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SYNTHESIS AND INVESTIGATION OF FUNCTIONALIZED ALKYL OLIGO(ETHYLENE OXIDES)

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The research was accomplished in State Research Institute (SRI) Center for Physical Sciences and Technology in the period of 2008 – 2011 and 2013 – 2015.

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Contents

1. Introduction

Self-assembled structures have attracted an enormous interest of different scientists. Self-assembled monolayers (SAMs) are the popular tool for tailoring the reactive properties of the surface. Due to their dense and stable structure, SAMs have been investigated for use in electrochemical sensors technology, electronics, nanodevices of any kind, and other areas [1-3]. Besides, the close similarity of the SAMs with biomembranes enables the use of them as model systems. Such a system allows the preparation of ultrathin and high-resistance lipid layers on metals or semiconductors and the incorporation of receptor proteins into these insulating layers in order to design biosensors, bioelectronic or other biomimetic devices [4-6]. It also opens new paths to investigate membrane-related processes (cell adhesion, photosynthesis, respiration, drug-protein interactions, etc.).

The stability, flexibility and other properties made tethered bilayer membranes (tBLMs) one of the most promising model systems on a solid support, which can be successfully investigated with a multitude of surface-sensitive analytical techniques for much biotechnological manipulation [7].

Neutral oligo ethylene glycol (OEG) units are important components of specific-binding SAMs and might be incorporated with a number of functional groups; they also serve as a spacer arm for tethering the lipid layer on the solid substrate. Because the formation of the sulphur-gold bond is a commonly used chemisorption method for self-assembly [8], oligo(ethylene oxide) thiols and sulphides are the main building blocks for tBLMs. Such derivatives, functionalized with lipid, biotin and cholesterol groups are the most popular tools used in attachments, further development and investigation of artificial membranes.

The goal and tasks of the present work

The goal of the work: to create bifunctional materials with oligo(ethylene oxide) chain suitable for tBLMs formation and investigation.

The tasks of the work:

1) to synthesize stable 1,2-ditetradecylglycerol, biotin, cholesterol bifunctional derivatives with organosulphur head groups, capable tether tBLMs on metal surface;
2) to synthesize functionalized biotins and cholesterols with label (fluorescent – stilbazolium, cyanine groups; redox – viologen group), capable for investigation of the properties of model membranes.

Scientific novelty of the work

The first step in the tBLM modeling process is preparation of SAM. Organosulphur molecules (thiols, sulphides) adsorb spontaneously from solution on the metal (Au, Ag, Cu) surface, form a highly reproducible, stable and densely packed monomolecular layer. Unfortunately, such compounds are uncomfortable in use because of their ability to oxidize, that leads to unacceptable formation of bilayer from oxidized forms. The solution for this problem might be stable derivatives with organosulphur head groups able for strong metal-support adsorption. Therefore bifunctional 1,2-ditetradecylglycerol, biotin and cholesterol organosulphur tethers were synthesized. New synthesis routs were created by using inexpensive, commercially available reagents. Moreover, almost all products were purified without column chromatography.

Another searching area – synthesis of stable labeled derivatives, suitable for investigation properties of model membranes. For this purpose, new functionalized biotin and cholesterol markers were synthesized and investigated by fluorescence, vibrational spectroscopy and electrochemical methods. The great binding affinity of biotins to glycoproteins (streptavidin, neutravidin, avidin) and effective cholesterol labels (fluorescent – cyanine, redox – viologen groups) opens future perspectives in investigation of model membranes.

Many analogs of mention bifunctional tethers and markers include a peptide bond, which joins the end group, OEG spacer arm and head group or label. We accepted it, to avoid possible hydrogen binding, which is not favorable for tBLM; besides, we saved the nature of bulky functional group as cholesterol in model membrane, providing system stability and flexibility.

Main statements for the defense

1) Optimization of glycerol monoprotection by *p*-methoxybenzylalcohol (PMBOH) in the presence of acidic catalyst refluxing in dichloromethane allows to isolate primary and secondary products with the best ratio (9.4:1).
2) Monoprotected glycerols are isolated by selective protection of primary PMB glycerol by cyclohexanone and followed acid hydrolysis in the presence of anh. $CaCl_2$.
3) Alkylation of primary monoPMB glycerol in NaOH/DMSO system leads to formation of product, which was successfully deprotected by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to form 1,2-ditetradecylglycerol – precursor of thiolipids.
4) Protection of PMB glycerol by *tret*-butyldimethylsilylchloride (TBSCl) in the presence of base at low temperatures selective forms monoTBS ethers.
5) New (chlorohexyl)triethylene glycol functionalized with biotin is convenient for the synthesis of biotinylated esters, which binds to streptavidine.
6) 4,4′-Disubstituted-1,2-dithiolanes adsorption on the gold surface occurs with decomposition of S-S bond (508 cm^{-1}) and formation of Au-S (255 cm^{-1}) bond.
7) 4,4′-Disubstituted-1,2-dithiolanes are stable anchors, convenient for the synthesis of bifunctional cholesterol tethers for model membranes.
8) Synthesized (cholesteryl)tetraethylene glycol without peptide bonds is a precursor in preparation of labeled with fluorescent and redox groups cholesterols.

2. Results and discussion

2.1 Monoprotection of glycerol by PMBOH

In our ongoing project of developing novel syntheses of different molecular systems based on tailoring the reactive properties of surfaces, we synthesized glycerol-based lipids for the farther tBLMs design and research.

In the synthesis of long glycerol-based lipids we have choose hydroxyl group protection with PMB group. PMB ethers are much less stable to acids than popular benzyl ethers. Aqueous mineral acids or camphorosulfonic acid in methanol removes them. They also can be removed under mildly oxidizing conditions using DDQ reagent that do not affect benzyl, silyl and some other ethers. Therefore, PMB ethers are used in the synthesis of functionally complex compounds, where extensive selective protection-deprotection protocols are required [9].

The most commonly employed methods for PMB protection of alcohols involve usage of *p*-methoxybenzyl chloride (PMBCl) or *p*-methoxybenzyl bromide (PMBBr), however both reagents are prone to decomposition [10]. We suggest monoprotection of glycerol hydroxyl group with commercially available, stable and easy to handle *p*-methoxybenzyl alcohol (PMBOH) in the presence of acidic resins Amberlyst-15 (A-15) and Amberlite-200C (A-200C).

