The design and synthesis of pnictogen cages for pnictogen bond driven self-assembly of reversed bilayer vesicles for aqueous environments

Serena Yeh,[1] Lina M. Delgado,[2] Anthony F. Cozzolino[2]

[1]BASIS Independent Silicon Valley, 1290 Parkmoor Avenue, San Jose, CA 95126, United States

[2]Department of Chemistry and Biochemistry, Texas Tech University, 1204 Boston Avenue, Lubbock, TX 79409-1061, United States

Keywords: synthetic vesicle formation, pnictogen bonding, water dispersible, non-covalent interactions, supramolecular chemistry

Abstract Pnictogen bonding, a type of non-covalent interaction, represents a new kind of interaction that can be used to program molecules with the ability to self-recognize and assemble into vesicles. Synthetic, water-dispersible bilayer vesicles are capable of greatly benefiting biomedical applications (e.g. drug delivery and disease monitoring). In this study, we look to take advantage of pnictogen bonding and synthesize reversed bilayer molecules, with an antimony-based “head” capable of pnictogen bonding and a hydrophilic PEG chain “tail”, with the goal of having water dispersible vesicles. Antimony dithiothreitol cages are produced and esterified with a [2-(2-methoxyethoxy)ethoxy]acetic acid PEG chain tail, in addition to a 1-adamantanol and [2-(2-methoxyethoxy)ethoxy]acetic acid ester as a control for confirming the role of pnictogen bonding in vesicle formation. A pure product is isolated in both cases and through the workup processes, we are able to confirm the solubility of our product in aqueous solutions, in addition to slightly polar ones like dichloromethane.

INTRODUCTION

Bilayer vesicles play an essential role in supporting biological functions, most importantly the transportation of materials (e.g. lipids and proteins).[1] Synthesizing such molecules would thus allow access to manipulating such functions and aid in numerous biomedical applications, such as targeted drug delivery and disease monitoring.[1,2] Currently, anthropogenic methods mainly branch off liposomes (composed of amphiphilic lipid building blocks naturally found in the body) or polymersomes (composed of amphiphilic adjustable copolymers).[3] Some have also looked towards non-covalent bonding solutions, including pnictogen bonding, which this paper takes advantage of to synthesize water dispersible molecules capable of self-assembling into reversed bilayer vesicles.

The ability of heavy pnictogens to participate in non-covalent bonding behavior, similar to that of hydrogen, chalcogen and halogen bonds,[4] makes it useful in vesicle formation. With three electronegative species bonded to the pnictogen atom, three intermolecular sites are introduced, allowing the creation of a sheet configuration consisting of multiple molecules capable of pnictogen bonding.[5,6] We are thus able to synthesize self-assembling reversed bilayer vesicles, with a mutable 'tail' and a hydrophobic 'head' consisting of the pnictogen bonding site (**Fig. 1**). The previous studies utilized hydrophobic tails and could therefore only form vesicles in organic solvents.

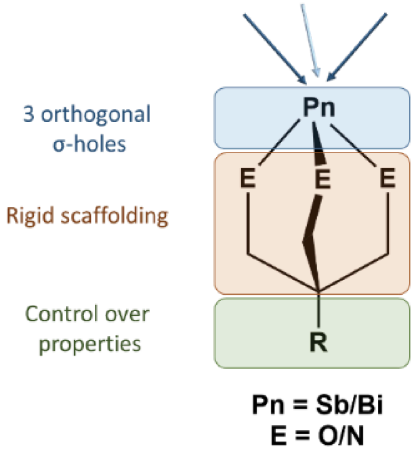


Figure 1. Pnictogen(III) design for self assembly.

