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BASE-LABILE PROTECTING GROUPS FOR STEPWISE PEG SYNTHESIS

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BASE-LABILE PROTECTING GROUPS FOR STEPWISE PEG SYNTHESIS

By

Logan D. Mikesell

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Chemistry

MICHIGAN TECHNOLOGICAL UNIVERSITY

2021

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This thesis has been approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE in Chemistry.

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Author Contribution Statement

The initial ideas of all research projects in this dissertation were directed under the supervision of Dr. Shiyue Fang. All the writing of this dissertation (Chapter 1 and 2) was carried out by Mr. Logan Mikesell and revised by Dr. Shiyue Fang.

In chapter 2, Ms. Adikari Mudiyanselage Dhananjani Nisansala Eriyagama did preliminary experimentation and acted as a mentor for Mr. Logan Mikesell. Mr. Yipeng Yin contributed to producing four compounds: 3d, 3e, 3g, and 3j. Mr. Logan Mikesell conducted all other experimentations, purification, and data analysis. Work will be submitted for publication in a journal within a month.

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I am thankful for all the chemistry faculty that I have met and learned from at Michigan Tech, such as Mr. Andrew Galerneau, Dr. Loredana Valenzano-Slough, and many others; your teachings will always be remembered. Moreover, I am thankful to Dr. Rudy Luck, Dr. Marina Tanasova, and Dr. Tarun Dam for their time, support, and input regarding my thesis and defense. I also would like to thank the NSF funding agency for providing financial assistance throughout this journey.

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List of Abbreviations

Ac	acetate
Bn	benzyl
DCM	dichloromethane
DMF	N,N-dimethylformamide
DMTr	4,4'-dimethoxytrityl
Eq	equivalent
ESI	electrospray ionization
Et	ethyl
FDA	Food and Drug Administration
g	gram
h	hour(s)
LC	liquid chromatography
LDA	lithium diisopropylamide
LRMS	low resolution mass spectrocopy
HRMS	high resolution mass spectroscopy

Hz	hertz
J	coupling constant
KHMDS/KN(TMS)$_2$	potassium bis(trimethylsilyl)amide
M	molar
MALDI	matrix-assisted laser desorption/ionization
Me	methyl
mg	milligram
MHz	megahertz
mL	milliliter
mmol	millimole
MS	mass spectroscopy
NMR	nuclear magnetic resonance
PEG	polyethylene glycol(s)
Ph	phenyl
*t*Bu	tert-butyl
TFA	trifluoroacetic acid

THF	tetrahydrofuran
TLC	thin layer chromatography
Ts	tosyl

Abstract

Stepwise synthesis of monodisperse polyethylene glycols (PEGs) and their derivatives usually involves using an acid-labile protecting group such as DMTr and coupling two PEG moieties together under the basic Williamson ether formation conditions. Using this approach, each elongation of PEG is achieved in three steps – deprotection, deprotonation, and coupling – in two pots. Here, we report a more convenient approach for PEG synthesis featuring the use of a base-labile protecting group such as the phenethyl group. Using this approach, each elongation of PEG can be achieved in only two steps – deprotection and coupling – in one pot. The deprotonation step and the isolation and purification of the intermediate product after deprotection using existing approaches are not needed when the new one-pot approach is used. Because stepwise PEG synthesis usually requires multiple PEG elongation cycles, the new PEG synthesis method is expected to significantly lower PEG synthesis cost and reduce the use of harmful solvents and other chemicals.

Chapter 1 Background

1.1 Introduction

This thesis is based on my work in Dr. Fang's laboratory. The focal point is on the multi-step synthesis of Polyethylene glycols (PEGs) using a base-labile protecting group and its application in making longer PEGs. The beginning of the work consisted of making a monomer and using it to systematically build PEG compounds by a bidirectional approach to become longer using Williamson ether formations. This chapter is an introduction to that work, focusing on what PEG is and its application in the world of chemistry, and how it is produced.

1.2 PEG and Its Applications

PEGs are polymer compounds that can come in different sizes of ethylene glycol units (Figure 1.1.). These compounds have physical and chemical properties that have a stable, flexible, and neutral backbone. The compound is also soluble in water and many other organic solvents. This range of properties allows it to be a gold standard for biopharmaceuticals and various other applications. [1]

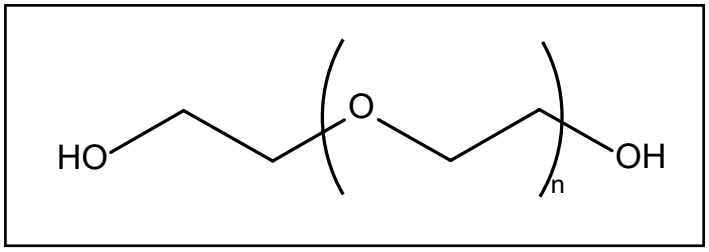


Figure 1.1. A structural representation of PEG.

PEGylation is the process of coupling PEG to biomolecules. PEGylation helps to improve solubility and stability as well as to decrease immunogenicity and dosing

frequency in biomacromolecules.[2] Other applications of PEGs consist of being used as linkers in organic synthesis, ingredients in nanomedicines that are used to stabilize nanoparticles and assist cell entry.[5] Moreover, they are used to evade undesired immune responses during drug delivery. PEGs are also being used as drug carriers for the new COVID-19 mRNA vaccines.[4]

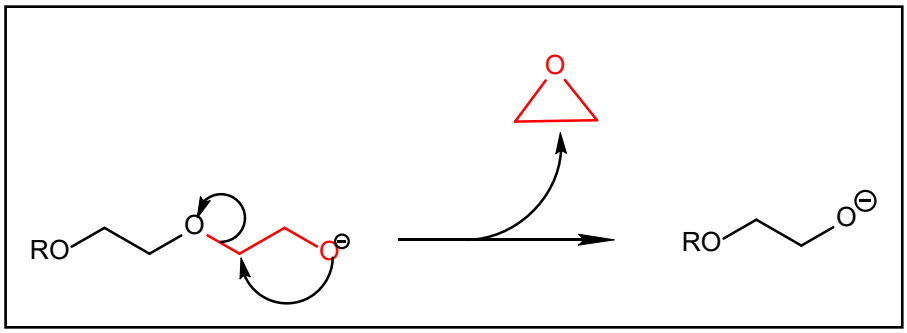
1.3 Known Methods for Mono-dispersed PEG Synthesis

The simple method for producing PEGs is the polymerization of ethylene oxide. The drawback of this method is that it makes PEGs with admixtures of length and molecular weights.[7] Purification and separation of these polydisperse PEGs is impossible, so often, it is characterized, weighted, and numbered by its average molecular weight.[8] When preparing drugs, if there are polydisperse PEGs, there can be a problem in having a consistent composition, which is essential for delivering the drug into the body. This heterogeneity can cause a loss in biological activities to result in different chemical and physical properties. These drawbacks make polydisperse PEG not preferred in drug delivery.

There has been a high demand for mono-dispersed PEG compounds as future crosslinking agents for biomolecules. The advantages of these mono-dispersed PEGs are their uniform size and distinct structure, which is highly important in the pharmaceutical industry.[3] This causes the uniformation of the PEG chain to be valuable for the drug registration process.

Although, PEGs are structurally simple molecules. They are a challenge to synthesize. One of the main challenges dealing with depolymerization occurs when a

deprotonated PEG reacts with itself by undergoing anionic depolymerization to produce ethylene oxide, the depolymerized product of PEG. (Scheme 1.1). After depolymerization, it is practically impossible to separate the depolymerized PEG by any purification techniques.



Scheme 1.1. Representation of the depolymerization of PEG

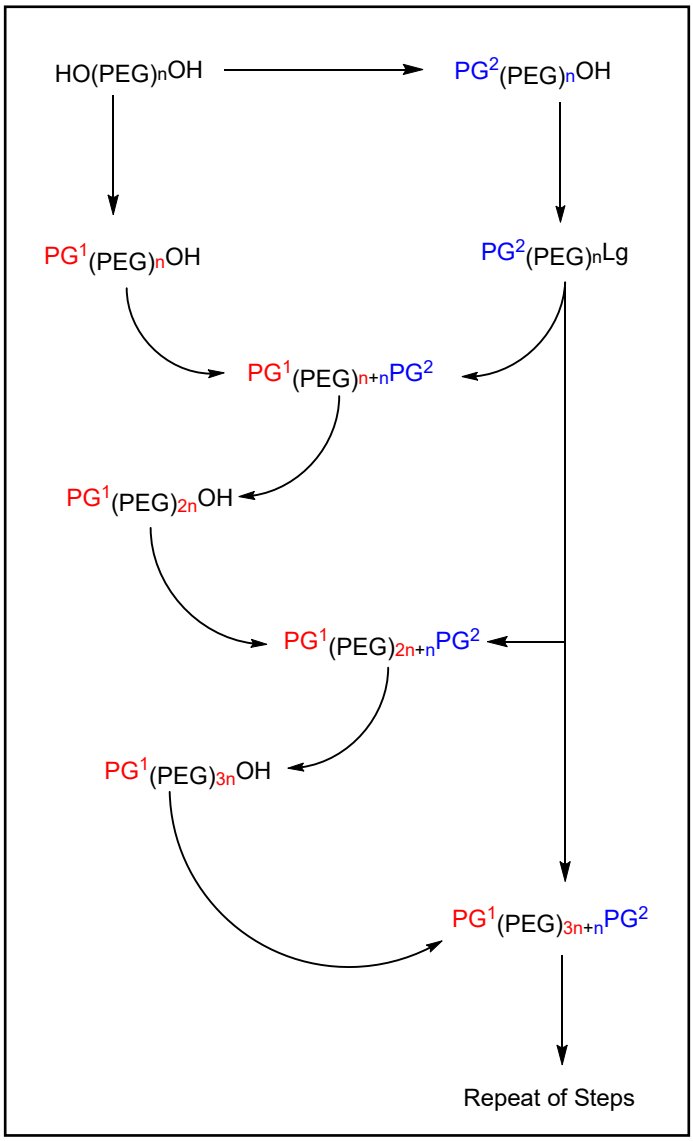
Over the years, research groups around the world have been developing ways to make PEG more uniform. One of the ways for synthesizing uniform PEG is stepwise solution-phase synthesis. Most methods undergo Williamson ether formation reaction. This synthetic route typically begins when an alkylating agent containing a leaving group reacts with an alkoxide ion. The common leaving groups used today for this synthesis are primary halides and alkyl sulfonates, such as chloride (–Cl), bromide (–Br), methanesulfonate (or mesyl, –OMs), and toluenesulfonate (or tosyl, –Ts).[9]

Using the Williamson ether synthetic method, there have been various strategies in producing high purity monodispersed PEGs.[1-3, 6-8] The common strategies used today for mono-dispersed PEG synthesis are unidirectional and bidirectional PEG elongation.[9]

In unidirectional PEG elongation, it builds upon one side of the polymer (Scheme 1.2.). The PEG compound relies on two protecting groups (PG) and a leaving group (Lg).

When comparing the two PGs, one is temporary, where the other is short-term throughout the synthetic cycle. The temporary PG is removed to extend the PEG further using a monomer of n-length in which has an Lg and the temporary PG. When undergoing this route, the short-term PG needs to be stable under conditions when removing the temporary PG. This process is repeated to achieve the desired PEG chain length. However, often unidirectional PEG elongation is not used for long-chain PEG elongation

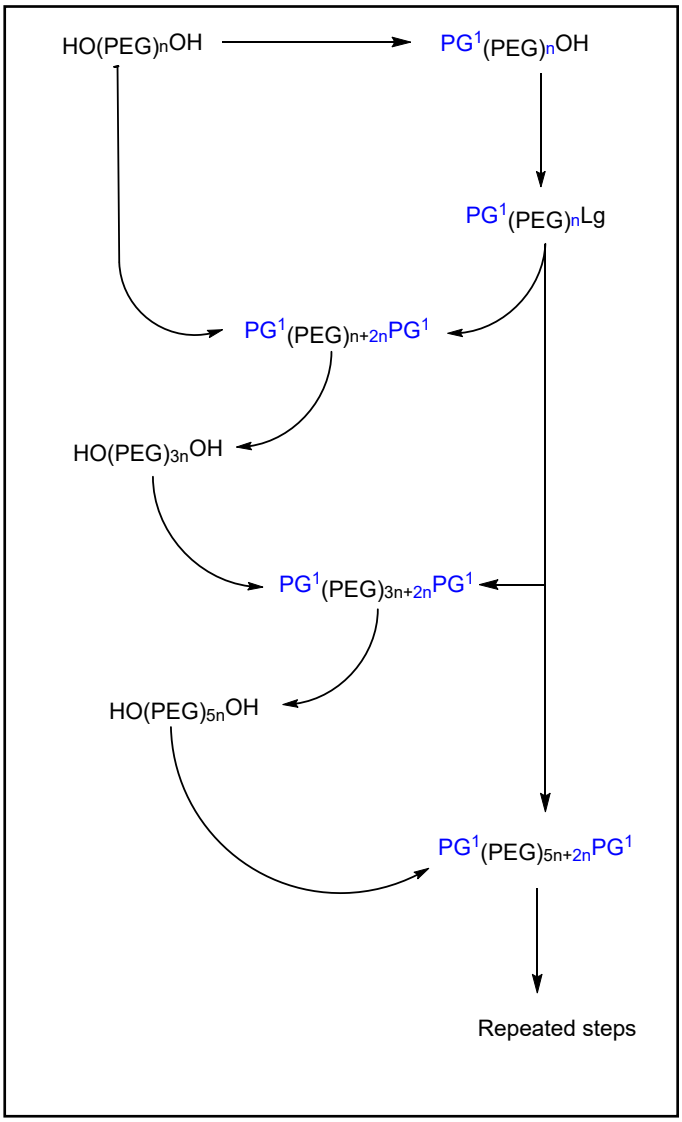
because of its inferior strategy to grow the polymer. Still, rather it is used as a monomer for other methods such as bidirectional PEG elongation.[9]



Scheme 1.2. Representation of unidirectional PEG elongation

In bidirectional PEG elongation, it involves extending the PEG on both sides of the chain (Scheme 1.3.). Unlike unidirectional PEG elongation, bidirectional extension relies

on a temporary PG rather than a short-term PG.[9] This allows PEG chain growth to increase two times faster, superior to the unidirectional route. One disadvantage this method has is that both sides of the product now have the same functionality. Moreover, this is the most commonly used strategy to make long-chain PEGs.[1, 3, 10, 11]



Scheme 1.3. Representation of bidirectional PEG elongation.

A typical synthetic pathway for the bidirectional approach uses an acid-labile protecting group such as 4,4'-dimethoxytriphenylmethyl (DMTr-) as the temporary PG and using -OTs as the Lg. The pathway goes through three steps: deprotonation, coupling, and deprotection. These steps occur in two pots in which yield homobifuctionalized DMTrO(PEG)$_{n+2n}$ODMTr.[8] This process is repeated to yield longer PEGs. However, as the PEG becomes longer, removing the DMTr- group becomes more difficult because the deprotection of the DMTr- is reversible. Another issue is that the reaction is difficult to complete when doing on a large scale.

The drawbacks of these multi-step synthetic strategies, such as the random polymerization of ethylene oxide, are not ideal for pharmaceutical applications. The difficulties for the unidirectional and bidirectional PEG elongation methods have problems as PEGs become longer. This is caused by the low efficiency of Williamson ether formation from the large size of the compound. Another problem is the need for multiple column chromatography uses to purify monomers, intermediates, and the final product, which entails heavy use of solvents.

1.4 One-Pot PEG Synthesis

In this thesis, I am going to describe a new method for the synthesis of monodisperse PEGs. A monomer with a base-labile protecting group instead of an acid-labile protecting group is used in this method. With monomers having a base-labile protection group, PEG elongation is achieved in two steps – deprotection and coupling in only one pot (Scheme 2.1.). There is no need to isolate and purify the intermediate between deprotection and coupling, and the deprotonation step is not needed. Our results

show that the synthesis is significantly more convenient than known methods, and high quality of monodisperse PEGs can be obtained in acceptable to high yields.

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Chapter 2

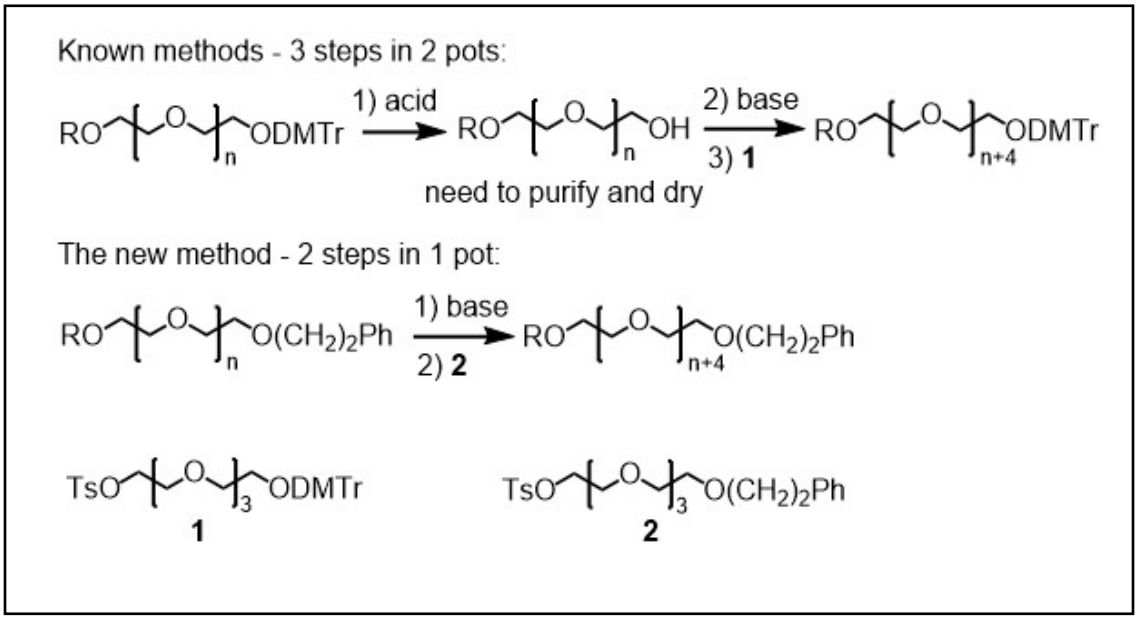
PEG Synthesis Featuring PEG Elongation in One Pot

By

Logan Mikesell, Adikari Mudiyanselage Dhananjani Nisansala Eriyagama, Yipeng Yin, Bao-Yuan Lu, and Shiyue Fang

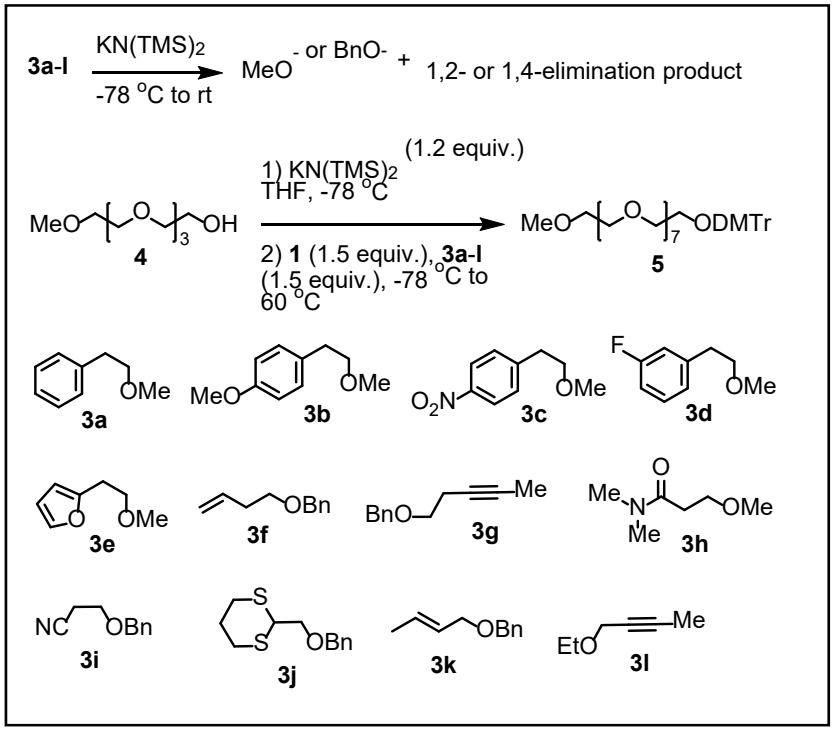
2.1 Introduction

Polyethylene glycols and derivatives (PEGs) have found wide applications in many areas.[1-6] For some applications, polydisperse PEGs are acceptable, although those with narrow molecular weight distribution are almost always desirable. These PEGs can be synthesized conveniently by polymerization of ethylene oxide under basic or acidic conditions.[7] The polymerization methods are inexpensive, and PEGs with high molecular weight can be obtained. However, for many other applications, which include as linkers in organic synthesis and bioconjugation,[8] as ingredients in nanomedicines to stabilize nanoparticles and to assist nanoparticle cell entry,[9-11] and as PEGylation agents to stabilize drugs based on biologic molecules such as peptides, proteins, and nucleic acids and to evade undesired immune responses, monodisperse PEGs are required or highly desirable.[12, 13]



Scheme 2.1. A comparison of the new PEG synthesis method using a base-labile protecting group with known PEG synthesis methods using an acid-labile protecting group.