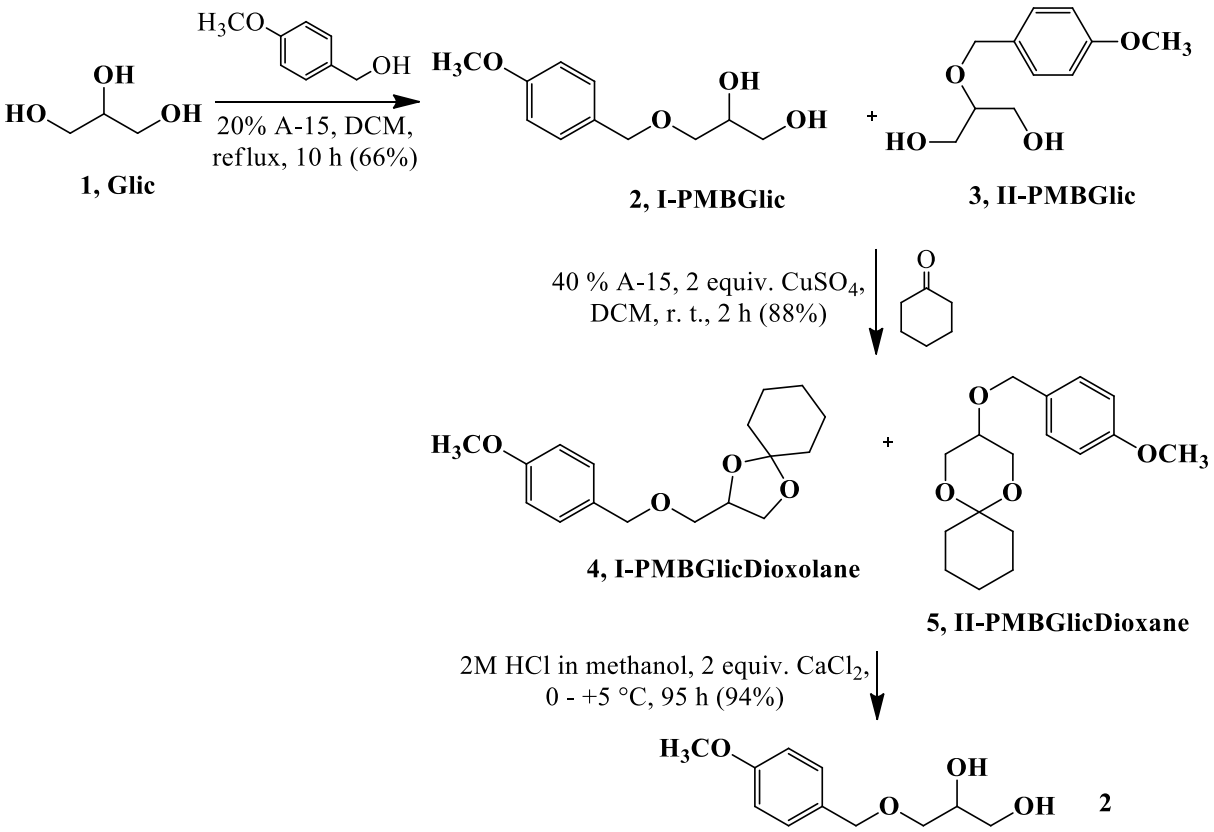
Firstly, glycerol was protected with PMBOH under several conditions (**Table 1**): in dioxane and glycerol mixture (under microwave irradiation (MW)), in dioxane (85-100 °C), in glycerol (70-90 °C), in benzene (reflux), in dichloromethane (reflux) using 10-30% A-15 and 20-60% A-200C. Gas Chromatograph-Mass Spectrometer (GC-MS) analysis allowed identifying two main monoprotected glycerols (compounds **2** and **3** in **Scheme 1**) and three additional products (diPMB ether, diPMB glycerols). The best result was obtained with 20% A-15 (yield 66%) in dichloromethane (DCM). The ratio of two monoprotected PMB glycerols was 9.4:1. Next, it was decided to do two simple reactions for farther separation of main isomers without column chromatography, using only extraction methods. Monoprotected glycerols were tried to selective coupling with acetone, cyclopentanone and cyclohexanone. The best selectivity achieved (only product **4** was formed) in reaction with cyclohexanone and anh. $CuSO_4$ in DCM at room temperature (yield 88%). Next, dioxolane fragment was decomposed with 2M HCl in

methanol and anh. $CaCl_2$ at 0 − +5 °C, yielding (94 %) corresponding monoglycerol **2**. Other tested conditions (1M and 5M HCl in methanol, -5 − +25 °C, using anh. $Na_2S_2O_5$, Na_2SO_3, Na_2SO_4, $CuSO_4$) of this reaction were less successful.

Isolated monoprotected PMB glycerol was used for the synthesis of thiolipids precursor – 1,2-ditetradecylglycerol.

Table 1. Optimization of glycerol monoprotection by PMBOH.

Solvent	**t (°C)**	**No.**	**Catalyst**	**Time (h)**	**Yield of both isomers (%)**	**Ratio of isomers (I/II) after extraction**
Dioxane/ Glycerol (1:2)	MW (180W)	**1.1**	10% A-15	0,16	43	2.8:1
Dioxane	85	**1.2**	10% A-15	7	48	3.6:1
	100	**1.3**		5	50	1.7:1
Benzene	80	**2.1**	20% A-15	8	57	5.4:1
		2.2	20% A-200C	8	28	5.9:1
		2.3	40% A-200C	2	47	4.6:1
Glycerol	70	**3.1**	20% A-15	3	29	3.5:1
		3.2	30% A-15	3	30	5.1:1
	80	**3.3**	20% A-15	2	55	3.8:1
		3.4	30% A-15	1.5	61	4.7:1
	90	**3.5**	20% A-15	1.5	48	5:1
		3.6	30% A-15	0.5	54	3.7:1
		3.7	40% A-200C	1	54	3.5:1
DCM	40-42	**4.1**	5% A-15	13	24	7.2:1
		4.2	10% A-15	13	59	6.8:1
		4.3	20% A-15	10	**66**	**9.4:1**
		4.4	10% A-200C	21	35	4.6:1
		4.5	20% A-200C	16	45	5.4:1
		4.6	40% A-200C	10	34	8.3:1
		4.7	60% A-200C	13	45	7.5:1



Scheme 1. Glycerol protection by PMBOH and separation of PMB monoprotected glycerols.

2.2 PMB glycerol protection with THP and TBS groups

Protection of PMB glycerol by tetrahydropyranyl (THP) and by *tret*-butyldimethylsilyl (TBS) groups was tried for main purpose – to synthesize precursors of unsymmetrical thiolipids. It also can be used for the synthesis of bifunctional derivatives with different substituents.

