

Solid-Phase Synthesis of 11-Mercapto-1-Undecanoic Acid and 1-Dodecanethiol for *In Situ* Assembly of Monolayers on Gold

Kseniya S. Deryckx,¹ Andrea E. Riegel,² Esmaeel D. Naeemi^{1,2}

¹Seattle Central Community College, Seattle, Washington 98122

²Asemblon, Inc., Redmond, Washington 98052

Abstract: *Synthesis and purification of thiols can be a time consuming and complicated process. Utilizing solid phase synthesis techniques, commonly used for peptide synthesis, could increase the purity of thiols and streamline the synthetic procedure. In situ assembly of monolayers from resin-bound thiols also prevents the unpleasant odor associated with thiols. In addition, in situ assembly facilitates the ultimate goal of the project: the easy to use self-assembly process making monolayers accessible for non-chemists. In this experiment, attempts to synthesize 11-mercapto-1-undecanoic acid and 1-dodecane thiol using thiol trityl resin are outlined, and identities of the compounds are explored using Nuclear Magnetic Resonance (NMR), Infrared Spectroscopy (IR), and Gas Chromatography/Mass Spectrometry (GC/MS). Thin layer chromatography (TLC) and Ellman's reagent are used as secondary thiol detectors.*

1. INTRODUCTION

Self-assembled monolayers (SAMs) have found their respected place in fields like bioengineering, physics, material sciences, and chemistry [1]. The most common goal for SAM development is an ability to control chemical and physical properties of the surface.

One way to obtain the surface of the desired quality is assembling the monolayer on gold from a solution of a purified thiolated compound of interest [1]. Although this method is commonly used, it poses complications for monolayer assembly by non-chemists. While the procedure may be easy to follow for people with experience in the lab, the number of potential people that can benefit from SAMs becomes limited. Many scientists are not interested in assembling monolayers as their goal, but rather using SAMs as a tool for studying surface properties.

Synthesis of purified thiolated compounds presents multiple challenges to even the seasoned organic chemist. In addition to the thiol moiety, synthesis often results in side products, which interfere with monolayer formation and are difficult to separate [1]. Impurities can compete with the thiol of interest for adsorption on gold, which becomes even more relevant if the impurity contains thiolated molecules [1].

Thiols are reactive molecules and are capable of intra- and intermolecular interactions in presence of oxygen to form disulfides [7]. These types of reactions may complicate or slow

the self-assembly process due to disulfide formation and possible crosslinking if the molecule of interest has more than one thiol functional group [2]. Moreover, thiols have a foul stench and are known to be components of skunk secretion [8]. Handling thiols without proper ventilation is not only unsafe but also highly objectionable.

In response to the concerns listed above, this project's final goal is the development of a simple procedure for the self-assembly of monolayers. As a final outcome, people with little or no organic chemistry knowledge could easily assemble monolayers for various applications like optics research, tissue growth studies, and simple educational demonstrations of surface properties [4].

Solid phase synthesis techniques were used to make a thiol of interest, and the solid support, thiol trityl resin, was used as a protecting group and as a key reagent in thiol synthesis (Figures 1, 2). Solid-phase synthesis was introduced by Merrifield in 1960s to make polypeptides [5]. For our purposes, solid phase synthesis was used as means of maximizing purity and decreasing the reactivity and odor of the thiol during storage. Also, the extraction and purification steps were limited to a simple washing of the resin. 1-Dodecanethiol and 11-mercapto-1-undecanoic acid were chosen as desired products for future monolayer assembly: 1-dodecanethiol for hydrophobic surfaces and 11-mercapto-1-undecanoic acid for hydrophilic surfaces.

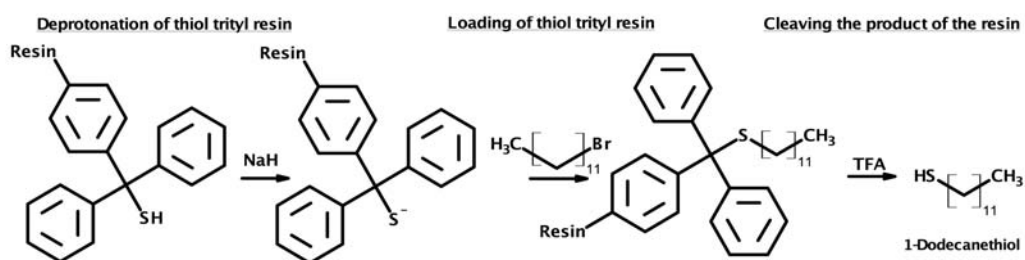


Figure 1 Reaction Scheme: Synthesis of 1-Dodecanethiol.

