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New syntheses for 11-(mercaptoundecyl)triethylene glycol and mercaptododecyltriethyleneoxy biotin amide

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Abstract—Novel syntheses for mercaptododecyltriethyleneoxy biotin amide and 11-(mercaptoundecyl)triethylene glycol are presented here. Such alkyl thiols are popular components in creating monolayers capable of specifically binding proteins. The development of a variety of functionalized alkyl thiol compounds has a great impact on biosensor substrate design. In our synthesis of mercaptododecyltriethyleneoxy biotin amide, we couple aminotriethyleneoxydodecane disulfide to the NHS-activated biotin; this technique is amenable to attaching a carboxylated molecule of interest in order to create the functionalized alkyl thiol of choice. The 11-(mercaptoundecyl)triethylene glycol synthesis presented here is an alternative method easily completed in three steps.

1. Introduction

Self-assembled monolayers (SAMs) are a popular tool for tailoring the reactive properties of a surface. These functionalized surfaces are used in liquid mediated assays such as DNA chips, protein chips, and carbohydrate chips. To make the monolayers, molecules may be physisorbed from solution or vapor or more tightly attached by covalent bonds, as with Au substrates and alkyl thiols. In this case, functionalized alkyl thiols adsorb as thin films onto Au substrates. Based on the functional group of the alkyl thiol, substrates may be made to bind or repel proteins,[1,2] carbohydrates, and peptides,[3–5] DNA,[6,7] or other small molecules.[8] SAMs based on alkyl thiols and disulfides and gold surfaces have been well studied and there are numerous literature reports.[9–12] For example, long all-*trans* alkyl chains ($n > 12$) of adjacent alkylthiolates align via van der Waals interactions to form a packing of molecules with a preferred orientation: the thiolate group is in contact with Au and the alkyl chains tilted at a 30° angle from normal.[13–15] However, alkyl chains shorter than 9–10 were found to form monolayers that lacked rigid orientation. Such SAMs were poorer at presenting functional groups to a substrate–liquid interface.

In our ongoing research project of developing novel SAM-based biosensors, we designed and synthesized a new linker containing alkyl thiols and triethylene glycol functionalized with biotin (compound **2**, Fig. 1). In this letter, we also report the modified procedures to synthesize the known SAM substrate 11-(mercaptoundecyl)triethylene glycol (**1**). Biotin is a small molecule with a great avidity and specificity for proteins such as avidin and streptavidin. The binding affinity of biotin to avidin ($K_a \sim 10^{15}\ M^{-1}$) is so great that the bond is considered irreversible. Biotin is also able to bind with high avidity to alternative proteins such as streptavidin ($K_a \sim 10^{13}\ M^{-1}$) and neutravidin ($K_a \sim 10^{11}\ M^{-1}$). Likewise, biotin derivatives are capable of binding with avidin-like proteins with various binding affinities. While the biotin–streptavidin interaction is strong with high specificity, these proteins have been shown to bind in a non-specific fashion when presented with a surface comprising only straight-chain alkyl thiolates.[16] In creating a reactive surface, it is important to promote only high affinity binding events while repelling non-specific ones.

In addition, attachment of polyethylene glycol (PEG) units on surfaces has been shown to reduce the non-specific binding of proteins and even cells to the

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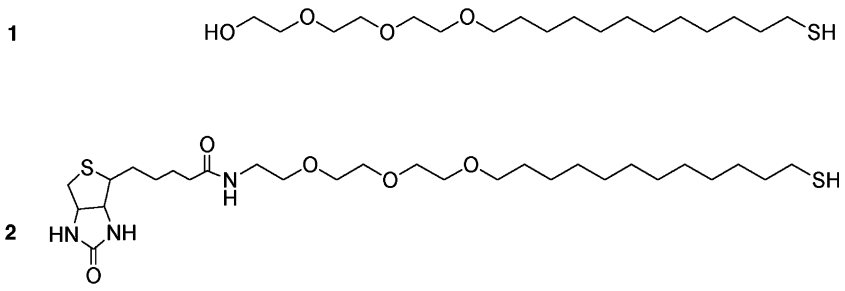

Figure 1. (1) Polyethylene glycol alkane thiol and (2) biotinylated alkane thiol.

substrates.[17–20] In protein binding assays, the biotinylated SAMs with the best avidity for streptavidin are those that present both biotin and PEG groups to the substrate–liquid interface. The mechanism for protein resistance by PEG is not fully understood, but a study by Grunze et al. suggests that it is related to the interfacial adsorption of water molecules with the PEG groups.[21] The uncharged hydrophilic PEG oligo groups adopt random conformations in aqueous solution and coordinate very well with water. When a protein approaches the PEG-covered surface, there is a loss in the available volume and conformations for each oligomer, creating a repulsive force. Since PEG is hydrophilic, the coordinated water cannot be displaced by proteins as they approach the surface, and so the proteins are repelled away.[22] However, in the presence of a surface receptor with high binding affinity to the solvated protein, the thermodynamic stability of the protein–receptor pair overcomes the repulsive effects of PEG and results in protein adsorption. PEG groups are an important and integral component of a functional and specific-binding SAM; they are mostly inert and therefore may be incorporated with a number of functional groups besides biotin. For example, substrates modified with PEG groups are incorporated with peptides, quinones, and carbohydrates. Many of these substrates are under development to create bio-sensing devices.