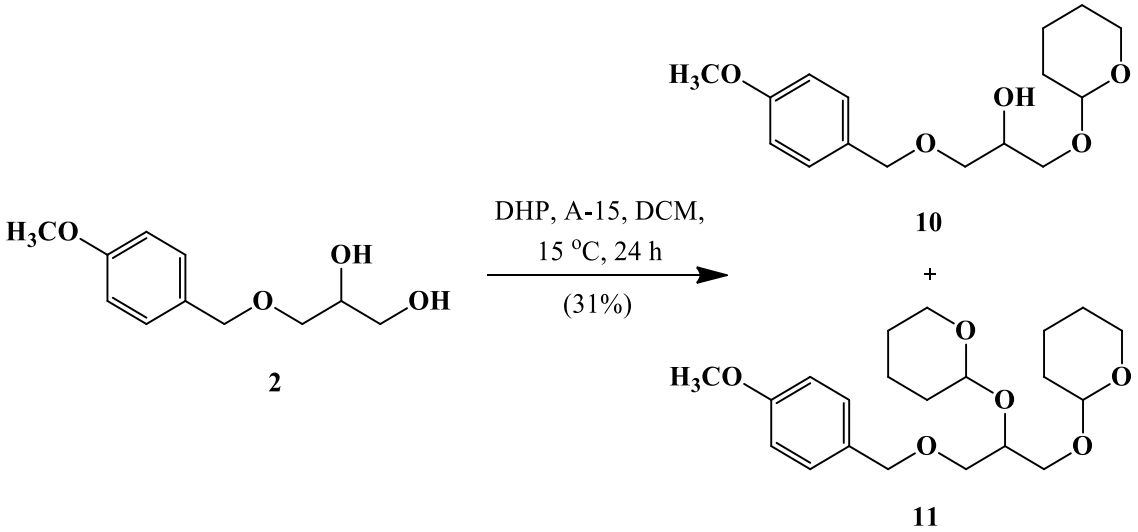
THP ethers were one of the first generally useful protecting groups for alcohol to be adopted. The ease introduction of THP group (generally in presence of TsOH or acidic resins), its stability under a wide range of reaction conditions and its ease removal (AcOH in water (4:1), acidic resins, pyridinium *p*-toluenesulfonate (PPTS) in ethanol, etc.) remain it still widely used protection group [9]. Moreover, 3,4-dihydro-2H-pyran (DHP) is low cost and commercially available protective reagent.

Our PMB glycerol protection by THP group experiments were done with TsOH, A-200C and A-15 catalysts under several conditions (**Table 2**). The best result was obtained with A-15 in DCM at 15 °C temperature (ratio of mono- and diproducts – 4.2:1). Unfortunately, we do not found convenient conditions for main synthesis of

monoprotected THP product (**Scheme 2**). So, only the mixture of mono- and diprotected THP ethers was isolated (yield 31%).

Table 2. Synthesis of THP ethers.

No.	Reagents (equiv.)			Solvent	t (°C)	Time (h)	Ratio of mono- and diproducts by GC-MS analysis (yield)
	PMB-Glic	DHP	Catalyst				
1.1	1	1	0.005 TsOH	DCM	5	1	1:1 after 1 h
1.2	1	1	0.005 TsOH	DCM	19	3	2:1 after 10 min
2	1	1	1% A-200C	DCM	15	24	2.6:1 after 1 h
3	1	1	2% A-15	DCM	15	24	4.2:1 after 24 h (**31%**)



Scheme 2. Protection of PMB glycerol by THP group.

Whereas the THP protection results were not excellent, we decided to protect PMB glycerol by bulky TBS group, using TBSCl reagent.

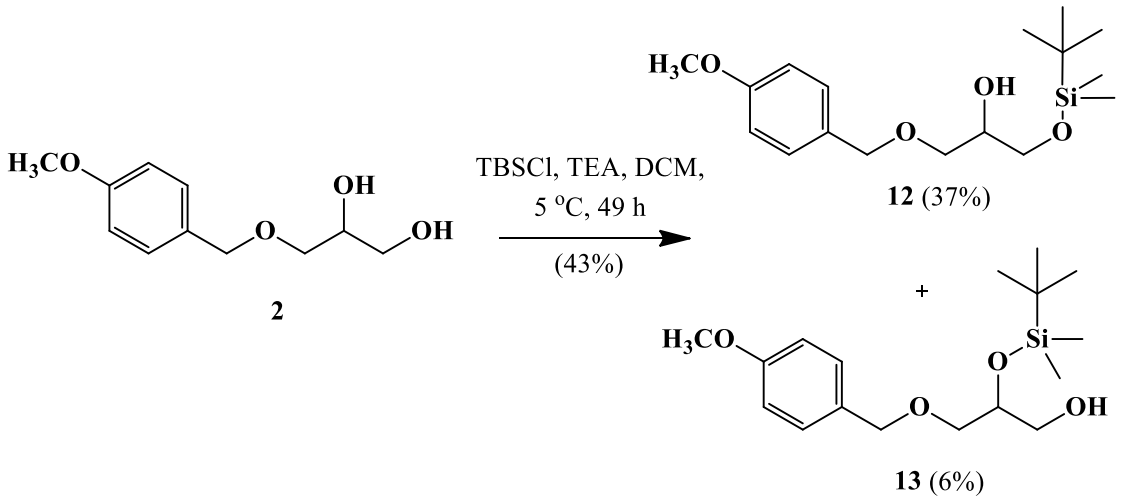
A simple deprotection procedure under several conditions (complexes of HF with amines, fluorides, PPTS, acidic resins, etc.) made TBS ethers the most popular silyl derivatives. The TBS ethers usually are formed in the presence of base-activator, such as imidazole, 4-dimethylaminopyridine (DMAP) [9], 1,8-diazabicycloundec-7-ene (DBU) [11] or triethylamine (TEA) [12, 13].

During experiment with DBU, it was found, that at 15 °C temperature react both primary and secondary hydroxyl groups of PMB glycerol (**Table 3**). Therefore, next we

tried TEA base without the DMAP catalyst. Optimization of mention conditions practically allows avoiding the formation of diTBS ether. The mixture of two monoproducts (**Scheme 3**) was isolated in 43% yield (37% primary TBS ether; 6% secondary TBS ether).

Table 3. Synthesis of TBS ethers.