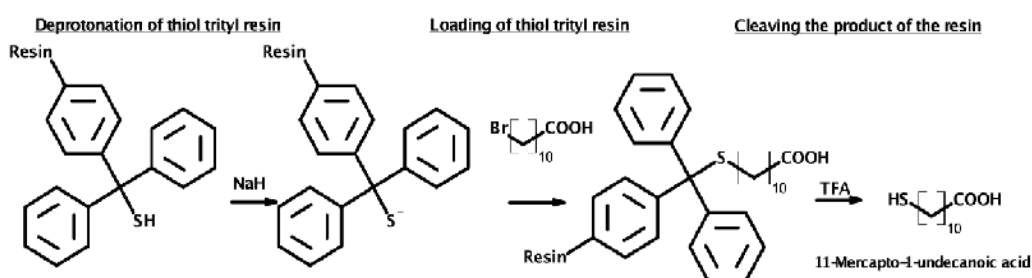


Figure 2 Reaction Scheme: Synthesis of 11-Mercapto-1-undecanoic Acid.

2. MATERIAL AND METHODS

The procedure design for synthesis of 1-dodecanethiol and 11-mercapto-1-undecanoic acid was based on the work of Inman *et al.* [4]. Although in their paper thiol trityl group was used in traditional solution-based synthesis, it proved to be helpful when working with thiol trityl resin.

Thiol trityl resin (0.7 mmol/g loading capacity) was obtained from Advanced ChemTech. Positive control for 1-dodecanethiol was synthesized and purified by Asemblon, Inc. 11-Bromo-undecanoic acid (95%), 1-bromododecane (97%), and sodium hydride (60% dispersion in mineral oil) were obtained from Aldrich. Tetrahydrofuran and anhydrous sodium sulfate were purchased from EMD. Methylene chloride and N,N'-Dimethylacetamide were purchased from JT Baker. Trifluoroacetic acid (99.5+%) was supplied by Alfa Aesar.

2.1 Loading Thiol Trityl Resin with 1-Dodecanethiol

Thiol trityl resin (1.4645 g) was washed with methylene chloride until a clear rinse was obtained. Tetrahydrofuran (35 ml) was added, and nitrogen was used to mix the flask contents (Figure 3). Sodium hydride (0.0285 g/ 7.13×10^{-4} mol) was dissolved in methylene chloride (15ml) under a nitrogen bath and added to the resin solution dropwise. The reaction was refluxed for 3 h then 1-bromododecane (1.0517 g/ 4.22×10^{-3} mol) was added, and reaction refluxed for ~20 h. Water (30 ml) was added to quench any excess sodium hydride. The resulting resin was filtered and washed with 10-15 ml portions of methylene chloride, water and methanol (1:1), methylene chloride and methanol (1:1) methylene chloride (twice) consecutively. The washed resin was dried and separated into two batches.

2.2 Cleaving 1-Dodecanethiol off Thiol Trityl Resin (first batch)

The loaded resin 1 (0.5800 g) was dissolved in methylene chloride (15 ml) and trifluoroacetic acid (1 ml) was added dropwise to this solution and mixed with nitrogen (Figure 3) for 30 min.

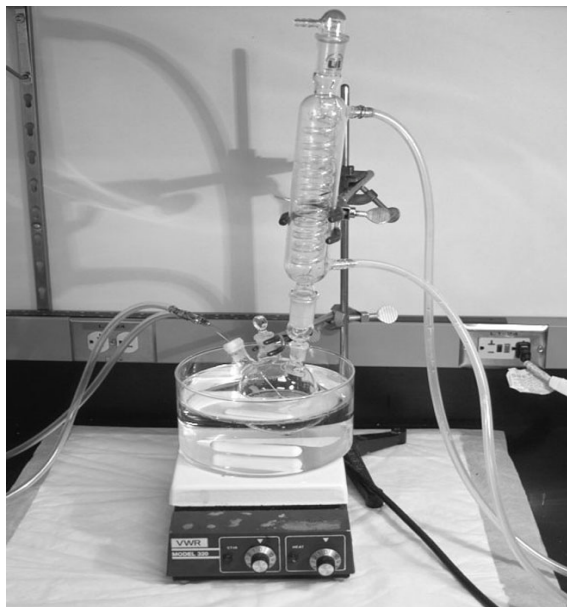


Figure 3 Reaction set up: The nitrogen bubbles into the reaction through two needles inserted into the septum on the three-neck flask and leaves through the outlet on the top of condenser