2. Synthesis

Literature reports the use of radical chemistry initiated by photolysis to achieve the synthesis of compound **1** in decent yield.[23,24] The results in our lab, however, were inconsistent. Instead, we developed the synthesis shown in Figure 2 to produce compound **1**. In the first step, triethylene glycol is deprotonated in anhydrous DMF by NaH, then reacted to large excess with 1,11-dibromoundecane to yield 11-(bromoundecyl)triethylene glycol (41% yield). The introduction of thioacetate was achieved by reacting 11-(bromoundecyl)triethylene glycol to sodium thioacetate, prepared by a reaction of sodium methoxide and thioacetic acid, giving the desired product, 11-(thioacetylundecyl)triethylene glycol, in excellent yield (93%). The deprotonation of thiol ester was straightforward, via ammonia hydrolysis in methanol as a solvent (54% yield).

A biotinylated alkylthiol reagent in the literature is depicted as compound **3**[24,25] (Fig. 3). The body of the compound consists of three sections: biotin, PEG, and alkyl chain. In compound **3**, an amide bond connects the dodecyl chain and the ethylene glycol groups. Compound **2** presented here has an ether bond connecting the alkyl chain to the ethylene glycol group, more closely mimicking the structure of **1**. Another difference between the syntheses of **2** and **3** is the direction in which the compounds are made. As outlined in the procedures by Nelson et al., the molecule is constructed by the attachment of the PEG group to the biotin, followed by the attachment of the alkyl chain to the PEG group.

The synthesis of compound **12** is described in Figure 4. The selective tosylation of triethylene glycol in two steps using NaH and *p*-toluenesulfonyl chloride in

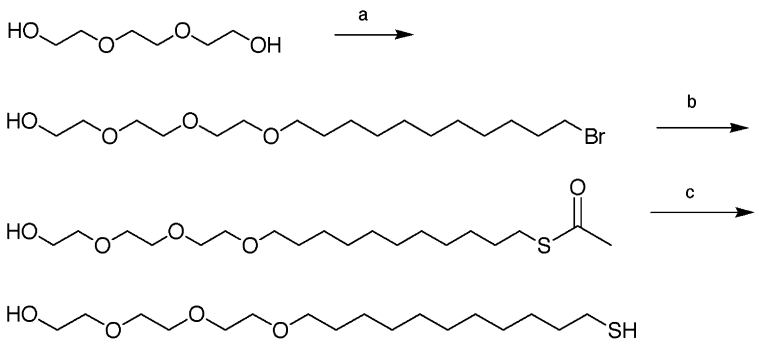

Figure 2. (a) (i) NaH/DMF, (ii) 1,11-dibromoundecane; (b) (i) NaH/CH_3OH, (ii) CH_3COSH; (c) NH_4OH/CH_3OH, 40 °C.

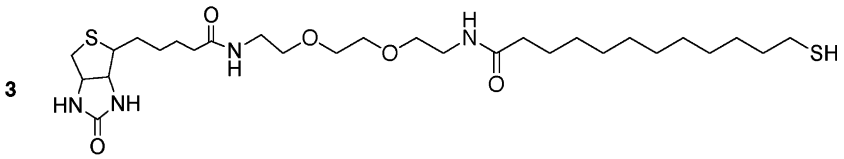

Figure 3. Biotinylated alkane thiol described by Knoll and Nelson et al.

