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Synthesis of Conjugated Diacetylene, Metal-Chelating Monomers for Polymerizable Monolayer Assemblies

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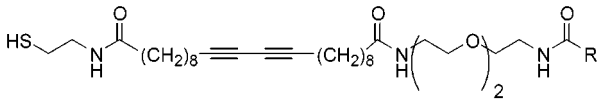
ABSTRACT



Self-assembled monolayers (SAMs) of thiols on gold have been used for numerous applications. For protein targeting applications, one successful strategy is to use a metal-chelating SAM. It has also been demonstrated that polymerized SAMs are much more stable than non-polymerized counterparts. We report herein, the synthesis of several polymerizable, metal-chelating thiols capable of complexing luminescent lanthanide ions.

Self-assembled monolayers (SAMs) of organic thiols and sulfides on solid substrates is an area of intense research.[1] These systems have been used as sensors for metal ions,[2] oligonucleotides,[3] proteins,[4] etc. Usually, for most of the applications reported, thiols are self-assembled on a gold surface. The strong interaction of sulfur with gold and the van der Waals interactions among the hydrophobic hydrocarbon moieties provide the stabilization of SAMs (40−45 kcal/mol).[5]

Several technological applications (for corrosion inhibition, lubrication, adhesion, etc.) of SAMs (self-assembled monolayers) require harsh conditions. The need for increased stability has led to the synthesis of polymerizable SAMs.[6] The molecules can be polymerized by employing diacetylene groups at the center of the hydrophobic chain. The resultant polymerized SAMs are much more stable than nonpolymerized counterparts and have been used as ultrathin photoresists[7] and as rugged adhesion layers.[8] Stable polymerized SAMs can also be prepared by polymerization of the termini of the long chains containing epoxides,[9] pyrroles,[10] or aniline[11] groups.

Protein targeting to membranes is important for 2D protein crystallization, drug delivery, diagnostic imaging, and protein sensing.[12] Interaction between transition metal ions (e.g., Cu^{2+}, Ni^{2+}) and histidine residues on the protein surface has

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been employed for active protein targeting to a monolayer. The monolayer could be either a Langmuir film[13] at the air−water interface or SAMs incorporating metal-chelating moieties on a solid support.[14] The resultant SAMs can be used as enzyme immobilization agents (without loss of activity)[14a,15] and biosensors.[16] Metal-chelating SAMs have also been used for trace detection of metal ions.[17]

We are interested in protein immobilization on the gold surface of a Quartz Crystal Microbalance (QCM)[18] using metal−ligand interactions. For this purpose, a nonpolymerizable, metal-containing thiol, **5** (Figure 1), was synthesized

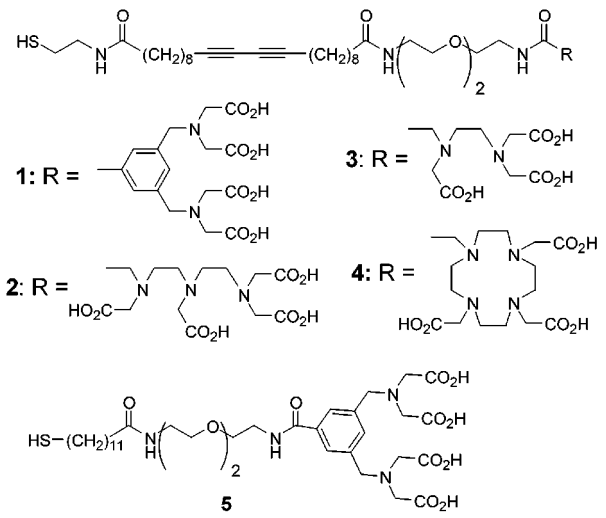

Figure 1. Structures of the polymerizable (**1**, **2**, **3**, and **4**) and nonpolymerizable (**5**) metal-chelating thiols synthesized.

and the SAM was fabricated. Proteins (myoglobin from horse heart and carbonic anhydrase from bovine erythrocyte) were found to bind to the metal ions of the surface of the SAM and also to get embedded inside the monolayer. This complicated the data analysis from QCM.[19]

It will be advantageous to combine the usefulness of metal-chelating SAMs with the cross-linked structure of polymerized monolayers. This is expected to prevent the proteins from getting embedded in the monolayer. Herein, we report the syntheses of several metal-chelating, polymerizable thiols. To our knowledge, this is the first report of this type of thiol. In addition, if a luminescent lanthanide ion (e.g., Eu^{3+}, Tb^{3+}, etc.)[20] is used to complex the headgroup, protein binding to the monolayer can be probed by fluorescence energy transfer.[13a,b,21]