The resin changed colors from yellow to burgundy. Water (~20 ml) was added to the mixture and the resin was removed by vacuum filtration. The product was extracted out of filtrate with two portions of methylene chloride (~10 ml each). The methylene chloride layer was dried with sodium sulfate, filtered, and condensed by evaporation at reduced pressure.

Ellman's Reagent: solution turned yellow.

2.3 Cleaving 1-Dodecanethiol off Thiol Trityl Resin (second batch)

The loaded resin 2 (~0.8 g) was dissolved in methylene chloride (~30 ml) and treated with trifluoroacetic acid (2 ml), and the mixture was shaken for 20 min. Water (20 ml) was added (turning burgundy resin into yellow resin). The aqueous layer was then separated. The organic layer was dried with sodium sulfate and filtered. The final solution was condensed by evaporation at reduced pressure.

Ellman's Reagent: solution turned bright yellow.

2.4 Loading Thiol Trityl Resin with 11-Mercapto-1-undecanoic acid

Thiol trityl resin was washed by Soxhlet extraction with methylene chloride for 5 h and with a mixture of methanol and methylene chloride in a peptide synthesis vessel for 2 h. Sodium hydride (0.0734 g/ 1.84×10^{-3} mol) was dissolved in methylene chloride (12.5 ml), and added dropwise to a methylene chloride (12.5 mL) solution of 11-bromo-1-undecanoic acid (0.5651 g/ 2.13×10^{-3} mol). After all gas production ceased, the mixture was added to a solution of thiol trityl resin (1.4645 g) in N,N-dimethylacetamide (35ml), backfilled with nitrogen and placed on the shaker for 30 min. The excess of gas was released from the vessel and the reaction again backfilled with nitrogen and placed on the shaker for 28 h. Water (~20 ml) was added to quench any excess sodium hydride. The resin was filtered and washed with N,N-dimethylacetamide and methylene chloride (1:1, 1:3, 2:3 consecutively) using peptide synthesis apparatus for 20 min for each solvent system. The resin was air dried.

2.5 Cleaving 11-Mercapto-1-undecanoic acid off Thiol Trityl Resin

The loaded resin (~1 g) was dissolved in methylene chloride (~30 ml) and treated with trifluoroacetic acid (2 ml), and the mixture was shaken for 20 min. Water (20 ml) was added (turning burgundy resin into yellow resin). The aqueous layer was then separated. The organic layer was dried with sodium sulfate and filtered. The final solution was condensed by evaporation at reduced pressure.

Ellman's Reagent: solution turned faint yellow.
NMR: no peaks at 2.5 or 2.7 ppm.

3. RESULTS AND DISCUSSION

Traditional solid-phase synthesis requires agitation by shaking the vessel with reactants in an appropriate solvent, bubbling nitrogen into the mixture or by conventional stirring with magnetic bar [3]. The apparatus for shaking was not available during first three months of the research, and magnetic stirring was grinding the resin into a fine powder, which clogged the filtration apparatus. Because of these difficulties, an alternate method of agitation was developed (Figure 3).

Nitrogen gas bubbled into solution through two needles facilitated continuous mixing of reagents in the solution. The needles were inserted in the septum so that the two needle ends would point in opposite directions, which was necessary for uniform mixing throughout the flask.

Although 1-dodecanthiol was synthesized using the setup described above, the setup appeared to have several drawbacks. During reflux, some solvent still escaped through the condenser, which obligated addition of extra solvent. Although it may not be crucial for single solvent systems, a particular mix of solvent could not stay constant in the set up described above. Also, addition of fresh solvent temporarily changed the temperature of reaction, which may be relevant for optimization of the reaction. In addition, the setup was not completely safe for leaving unattended over long periods of time. Running water in the condenser, hot plate, change of pressure in nitrogen inlet, and spontaneous bumping in the reaction vessel could prove to be a hazard in the lab. It was concluded that using a shaking apparatus for reactions longer than ten h is safer and more appropriate for optimization of the system.