Considering the cost effectiveness and environmental friendliness of water as a solvent, as well as the importance of water dispersibility for applications in the body, [2-(2-methoxyethoxy)ethoxy]acetic acid was chosen for its hydrophilic and biocompatible properties as a polyethylene glycol (PEG) chain.[7] As a pnictogen with well-studied properties, antimony was used to make pnictogen bonding possible and was

configured into a cage formation, creating sufficient space around the atom for pnictogen bonds.[4,6,8] The antimony is bonded to two sulfur atoms and one oxygen atom, benefitting from the stability and water insensitivity of the antimony-sulfur bonds, and from the large electronegativity difference present in the antimony-oxygen bond. This antimony-dithiothreitol (Sb-DTT) cage structure represents the heads of the bilayer vesicle, and the PEG chain represents the tail of the bilayer vesicle, as shown in **Fig. 2**.

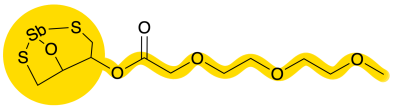


Figure 2. Synthetic reversed bilayer 'lipid' for this study, depicting the head (Sb-DTT cage) and the tail (PEG chain).

This paper reports on the use of a Steglich esterification, a common mechanism for reacting an alcohol and a carboxylic acid,[9,10] to esterify the Sb-DTT cages and the PEG chain. This process is accelerated by a carbodiimide coupling reagent (dicyclohexylcarbodiimide, or DCC) and an acylation catalyst (dimethylaminopyridine, or DMAP), and can take place under mild conditions.[9,10]

MATERIALS AND METHODOLOGY

General Methods.

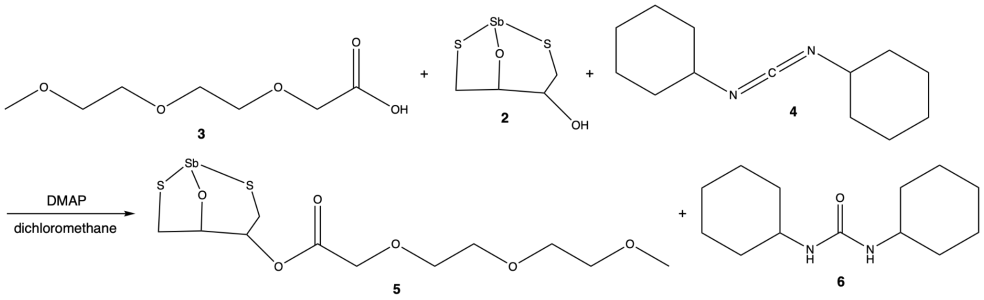
The starting materials DTT (98%, Oakwood Chemical), [2-(2-methoxyethoxy)ethoxy]acetic acid (>95.0%, Tokyo Chemical Industry), 1-adamantanol (99%, Acros Organics), DMAP (99%, Acros Organics) and DCC (99.42%, Chem-Impex International) were used as purchased. Antimony triethoxide was produced with reference to previously reported methods of synthesizing bismuth triethoxide.[11] Esterification reactions were conducted by adapting previous methods.[9,10]

Dry tetrahydrofuran (THF) and dry dichloromethane (DCM) were prepared by passing HPLC grade THF and DCM over activated molecular sieves in an LC Technologies Solutions Inc. solvent purification system. Deuterated chloroform ($CDCl_3$, Cambridge Isotopes Laboratory) was poured over activated molecular sieves.

Reactions under a nitrogen atmosphere were performed in an N_2 purged inert atmosphere glove box from LC Technology Solutions Inc. or with Schlenk line techniques. NMR spectroscopy was performed with a JEOL ECS 400 MHz NMR spectrometer. IR spectroscopy data were collected from a Nicolet iS 5 FT-IR spectrometer equipped with a Specac Di Quest ATR accessory.

Synthesis of Sb-DTT Cages.