No.	Reagents (equiv.)			Solvent	t (°C)	Time (h)	The best ratio of mono- and diproducts by GC-MS analysis (yield of monoproducts)
	PMB-Glic	TBS-Cl	Base				
1	1	1	1.1 DBU	C_6H_6	15	2	2.3:1 after 0.5 h
2.1	1	1	1.1 TEA	DCM	-8 – 18	16	PMB decomposition products after 16 h
2.2	1	2	6 TEA	DCM	-8 – 18	141	1:1 after 117 h
2.3	1	2	6 TEA	DCM	5	49	8.9:0.1 after 49 h (**43%**)



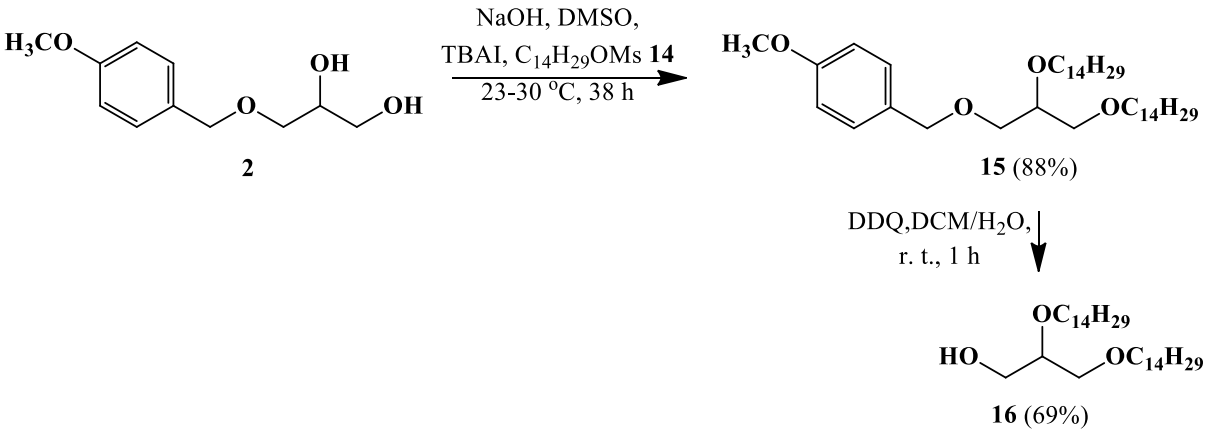
Scheme 3. Monoprotection of PMB glycerol by TBSCl.

2.3 Synthesis of thiolipids

We have developed an efficient synthesis of the glycerol-based thiolipids. Two compounds were made from the same parts as natural membrane lipids: a hydrophilic head group with a neutral spacer unit, hydrophobic tails and a linker – glycerol substructure that tethers two parts. We have decided to synthesize thiol and cyclic disulfide with saturated

long chains for the future possibility to compare their stability and suitability for tBLM modeling and research. Our novelty is in determination of interesting alternative synthesis route avoiding so popular amide and peptide bounds with possible hydrogen binding that is not acceptable during the tBLM formation process. So, two thiolipids containing neutral and flexible tetraethylene glycol (OEG4) chain with thiol and cyclic disulphide anchors for attachment onto a gold support were synthesized. Some working groups reported synthesis of similar thiolipids [14-16]. We suggest a new simpler way for the synthesis of analogue compounds. All components to our synthesis are inexpensive and commercially available. Moreover, all products, except isothiouronium salt, were purified without column chromatography in good yields (59-99%).

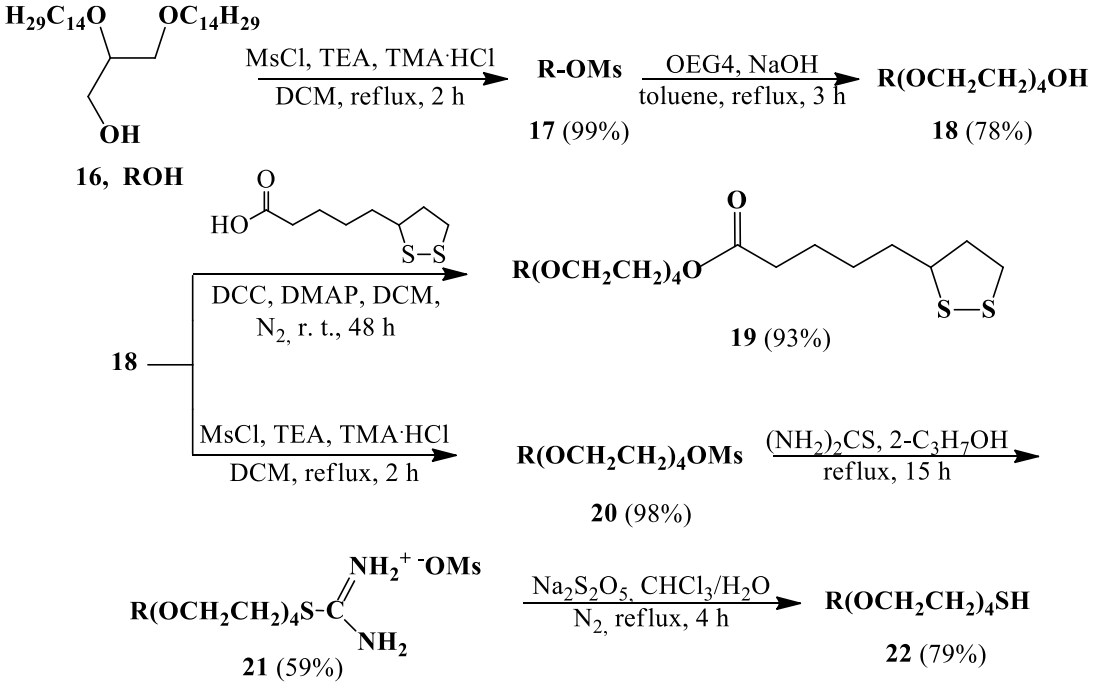
The starting reagent of thiolipids synthesis – 1,2-ditetradecylglycerol (**16**) was synthesized (**Scheme 4**) from monoprotected PMB glycerol (**2**) coupled with tridecylmethanesulfonate ($C_{14}H_{29}OMs$ **14**). Firstly, DMSO/KOH or NaOH system was tried. It was noticed, that hydrolysis of mention mesylate occurs at 40-50 °C temperature. Therefore, tetrabutylammonium iodide (TBAI) catalyst was used. It allows reducing temperature of reaction and increasing yield up to 88%. Next, PMB group was deprotected with DDQ in DCM and water mixture (10:1).