To meet the needs of monodisperse PEGs, many efforts have been made to develop stepwise methods for their synthesis.[14-26] Perhaps, the most widely used method in academia and in the industry involves using a monomer such as compound 1, which contains the acid-labile DMTr protecting group. PEG elongation is achieved by deprotection under acidic conditions, purifying the intermediate, and setting up separate reactions to carry out the deprotonation and Williamson ether formation reactions under basic conditions (Scheme 2.1.).[17, 19, 20, 22, 26] In this letter, we report the use of monomers containing a base-labile protecting group such as 2 with the phenethyl group for stepwise monodisperse PEG synthesis. With monomers having a base-labile protection group, PEG elongation is achieved in two steps – deprotection and coupling in only one pot (Scheme 2.1). There is no need to isolate and purify the intermediate between deprotection and coupling, and the deprotonation step is not needed. Our results show that the synthesis is significantly more convenient than known methods, and high quality of monodisperse PEGs can be obtained in acceptable to high yields.



Scheme 2.2. Screening base-labile protecting groups for stepwise PEG synthesis.

2.2 Results and Discussion

For a base-labile protecting group to be helpful in PEG synthesis using the one-pot PEG elongation approach, it needs to meet two criteria: (1) The protecting group can be removed under basic conditions. (2) The protecting group is stable under the basic Williamson ether formation conditions. For this reason, we screened several potentially functional protecting groups against these two criteria using compounds **3a**-**l**.

For criterium (1), we subjected the compounds to basic conditions and used TLCs to monitor the progress of the 1,2-elimination (**3a**-**j**) or 1,4-elimination (**3k**-**l**) reactions. Initially, compound **3a** (1 equiv.) was treated with LDA (1 equiv.) with a catalytic

amount of *t*BuOK (0.2 equiv.) in THF at -78 ºC.[27, 28] Complete consumption of **3a** to give methoxide and styrene was observed after warming the reaction mixture to -50 ºC and stirring at the temperature for less than two hours. Because LDA has a short shelf life and has to be stored at low temperature, we were curious if $(TMS)_2NK$ (pKa of conjugate acid, 26), which is a weaker base than LDA (pKa of conjugate acid, 36)[29] and can be stored at room temperature for a long period of time, could also bring about the reaction. Surprisingly, we found that the reaction occurred with high efficiency even without using any catalysts. Therefore, $(TMS)_2NK$ was used for screening the rest of the compounds (**3b**-**j**). Gratifyingly, all the compounds underwent 1,2-elimination (**3b-i**) or 1,4-elimination (**3j**) readily using this weaker base, and according to TLC, the reactions had 100% conversion after stirring at 0 ºC for less than two hours (appendix A, Figures A.1.-A.12.). Thus, we concluded that all the protecting groups in compounds **3a-j** meet criterium (1).

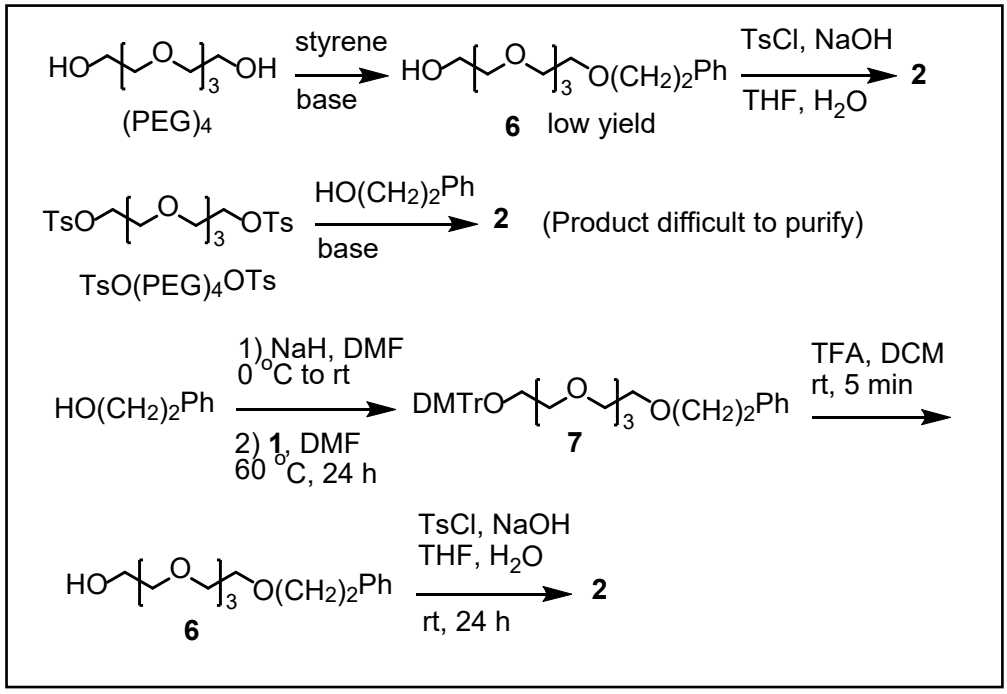
For criterium (2), we conducted the Williamson ether formation reaction between compounds **4** and **1** to form compound **5** using $KN(TMS)_2$ as the base in the presence of compounds **3a**-**j**. Compound **4** (1 equiv.) in THF was deprotonated with $KN(TMS)_2$ (1.2 equiv.). The mixture was cooled to -78 ºC, and the solution of **1** (1.5 equiv.) and **3a**-**i** or **3j** (1.5 equiv.) in THF was added. The reaction mixture was warmed to room temperature gradually, and then heated to 60 ºC. TLC analysis was performed to determine if the product **5** could be formed without causing the elimination reaction of **3**. The addition of excess base for the deprotonation of **4** was to ensure complete deprotonation in the event of inadvertent moisture. Cooling the solution of the deprotonated **4** to low temperature before addition of **1** and **3** and gradually warming the mixture to room temperature before

heating was to prevent the removal of the base-labile protecting group in **3** by the excess strong base by allowing the excess base to be consumed selectively via β-elimination of the tosylate in **1**. The product of premature removal of the base-labile protecting group – an alkoxide – would complicate the reaction, while the product of β-elimination of the tosylate – a vinyl ether – is inert under the reaction conditions. Compounds **3a-j** were subjected to the study. All were found to be stable under the coupling conditions, while product **5** was formed as indicated by TLC analysis (appendix A, Figures A.13 – A.24.). Therefore, we concluded that all the protecting groups in compounds **3a-j** meet criterium (2) under the conditions used for the screening studies.

Among the groups studied, the phenethyl group (the -$(CH_2)_2$Ph group) is one of the simplest. In addition, when the proposed one-pot PEG elongation approach is used for the synthesis of long PEGs, higher temperature and longer reaction time are usually needed for the Williamson ether formation reaction.[17] This requires the protecting groups to be stable under conditions harsher than those used in our screening studies. Therefore, it is preferable to choose a relatively more stable group than a less stable one for the one-pot PEG elongation application. Among the groups studied, the phenethyl group belongs to the more stable ones. With these considerations, the phenethyl group was chosen for the development of the one-pot PEG elongation approach for PEG synthesis.

The required monomer is **2**. The simplest method for its synthesis would be to react $(PEG)_4$, which is commercially available and inexpensive, with styrene to give **6**,[30] and tosylation of **6** to give the monomer (Scheme 2.3). However, the reported conditions for the synthesis of **6** without using an expensive catalyst gave low yields. We did not test

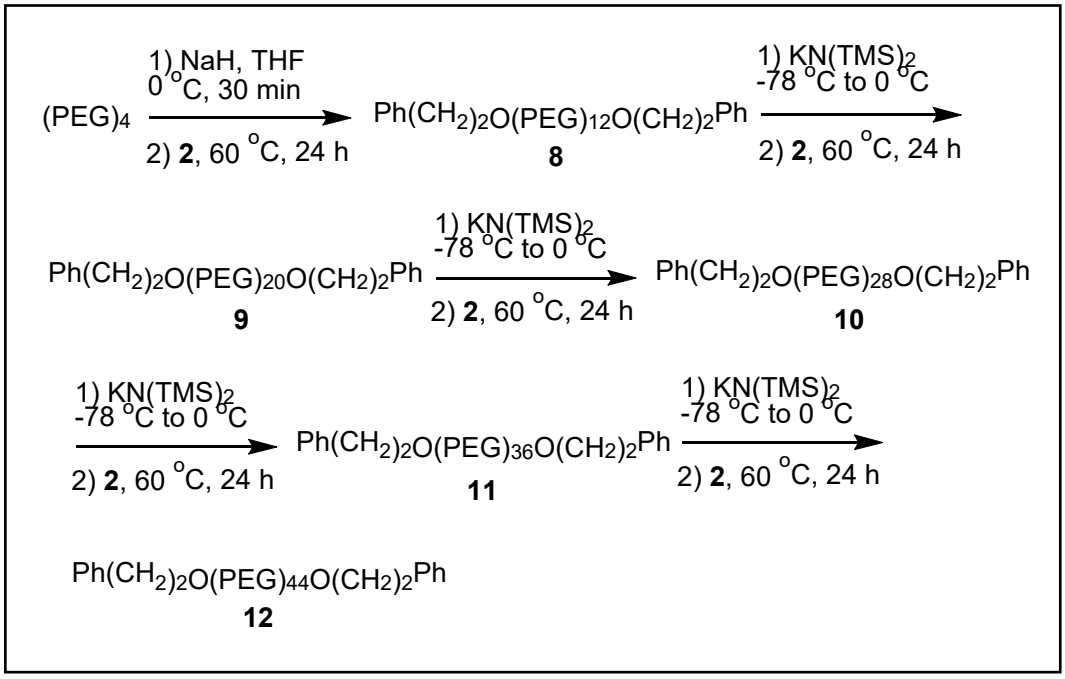
the conditions using the expensive catalyst that was used in the literature[30] due to cost considerations in practical applications although there is a possibility to obtain acceptable yields under those conditions. Another method we tried was to react excess $TsO(PEG)_4OTs$ with 2-phenylethan-1-ol under basic conditions to give **2** (Scheme 2.3). However, separation of **2** from $TsO(PEG)_4OTs$ and $TsO(PEG)_4O(CH_2)_2Ph$ required extensive chromatography. Thus, this method had been put aside. In our lab, we can produce **1** in large quantities without any chromatography,[26] and therefore, we decided to use a route for the synthesis of **2** using **1** as the starting material. As shown in Scheme 2.3, 2-phenylethan-1-ol was reacted with **1** under basic conditions to give **7**. Removal of the DMTr group of **7** under acidic conditions gave **6**. Tosylation of **6** under reported conditions gave **2**. This route is longer than the other two, but the products of all the steps are easy to purify, and it is our preferred route.



Scheme 2.3. Synthesis of monomer **2**.

With the monomer **2** in hand, the stepwise synthesis of monodisperse PEG using the one-pot elongation approach was investigated using the route in Scheme 4. The commercially available and inexpensive $(PEG)_4$ was deprotonated with excess NaH and reacted with monomer **2**. This gives the $(PEG)_{12}$ derivative **8**. The next reactions can elegantly show the convenience of the one-pot PEG elongation approach. The phenethyl groups in **8** was removed with $KN(TMS)_2$ and the intermediate alkoxide was reacted directly with **2** in one pot to give the $(PEG)_{20}$ derivative **9**. The same procedure was simply repeated to give PEG derivatives $Ph(CH_2)_2O(PEG)_{28}O(CH_2)_2Ph$ (**10**), $Ph(CH_2)_2O(PEG)_{36}O(CH_2)_2Ph$ (**11**), and $Ph(CH_2)_2O(PEG)_{44}O(CH_2)_2Ph$ (**12**). In the PEG elongation process, we used excess $KN(TMS)_2$ (2.5 equiv.) for the deprotection to overcome inadvertent moisture. To prevent the excess base from deprotecting the phenethyl groups in the monomer, before adding the monomer, the reaction mixture was cooled to -78 ^oC, and then the monomer solution was added, and the reaction mixture was warmed to room temperature slowly before heating the 60 ^oC. The careful manipulation of the temperature allowed the excess base to be selectively consumed via β-elimination of the tosylate of the monomer instead of removing its protecting group. As noted earlier, the side product of β-elimination of the tosylate does not affect the reaction while the side product of premature deprotection of the monomer would cause problems. The yields of the one-pot PEG elongation reactions were not optimized. They ranged from 25% to 86%. We believe that the yields can be improved by careful reaction workup and product purification, which is especially true for long PEG synthesis when the relatively hydrophobic phenethyl groups in the molecules are less capable to curtail the hydrophilicity of PEG moiety and to bring the product to organic phase during aqueous

workup. We also believe that the one-pot approach can be readily adopted for the synthesis of PEGs longer then $(PEG)_{44}$. Two facts are supportive of this speculation. One is that PEG depolymerization did not appear to be a significant problem according to MS (appendix A).[31] The other is that according to TLC (appendix A), the PEG products we made were not difficult to purify, and it is reasonable to predict that PEGs significantly longer than the ones we made will behave similarly.



Scheme 2.4. PEG synthesis using the one-pot PEG elongation approach.

The major advantage of using a base-labile protecting group such as the phenethyl group for stepwise monodisperse PEG synthesis is reducing the PEG elongation process from three steps in two pots to two steps in one pot. There is no need to isolate and purify the intermediate product after the deprotection step using the new approach. Because stepwise PEG synthesis requires repeating the PEG elongation process multiple times, shortening the process can make PEG synthesis significantly more convenient and

monodisperse PEG potentially much more affordable. In the literature, besides the DMTr group, other protecting groups, including benzyl and silyl groups, have also been used for PEG synthesis.[16, 19, 23, 25] However, like the DMTr group, when they were used, all required three steps – deprotection, deprotonation and coupling – in two pots for each PEG elongation. Therefore, the base-labile group is not only a better choice than the DMTr group, but also a better choice than any known protecting groups.

In addition to shortening the PEG elongation process from two pots to one pot, the base-labile protecting group is also easier to remove than other protecting groups. For example, the reaction to remove the DMTr group is reversible, and thus the reaction is difficult to complete when it is carried out at large scales. It is also reported that removing the group from PEGs can become more and more difficult as PEGs become longer.[17] For removing the benzyl group, palladium is usually needed for the hydrogenation reaction. Palladium is expensive, and more problematically, it is difficult to remove from the product.[16, 25] In contrast, the base-labile groups can be removed with an environmentally benign base at 0 ºC, and the reaction is irreversible and fast.[30]

The discovery that $KN(TMS)_2$ can efficiently remove phenethyl and other groups at 0 ºC is remarkable. In the literature, the presentiment suggested by data is that a strong base is needed for the reaction, and even with a base as strong as LDA, tBuOK has to be used to catalyze the reaction. Otherwise, the reaction would not occur. Our finding that $KN(TMS)_2$ alone can remove the phenethyl group and other groups is remarkable because this base is significantly less basic than LDA, and its solution in toluene and THF can be stored at room temperature for long period of time. In contrast, LDA has to

be stored at low temperatures and has a short lifetime. Usually, freshly preparing them before each use is preferred by most chemists.

There are several different routes for stepwise monodisperse PEG synthesis.[19] The base-labile protecting strategy can be easily incorporated into all those routes, and the routes can be shortened significantly by carrying out deprotection and coupling in one pot. We demonstrated the convenience of the one-pot PEG elongation approach using the route in Scheme 2.3. This route has the advantage of using the same monomer in each elongation cycle. In addition, the length of the monomer is significantly shorter than that of the product, and therefore, excess monomer can be used to drive the PEG elongation reactions to completion because the excess monomer can be easily removed from the product using chromatography. However, for the synthesis of PEGs longer than $(PEG)_{60}$ or asymmetric PEGs, routes using two different protecting groups such as the phenethyl and DMTr groups involving converting $DMTrO(PEG)_nO(CH_2)_2Ph$, where n is an integer, to $HO(PEG)_nO(CH_2)_2Ph$ and then to $TsO(PEG)_nO(CH_2)_2Ph$, and coupling $DMTrO(PEG)_nO(CH_2)_2Ph$ with $TsO(PEG)_nO(CH_2)_2Ph$ in one pot to give $DMTrO(PEG)_{2n}O(CH_2)_2Ph$ would be preferred. This route can make the length of the PEG double in each elongation cycle and the PEG product is asymmetric.

It is noted that the use of base-labile protecting groups or linkers in organic synthesis involving carrying out reactions under less basic reactions and removing the protecting group or cleaving the linker under more basic conditions is not common. In contrast, the use of acid-labile protecting groups or linkers involving carrying out reactions under less acidic conditions and removing the protecting group or cleaving the

linker under more acidic conditions is more frequently adopted. For example, in peptide synthesis, the trityl group can be selectively removed under acidic conditions in the presence of the acid-sensitive BAL linker,[32] the Boc group can be selectively removed in the presence of the acid-sensitive PAM linker,[33] and protected peptides can be cleaved from the acid-labile 2-chlorotrityl resin with dilute TFA without affecting acid-labile side-chain protecting groups.[34] In solid-phase RNA synthesis, the acid-sensitive 2'-TOM protecting groups can survive the acidic conditions for removing the 5'-DMTr groups.[35] The one-pot PEG elongation approach using a base-labile protecting group is feasible only if there is a large difference between the pKa of the hydroxyl group of PEG and that of the hydrogen in the protecting group that is needed to be removed by a base to initiate the deprotection via β-elimination. The pKa of the hydroxyl group of PEG is less than 15, while that of the β-hydrogen in the phenethyl group is probably over 40. The large difference between the pKa values ensures that the base-labile protecting group is stable under the basic Williamson ether formation reaction conditions. While the pKa of the β-hydrogen in the phenethyl group is over 40, β-elimination during deprotection can still occur readily at 0 ^{o}C even with the relatively weak base $KN(TMS)_2$, the conjugated acid of which has a pKa of only 26.[29] The reasons for this to happen may be attributed to the irreversibility of the β-elimination reaction, the low kinetic barrier of the reaction, and probably the inaccuracy of the pKa values as pKa values vary widely under different conditions.

2.3 Conclusion

In summary, a one-pot PEG elongation approach has been developed for stepwise monodisperse PEG synthesis. By using a base-labile protecting group such as the

phenethyl group instead of the commonly used groups such as the acid-labile DMTr group, the deprotection and coupling steps in PEG synthesis can be carried out in one pot instead of two pots. The deprotonation step is not needed using the new approach. In addition, due to the irreversibility of the reactions for their deprotection, the new protecting groups are also easier to remove. Our results showed that the PEG synthesis method is convenient to execute, and acceptable to high yields of PEG products can be obtained. We expect that the one-pot PEG elongation approach will be helpful to make monodisperse PEGs more affordable and have a positive impact in areas where monodisperse PEGs are needed.