The structures of metal-chelating, polymerizable thiols synthesized (**1**, **2**, **3**, and **4**) are shown in Figure 1. A conjugated diacetylene was used as the polymerizable moiety. This group can be efficiently polymerized by UV light when the molecules are self-assembled on a gold surface.[6] A hydrophilic triethylene glycol unit was introduced as a spacer[4a,14b] between the polymerizable and metal-chelating groups. Compound **1** has iminodiacetate (IDA) as the metal-chelating moiety. IDA has a strong affinity ($>10^{10}$ M^{-1}) for various transition metal ions (e.g., Cu^{2+}, Ni^{2+}, Co^{2+}, etc.).[22] IDA−Cu^{2+} complexes have been used to target proteins to Langmuir monolayer films.[13c,d] The two IDA groups of **1** can position two transition metal ions ∼8 Å apart.[23]

Compounds **2**, **3**, and **4** were designed to complex a lanthanide ion. Compound **2** has DTPA (diethylenetriaminepentaacetic acid) and compound **3** has EDTA as the metal-chelating moiety. These ligands complex lanthanide ions with a strong affinity ($>10^{15}$ M^{-1})[24] and have defined structures.[25] The resultant lanthanide complexes have been used to label proteins[26] and for protein detection by fluorescence spectroscopy.[27] Thiol **4** incorporates the widely used[20] DOTA as the metal-chelating group. This ligand has a very strong affinity ($>10^{25}$ M^{-1}) for the lanthanide ions,[24] and the lanthanide complexes have been used as magnetic resonance contrast agents.[20]

Syntheses of the polymerizable thiols **1**, **2**, **3**, and **4** are depicted in Scheme 1.[28] N-Boc-protected 2-bromoethylamine (**6**)[29] was combined with potassium thioacetate to afford **7** after deprotection. Compound **7** was combined with com-

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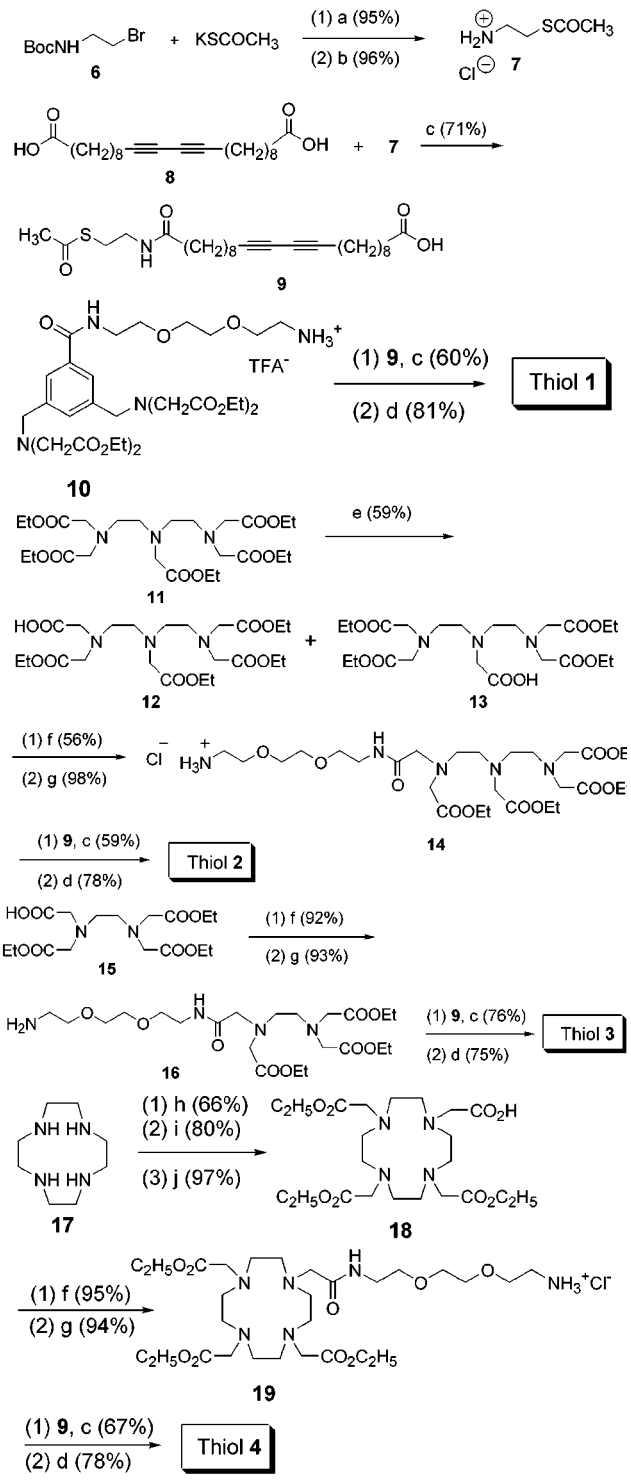
Scheme 1. Synthesis of the Polymerizable, Metal-Chelating Thiols[a]