3.1 Synthesis of 1-Dodecanethiol

The ability of the resin to swell relies on the solvent used during reaction and influences the reactivity of the resin itself [6]. Methylene chloride and tetrahydrofuran were used as solvents to allow full swelling of the resin and facilitate safe dissolution of sodium hydride. However, as mentioned before in the setup description, the ratio of methylene chloride to tetrahydrofuran did not stay constant because of evaporation during the reflux.

During cleaving, some starting material and aromatic side products were released into solution. At this stage, cleaving was used as a way to test the successful loading of the resin, so the final product was not further purified. The NMR data was used as means of verifying the presence of thiols and disulfides. NMR data obtained from the resin-cleaved 1-dodecanethiol (Figure 4) was compared to positive control (previously purified 1-dodecanethiol). Although the resin-cleaved product showed a great amount

of impurities, both the positive control and resin-cleaved 1-dodecanethiol showed a quartet at 2.5 ppm, which corresponds to protons on carbon attached to thiol functional group. Also, the resin-cleaved product had another important peak at 2.7 ppm indicating the presence of disulfide. Because the thiol moiety was introduced using the resin itself, these results were very encouraging, suggesting successful loading and cleaving.

In order to verify that the constituent of interest was in fact 1-dodecanthiol, GC/MS data was used to pinpoint specific identity of the final product, and a positive control was used to assist identification process (Figure 5). GC data of the resin-cleaved product and the positive control, showed a specific peak with a retention time from 7.05-7.15 min. The unique mass spectrum that appeared for resin-cleaved 1-dodecanethiol matched the positive control of 1-dodecanethiol.

3.2 Synthesis of 11-Mercapto-1-undecanoic Acid

In order to design a procedure for loading thiol trityl resin with 11-mercapto-1-undecanoic acid several factors were addressed. As one of the factors, an excess of sodium hydride was used to assure complete deprotonation of 11-bromo-1-undecanoic acid and thiol trityl resin, in order to smooth the progress of a bimolecular substitution reaction. N,N-dimethylacetamide was chosen to facilitate dissolution of the salt of 11-bromo-1-undecanoic acid that precipitated out when sodium hydride was added. However, N,N-dimethylacetamide did not swell the resin sufficiently, so methylene chloride was added in attempt to make a solvent system that could swell the resin and dissolve the reactants.

The treated resin was analyzed using FT-IR. Due to the intense carbonyl peak typical of carboxylic acids, successfully loaded resin with 11-mercapto-1-undecanoic acid could be differentiated from untreated resin. Based on the IR data collected, the resin was not loaded successfully because the spectrum of the loaded resin does not show any carbonyl peaks. In fact the IR data of both the untreated resin and the treated resin look almost identical. NMR data did not show any peaks typical of thiols or disulfides.