2.4 Experimental Section

General information: All compounds from commercial sources were used as received unless noted otherwise. THF was distilled over Na/benzophenone under nitrogen. Compounds **3d**,[37] **3e**,[38] **3g**[39] and **3j**[39] were synthesized following reported procedure. All reactions were carried out under nitrogen using oven-dried glassware. Thin layer chromatography (TLC) was performed using Sigma-Aldrich TLC plates, silica gel 60F-254 over glass support, 250 μm thickness. ^{1}H and ^{13}C NMR spectra were obtained on a Varian UNITY INOVA spectrometer at 400 and 100 MHz, respectively. Chemical shifts (δ) were reported in reference to solvent peaks (residue $CHCl_3$ at δ 7.24 ppm for ^{1}H and $CDCl_3$ at δ 77.00 ppm for ^{13}C). HRMS was obtained on a Thermo HR-Orbitrap Elite Mass Spectrometer. LRMS was obtained on a Thermo Finnigan LCQ Advantage Ion Trap Mass Spectrometer.

*Screening base-labile protecting groups for PEG synthesis – Testing if the groups in **3a-l** can be removed under basic conditions*: In an oven dried 25 mL flask, **3a**-**k** or **3l** (0.734 mmol, 1 equiv.) was dissolved in THF (4 mL). The solution was cooled to -78 ºC. $KN(TMS)_2$ (1 M in THF, 1.4 mL, 1.468 mmol, 2 equiv.) was added via a syringe. The reaction mixture was stirred while warming to 0 ºC gradually. After 2 h, TLC analyses (see supporting information) were carried out. All compounds **3a**-**l** were found to be consumed. Thus, the base-labile protecting groups in them meet the criterium of being deprotectable under basic conditions required for PEG synthesis. Compound **3a** was also tested using the base tBuOK/LDA and found deprotectable under the conditions.[27, 28]

Screening base-labile protecting groups for PEG synthesis – Testing the stability of protecting groups under the basic Williamson ether formation conditions: Compounds $DMTrO(PEG)_4OTs$ (**1**)[26] and $MeO(PEG)_4OH$ (**4**) were dried over P_2O_5 in a desiccator under vacuum for 2 days. Compound **4** (41 mg, 0.201 mmol, 1 equiv.) was dissolved in THF (200 µL) under nitrogen. The solution was cooled to -78 ºC, and $KN(TMS)_2$ (0.241 mL, 1 M in THF, 1.2 equiv.) was added dropwise via a syringe. After addition, the reaction flask was placed in an ice bath for ~30 min. The mixture was then cooled to -78 ºC. The solution of **1** (195 mg, 0.301 mmol, 1.5 equiv.) and **3a**-**k** or **3l** (0.301 mmol, 1.5 equiv.) in THF (500 µL) was added via a cannula dropwise over ~1 min. The reaction mixture was warmed to rt gradually over ~3 h. After stirring at rt for ~30 min, the mixture was heated to 60 ºC and stirred vigorously at the temperature for 24 h. TLC analyses (see supporting information) were carried out to determine if the Williamson ether formation reaction could proceed to form product **5** without any consumption of the compound **3a**-**k** or **3l**. All the compounds were found to be able to survive the basic

Williamson ether formation reaction conditions. Thus, the base-labile protecting groups in them meet the criterium of being stable under basic coupling conditions required for PEG synthesis.

*DMTrO(PEG)$_4$O(CH$_2$)$_2$Ph (**7**)*: The suspension of NaH (60% in mineral oil, 716 mg, 17.92 mmol, 2.5 equiv.) in anhydrous DMF (25 mL) in a 1 L 2-neck round bottom flask under nitrogen was cooled on an ice bath. The solution of Ph(CH$_2$)$_2$OH (2.14 mL, 17.92 mmol, 2.5 equiv.) in anhydrous DMF (15 mL) was added dropwise via a cannula over ~1 h. After addition, the reaction mixture was stirred at 0 °C for ~1 h. The ice bath was removed. This gave the solution of NaO(CH$_2$)$_2$Ph. Compound **1** (contaminated with DMTrO(PEG)$_4$ODMTr; total 4.66 g; assumed 7.17 mmol as if it were pure, 1 equiv.), which had been dried over P_2O_5 under high vacuum overnight, was dissolved in anhydrous DMF (15 mL). The solution was added to the solution of NaO(CH$_2$)$_2$Ph dropwise via a cannula. After addition, the mixture was stirred vigorously at 60 °C for 24 h. After cooling to rt, the reaction was quenched with EtOH. DMF was removed on a rotary evaporator under high vacuum. The residue was partitioned between EtOAc (250 mL) and 5% K_2CO_3 (100 mL). The organic phase was washed with 5% K_2CO_3 (100 mL × 3), dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to dryness under reduced pressure and further dried under high vacuum. The residue was purified with flash chromatography (SiO_2, 10% Et_3N/hexanes) to give compound **7** (4.02 g, 95.6%), as a yellow oil: TLC R_f = 0.3 (SiO_2, hexanes/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.47 (d, 2H), 7.37-7.35 (d, 4H), 7.29-7.18 (m, 8H), 6.83-6.80 (m, 4H), 2.76-2.69 (m, 8H), 3.74 (s, 6H), 3.68-3.59 (m, 16H), 3.25-3.23 (t, 2H), 2.91-2.87 (t, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 145.3, 139.1, 136.5, 130.3, 129.1, 128.5, 128.4,

127.9, 126.8, 126.3, 113.2, 86.2, 72.6, 71.0, 70.5, 63.5, 55.5, 36.6; HRMS (ESI) calcd for $C_{37}H_{43}O_7Na$ $[M+Na]^+$ 623.2985, found 623.2971.

*$Ph(CH_2)_2O(PEG)_4$ (**6**)*: Compound **7** (2.17 g, 3.62 mmol, 1 equiv.) was dissolved in dry DCM (10 mL). To the solution was added TFA (433 μL, 3.62 mmol, 1 equiv.). The reaction mixture was stirred vigorously. After ~5 mins, TLC indicated that compound **7** was consumed. The reaction was quenched with solid NaOH and low quantity of water until pH ~9. The mixture was then partitioned between DCM (total about 200 mL) and brine (75 mL). The aqueous phase was washed with DCM (100 mL x 3). The combined organic phase was dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to dryness, and the residue was purified with flash chromatography (SiO_2, EtOAc) to give compound **6** (568 mg, 77.4%) as a yellow oil: TLC R_f = 0.10 (SiO_2, hexanes/EtOAc 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25-7.10 (m, 5H), 3.67-3.64 (t, 2H), 3.62-3.53 (m, 16H), 2.87-2.83 (t, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9, 129.1, 128.5, 126.3, 72.8, 70.8, 70.5, 91.9, 36.5; HRMS (ESI) calcd for $C_{16}H_{26}O_5H$ $[M+H]^+$ 299.1859, found 299.1847; $C_{16}H_{26}O_5Na$ $[M+Na]^+$ 321.1678, found 321.1662.

*$Ph(CH_2)_2O(PEG)_4OTs$ (**2**)*: The compound was synthesized using a reported procedure with modifications.[36] The solutions of **6** (9.22 g, 46.5 mmol, 1 equiv.) in THF (50 mL) and NaOH powder (22.3 g, 558 mmol, 12 equiv.) in water (50 mL) were combined and stirred at 0 °C for 5 min. The solution of TsCl (26.5 g, 139.5 mmol, 3 equiv.) in THF (50 mL, note that it is important to keep the ratio of total THF and water at around 2:1 v/v) was added dropwise over 10 min while the reaction mixture was stirred at 0 °C. After addition, stirring was continued while the temperature was raised to rt gradually. The

progress of the reaction was monitored by TLC, and complete reaction was observed within 24 h. The mixture was partitioned between 5% Na_2CO_3 (300 mL) and EtOAc (500 mL). The aqueous phase was extracted with EtOAc (200 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4 and filtered. Volatiles were removed under reduced pressure, and the residue was further dried under vacuum from an oil pump. Compound **2** (12.7 g, 60%) was obtained as a colorless oil after flash chromatography purification (SiO_2, hexanes/EtOAc 1:0 to 2:1): TLC R_f = 0.30 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.76 (d, 2H), 7.32-7.30 (d, 2H), 7.27-7.16 (m, 5H), 4.14-4.12 (t, 2H), 3.68-3.59 (m, 16H), 2.89-2.86 (t, 2H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.9, 139.0, 133.2, 129.9, 129.1, 128.5,128.1, 126.3, 72.5, 70.9, 70.8, 70.7, 70.5, 69.5, 68.9, 36.5, 21.9; HRMS (ESI) calcd for $C_{23}H_{31}O_7SH$ $[M+H]^+$ 453.1942, found 453.1953; $C_{23}H_{31}O_7SNH_4$ $[M+NH_4]^+$ 470.2207, found 470.2216; $C_{23}H_{31}O_7SNa$ $[M+Na]^+$ 474.1761, found 475.1775.

*$Ph(CH_2)_2O(PEG)_{12}O(CH_2)_2Ph$ (**8**)*: Compound **2** (2.19 g, 4.83 mmol, 2.5 equiv.) was dried over P_2O_5 under vacuum in a desiccator overnight. A suspension of NaH (60% in mineral oil, 193 mg, 4.83 mmol, 2.5 equiv.) in dry THF (5 mL) under nitrogen was cooled on an ice bath. The solution of $(PEG)_4$ (333 µL, 1.93 mmol, 1 equiv.) in dry THF (10 mL) was added via a cannula dropwise over ~20 min. After addition, the reaction was allowed to proceed for ~30 min. The ice bath was removed, and compound **2** in THF (10 mL) was added via a cannula dropwise over ~10 min. After addition, the mixture was stirred vigorously at 60 ºC for 24 h. The reaction was quenched with EtOH. THF was removed under reduced pressure. The residue was partitioned between DCM (100 mL) and saturated NH_4Cl (50 mL). The aqueous phase was washed with DCM (100 mL × 3).

The combined organic phase was dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to dryness, and compound **8** was purified with flash chromatography (SiO_2, EtOAc/MeOH 100:0 to 100:3) to give a colorless oil (1.4 g, 97%): TLC R_f = 0.50 (SiO_2, DCM/Et_2O/MeOH 3:0.6:0.6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.712 (m, 10H), 3.64-3.55 (m 51H), 2.87-2.83 (t, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.9, 129.02, 128.44, 126.28, 72.52, 70.80, 70.90, 36.54 HRMS (ESI) calcd for $C_{40}H_{66}O_{13}Na$ $[M+Na]^+$ 777.4401, found 777.4436; $C_{40}H_{66}O_{13}Na_2$ $[M+2Na]^{2+}$ 400.2150, found 400.2112.

*$Ph(CH_2)_2O(PEG)_{20}O(CH_2)_2Ph$ (**9**)*: Compounds **2** and **8** were dried over P_2O_5 in a desiccator under vacuum for 2 days. Compound **8** (1.3 g, 1.8 mmol, 1 equiv.) was dissolved in dry THF (5 mL) under nitrogen. The solution was cooled to -78 ^{o}C, and $KN(TMS)_2$ (4.6 mL, 1 M in THF, 2.5 equiv.) was added dropwise via a syringe. After addition, the reaction flask was placed in an ice bath for ~3 h. TLC analysis indicated that both **8** and $Ph(CH_2)_2O(PEG)_{12}$ were not in the reaction mixture. The mixture was then cooled to -78 ^{o}C for ~10 min, and the solution of **2** (3.8 g, 8.3 mmol, 4.5 equiv.) in THF (10 mL) was added dropwise via a cannula over ~10 min. The reaction mixture was allowed to warm up to room temperature gradually over a period of ~3 h. After stirring at room temperature for ~30 min, the mixture was heated to 60 ^{o}C and stirred vigorously at the temperature for 24 h. THF was removed under reduced pressure. The residue was partitioned between DCM (100 mL) and saturated NH_4Cl (20 mL). The aqueous phase was washed with DCM (100 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4 and filtered. Flash chromatography (SiO_2, EtOAc to DCM/Et_2O/MeOH 100:8:4) gave compound **9** (1.765 g, 86%) as a yellow waxy solid: TLC R_f = 0.40 (SiO_2, DCM/Et_2O/MeOH 6:0.6:0.6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-

7.15 (m, 10H), 3.67-3.57 (m 81H), 2.90-2.88 (t, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.97, 129.08, 128.50, 126.34, 72.60, 70.80, 70.50, 36.55; HRMS (ESI) calcd for $C_{56}H_{98}O_{21}Na$ $[M+Na]^+$ 1129.6499, found 1129.6533; $C_{56}H_{98}O_{21}H_2$ $[M+2H]^{2+}$ 554.3379, found 554.3390. Compound **9** was also synthesized using tBuOK/LDA instead of $KN(TMS)_2$ as the base under otherwise identical conditions. Similar yields were obtained.

*$Ph(CH_2)_2O(PEG)_{28}O(CH_2)_2Ph$ (**10**)*: Synthesized using the procedure for the synthesis of **9**. Compound **9** (1.77 g, 1.59 mmol, 1 equiv.) in THF (10 mL), $KN(TMS)_2$ (3.39 mL, 1 M in THF, 2.5 equiv.), and **2** (3.24 g, 7.15 mmol, 4.5 equiv.) in THF (10 mL) gave the crude product, which was subjected to aqueous workup and chromatography purification as describe for **9**. Compound **10** (1.6 g, 70%) was obtained as a yellow waxy solid: TLC R_f = 0.40 (SiO_2, DCM/Et_2O/MeOH 6:0.6:0.6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.15 (m, 10H), 3.67-3.56 (m 116H), 2.89-2.85 (t, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.02, 129.04, 128.46, 126.30, 72.54, 70.80, 36.56; HRMS (ESI) calcd for $C_{74}H_{134}O_{30}Na$ $[M+Na]^+$ 1481.8596, found 1481.8571; $C_{74}H_{134}O_{30}Na_2$ $[M+2Na]^{2+}$ 752.4247, found 752.4247; $C_{74}H_{134}O_{30}H_3$ $[M+3H]^{3+}$ 487.2977, found 487.2971.

*$Ph(CH_2)_2O(PEG)_{36}O(CH_2)_2Ph$ (**11**)*: Synthesized using the procedure for the synthesis of **9**. Compound **10** (1.375 g, 0.942 mmol, 1 equiv.) in THF (10 mL), $KN(TMS)_2$ (2.4 mL, 1 M in THF, 2.5 equiv.), and 2 (1.7 g, 3.8 mmol, 4 equiv.) in THF (10 mL) gave the crude product, which was subjected to aqueous workup and chromatography purification as describe for **9**. Compound **11** (436 mg, 25%) was obtained as a yellow waxy solid: TLC R_f = 0.40 (SiO_2, DCM/Et_2O/MeOH 6:0.6:0.6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25-7.15

(m, 10H), 3.65-3.59 (m 148H), 2.87-2.83 (t, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.00, 129.02, 128.44, 126.29, 72.53, 70.79, 70.49, 36.54; HRMS (ESI) calcd for $C_{88}H_{162}O_{37}N_2H_8$ $[M+2NH_4]^{2+}$ 923.5742, found 923.5701; $C_{88}H_{162}O_{37}N_3H_{12}$ $[M+3NH_4]^{3+}$ 621.7276, found 621.7269.

*$Ph(CH_2)_2O(PEG)_{44}O(CH_2)_2Ph$ (**12**)*: Synthesized using the procedure for the synthesis of **9**. Compound **11** (386 mg, 0.241 mmol, 1 equiv.) in THF (10 mL), $KN(TMS)_2$ (0.532 mL, 1 M in THF, 2.5 equiv.), and **2** (436 mg, 0.964 mmol, 4 equiv.) in THF (10 mL) gave the crude product, which was subjected to aqueous workup and chromatography purification as describe for **9**. Compound **12** (199 mg, 43%) was obtained as a yellow waxy solid: TLC, R_f = 0.50 (SiO_2, DCM/Et_2O/MeOH 6:1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.13 (m, 10H), 3.76-3.38 (m 179H), 2.85-2.81 (t, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.95, 129.02, 128.44, 126.29, 72.53, 70.77, 70.47, 36.52; HRMS (ESI) calcd for $C_{104}H_{194}O_{45}N_2H_8$ $[M+2NH_4]^{2+}$ 1099.6790, found 1099.6711; $C_{104}H_{194}O_{45}N_3H_{12}$ $[M+3NH_4]^{3+}$ 739.1308, found 739.1266; $C_{104}H_{194}O_{45}N_4H_{16}$ $[M+4NH_4]^{4+}$ 558.8663, found 558.8548.

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Appendix A. Supporting information for Chapter 2

PEG Synthesis Featuring PEG Elongation in One Pot

TLC images for screening base-labile protecting groups for PEG synthesis – Testing if the groups in 3a-l can be removed under basic conditions

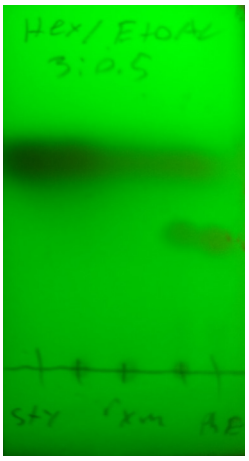


Figure A.1. TLC for testing if -$(CH_2)_2$Ph group can be deprotected using $KN(TMS)_2$. Left lane: styrene; middle lane: reaction mixture; right lane: Ph$(CH_2)_2$OMe (**3a**); 2nd and 4th lanes: co-spot of materials spotted on their adjacent lanes. Eluent: hexanes/EtOAc 3:0.5. The TLC indicates that **3a** was consumed and styrene was formed.

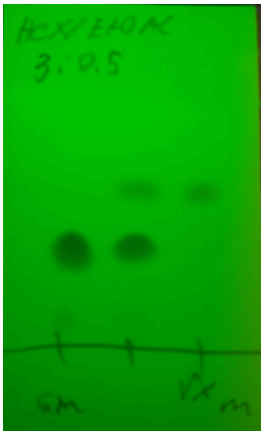


Figure A.2. TLC for testing if -$(CH_2)_2$Ph(4-OMe) group can be deprotected using $KN(TMS)_2$. Left lane: MeO$(CH_2)_2$Ph(4-OMe) (**3b**); middle lane: co-spot of materials on the left and right lanes; right lane: reaction mixture. Eluent: hexanes/EtOAc 3:0.5. The TLC indicates that **3b** was consumed.

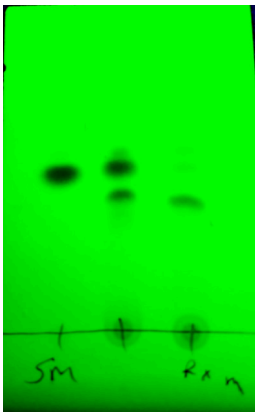


Figure A.3. TLC for testing if -$(CH_2)_2$Ph(4-NO_2) group can be deprotected by $KN(TMS)_2$. Left lane: MeO$(CH_2)_2$Ph(4-NO_2) (**3c**); middle lane: co-spot of materials on left and right lanes; right lane: reaction mixture. Eluent: hexanes/EtOAc 3:0.5. The TLC indicates that **3c** was consumed.

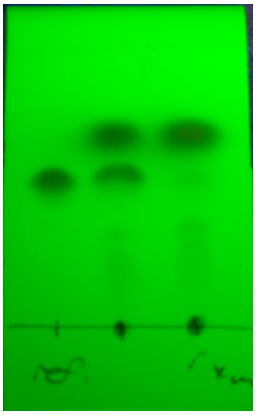


Figure A.4. TLC for testing if -$(CH_2)_2$Ph(3-F) group can be deprotected by $KN(TMS)_2$. Left lane: MeO$(CH_2)_2$Ph(3-F) (**3d**); middle lane: co-spot of materials on left and right lanes; right lane: reaction mixture. Eluent: hexanes/EtOAc 3:1. The TLC indicates that **3d** was consumed.

Figure A.5. TLC for testing if -$(CH_2)_2$-furan group can be deprotected by $KN(TMS)_2$. Left lane: $MeO(CH_2)_2$-furan (**3e**); middle lane: co-spot of materials on left and right lanes; right lane: reaction mixture. Eluent: hexanes/EtOAc 2:1. The TLC indicates that **3e** was consumed.

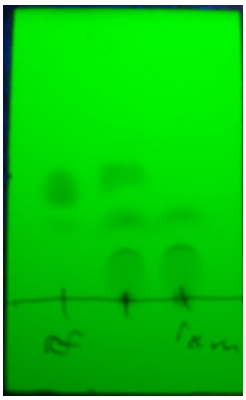


Figure A.6. TLC for testing if -$(CH_2)_2CH=CH_2$ group can be deprotected by $KN(TMS)_2$. Left lane: $BnO(CH_2)_2CH=CH_2$ (**3f**); middle lane: co-spot of materials on left and right lanes; right lane: reaction mixture. Eluent: hexanes/EtOAc 9:1. The TLC indicates that **3f** was consumed.