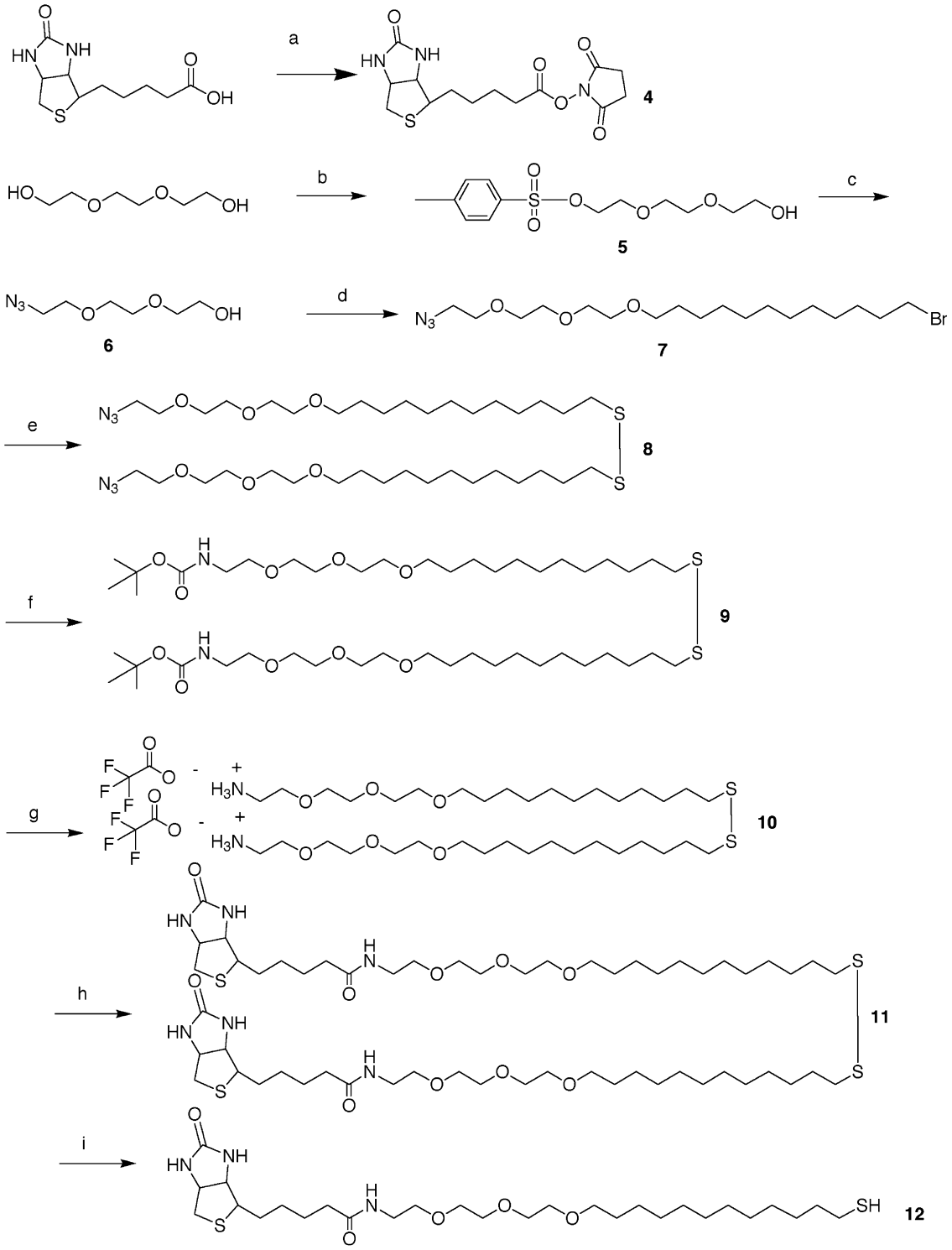

Figure 4. (a) *N*-hydroxysuccinimide/DCC/DMF; (b) (i) NaH/DMF, (ii) *p*-toluenesulfonyl chloride; (c) NaN_3/DMF/H_2O, 58 °C; (d) (i) NaH/DMF, (ii) 1,12-dibromododecane; (e) (i) thiourea/EtOH/H_2O, 88 °C (ii) NaOH; (f) (i) Ph_3P/THF/H_2O, (ii) ditertbutyl dicarbonate/THF/TEA; (g) TFA/CH_2Cl_2/triisopropyl silane; (h) 7/DMF/TEA; (i) DTT/THF/MeOH/TEA.

anhydrous DMF led to the formation of **5** (73% yield). The azide **6** was prepared in almost quantitative yield (99%) by stirring **5** in large excess of NaN_3 in DMF/H_2O. Reaction of this compound to excess amount of 1,12 dibromododecane resulted in the attachment of the azotriethylene glycol to the alkyl chain to yield 12-(azotriethyleneoxy)-1-bromododecane **7** in low yield (24% yield). The resulting bromoalkane **7** was refluxed with thiourea in ethanol, followed by hydrolysis by NaOH solution, affording the diazodo disulfide **8** (46% yield). The reduction of the azo group by triphenylphosphine in THF provided the corresponding amine; after protection by *tert*-butoxycarbonate, **9** was obtained in excellent yield (82% yield). The removal of the *tert*-butoxycarbonate group by trifluoroacetic acid (TFA) in CH_2Cl_2 gave ammoniumtriethyleneoxydodecane disulfide **10** (80% yield). The introduction of biotin to the linker through a reaction of the corresponding amine to the activated biotin derivative **4** was straightforward. The benefit of this reaction is its application for the attachment of a carboxylated molecule of choice.[26] The final compound **12** can be prepared by a simple reduction of the disulfide **11** by dithiothreitol (DTT) in basic medium.

In summary, we have designed and synthesized two biofunctionalized alkyl thiols. The synthesis of 11-(mercaptoundecyl)triethylene glycol **1** in three steps has provided a feasible alternative method. Furthermore, mercaptododecyltriethyleneoxy biotin amide **12** is novel and viable for the formation of SAMs. The intermediate aminotriethyleneoxydodecanethiol **11** will be very useful for attachment of any activated carboxyl groups in order to make novel receptors for biosensor development. Finally, the surface studies using these molecules will be published separately.

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Supplementary data

Materials, detailed synthesis, and product characterization can be found in the Supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.04.091.

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