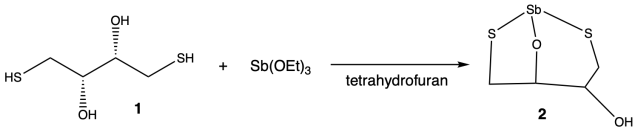
0.308 g DTT (2 mmol, 2 equiv) is dissolved in 2-5 mL of THF inside the glovebox under a nitrogen atmosphere. 0.514 g antimony triethoxide (2 mmol, 2 equiv) is dissolved in 4 mL of THF and added to the stirring solution of DTT. The mixture is stirred overnight and proceeds as shown in **Scheme 1**. The vial is removed from the glovebox, where the solid is then filtered and rinsed with acetone. 0.461 g Sb-DTT was collected as a white powder, for a percent yield of 84.4%.



Scheme 1. Esterification of Sb-DTT and [2-(2-Methoxyethoxy)ethoxy]acetic Acid (**5**)

Esterification of Sb-DTT Cages and [2-(2-Methoxyethoxy)ethoxy]acetic Acid (5).

0.3635 g [2-(2-methoxyethoxy)ethoxy]acetic acid (2.04 mmol, 1.2 equiv), 0.6231 g DMAP (5.1 mmol, 3 equiv), 0.5282 g DCC (2.56 mmol, 1.5 equiv) and 0.4641 g Sb-DTT (1.7 mmol, 1 equiv) are added to 30 mL dry DCM, as shown in **Scheme 2**. The reaction mixture is then stirred for 24 h at room temperature under nitrogen, turning milky white within minutes. The white precipitate (dicyclohexylurea, or DHU) is filtered off, and the residue is redissolved in DCM. Any additional DHU is filtered off again. The DCM solution is then washed with 0.1 M hydrochloric acid (two times), saturated sodium bicarbonate solution (two times) and water (three times). DCM is removed through evaporation, and the product is dissolved in diethyl ether. The diethyl ether solution is washed twice with water, and the resulting aqueous layer is washed twice with DCM and dried over magnesium sulfate. DCM is again removed through evaporation. Dichloroethane (DCE) is then added to the organic layer to further dry the product, after which it is removed through evaporation to give the final product **4**.

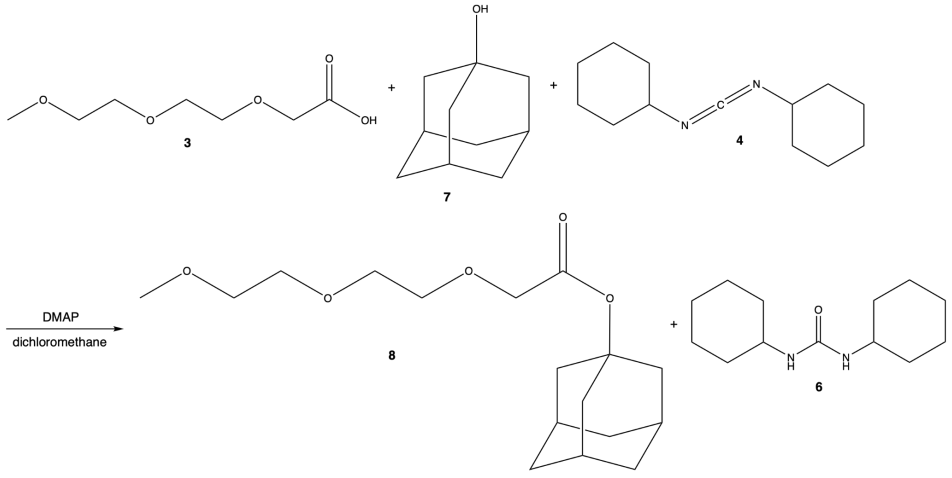


Scheme 2. Synthesis of Sb-DTT Cage (2)