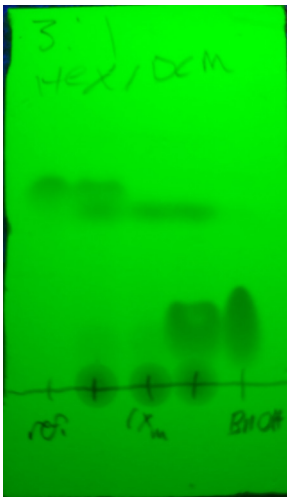


Figure A.7. TLC for testing if -$(CH_2)_2C{\equiv}CMe$ group can be deprotected by $KN(TMS)_2$. Left lane: $BnO(CH_2)_2C{\equiv}CMe$ (**3g**); middle lane, reaction mixture; right lane: BnOH; 2nd and 4th lanes, co-spot of materials spotted on their adjacent lanes. Eluent: hexanes/DCM 3:1. The TLC indicates that **3g** was consumed.

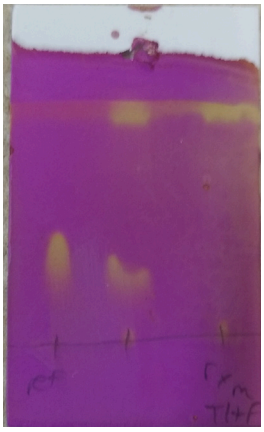


Figure A.8. TLC for testing if -$(CH_2)_2C(=O)NMe_2$ group can be deprotected by $KN(TMS)_2$. Left lane: $MeO(CH_2)_2C(=O)NMe_2$ (**3h**); middle lane: co-spot of materials on left and right lanes; right lane: reaction mixture. Eluent: EtOAc. The TLC indicates that **3h** was consumed.

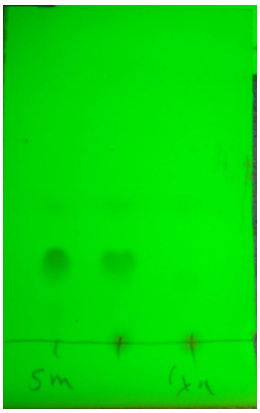


Figure A.9. TLC for testing if -$(CH_2)_2CN$ can be deprotected by $KN(TMS)_2$. Left lane: $BnOCH_2)_2CN$ (**3i**); middle lane: co-spot of materials on left and right lanes; right lane: reaction mixture. Eluent: hexanes/EtOAc 3:0.5. The TLC indicates that **3i** was consumed.

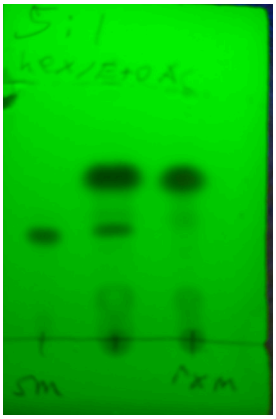


Figure A.10. TLC for testing if -$CH_2CH(SCH_2)_2CH_2$ group can be deprotected by $KN(TMS)_2$. Left lane: $BnOCH_2CH(SCH_2)_2CH_2$ (**3j**); middle lane: co-spot of materials on the left and right lanes; right lane: reaction mixture. Eluent: hexanes/EtOAc 5:1. The TLC indicates that **3j** was consumed.

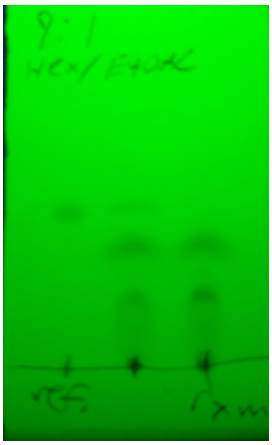


Figure A.11. TLC for testing if -CH_2CH=CHMe group can be deprotected by $KN(TMS)_2$. Left lane: $BnOCH_2CH$=CHMe (**3k**); middle lane: co-spot of materials on left and right lanes; right lane: reaction mixture. Eluent: hexanes/EtOAc 9:1. The TLC indicates that **3k** was consumed.

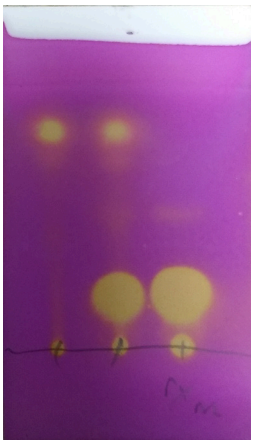


Figure A.12. TLC for testing if -CH_2C≡CMe group can be deprotected by $KN(TMS)_2$. Left lane: $EtOCH_2C$≡CMe (**3l**); middle lane: co-spot of materials on left and right lanes; right lane: reaction mixture. Eluent: hexanes/EtOAc 5:1. The TLC indicates that **3l** was consumed.

TLC images for screening base-labile protecting groups for PEG synthesis – Testing stability of protecting groups under the basic Williamson ether formation conditions

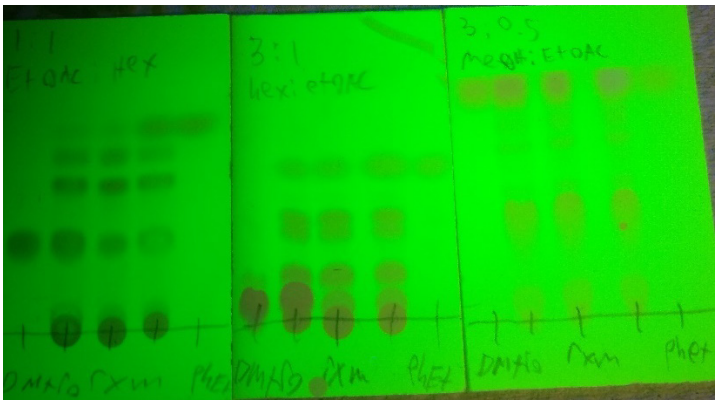


Figure A.13. TLC for testing the stability of -$(CH_2)_2$Ph group under Williamson ether formation conditions. For all three TLC: left lane, DMTrO$(PEG)_4$OTs (**1**); middle lane, reaction mixture; right lane, Ph$(CH_2)_2$OMe (**3a**); 2nd and 4th lanes, co-spot of materials spotted on their adjacent lanes. Eluent: left TLC, EtOAc/hexanes 1:1; middle TLC, EtOAc/hexanes 1:3; right TLC, EtOAc/MeOH 3:0.5. Left and middle TLC indicate that **3a** was not consumed. Right TLC indicates that DMTrO$(PEG)_8$OMe (**5**), which has a R_f of 0.40 and identified with ESI MS, was formed.

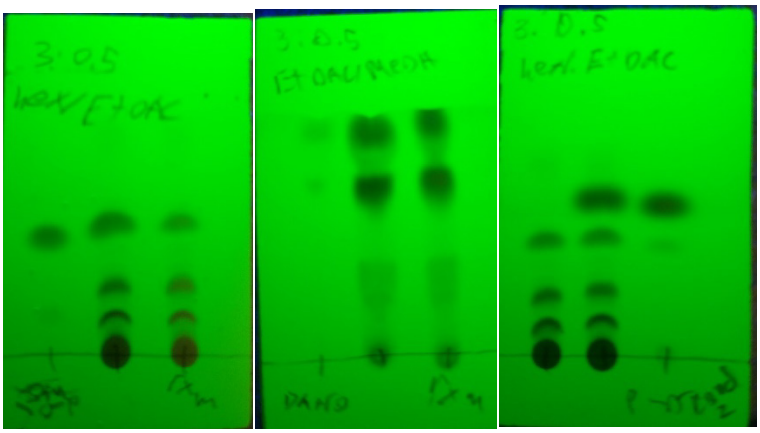


Figure A.14. TLC for testing the stability of -$(CH_2)_2$Ph(4-OMe) group under Williamson ether formation conditions. Left TLC: left lane, MeO$(CH_2)_2$Ph(4-OMe) (**3b**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that **3b** was not consumed. Middle TLC: left lane, DMTrO$(PEG)_8$OMe (**5**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that product **5** was formed. Right TLC: left lane, reaction mixture; middle lane, co-spot of materials on the left and right lanes; righ lane, reaction mixture of the β-elimination reaction of **3b**. The TLC shows that the β-elimination product of **3b** was not formed. Eluent: left TLC, hexanes/EtOAc 3:0.5; middle TLC, EtOAc/MeOH 3:0.5; right TLC, hexanes/EtOAc 3:0.5.

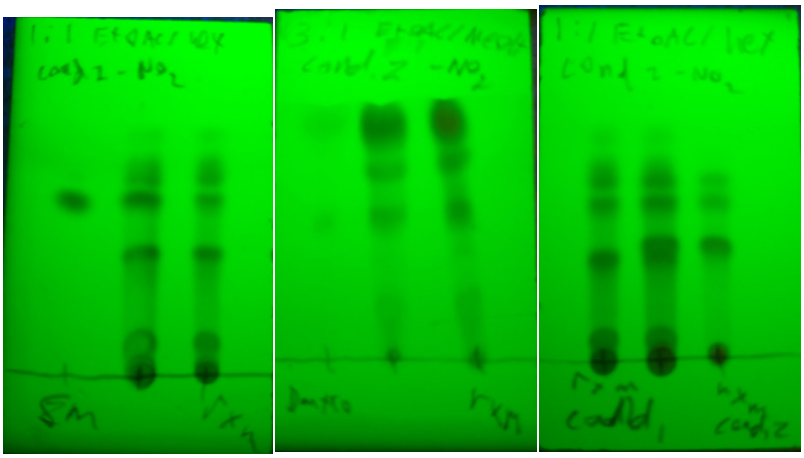


Figure A.15. TLC for testing the stability of -$(CH_2)_2$Ph(4-NO_2) group under Williamson ether formation conditions. Left TLC: left lane, MeO$(CH_2)_2$Ph(4-NO_2) (**3c**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that **3c** was not consumed. Middle TLC: left lane, DMTrO$(PEG)_8$OMe (**5**); middle lane, co-spot of material on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Right TLC: left lane, reaction mixture; middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture of the β-elimination reaction of **3c**. The TLC shows that the β-elimination product of **3c** was not formed. Eluent: left TLC, hexanes/EtOAc 1:1; middle TLC, EtOAc/MeOH 3:1; right TLC, hexanes/EtOAc 1:1.

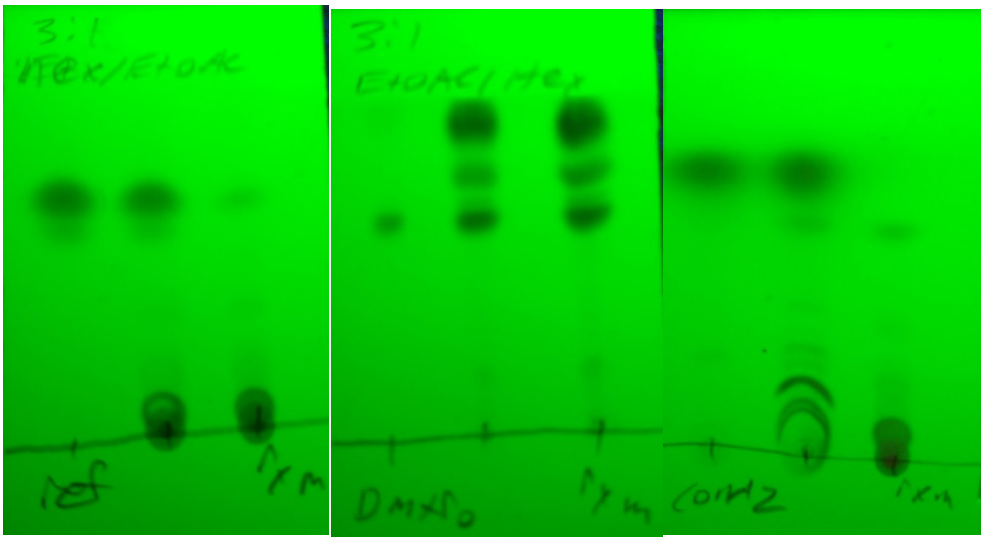


Figure A.16. TLC for testing the stability of -$(CH_2)_2$Ph(3-F) group under Williamson ether formation conditions. Left TLC: left lane, MeO$(CH_2)_2$Ph(3-F) (**3d**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that **3d** was not consumed. Middle TLC: left lane, DMTrO$(PEG)_8$OMe (**5**); middle lane, co-spot of material on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Right TLC: left lane, reaction mixture of the β-elimination reaction of **3d**; middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that the β-elimination product of **3d** was not formed. Eluent: left TLC, hexanes/EtOAc 3:1; middle TLC, EtOAc/MeOH 3:1; right TLC, hexanes/EtOAc 3:1.

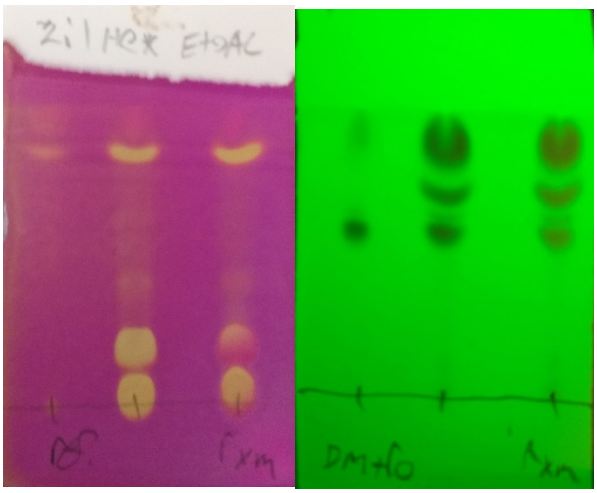


Figure A.17. TLC for testing the stability of -$(CH_2)_2$-furan group under Williamson ether formation conditions. Left TLC: left lane, MeO$(CH_2)_2$-furan (**3e**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that the **3e** was consumed. Right TLC: left lane, DMTrO$(PEG)_8$OMe (**5**); middle lane, co-spot of material on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Eluent: left TLC, hexanes/EtOAc 2:1; right TLC, EtOAc/MeOH 3:1.

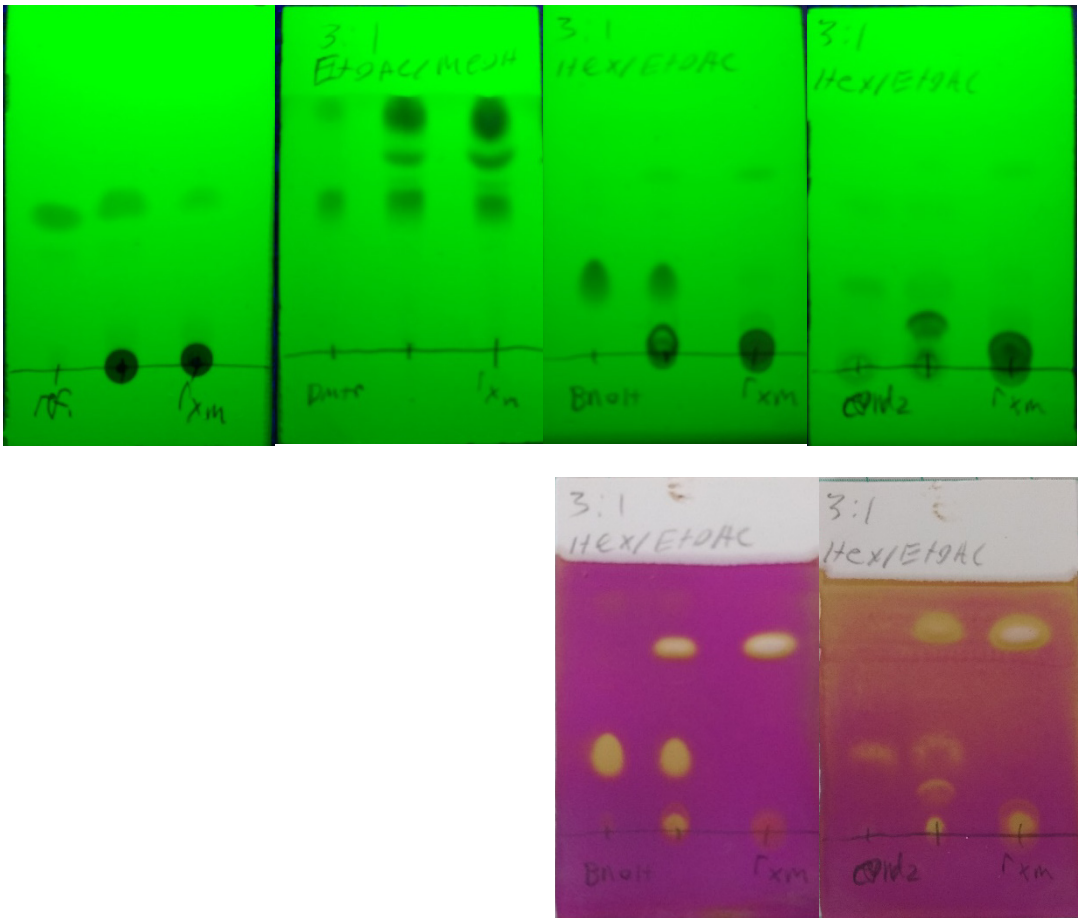


Figure A.18. TLC for testing the stability of -$(CH_2)_2CH=CH_2$ group under Williamson ether formation conditions. Reading from left to right, first TLC: left lane, $BnO(CH_2)_2CH=CH_2$ (**3f**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that **3f** was not consumed. Second TLC: left lane, $DMTrO(PEG)_8OMe$ (**5**); middle lane, co-spot of material on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Third TLC: left lane, BnOH; middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The $KMnO_4$ stain is below to further show that β-elimination product was not formed. Fourth TLC: left lane, reaction mixture of the β-elimination reaction of **3f**; middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that the β-elimination product of **3f** was not formed. The $KMnO_4$ stain is below to further show that β-elimination product was not formed. Eluent: first TLC, hexanes/EtOAc 9:1; second TLC, EtOAc/MeOH 3:1; third TLC, hexanes/EtOAc 3:1; fourth TLC, hexanes/EtOAc 3:1.