Scheme 4. The synthesis of 1,2-ditetradecylglycerol.

The route for the main synthesis of thiolipids started from alcohol **18**, which was prepared by a method of two steps as shown in the **Scheme 5**. Firstly, methanesulfonate **17** was synthesized from 1,2-ditetradecylglycerol and methanesulfonyl chloride (MsCl) under basic condition of tertiary amines. It was found, that the synthesis route depends on the humidity. The mesilation stage was made in a similar way in commercial DCM and

under absolute conditions using a conversed Dean-Stark receiver. So, the reaction yield increased from 69 up 99%, respectively. Secondly, OEG4 was deprotonated in anhydrous toluene by sodium hydroxide and then, reacted with compound **17**. This stage was repeated several times in benzene. However, product **18** was synthesized under reflux, it was noticed that reaction speed and yield depend on the solvent boiling point (b. p.). In toluene (b. p. 111 °C) the reaction was finished after 3 hours, yielding 78% of alcohol **18**. The same conditions in benzene (b. p. 80.1 °C) allowed us to obtain 68% of the suspected product. Only triple reaction time made possible to increase the yield up to 80%. Next, alcohol **18** was coupled with *N*,*N*′-dicyclohexylcarbodiimide (DCC) activated lipoic acid in the presence of the catalytic amount of DMAP, with formation of cyclic disulphide **19**. Another direction of synthesis was made by using mesyl isothiouronium salt **21**, which was purified by column chromatography. The final thiol **22** was prepared by mild hydrolysis of sodium metabisulfite (yield 79%).



Scheme 5. The synthesis route of thiolipids.

Synthesized thiol **22** was self-oxidized after two weeks standing at a freezer (-12 °C). Synthesized cyclic disulphide **19** was being kept under the same conditions without changes during several months. However, we recommend further keeping of these thiolipids under inert atmosphere of argon or nitrogen at low temperatures. Both synthesized final products **19** and **22** showed the ability to form tBLM.

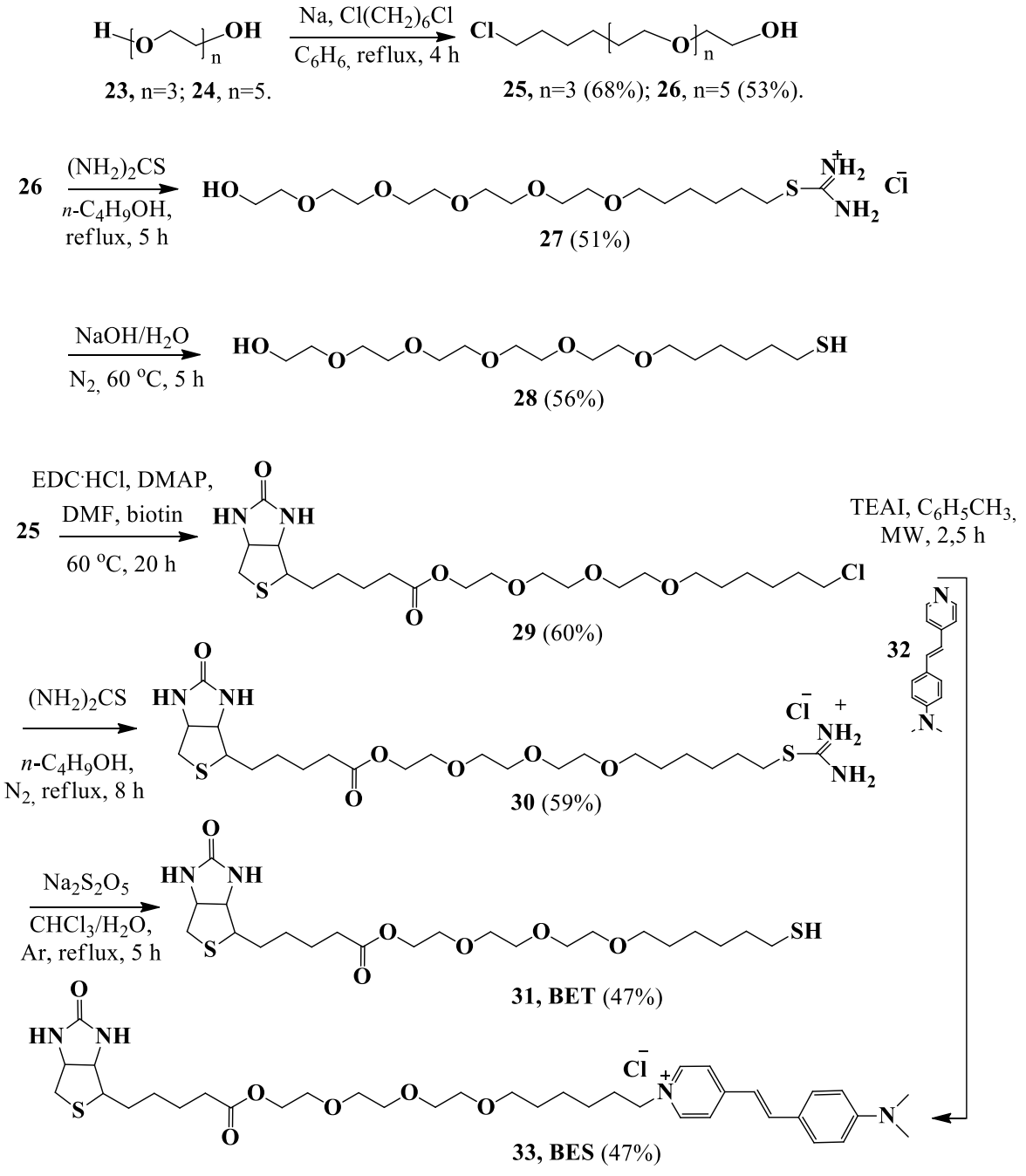
2.4 Synthesis of functionalized biotins

Biotin is one of the most used tools to immobilize antibodies (antigens, enzymes, DNA, etc.) onto surfaces through the biotin-protein (streptavidin, neutravidin, avidin) pair. Optimal biotin binding capabilities can be obtained by using a biotin derivative that has an extended spacer arm, which reduces the steric hindrance effect. The spacer arm also improves the complex formation of biotin with the deep biotin-binding site of protein. For this purpose, generally biotinylated systems include OEG groups. The properties of OEG such as chemical stability, water solubility, flexibility and low cytotoxity help to avoid the non-specific adsorption of proteins onto the surface and provide possibility for appropriate orientation of end group for interaction with the solution species.

