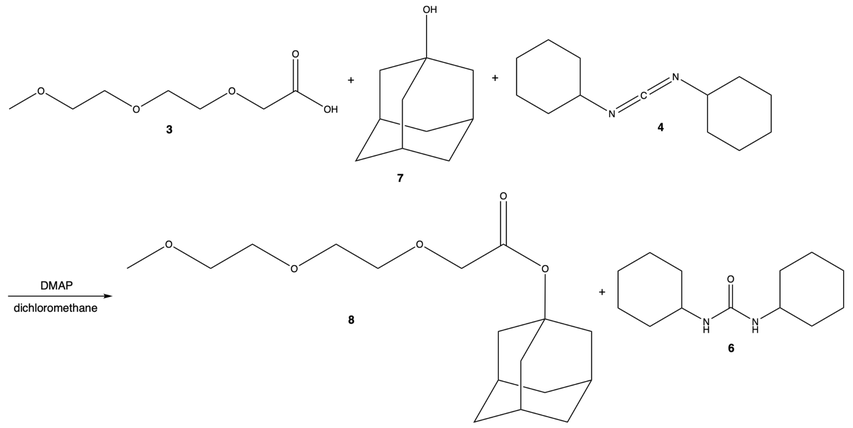
- Yield of 84.4% as a white solid
- Insoluble in water
- $Sb(OEt)_3$ produced based on previously reported methods of synthesizing $Bi(OEt)_3$[11]

ESTERIFICATION OF Sb-DTT CAGES AND [2-(2-METHOXYETHOXY)ETHOXY]ACETIC ACID



- Desired product (**5**) to participate in pnictogen bonding
- **6** filtered out as a white precipitate (indication of successful reaction)
- DMAP, excess **3** removed through acid-base extraction of DCM layer (HCl, $NaHCO_3$, H_2O)
- Excess **4** removed through liquid-liquid extraction (diethyl ether, water)
- Final product (**5**) transferred from aqueous to DCM layer, purified with dichloroethane
- Yield of 16.3% as a dark colored wax (**Figs. 3 and 4**)

ESTERIFICATION OF 1-ADAMANTANOL AND [2-(2-METHOXYETHOXY)ETHOXY]ACETIC ACID



- Control to show capabilities of pnictogen bonding for vesicle formation (hydrocarbon head incapable of PnB and self-assembling)
- **6** filtered out as a white precipitate
- DMAP, excess **3** removed through acid-base extraction of DCM layer (HCl, $NaHCO_3$, H_2O)
- Excess **4** removed through wash with cold ethanol
- Yield of 54.0% as a yellow oil (**Figs. 5 and 6**)

SPECTRAL ANALYSIS OF 5

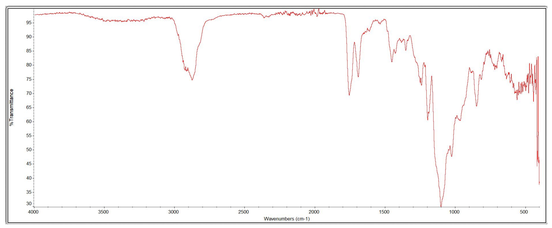


Figure 3. FTIR of **5**, with the expected C–H, C=O, C–O stretches and lacking any O–H stretch associated with unreacted **2** or **3**. The Sb–O peak is around 400 cm^{-1}.

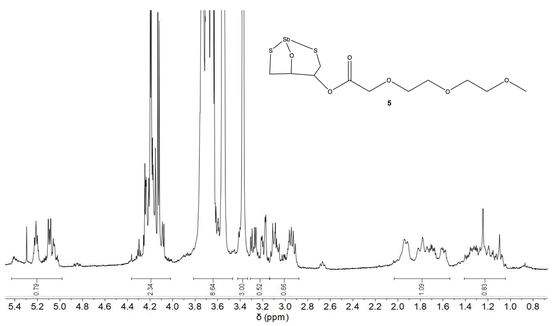


Figure 4. ^1H NMR of **5** in $CDCl_3$, showing peaks associated with expected ratios with marked shifts from starting materials. The peak at 3.63 ppm corresponds to 8 hydrogens from the PEG chain and 1 hydrogen from the Sb-DTT cage.

SPECTRAL ANALYSIS OF 8

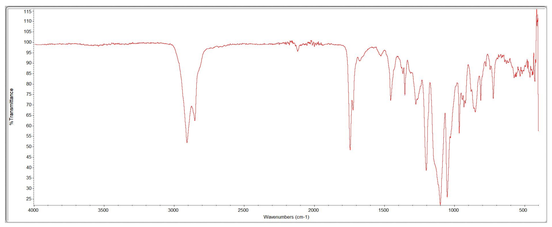


Figure 5. FTIR of **8**, with the expected C–H, C=O, C–O stretches and lacking any O–H stretch associated with unreacted **7** or **3**.

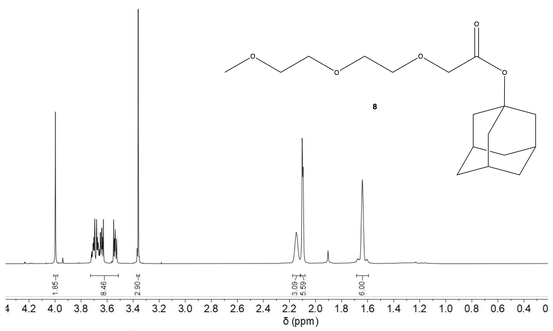


Figure 6. ^1H NMR of **8** in $CDCl_3$, showing peaks associated with expected ratios with marked shifts from starting materials.