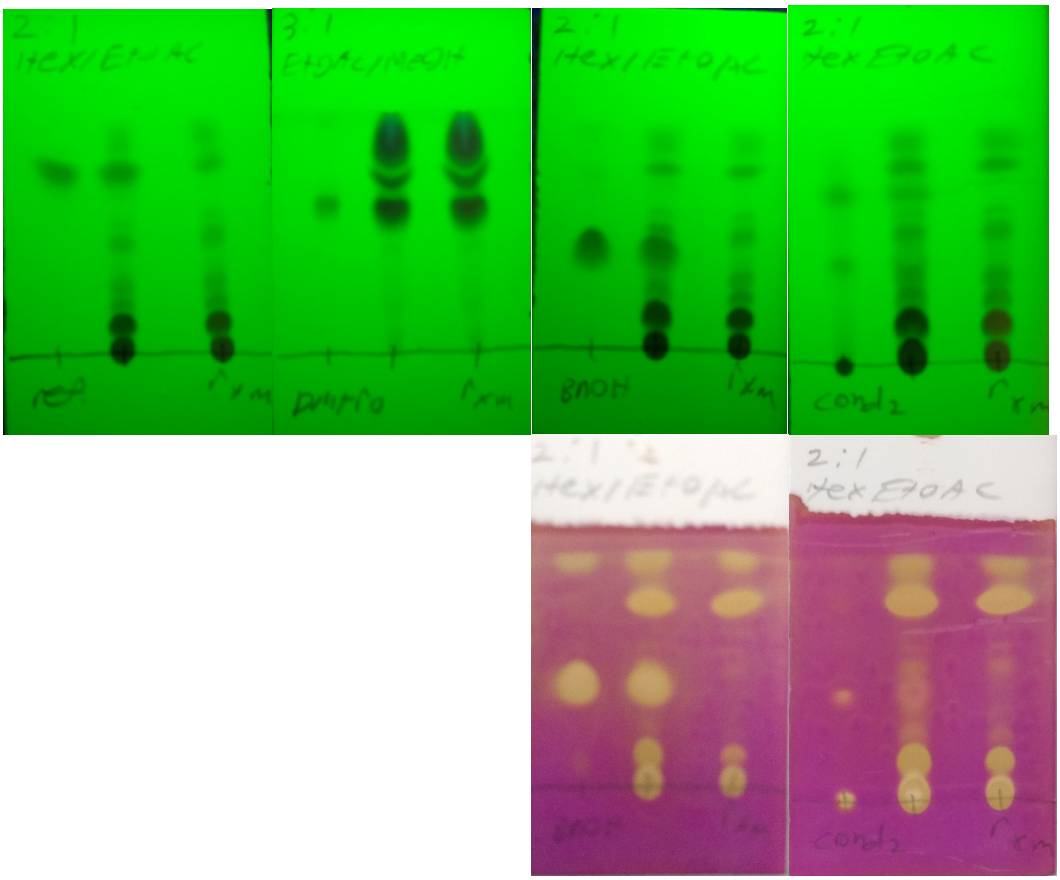


Figure A.19. TLC for testing the stability of -$(CH_2)_2C{\equiv}CMe$ group under Williamson ether formation conditions. Reading from left to right, first TLC: left lane, $BnO(CH_2)_2C{\equiv}CMe$ (**3g**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that **3g** was not consumed. Second TLC: left lane, $DMTrO(PEG)_8OMe$ (**5**); middle lane, co-spot of material on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Third TLC: left lane, BnOH; middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The $KMnO_4$ stain is below to further show that β-elimination product was not formed. Fourth TLC: left lane, reaction mixture of the β-elimination reaction of **3g**; middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that the β-elimination product of **3g** was not formed. The $KMnO_4$ stain is below to further show that β-elimination product was not formed. Eluent: first TLC, hexanes/EtOAc 3:1; second TLC, EtOAc/MeOH 3:1; third TLC, hexanes/EtOAc 3:1; fourth TLC, hexanes/EtOAc 3:1.

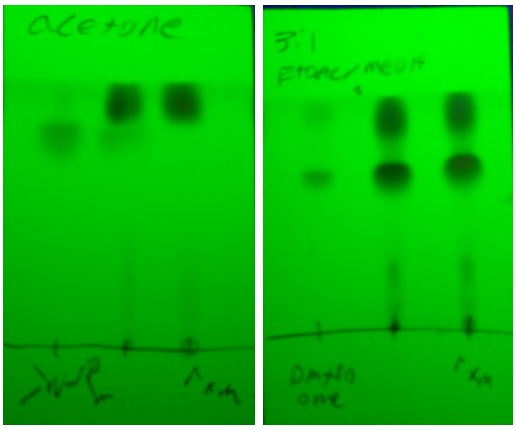


Figure A.20. TLC for testing the stability of -$(CH_2)_2C(=O)NMe_2$ group under Williamson ether formation conditions. Left TLC: left lane, $MeO(CH_2)_2C(=O)NMe_2$ (**3h**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that the **3h** was consumed. Right TLC: left lane, $DMTrO(PEG)_8OMe$ (**5**); middle lane, co-spot of material on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Eluent: left TLC, acetone; right TLC, EtOAc/MeOH 3:1.

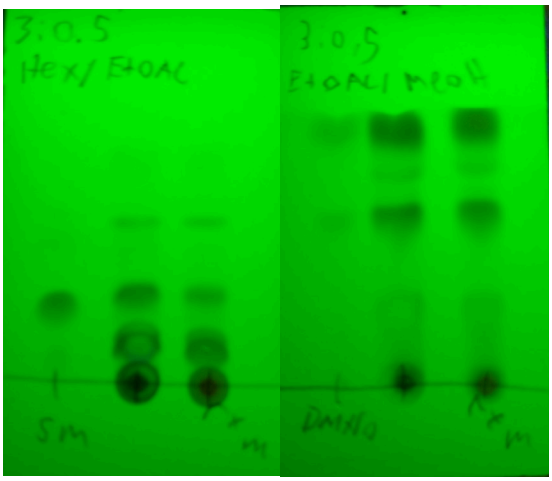


Figure A.21. TLC for testing the stability of -$(CH_2)_2CN$ group under Williamson ether formation conditions. Left TLC: left lane, $BnO(CH_2)_2CN$ (**3i**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that the **3i** was not consumed. Right TLC: left lane, $DMTrO(PEG)_8OMe$ (**5**); middle lane, co-spot of material on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Eluent: left TLC, hexanes/EtOAc 3:0.5; right TLC, EtOAc/MeOH 3:0.5.

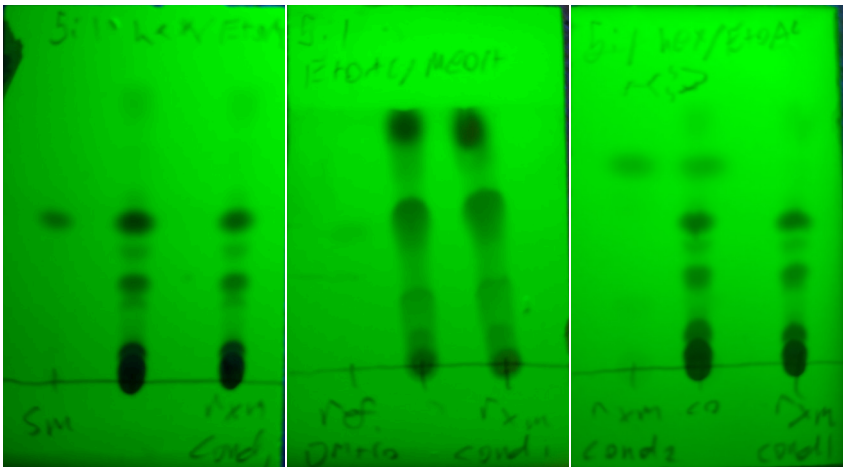


Figure A.22. TLC for testing the stability of -$CH_2CH(SCH_2)_2CH_2$ group under Williamson ether formation conditions. Left TLC: left lane, $BnOCH_2CH$-$(SCH_2)_2CH_2$ (**3j**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that **3j** was not consumed. Middle TLC: left lane, $DMTrO(PEG)_8OMe$ (**5**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Right TLC: left lane, reaction mixture of the β-elimination reaction of **3j**; middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that the β-elimination product of **3j** was not formed. Eluent: left TLC, hexanes/EtOAc 5:1; middle TLC, EtOAc/MeOH 5:1; right TLC, hexanes/EtOAc 5:1.

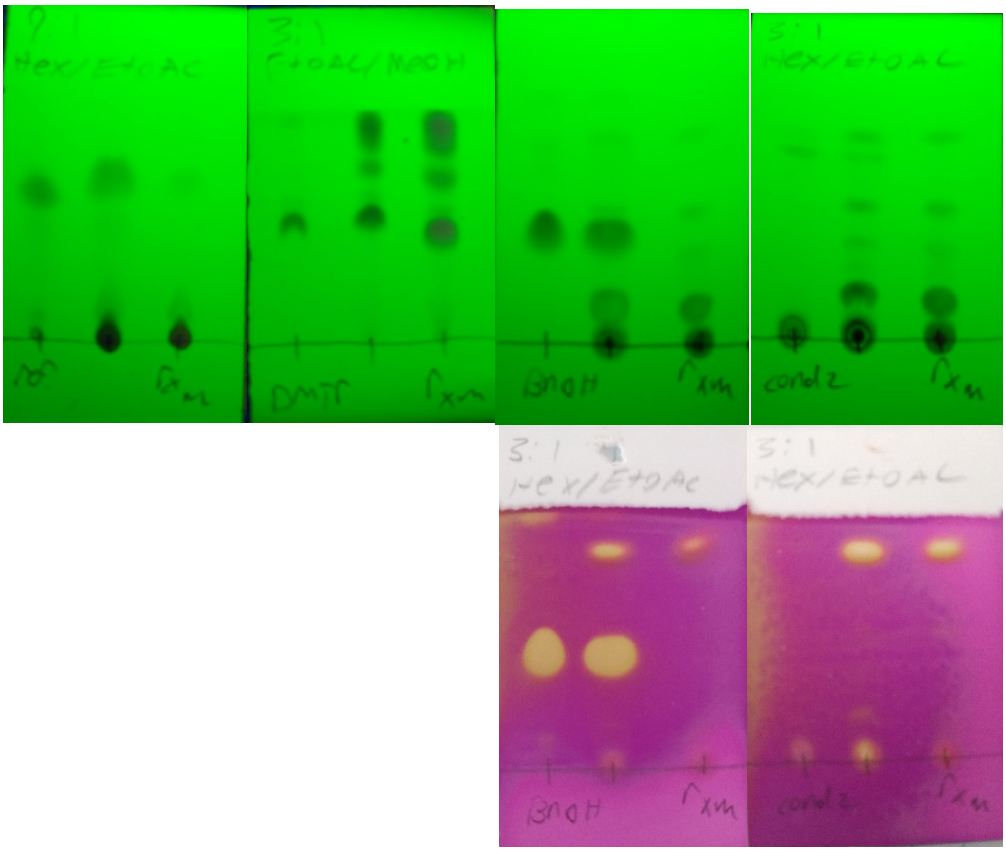


Figure A.23. TLC for testing the stability of -CH_2CH=$CHMe$ group under Williamson ether formation conditions. Reading from left to right, first TLC: left lane, $BnOCH_2CH$=$CHMe$ (**3k**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that **3k** was not consumed. Second TLC: left lane, $DMTrO(PEG)_8OMe$ (**5**); middle lane, co-spot of material on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Third TLC: left lane, BnOH; middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The $KMnO_4$ stain is below to further show that β-elimination product was not formed. Fourth TLC: left lane, reaction mixture of the β-elimination reaction of **3k**; middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that the β-elimination product of **3k** was not formed. The $KMnO_4$ stain is below to further show that β-elimination product was not formed. Eluent: first TLC, hexanes/EtOAc 9:1; second TLC, EtOAc/MeOH 3:1; third TLC, hexanes/EtOAc 3:1; fourth TLC, hexanes/EtOAc 3:1.

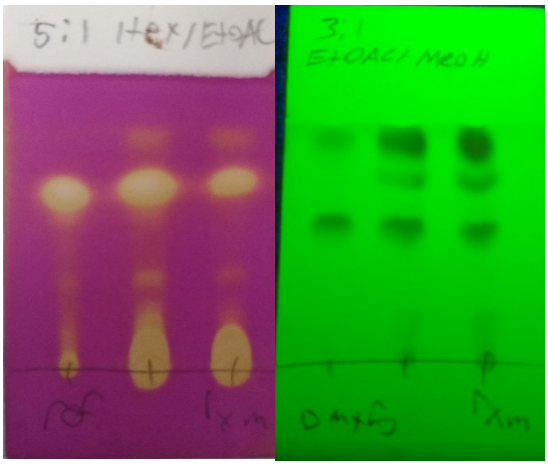
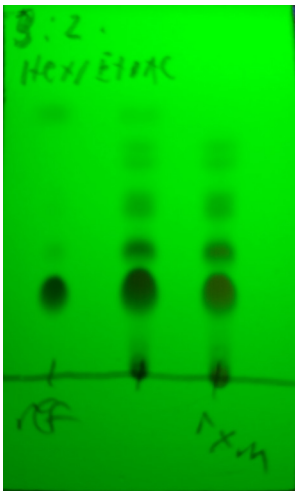


Figure A.24. TLC for testing the stability of -$CH_2C{\equiv}CMe$ group under Williamson ether formation conditions. Left TLC: left lane, EtOCH2C≡CMe (**3l**); middle lane, co-spot of materials on the left and right lanes; right lane, reaction mixture. The TLC shows that the **3l** was not consumed. Right TLC: left lane, $DMTrO(PEG)_8OMe$ (**5**); middle lane, co-spot of material on the left and right lanes; right lane, reaction mixture. The TLC shows that **5** was formed. Eluent: left TLC, hexanes/EtOAc 5:1; right TLC, EtOAc/MeOH 3:1.



DMTrO(PEG)$_4$O(CH$_2$)$_2$Ph (**7**)

Figure A.25. TLC of crude **7**. Eluent: hexanes/EtOAc 3:2. Left lane, DMTrO(PEG)$_4$OTs (**1**); middle lane, co-spot of materials on left and right lanes; right lane, crude **7**.

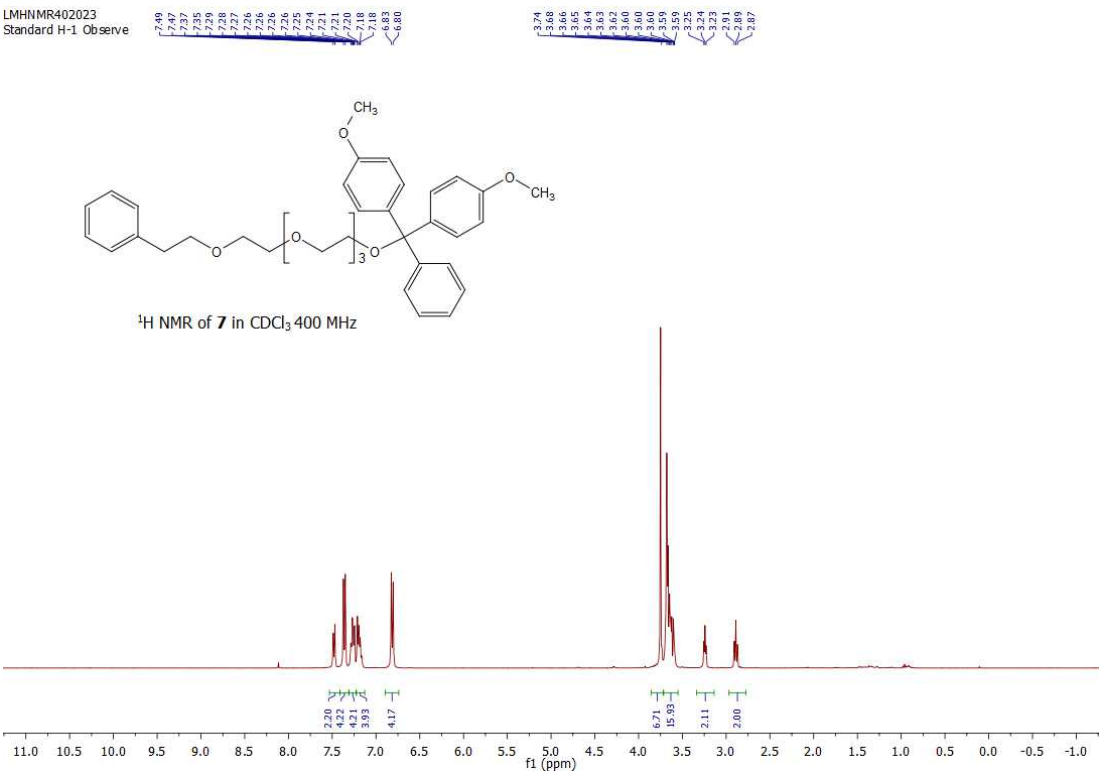


Figure A.26. ^{1}H NMR of Ph(CH$_2$)$_2$O(PEG)$_4$ODMTr (**7**).

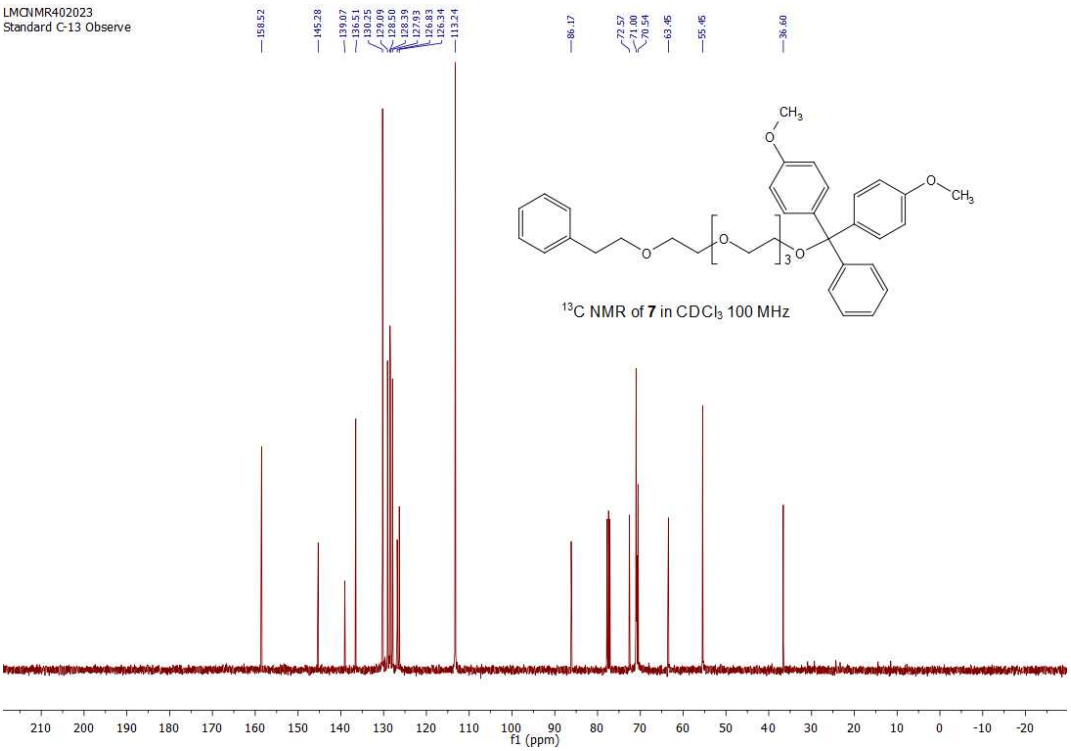


Figure A.27. ^{13}C NMR of $Ph(CH_2)_2O(PEG)_4ODMTr$ (**7**).

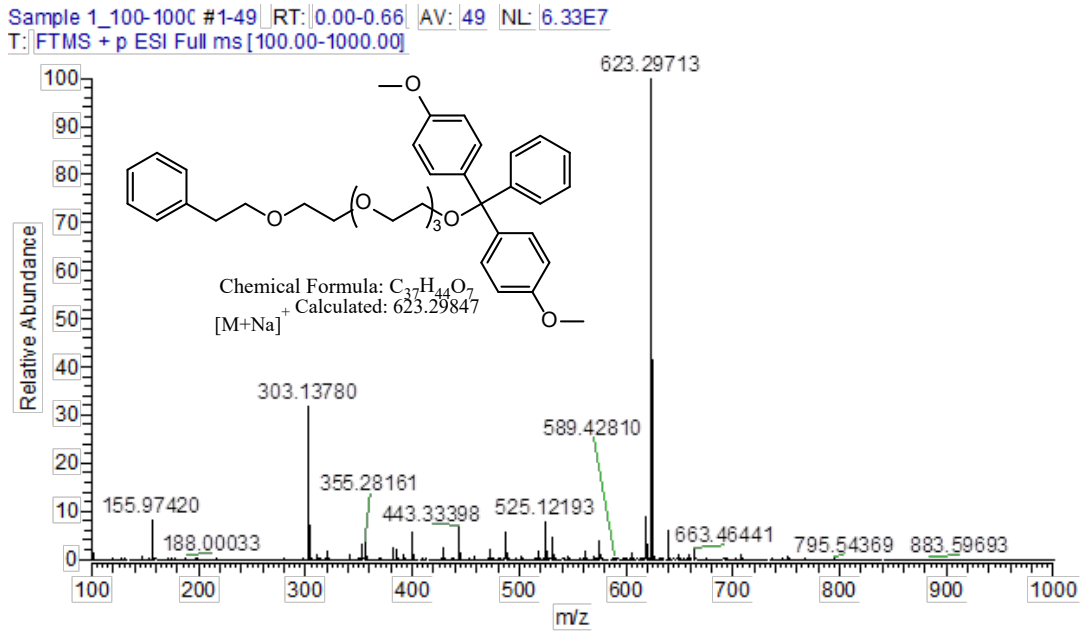


Figure A.28. ESI-MS of $Ph(CH_2)_2O(PEG)_4ODMTr$ (**7**).