Among various biotinylated derivatives reported in literature, the most popular are biotin alkyl thiols (BATs). They consist of three main parts: biotin, OEG and alkyl chains, generally joined with peptide bond [17]. Our purpose in this synthesis was to produce a derivative without peptide bounds to avoid possible hydrogen binding between biotinylated system and protein. Firstly, we have designed an efficient synthesis of halogenide containing (hexyl)triethylene glycol chain functionalized with biotin, which is a building block for the synthesis of biotinylated compounds. To prove this, two biotinylated esters were successful synthesized in several steps. Stilbazolium fluorophore was selected because of successful application of this dye as a voltage-sensitive fluorescent membrane probe and our intention to use stilbazolium group labeled proteins for studies of interactions between the proteins and sparsely tBLMs. We also described a novel synthesis strategy of the functionalized thiol that can be widely used to produce monolayers for different surface modifications and biological applications. The useful properties of synthesized biotinylated esters for surface modifications and protein marking were investigated using the surface plasmon resonance ellipsometry (SPRE) and fluorescence spectroscopy at Vilnius University Institute of Biochemistry.

The synthesis route started with the commercially available triethylene (OEG3) and pentaethylene (OEG5) glycols as shown in **Scheme 6**. In the first step, OEG3 was deprotonated in anhydrous benzene by sodium, then reacted with 1,6-dichlorohexane to yield 68% of 6-(chlorohexyl)triethylene glycol (**25**). The compound **26** was prepared similarly to give 53% yield. Diluent compound **28** for SPRE measurements was prepared by method of two steps. Firstly, isothiouronium chloride **27** was synthesized from

compound **26** and thiourea (yield 51%). Then, it was hydrolyzed with sodium hydroxide yielding 56% of 6-(mercaptohexyl)pentaethylene glycol (**28**). Next, we decided to choose strategy of biotinylated linker's **29** synthesis through carbodiimide activated biotin. The resulting product was recrystallized in good yield (60%) and used for isothiouronium salt **30** formation (yield 59%). Then we choose mild hydrolysis by sodium metabisulfite in chloroform and water solution to provide the corresponding thiol **31** (yield 47%). Finally, stilbazolium salt **33** was prepared from compound **29** and dimethylaminostilbazole by MW (yield 47%).



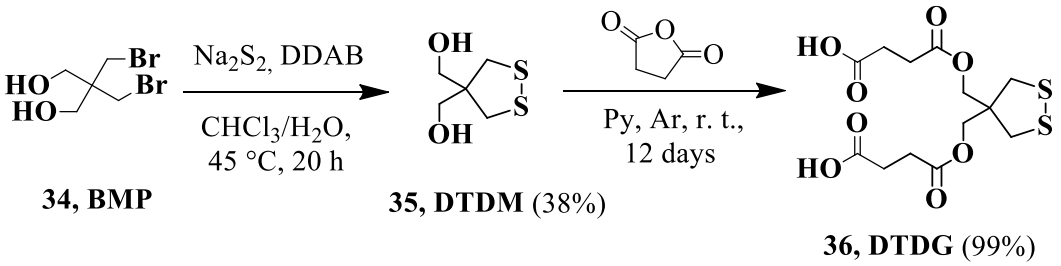
Scheme 6. The synthesis route of functionalized biotins.

2.5 Synthesis and Raman spectra analysis of 1,2-dithiolane derivatives

The disulfidic bridge improves stability of attachment on the surface by two sulfur atoms per one component upon adsorption to the substrate. Therefore, we synthetically design not oxidizing after several years and stable anchor formed from (1,2-dithiolane-4,4-diyl)dimethanol (DTDM).

We choose convenient way and synthesized cyclic disulphide **35** (DTDM) in a 38% yield from 2,2-bis(bromomethyl)-1,3-propanediol (BMP) and Na_2S_2 using di-*n*-decyldimethylamonium bromide (DDAB) catalyst (**Scheme 7**). Next, it was coupling with excess of succinic anhydride in dry pyridine at room temperature to give 99% of product **36** (DTDG).

DTDG was used as diluent compound for SAMs and as anchor in the synthesis of functionalized cholesterols.



Scheme 7. Synthesis of cyclic anchors.

Theoretical modeling of DTDM and DTDG structures was performed using Gausian 03W and 09 programs [18]. Part of computations were performed on resources at the High Performance Computing Center HPC Sauletekis in Vilnius University Faculty of Physics. Geometry optimization and frequency calculations were accomplished with the density functional theory (DFT) method using B3LYP functional and 6-311++G(d,p) basis set for DTDG, PBE1PBE functional and 6-311++G(2d,p) basis set for DTDM and B3LYP functional and LanL2DZ ECP basis set for Au atoms (DTDM on 9 Au cluster). Calculated Raman scattering activities were scaled by conversion them to the Raman cross sections, which are proportional to the Raman intensities and can be compared with the experimental data. Predicted spectra were generated by using Lorentzian function for broadening of Raman lines with 20 cm^{-1} full width at half-maximum values.

So, DTDM (**Figure 1**), DTDM on Au (**Figure 2**), DTDG, reduced (by PBu_3) DTDG – DTDG-H (**Figure 3**) Raman spectra were recorded. Comparison of calculated

and experimental Raman or SERS spectra of DTDG, DTDM and DTDM on Au and analysis of potential energy distribution led to assignment of vibrational bands. It was noticed, that adsorption of 1,2-dithiolane ring occurs with decomposition of S-S bond (508 cm^{-1}) and formation of Au-S (255 cm^{-1}) bond.