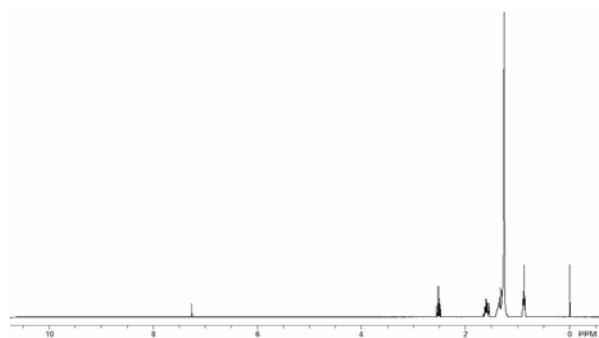


Figure 4a

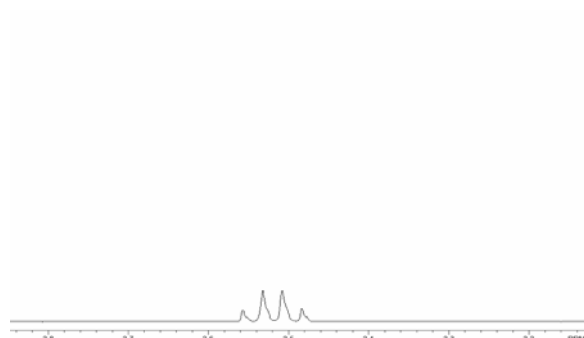


Figure 4b

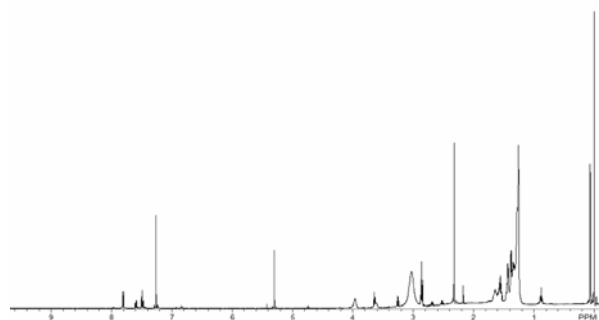


Figure 4c

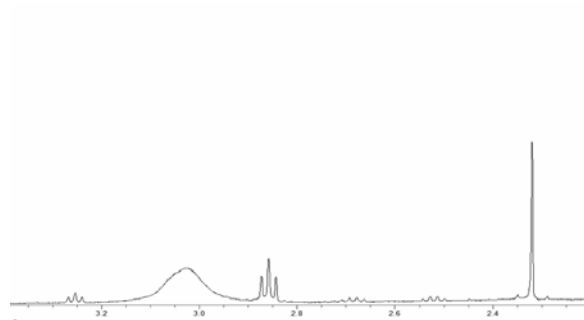


Figure 4d

Figure 4 NMR Analysis of 1-Dodecanethiol: a) Positive Control for 1-Dodecanethiol; b) Positive Control for 1-Dodecanethiol: close up of the thiol peak area; c) Resin-cleaved 1-Dodecanethiol (second batch); d) Resin-cleaved 1-Dodecanethiol (second batch): close up of the thiol peak area.

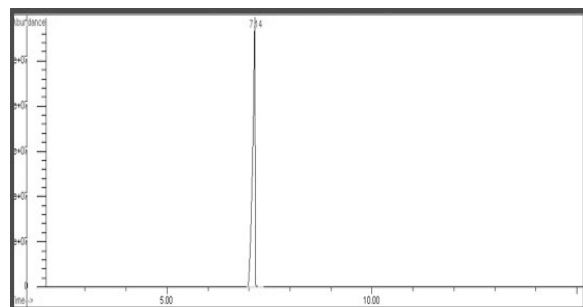


Figure 5a

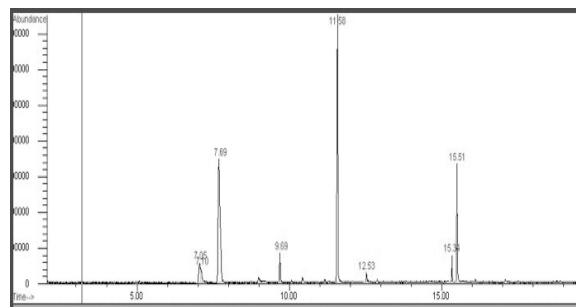


Figure 5b

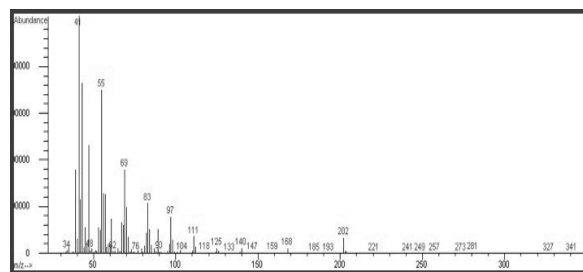


Figure 5c

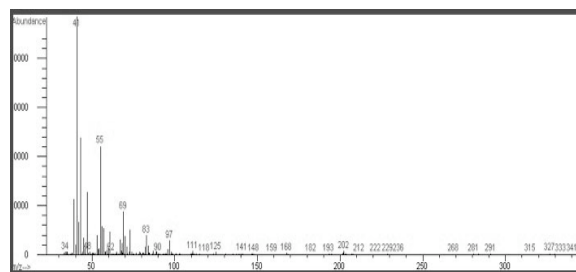


Figure 5d

Figure 5 GC/MS Analysis of 1-Dodecanethiol: a) GC: Positive Control for 1-Dodecanethiol b) GC: Resin-cleaved 1-Dodecanethiol (second batch); c) MS: Positive Control for 1-Dodecanethiol; d) MS: Resin-cleaved 1-Dodecanethiol (second batch).