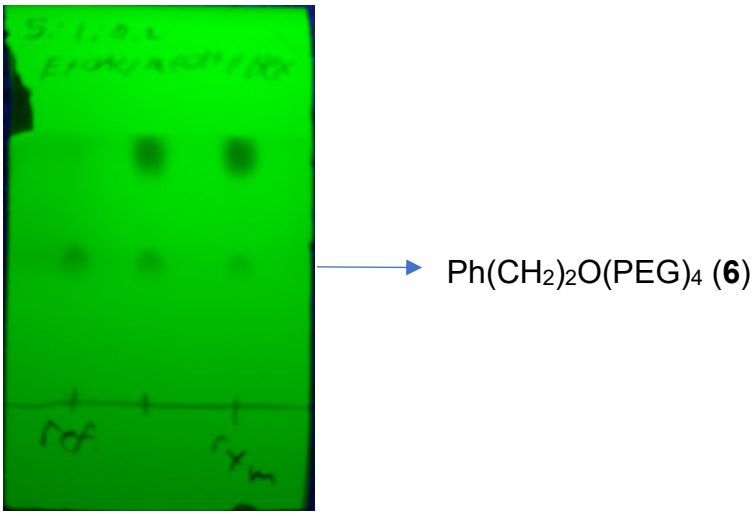


Figure A.29. TLC of crude **6**. Eluent: EtOAc/MeOH/hexanes 5:1:0.2. Left lane, purified **6**; middle lane, co-spot of materials on left and right lanes; right lane, crude **6**.

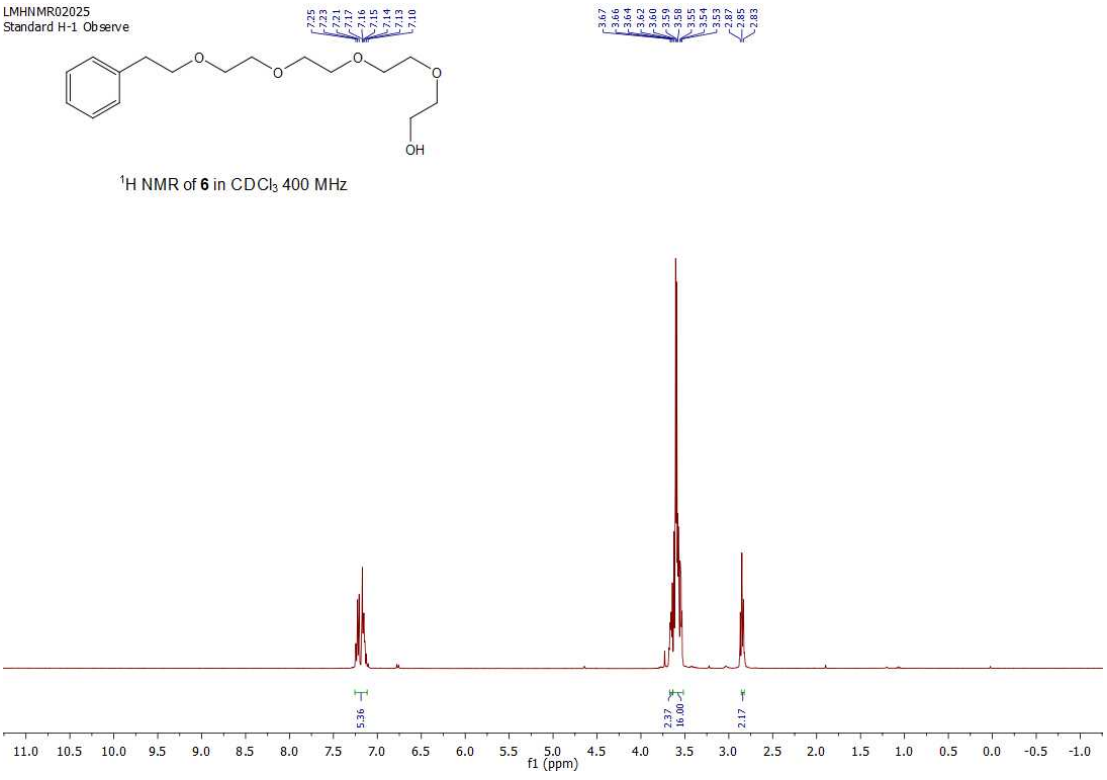


Figure A.30. ^{1}H NMR of $Ph(CH_2)_2O(PEG)_4$ (**6**).

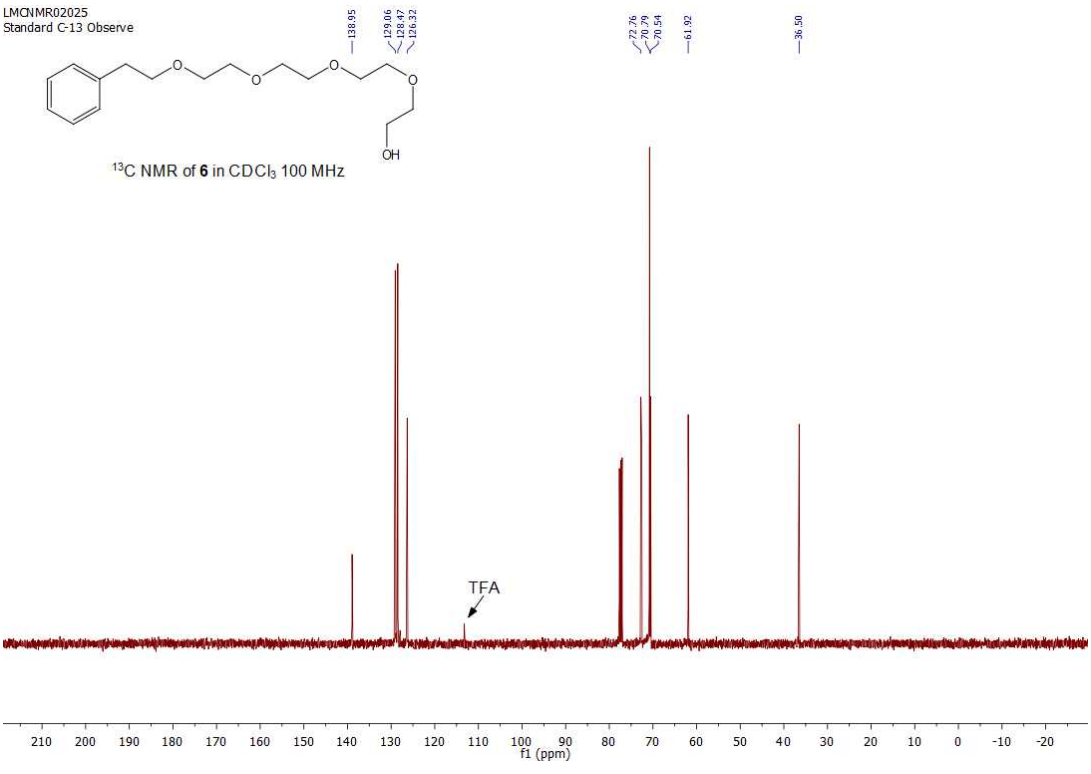


Figure A.31. ^{13}C NMR of $Ph(CH_2)_2O(PEG)_4$ (**6**).

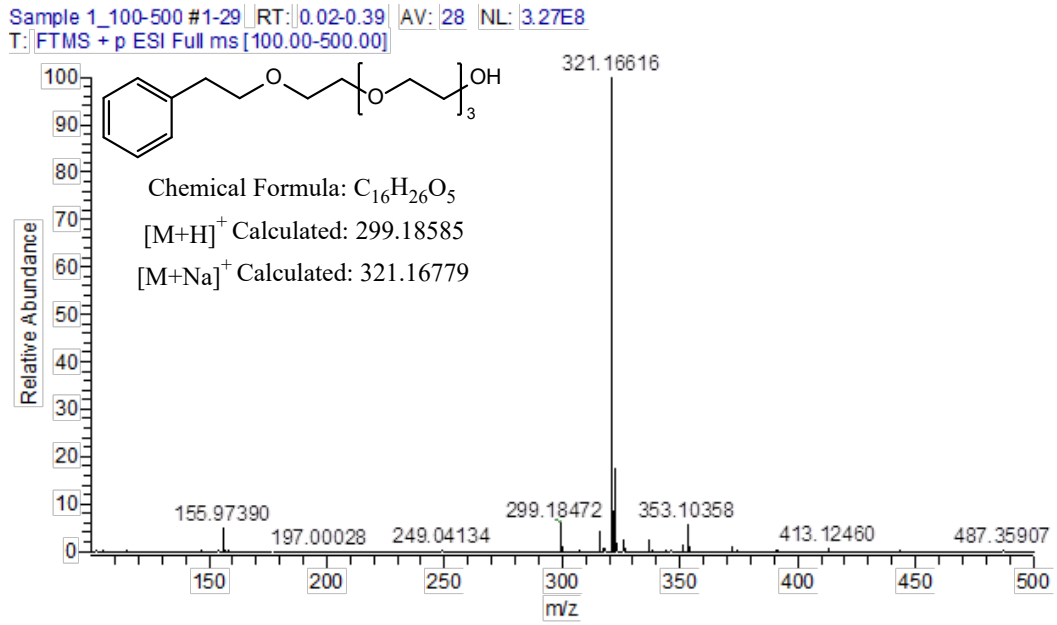


Figure A.32. ESI-MS of $Ph(CH_2)_2O(PEG)_4$ (**6**).

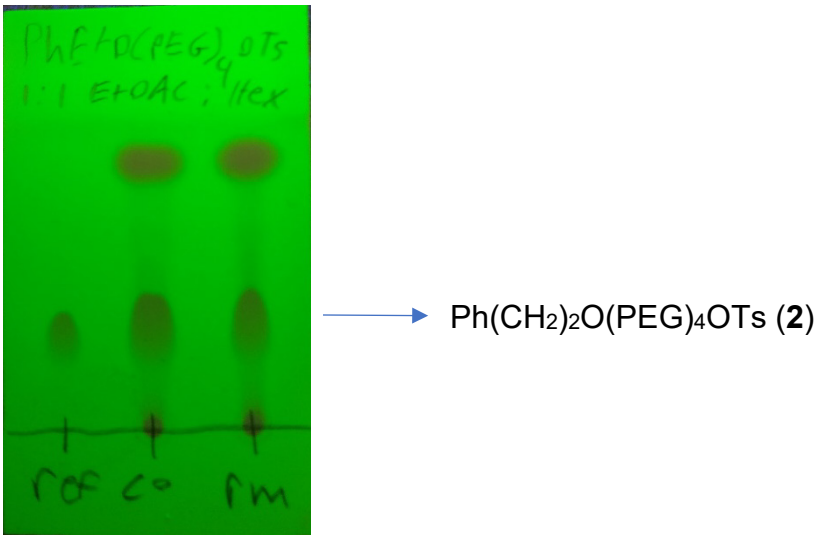


Figure A.33. TLC of crude **2**. Eluent: hexanes/EtOAc 1:1. Left lane, purified **2**; middle lane, co-spot of materials on left and right lanes; right lane, crude **2**.

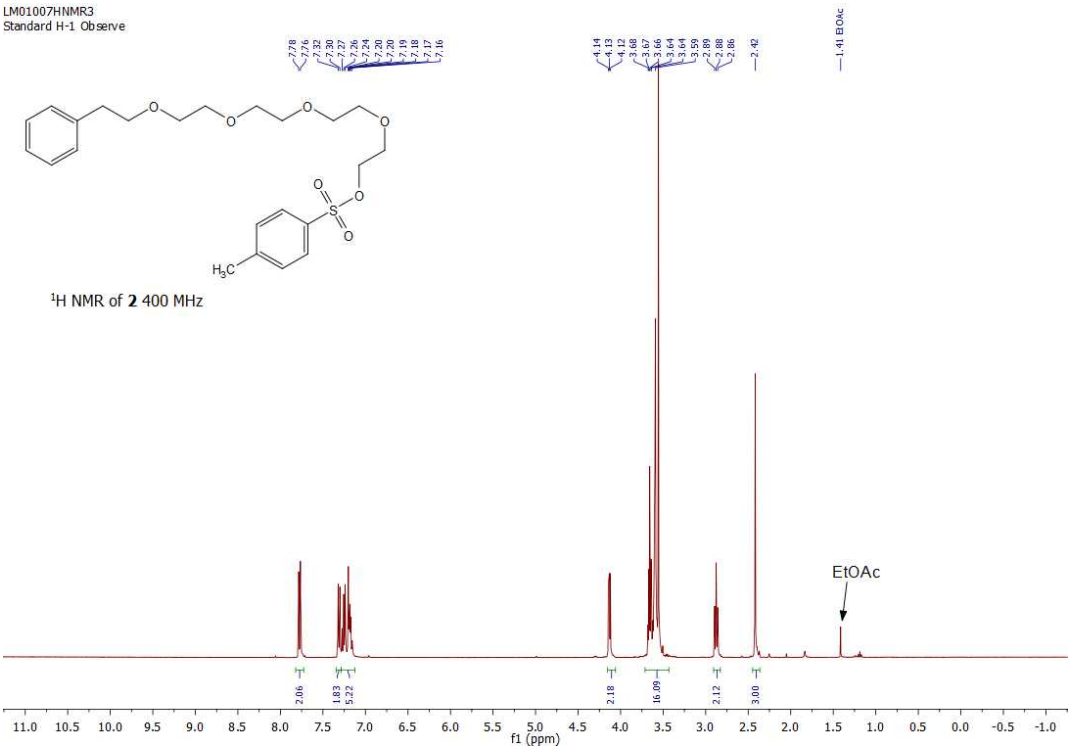


Figure A.34. ^{1}H NMR of $Ph(CH_2)_2O(PEG)_4OTs$ (**2**).

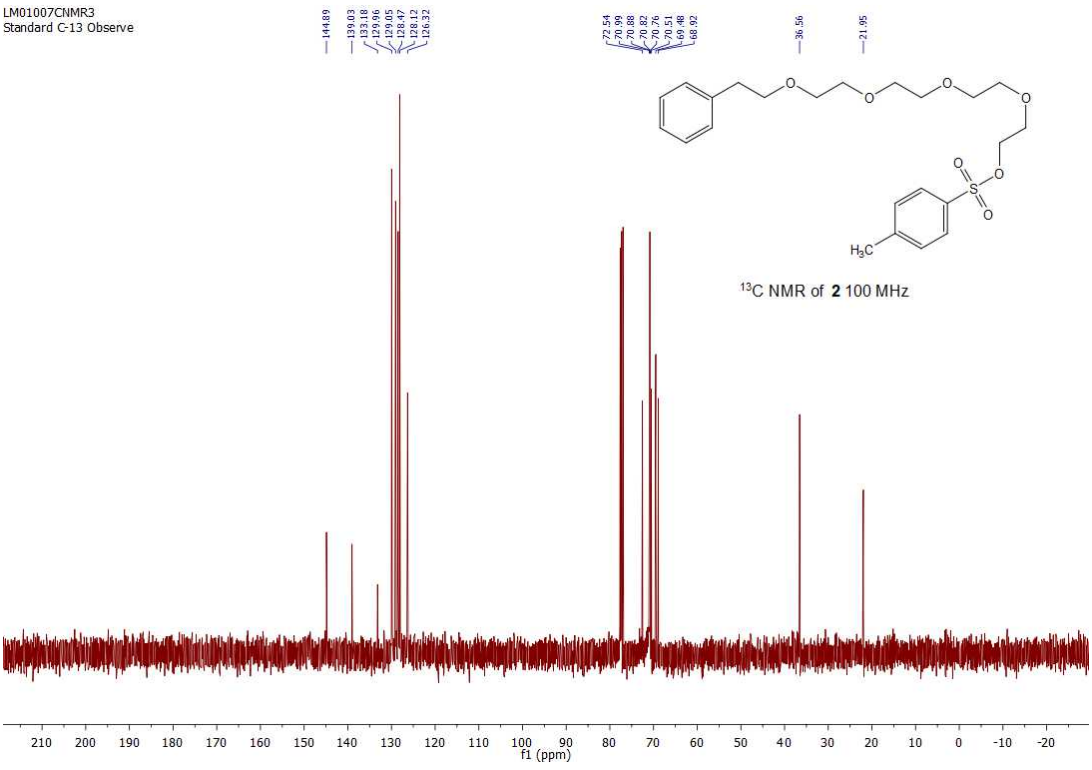


Figure A.35. ^{13}C NMR of $Ph(CH_2)_2O(PEG)_4OTs$ (**2**).

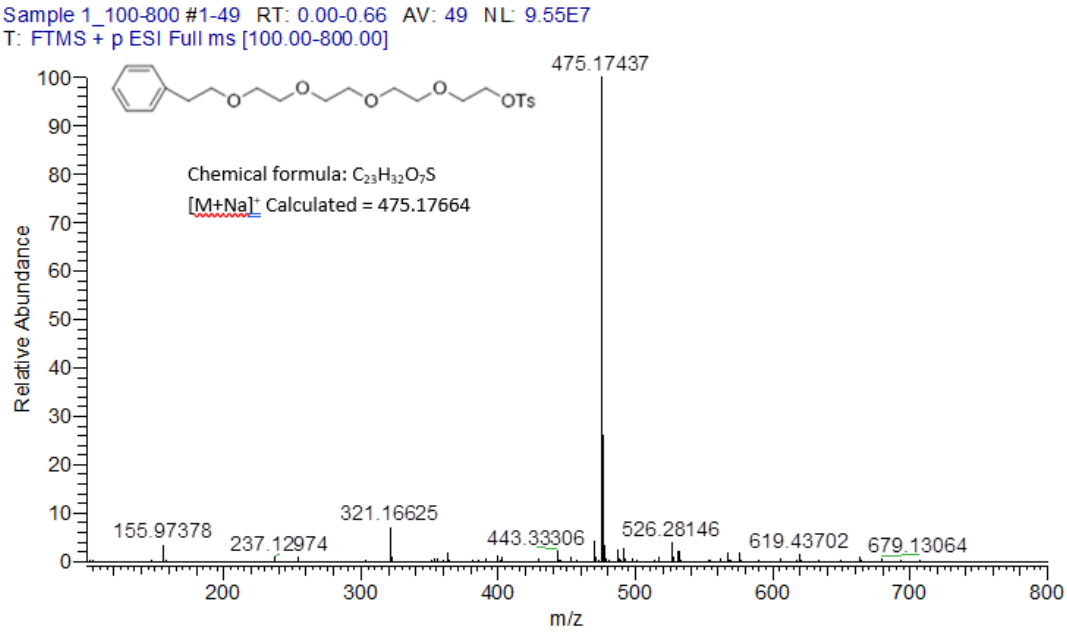
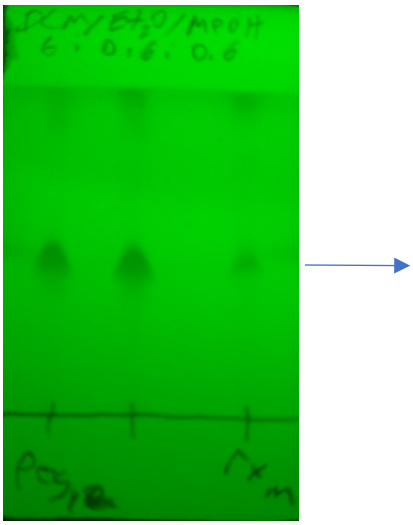
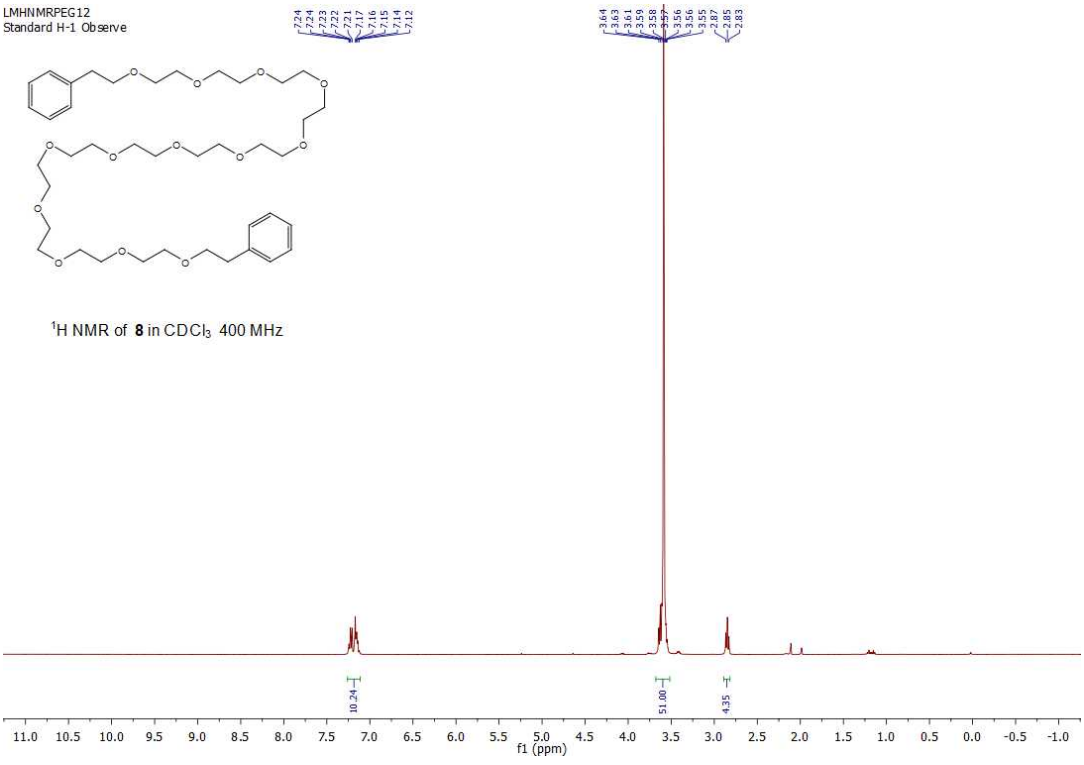


Figure A.36. ESI-MS of $Ph(CH_2)_2O(PEG)_4OTs$ (**2**).