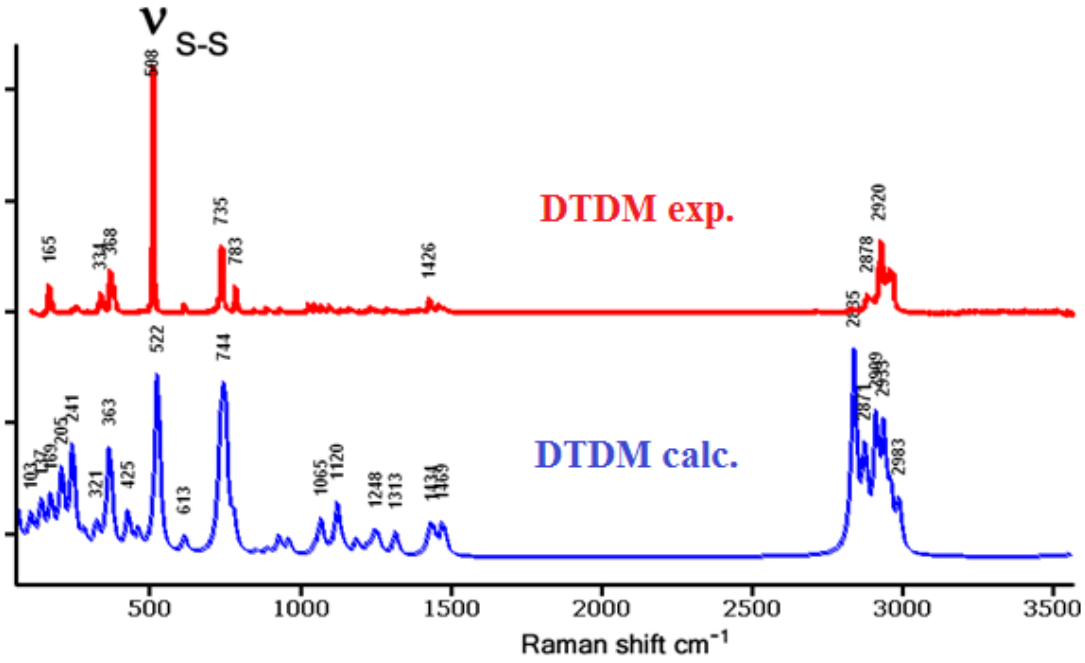


Figure 1. Experimental (red) and calculated (blue) Raman spectra of DTDM.

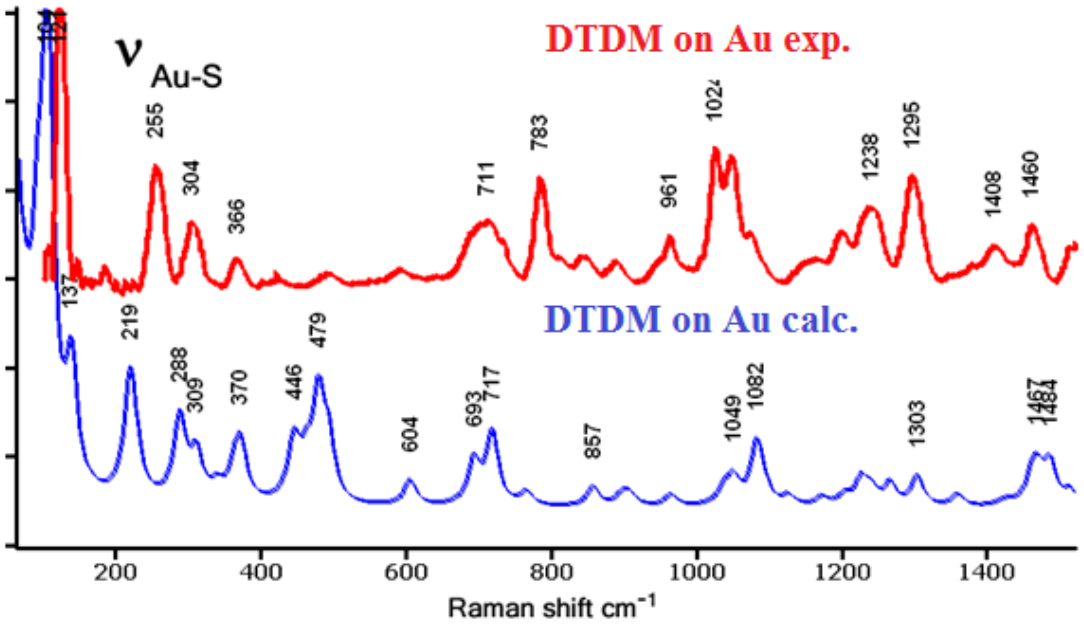


Figure 2. Experimental (red) and calculated (blue) SERS spectra of DTDM on Au.

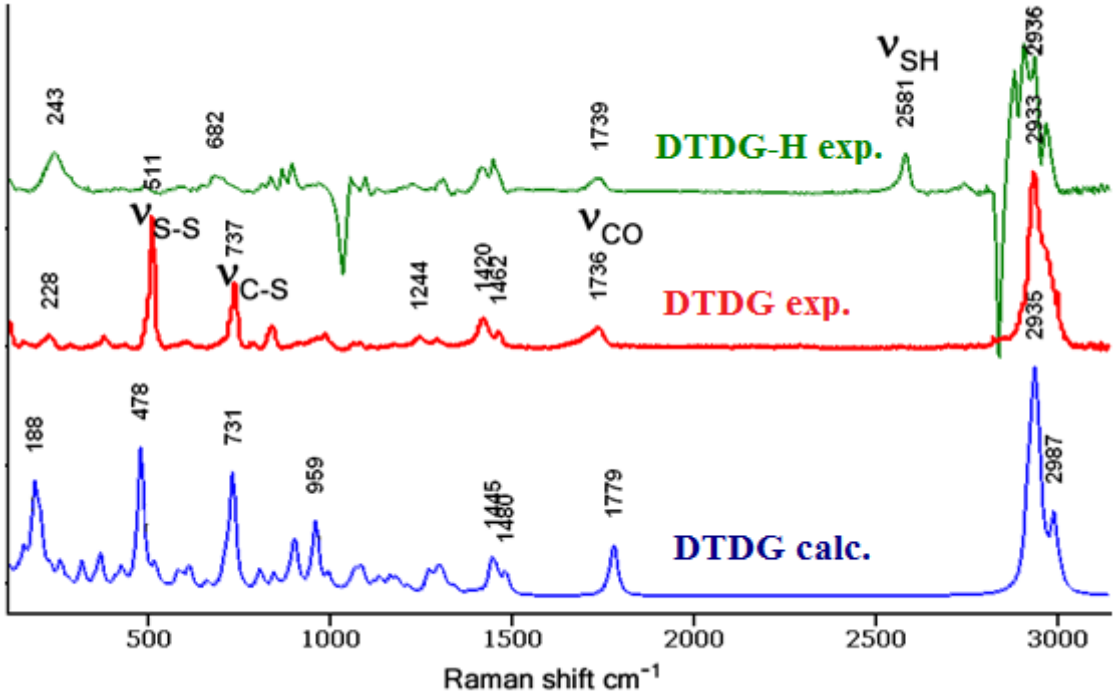


Figure 3. Experimental (green) Raman spectrum of DTDG-H; experimental (red) and calculated (blue) Raman spectra of DTDG.