[a] Reagents and conditions: (a) MeOH, reflux, 5 h; (b) 4 N HCl in dioxane, 25 °C, 3 h; (c) BOP, Et_3N, CH_3CN, 25 °C, 15 h; (d) LiOH, MeOH−THF (1:1), 25 °C, 15 h, then pH = 3.0 with 6 N HCl; (e) $CuCl_2 \cdot 2H_2O$, NaOH−H_2O, H_2S, pH = 5.0, 25 °C, 12 h; (f) $H_2NCH_2CH_2OCH_2CH_2OCH_2CH_2NHCbz$ (**20**), BOP, Et_3N, CH_3CN, 25 °C, 16 h; (g) H_2, Pd-black, MeOH, HCl (1 equiv), 25 °C, 10 h; (h) $BrCH_2CO_2Et$ (2.4 equiv), K_2CO_3, CH_3CN, reflux 16 h; (i) $BrCH_2CO_2Bn$, K_2CO_3, CH_3CN, sonication, 12 h; (j) Pd-black, H_2, MeOH, 25 °C, 10 h.

mercially available[30] polymerizable diacid **8** to give **9**. This compound was used as a common intermediate for the syntheses of the polymerizable thiols.

For polymerizable thiol **1**, compound **9** was reacted with the previously reported IDA derivative **10**.[32] Subsequent hydrolysis of the ester groups was performed using LiOH in a mixed solvent system (MeOH−THF, 1:1, to ensure solubility of all the reactants) to afford the thiol **1**. The thiol was precipitated and then isolated by lowering the pH of the reaction medium (after removal of THF) to 3.0 by dilute HCl. The resultant white solid was found to be pure by ^1H and ^{13}C NMR spectra and elemental analysis.[28]

Synthesis of thiol **2** began with selectively hydrolyzing one of the ester groups of DTPA pentaethyl ester **11**[31] to produce a mixture of the two carboxylic acids **12** and **13**. These two compounds were difficult to separate and were taken to the next step (combined with the monoprotected diamine **20**[32] using BOP as the peptide-coupling reagent) without separation. The two resultant amides can be separated by chromatography. After chromatographic separation of these two compounds, the Cbz group was removed to produce pure **14**. Amine **14** was then coupled with the acid **9**. Hydrolysis of the ester groups and subsequent precipitation (by lowering the pH to 3.0) provided thiol **2**.

To synthesize **3**, compound **15** was prepared by a literature procedure[33] of selective EDTA tetraethyl ester hydrolysis (by pig liver esterase). This was combined with monoprotected diamine **20**,[32] and the Cbz group was removed to yield **16**. Amine **16** was coupled with acid **9**, and subsequent hydrolysis of the ester groups afforded thiol **3**.

For the synthesis of DOTA-based thiol **4**, cyclen (**17**) was selectively functionalized with ethyl 2-bromoacetate following a modification of a reported procedure.[34] The diethyl glycol spacer was introduced next. Reaction with acid **9** and subsequent hydrolysis produced polymerizable thiol **4**.

Overall yields were 49% for **1**, 15% for **2**, 49% for **3,** and 24% for **4** starting from the common intermediate **9**. The polymerizable thiols were prepared in 100−150 mg quantitys and stored under nitrogen at −20 °C, protected from light. Studies are currently underway to study the interaction of proteins with the polymerized SAMs fabricated with the Tb(III) complexes of these thiols.

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Supporting Information Available: Characterization data (^1H, ^{13}C, and elemental analyses) for compounds **1**, **2**, **3**, **4**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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