$Ph(CH_2)_2O(PEG)_{12}O(CH_2)_2Ph$ (**8**)

Figure A.37. TLC of crude **8**. Eluent: DCM/MeOH/Et_2O 6:0.6:0.6. Left lane, purified **8**; co-spot of materials on left and right lanes; right lane, crude **8**.



^1H NMR of **8** in $CDCl_3$ 400 MHz

Figure A.38. ^1H NMR of $Ph(CH_2)_2O(PEG)_{12}O(CH_2)_2Ph$ (**8**).

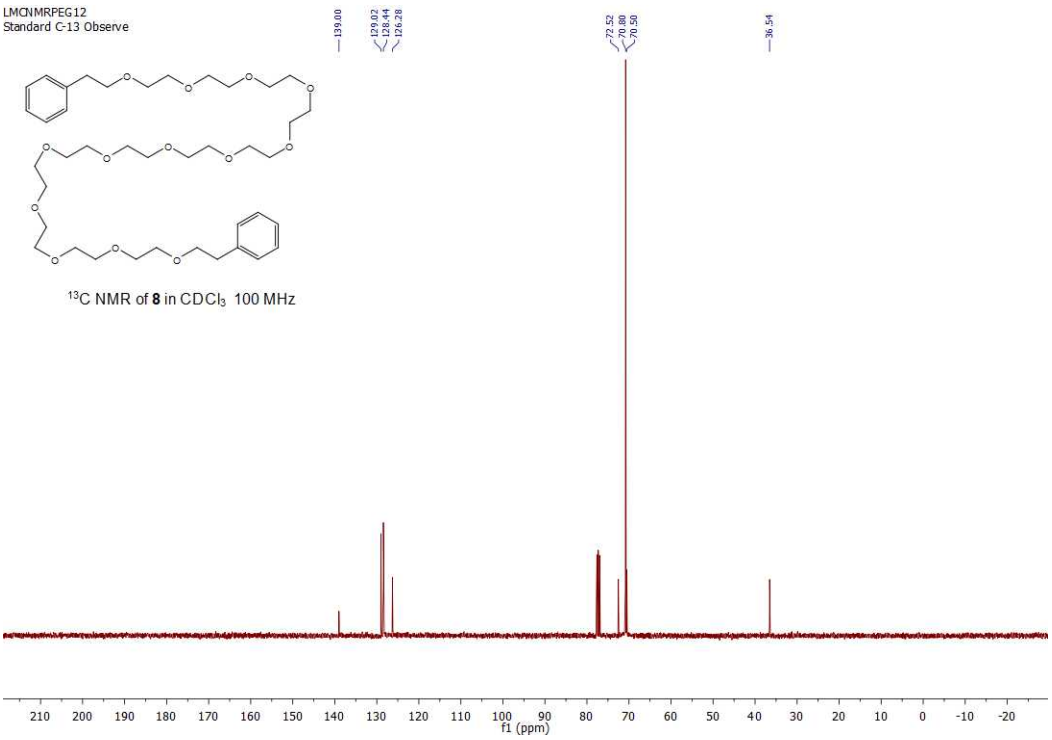


Figure A.39. ^{13}C NMR of $Ph(CH_2)_2O(PEG)_{12}O(CH_2)_2Ph$ (**8**).

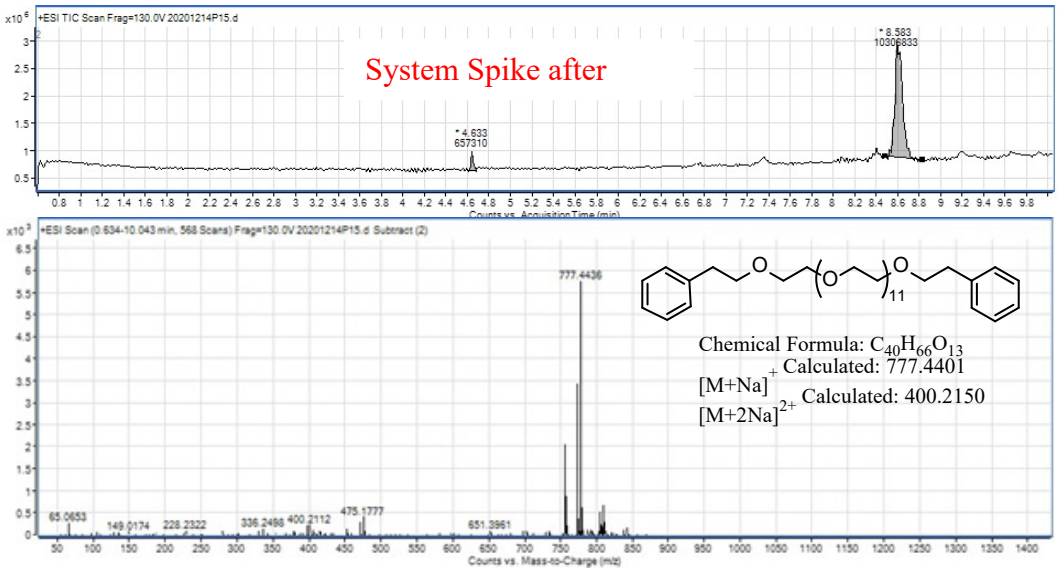


Figure A.40. LC-MS of $Ph(CH_2)_2O(PEG)_{12}O(CH_2)_2Ph$ (**8**).

$Ph(CH_2)_2O(PEG)_{20}O(CH_2)_2Ph$ (**9**)

Figure A.41. TLC of crude **9**. Eluent: DCM/MeOH/Et_2O 6:0.6:0.6. Left lane, purified **9**; co-spot of materials on left and right lanes; right lane, crude **9**.

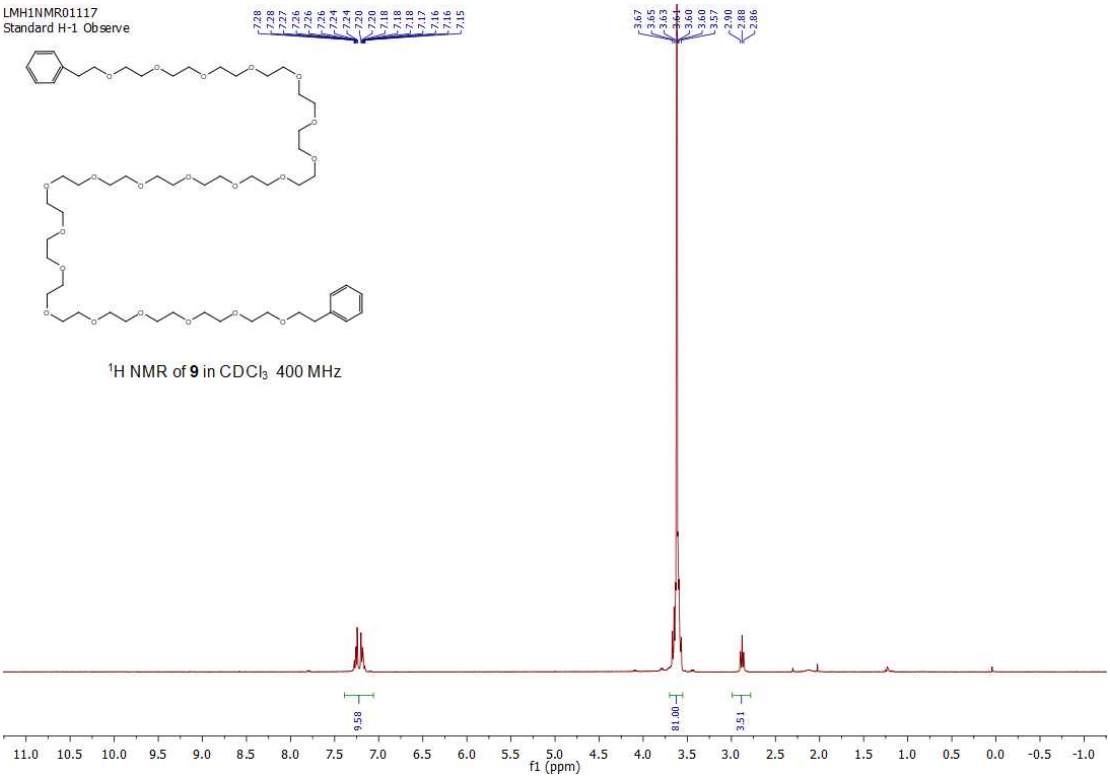


Figure A.42. ^{1}H NMR of $Ph(CH_2)_2O(PEG)_{20}O(CH_2)_2Ph$ (**9**).

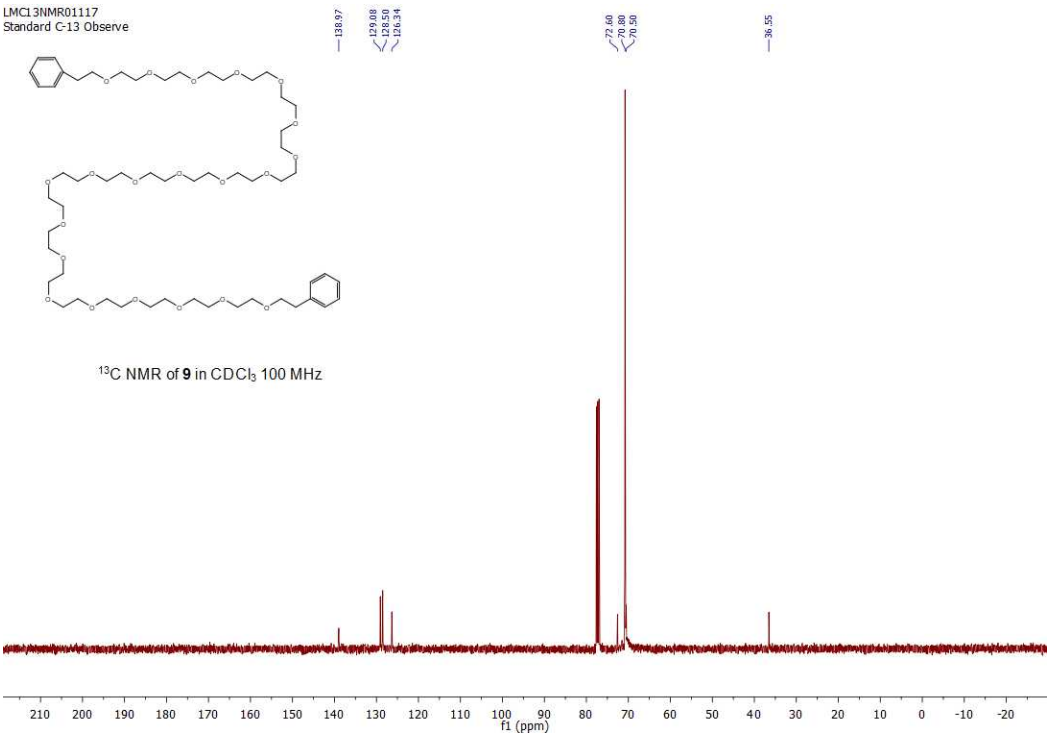


Figure A.43. ^{13}C NMR of $Ph(CH_2)_2O(PEG)_{20}O(CH_2)_2Ph$ (**9**).

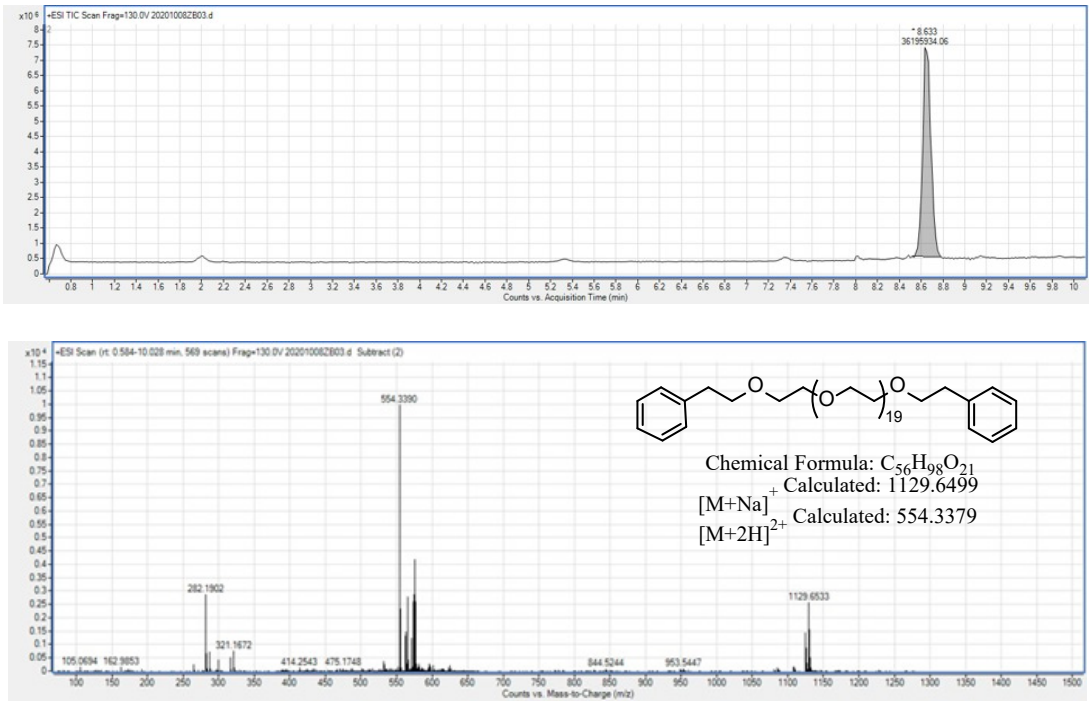
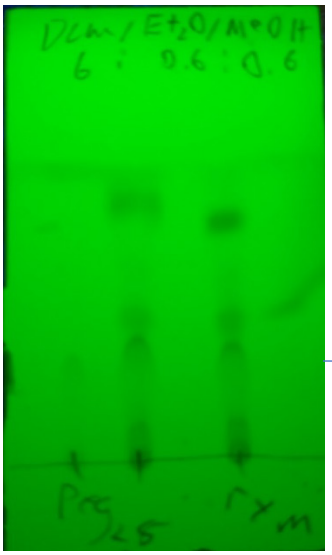


Figure A.44. LC-MS of $Ph(CH_2)_2O(PEG)_{20}O(CH_2)_2Ph$ (**9**).



$Ph(CH_2)_2O(PEG)_{28}O(CH_2)_2Ph$ (**10**)

Figure A.45. TLC of crude **10**. Eluent: DCM/MeOH/Et_2O 6:0.6:0.6. Left lane, purified **10**; middle lane, co-spot of materials on left and right lanes; right lane, crude **10**.

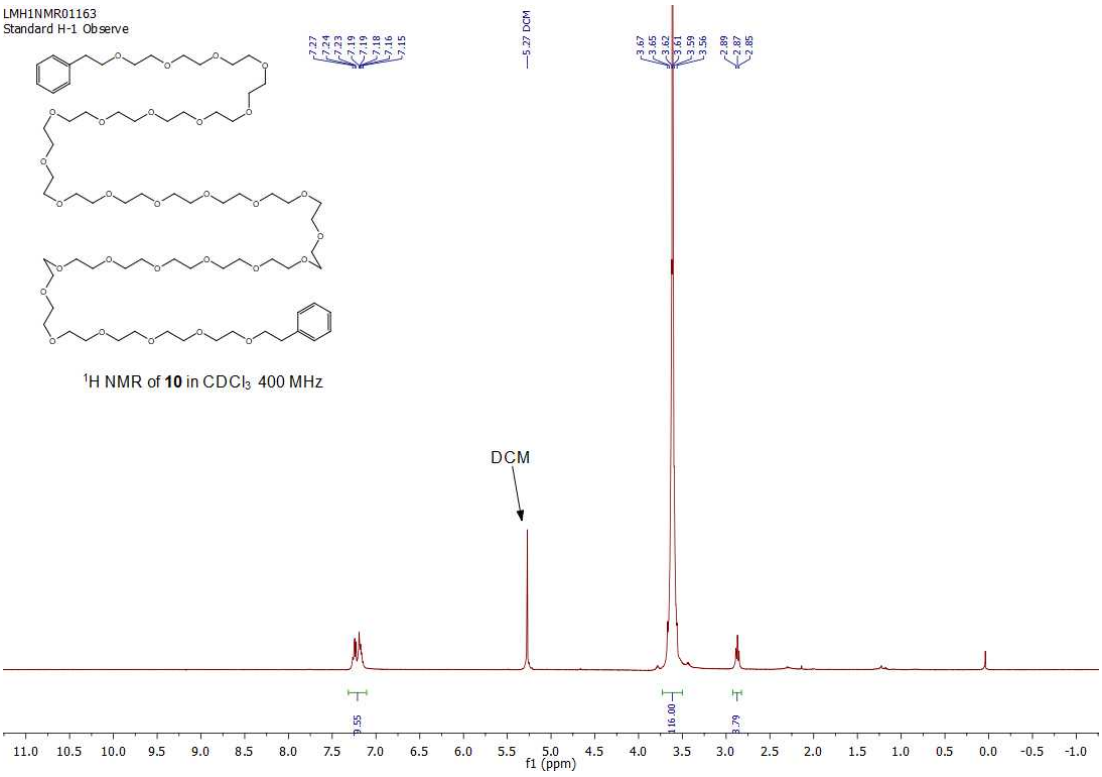


Figure A.46. ^1H NMR of $Ph(CH_2)_2O(PEG)_{28}O(CH_2)_2Ph$ (**10**).