CONCLUSION AND DISCUSSION

- Successful synthesis and purification of **5** (for pnictogen bonding to form vesicles in aqueous environments) and **8** (as a control)
- Low yield of **5**
  - Difficulties with diethyl ether and water extraction step (formed an emulsion, hard to separate layers)
  - Small amounts left in each layer after extractions
- Addition of PEG tail to Sb-DTT cage allowed for water solubility
  - Preferred DCM over water over diethyl ether
  - Important for biomedical applications
- **8** insolubility in water contributed to high yield

FUTURE WORK

- EDC hydrochloride (polar, water soluble) instead of DCC (non-polar) to eliminate need for diethyl ether and water extraction step
- Testing vesicle formation abilities of **5** and **8** when dispersed in water
  - Sonication and dynamic light scattering for size
  - Electron microscopy for shape and solidity (**Fig. 7**)

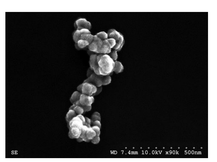


Figure 7. Scanning electron microscopy image of a synthetic vesicle dispersed in an organic solvent after sonication. (Source: Moaven et. al)

- Obtaining single crystal structures
- Experimentation with other heavy pnictogens
  - Bismuth (nontoxic, used in medicine)
- PEG chains of longer lengths to possibly increase polarity

REFERENCES

(1) Kwok, Z. H.; Wang, C.; Jin, Y. Extracellular Vesicle Transportation and Uptake by Recipient Cells: A Critical Process to Regulate Human Diseases. Processes 2021, 9 (2), 273. https://doi.org/10.3390/pr9020273.

(2) Datta, B.; Paul, D.; Pal, U.; Rakshit, T. Intriguing Biomedical Applications of Synthetic and Natural Cell-Derived Vesicles: A Comparative Overview. ACS Appl. Bio Mater. 2021, 4 (4), 2863–2885. https://doi.org/10.1021/acsabm.0c01480.

(3) Shin, J.; Cole, B. D.; Shan, T.; Jang, Y. Heterogeneous Synthetic Vesicles toward Artificial Cells: Engineering Structure and Composition of Membranes for Multimodal Functionalities. Biomacromolecules 2022, 23 (4), 1505–1518. https://doi.org/10.1021/acs.biomac.1c01504.

(4) Mahmudov, K. T.; Gurbanov, A. V.; Aliyeva, V. A.; Resnati, G.; Pombeiro, A. J. L. Pnictogen Bonding in Coordination Chemistry. Coord. Chem. Rev. 2020, 418, 213381. https://doi.org/10.1016/j.ccr.2020.213381.

(5) Moaven, S.; Yu, J.; Vega, M.; K. Unruh, D.; F. Cozzolino, A. Self-Assembled Reversed Bilayers Directed by Pnictogen Bonding to Form Vesicles in Solution. Chem. Commun. 2018, 54 (64), 8849–8852. https://doi.org/10.1039/C8CC05187A.

(6) Ensing, B.; Tiwari, A.; Tros, M.; Hunger, J.; Domingos, S. R.; Pérez, C.; Smits, G.; Bonn, M.; Bonn, D.; Woutersen, S. On the Origin of the Extremely Different Solubilities of Polyethers in Water. Nat. Commun. 2019, 10 (1), 2893. https://doi.org/10.1038/s41467-019-10783-z.

(7) Moaven, S.; Andrews, M. C.; Polaske, T. J.; Karl, B. M.; Unruh, D. K.; Bosch, E.; Bowling, N. P.; Cozzolino, A. F. Triple-Pnictogen Bonding as a Tool for Supramolecular Assembly. Inorg. Chem. 2019, 58 (23), 16227–16235. https://doi.org/10.1021/acs.inorgchem.9b02761.

(8) Moaven, S.; T. Watson, B.; B. Thompson, S.; J. Lyons, V.; K. Unruh, D.; J. Casadonte, D.; Pappas, D.; F. Cozzolino, A. Self-Assembly of Reversed Bilayer Vesicles through Pnictogen Bonding: Water-Stable Supramolecular Nanocontainers for Organic Solvents. Chem. Sci. 2020, 11 (17), 4374–4380. https://doi.org/10.1039/D0SC00206B.

(9) Neises, B.; Steglich, W. Simple Method for the Esterification of Carboxylic Acids. Angew. Chem. Int. Ed. Engl. 1978, 17 (7), 522–524. https://doi.org/10.1002/anie.197805221.

(10) Lutjen, A. B.; Quirk, M. A.; Kolonko, E. M. Synthesis of Esters Via a Greener Steglich Esterification in Acetonitrile. JoVE J. Vis. Exp. 2018, No. 140, e58803. https://doi.org/10.3791/58803.

(11) Chapter 2 - Organobismuth(III) Compounds. In Organobismuth Chemistry; Suzuki, H., Matano, Y., Eds.; Elsevier Science: Amsterdam, 2001; pp 21–245. https://doi.org/10.1016/B978-044420528-5/50004-5.