0.120 g of **5** was recovered as a dark colored wax for a yield of 16.3%. FTIR (ATR, cm^{-1}): 2873 (m), 1755 (m), 1690 (m), 1102 (s). ^1H NMR (400 MHz, $CDCl_3$, δ): 1.26 (m, 1H, Sb(SC***H***$_2$CH(O)CHCH$_2$S)O(C=O)CH$_2$(OCH$_2$CH$_2$)$_2$OCH$_3$), 1.78 (m, 1H, Sb(SCH$_2$CH(O)CHC***H***$_2$S)O(C=O)CH$_2$(OCH$_2$CH$_2$)$_2$OCH$_3$), 2.95 (m, 1H, Sb(SC***H***$_2$CH(O)CHCH$_2$S)O(C=O)CH$_2$(OCH$_2$CH$_2$)$_2$OCH$_3$), 3.27 (m, 1H, Sb(SCH$_2$CH(O)CHC***H***$_2$S)O(C=O)CH$_2$(OCH$_2$CH$_2$)$_2$OCH$_3$), 3.37 (s, 3H, Sb(SCH$_2$CH(O)CHCH$_2$S)O(C=O)CH$_2$(OCH$_2$CH$_2$)$_2$OC***H***$_3$), 3.63 (m, 9H, Sb(SCH$_2$C***H***(O)CHCH$_2$S)O(C=O)CH$_2$(OC***H***$_2$C***H***$_2$)$_2$OCH$_3$), 4.18 (m, 2H, Sb(SCH$_2$CH(O)CHCH$_2$S)O(C=O)C***H***$_2$(OCH$_2$CH$_2$)$_2$OCH$_3$), 5.18 (m, 1H, Sb(SCH$_2$CH(O)C***H***CH$_2$S)O(C=O)CH$_2$(OCH$_2$CH$_2$)$_2$OCH$_3$).

Esterification of 1-Adamantanol and [2-(2-Methoxyethoxy)ethoxy]acetic Acid (8).

0.3635 g [2-(2-methoxyethoxy)ethoxy]acetic acid (2.04 mmol, 1.2 equiv), 0.6231 g DMAP (5.1 mmol, 3 equiv), 0.5282 g DCC (2.56 mmol, 1.5 equiv) and 0.2588 g 1-adamantanol (1.7 mmol, 1 equiv) are added to 30 mL dry DCM, as shown in **Scheme 3**. The reaction mixture is then stirred for 24 h at room temperature under nitrogen, turning faintly yellow within an hour. The white precipitate (DHU) is filtered off and the residue is redissolved in DCM. Any additional DHU is filtered off again. The DCM solution is then washed with 0.1 M hydrochloric acid (two times), saturated sodium bicarbonate solution (two times) and water (three times). DCM is removed through evaporation. To remove leftover DCC, the crude product is washed with cold ethanol, and any solids are filtered off. The ethanol is removed through evaporation to give the final product **8**.



Scheme 3. Esterification of 1-Adamantanol and [2-(2-Methoxyethoxy)ethoxy]acetic Acid (**8**)

0.287 g of **8** was recovered as a yellow liquid for a yield of 54.0%. FTIR (ATR, cm^{-1}): 2908 (m), 2853 (m), 1744 (m), 1724 (m), 1202(m), 1102 (s), 1052 (s). ^1H NMR (400 MHz, $CDCl_3$, δ): 1.64 (s, 6H,

$[(C\boldsymbol{H_2})_3(CH)_3(CH_2)_3]CO(C{=}O)CH_2(OCH_2CH_2)_2OCH_3$), 2.09 (s, 6H, $[(CH_2)_3(CH)_3(C\boldsymbol{H_2})_3]CO(C{=}O)CH_2(OCH_2CH_2)_2OCH_3$), 2.15 (s, 3H, $[(CH_2)_3(C\boldsymbol{H})_3(CH_2)_3]CO(C{=}O)CH_2(OCH_2CH_2)_2OCH_3$), 3.35 (s, 3H, $[(CH_2)_3(CH)_3(CH_2)_3]CO(C{=}O)CH_2(OCH_2CH_2)_2OC\boldsymbol{H_3}$), 3.61 (m, 8H, $[(CH_2)_3(CH)_3(CH_2)_3]CO(C{=}O)CH_2(OC\boldsymbol{H_2}C\boldsymbol{H_2})_2OCH_3$), 4.00 (s, 2H, $[(CH_2)_3(CH)_3(CH_2)_3]CO(C{=}O)C\boldsymbol{H_2}(OCH_2CH_2)_2OCH_3$).