During the experiment, it was noted that the reagents used for the synthesis of 11-mercapto-1-undecanoic acid were not fully soluble in the solvent system designed. Achieving complete solubility appeared to be complicated. Methylene chloride was suitable for swelling the resin, and N,N-dimethylacetamide served well in dissolving deprotonated 11-bromo-1-undecanoic acid. However, when mixed together (~1:1), the solvents did not prove to be the system required for successful swelling of the resin and dissolution of 11-bromo-1-undecanoic acid because reagents precipitated out in small portions. It is possible that the solvent system used was one of the reasons why the synthesis was not successful. Also, the fact that the active sites on the resin are highly hindered, higher molar ratios of sodium hydride and 11-bromo-1-undecanoic acid to the resin's loading capacity could be used to explore further the synthesis of 11-mercapto-1-undecanoic acid.

3. CONCLUSION

Preliminary studies show that 1-dodecanethiol was synthesized and cleaved from the solid support using nitrogen to mix the reagents. This conclusion is supported by NMR and GC/MS data. Further optimization of the procedure involving 1-dodecanethiol has to be explored.

IR data shows that 11-mercapto-1-undecanoic acid was not bound to the resin successfully. Further procedure corrections and optimization of 11-mercapto-1-undecanoic acid loading and cleaving has to be researched.

Future work should include use of a shaker apparatus for solid-phase synthesis to avoid evaporation of solvent through nitrogen bubbling into reaction and to provide stability of solvent system (if several solvents are mixed), use solid phase synthesis glassware with frit to minimize the loss of resin and subsequently increase amount of a thiol produced (particularly relevant for 1-dodecanethiol synthesis, since only a nitrogen bubbling based procedure was attempted). In addition to synthesis improvement, future goals of the project have to be researched including monolayer assembly *in situ* from resin-bound thiols and analysis of resulting monolayers using Contact Angle Goniometry and X-ray Photoelectron Spectroscopy.

ACKNOWLEDGEMENTS

This research could not have been done without consultations with Benjamin Roberts and Dr. Maxi Boeckl of Asemblon, Inc. Jim Roe of the University of Washington and Maitham Naeemi of Asemblon, Inc. provided help with instrumentation, which was appreciated immensely. Photos were taken by Bart Norton of Asemblon, Inc. The research was funded through UWEB-RECCS by National Science Foundation (USA), EEC 9529161.

REFERENCES

1. Asemblon, Inc catalog, Version 4.0. 13 April 2006. [http://www.asemblon.com/files/Asemblon-Catalog 5.0.pdf](http://www.asemblon.com/files/Asemblon-Catalog%205.0.pdf).
2. Bain CD, Biebuyck HA, Whitesides GM. Comparison of self-assembled monolayers on gold: coadsorption of thiols and disulfides. *Langmuir* 1989;5:723-727.
3. Bing Y, Dunn RF, Tang Q, Coppola GM, Vlattas I. Optimization of solid phase synthesis monitoring reactions on single resin beads using FTIR microspectroscopy. In: Bing Y, Czarnic AW, editors. Optimization of solid-phase combinatorial synthesis. New York: Marcel Dekker, Inc.; 2002. p 129-164.
4. Inman CE, Reed SM, Hutchison JE. In situ deprotection and assembly of S-tritylalkanethiols on gold yields monolayers comparable to those prepared directly from alkanethiols. *Langmuir* 2004;20:9144-9150.
5. Merrifield RB. Solid-phase peptide synthesis. I. The synthesis of a tetrapeptide. *Journal Am. Chem. Soc.* 1963;85:2149-2154.
6. Seneci P. Solid-phase synthesis and combinatorial technologies. New York: John Wiley and Sons, Inc.; 2000. p 2.
7. Tarbell DS. The mechanism of oxidation of thiols to disulfides. In: Kharasch N, editor. Organic sulfur compounds. New York: Pergamon Press; 1961. p 97-102.
8. Wood WF. New components in defensive secretion of the striped skunk, *Mephitis mephitis*. *Journal of Chemical Ecology* 1990;16:2057-2065.