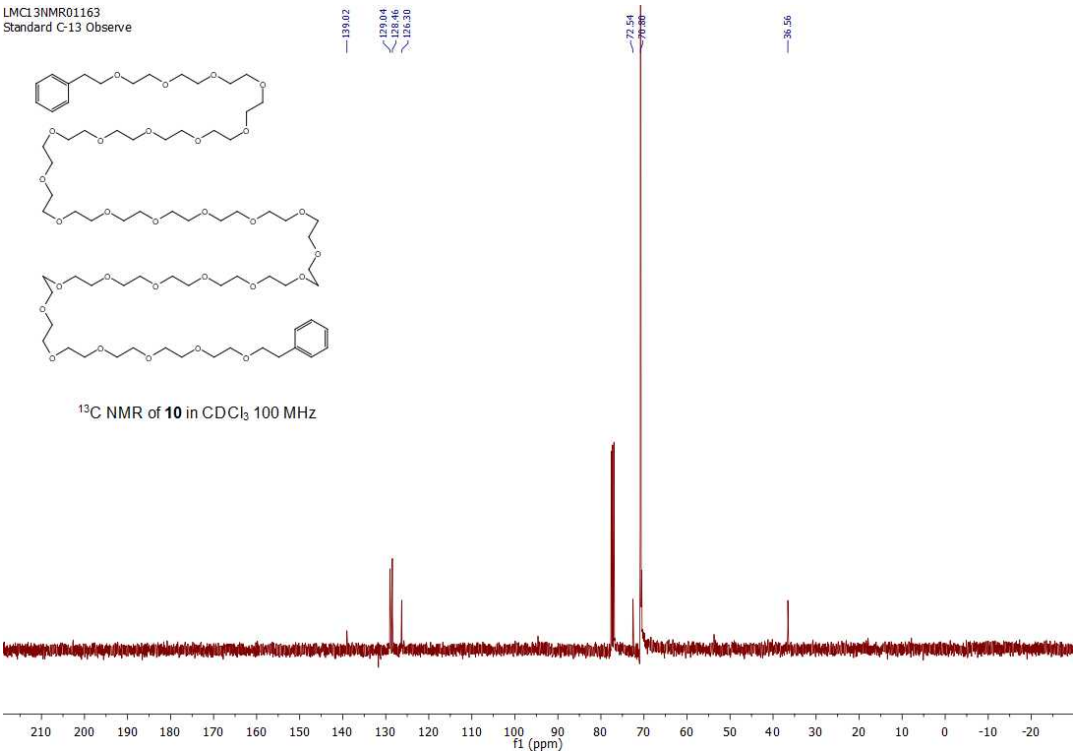


Figure A.47. ^{13}C NMR of $Ph(CH_2)_2O(PEG)_{28}O(CH_2)_2Ph$ (**10**).

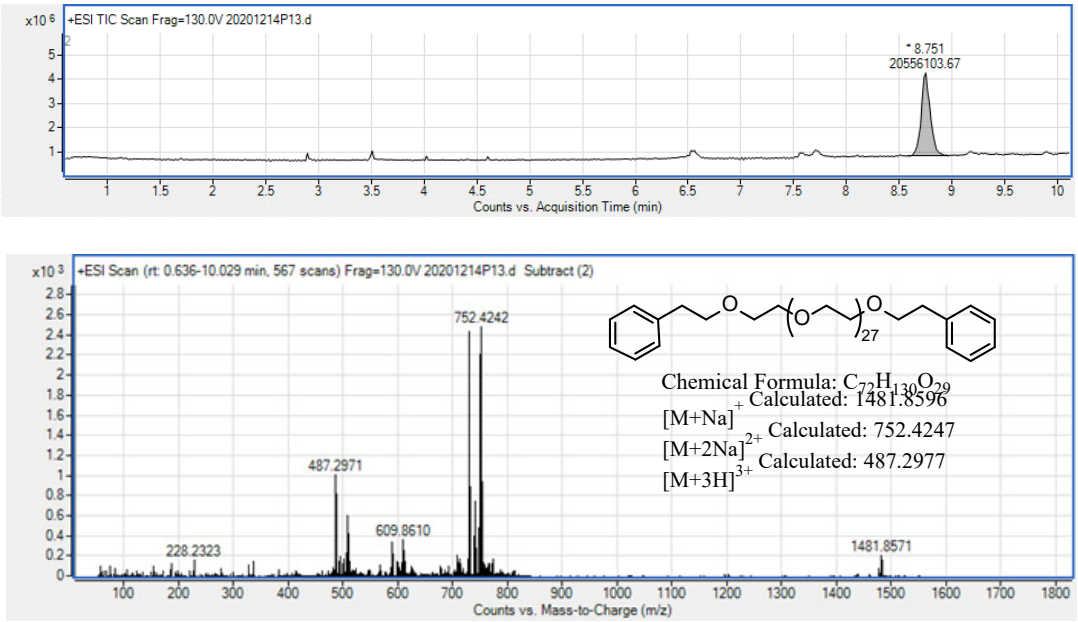
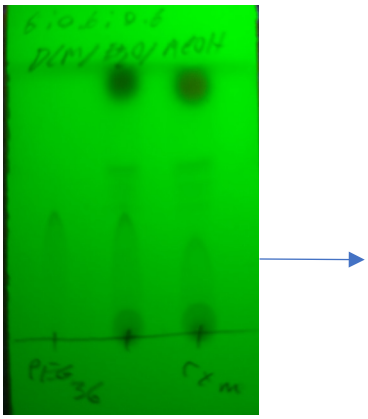


Figure A.48. LC-MS of $Ph(CH_2)_2O(PEG)_{28}O(CH_2)_2Ph$ (**10**).



$Ph(CH_2)_2O(PEG)_{36}O(CH_2)_2Ph$ (**11**)

Figure A.49. TLC of crude **11**. Eluent: DCM/MeOH/Et_2O 6:0.6:0.6. Left lane, starting material **10**; middle lane, co-spot of materials on the left and right lanes; right lane, crude **11**.

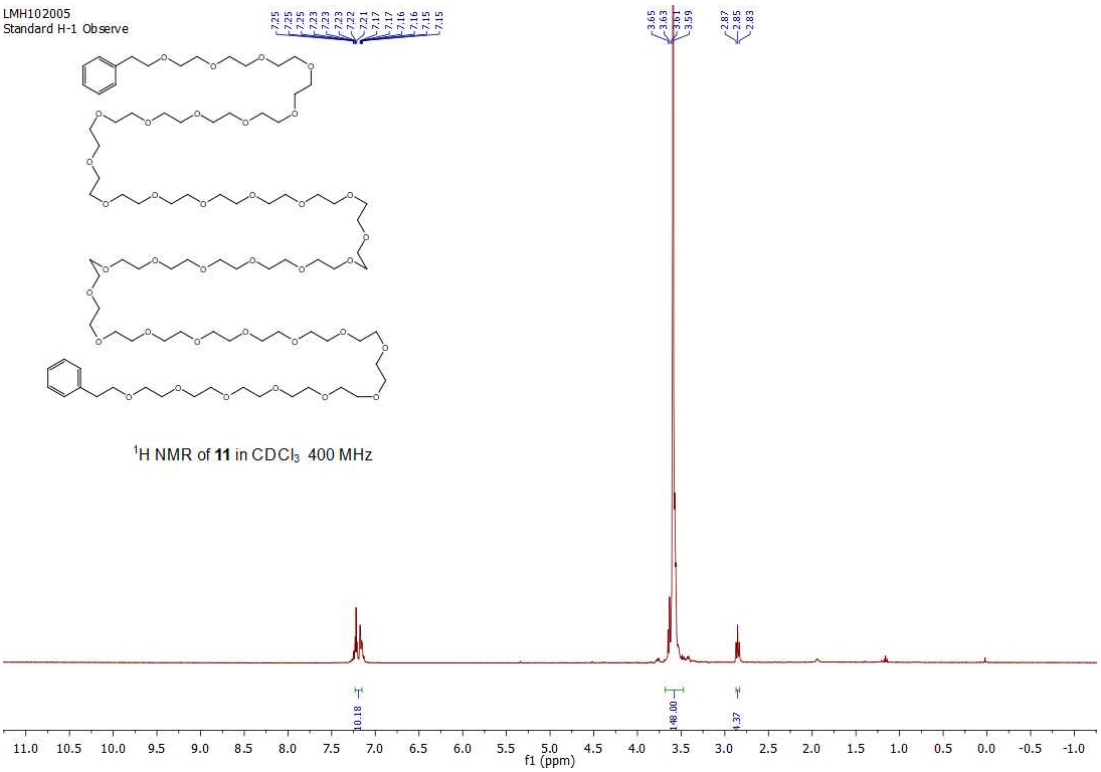


Figure A.50. ^1H NMR of $Ph(CH_2)_2O(PEG)_{36}O(CH_2)_2Ph$ (**11**).

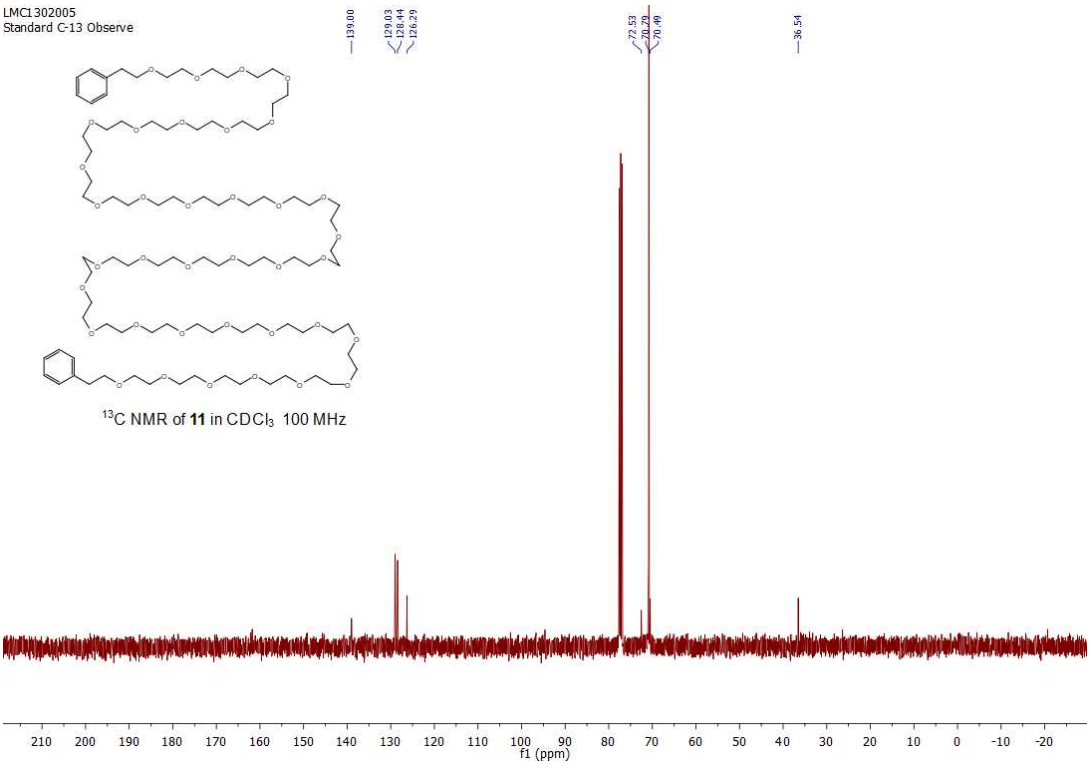


Figure A.51. ^{13}C NMR of $Ph(CH_2)_2O(PEG)_{36}O(CH_2)_2Ph$ (**11**).

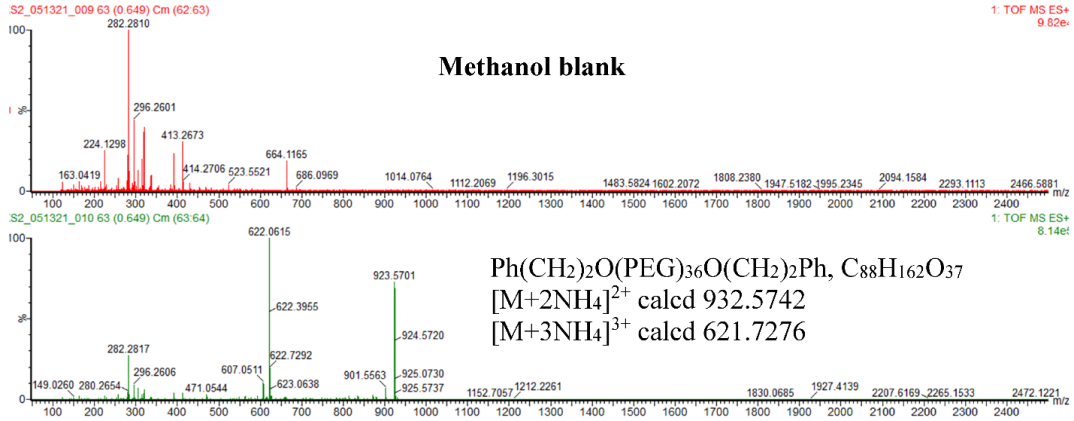
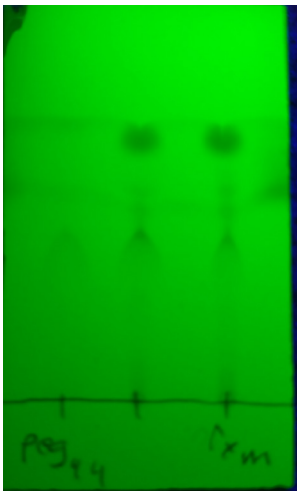


Figure A.52. ESI-MS of $Ph(CH_2)_2O(PEG)_{36}O(CH_2)_2Ph$ (**11**).



$Ph(CH_2)_2O(PEG)_{44}O(CH_2)_2Ph$ (**12**)

Figure A.53. TLC of crude **12**. Eluent: DCM/MeOH/Et_2O 6:1:1. Left lane, purified **12**; co-spot of materials on left and right lanes; right lane, crude **12**.

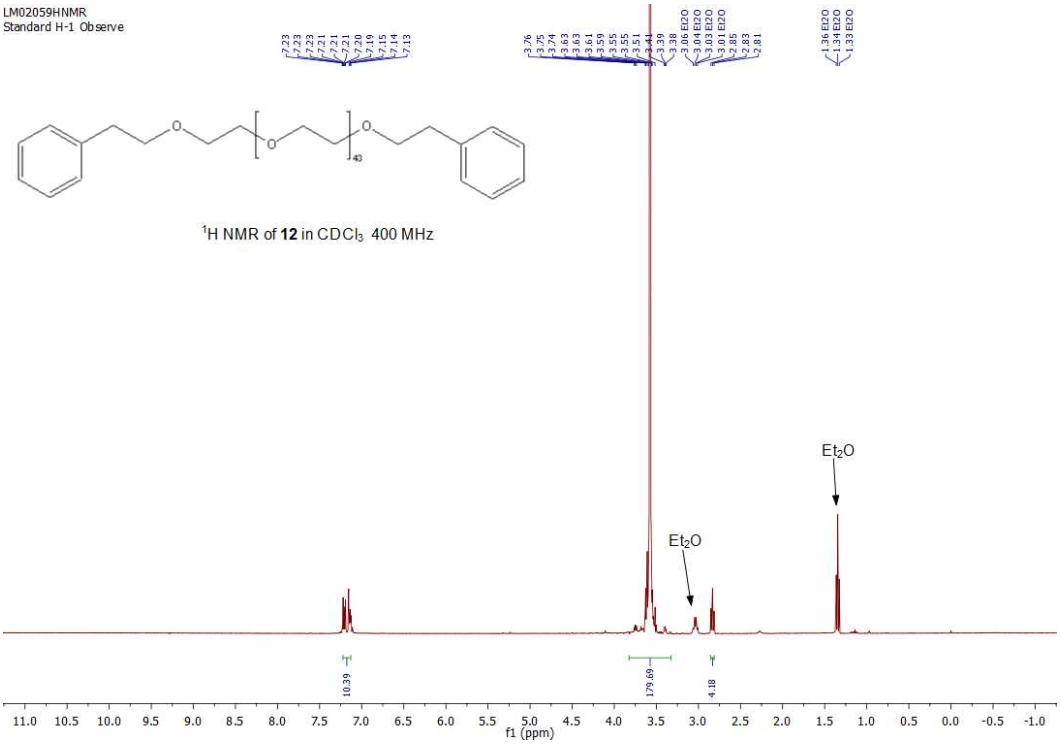


Figure A.54. ^{1}H NMR of $Ph(CH_2)_2O(PEG)_{44}O(CH_2)_2Ph$ (**12**).

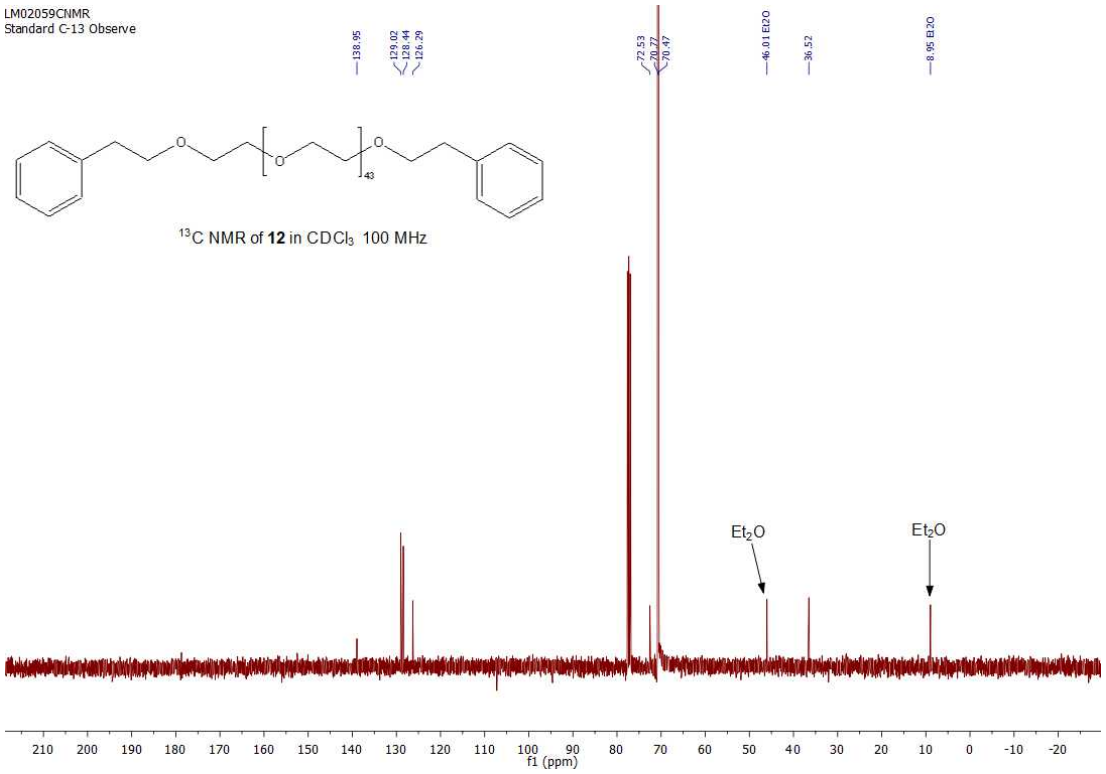


Figure A.55. ^{13}C NMR of $Ph(CH_2)_2O(PEG)_{44}O(CH_2)_2Ph$ (**12**).

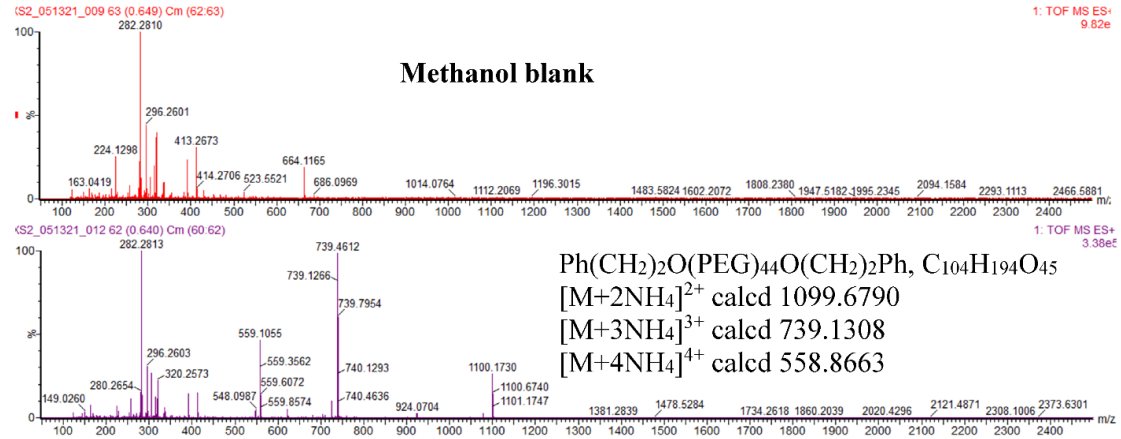


Figure A.56. HRMS of $Ph(CH_2)_2O(PEG)_{44}O(CH_2)_2Ph$ (**12**).