RESULTS AND DISCUSSION

Product **5** was recovered as a dark colored waxy substance with a relatively low yield (16.3%) as a result of challenges associated with purification. The final purified product was characterized by FTIR (**Fig. 6**) and was found to have the expected C–H, C=O and C–O stretches and lacked any O–H stretch associated with the unreacted alcohol (**2**) or carboxylic acid (**3**). The ^{1}H NMR spectrum (**Fig. 7**) showed peaks associated with both fragments in the expected ratios with marked shifts from the starting materials. It was noted during the workup, that compound **5** displayed increased solubility in water while retaining solubility in organic solvents (dichloromethane and ether).

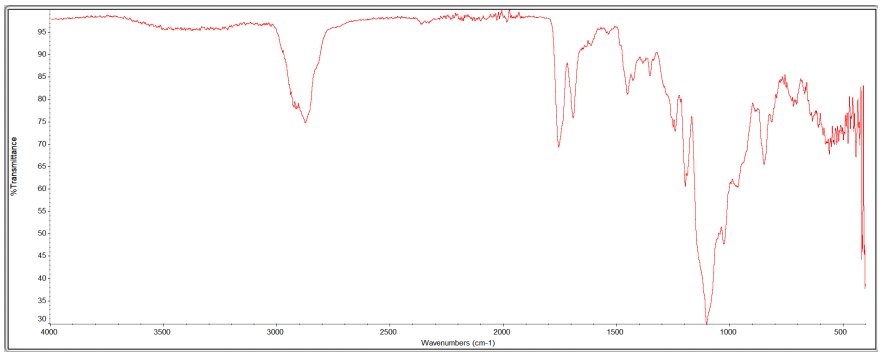


Figure 6. FTIR of **4**. Sb-O peak is around 400 cm^{-1}.

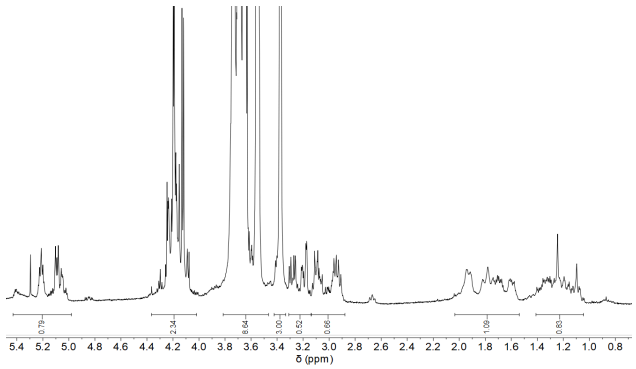


Figure 7. ^{1}H NMR of **4** in $CDCl_3$. The peak at 3.63 ppm corresponds to 8 hydrogens in the PEG chain and 1 hydrogen in the Sb-DTT cage.

Although the purification process continues to be a challenge, these results provide a big indication of future possible vesicle formation and the applications of water dispersibility.

To show the capability of pnictogen bonding to form vesicles, 1-adamantanol and [2-(2-methoxyethoxy)ethoxy]acetic acid esters were used as a control. These were esterified with the same Steglich reaction mechanism as the previous Sb-DTT esters. Product **8** was recovered as a yellow oil with a relatively good percent of yield (54%). The successful synthesis of this product also represents progress in the development of a control to demonstrate the importance of the pnictogen bonding in the vesicle formation process, due to the inability of hydrocarbon "heads," to form pnictogen bonds and the resulting self-assembly process. They were also insoluble in water, which would explain the notable differences in yield between **5** and **8** during the extractions process because of the successful obtention of the product in only the organic layers. The final purified product was characterized by FTIR (**Fig. 8**) and was found to have the expected C–H, C=O and C–O stretches and lacked any O–H stretch associated with the unreacted alcohol (**7**) or carboxylic acid (**3**). The ^{1}H NMR spectrum (**Fig. 9**) showed peaks associated with both fragments in the expected ratios with marked shifts from the starting materials.

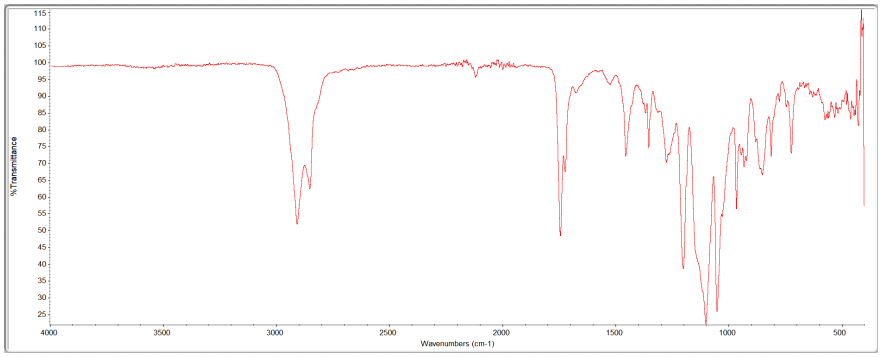


Figure 8. FTIR of **8**

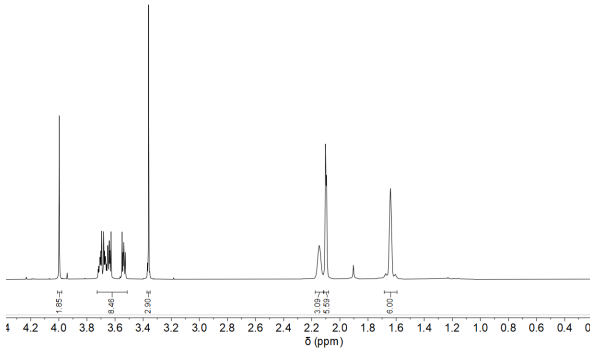


Figure 9. ^{1}H NMR of **8** in $CDCl_3$.

CONCLUSION

In summary, the esterification of Sb-DTT and the PEG chain [2-(2-methoxyethoxy)ethoxy]acetic acid to form synthetic bilayer vesicles, in addition to the control with 1-adamantanol, was successful. The addition of the hydrophilic PEG chain to the Sb-DTT cage allowed for the desired water solubility, with **5** preferring water over nonpolar solvents like diethyl ether but not mildly polar ones like DCM. This preference for more polar solvents suggests its capability to form vesicles in water, a necessary aspect of synthetic vesicles to be used in the therapeutic applications for drug delivery or radioactive labeling.

Future Work.

While the yield of **5** was on the low end, the purification process can still be refined, as we found small amounts of our product in multiple layers throughout the extraction work up process. The poor separation of the diethyl ether and aqueous layers during the second extraction, even in trials where saturated NaOH is added to alter the pH, was likely a major factor in lowering the yield. Using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, a water soluble carbodiimide coupling reagent, instead of DCC could make the diethyl ether-water extraction unnecessary and possible thus improve yield.

While past literature provides examples for esters, like the previously produced **5**, to self-assemble into vesicles held together by pnictogen-bonding, testing these capabilities is still necessary, especially when dispersed in water. For example, through sonication and dynamic light scattering, we will be able to analyze the size of any formed vesicles, and through electron microscopy, we would obtain more information about their shape and solidity. **6** also needs to be tested as a control to connect vesicle formation with pnictogen bonding rather than the head and tail structure present in both esters. Obtaining the single crystal structure is also necessary to confirm both structures.

Experimentation with using other heavy pnictogens, like bismuth with its nontoxic properties and its current use in medicine, and with PEG chains of different lengths to possibly increase polarity would further solidify synthesizing water dispersible reversed bilayer vesicles capable of self-assembly.

ACKNOWLEDGEMENTS

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

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) 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.

(5) 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.

(6) 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.

(7) 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.

(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.