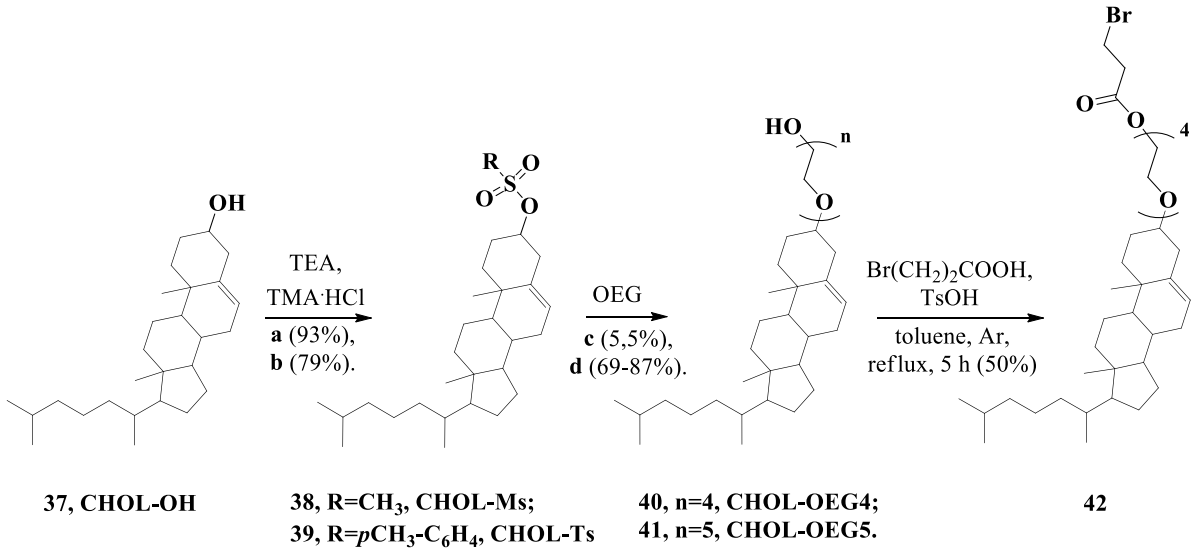
2.6 Synthesis of functionalized cholesterols

Modification of model molecular systems with cholesterol moiety may be of interest, since cholesterol is essential component of mammalian membranes. Many properties of biological membranes depend on a well-defined amount of cholesterol [19]. Considering to this, our working group choose bifunctional cholesterol compounds as main synthetic target for future tBLMs design and research.

There are many examples of tethers and labels for lipids and only some cholesterol analogues in literature; commonly ester or peptide bond are near cholesterol side in such structures [20, 21]. We synthetically designed several new structures, were cholesterol side was spaced from anchor, fluorophore or redox groups with an oligo(ethylene oxide) spacer arm via ether linkage to save the nature of cholesterol during embedding into a lipid bilayer. These also help to avoid possible hydrogen binding that can negatively influenced tBLM formation and properties. Cholesterol compounds with cyclic disulphide or thiol groups as anchors were used for tethering the lipid bilayer to the gold substrate and show good ability to form tBLMs. The former derivatives displayed extended stability in the air. We also successfully demonstrated that our synthesized cholesterol compounds labeled with cyanine dyes can be use as fluorescent tools for monitoring of cholesterol traffic in

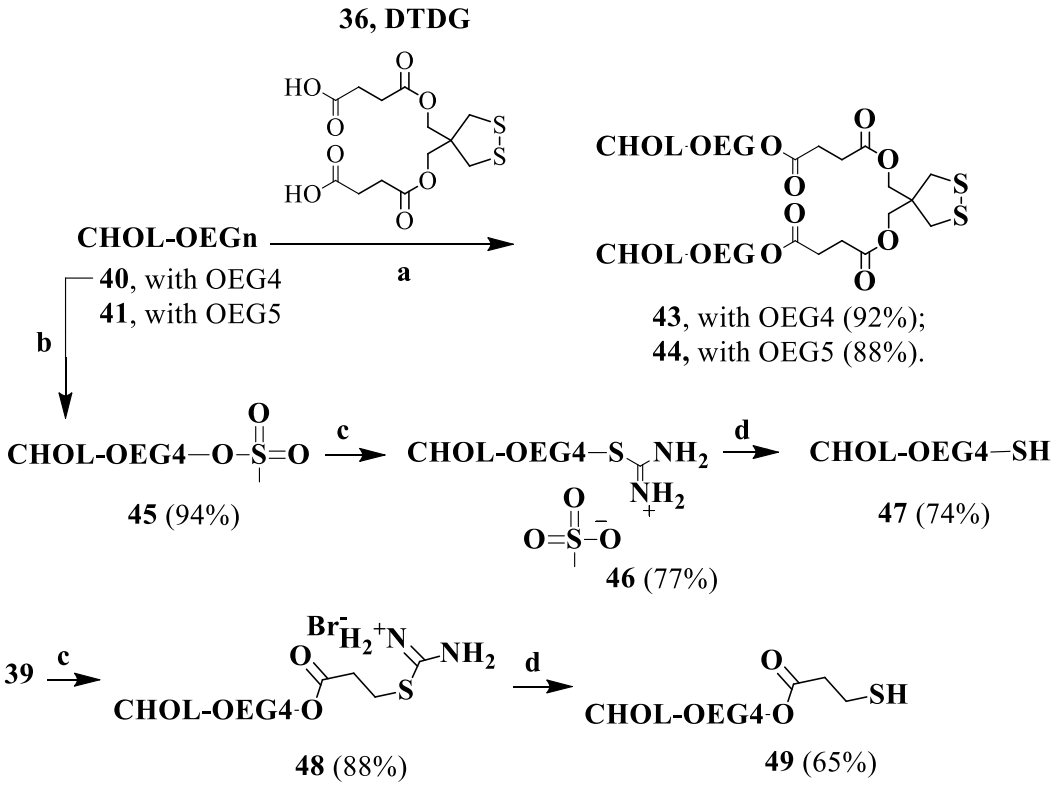
membrane. Viologen labeled cholesterol showed utility as an electron carrier, which can be use in artificial bioelectrochemical systems. Besides, modification with cyano group allows shifting the redox potential to more positive values, which opens more comfortable opportunity for tBLMs electrochemical research.

Firstly, we designed synthesis route for cholesterol precursors. We have synthesized the series of cholesterol derivatives by converting cholesterol into the corresponding mesylate or tosylate under basic conditions (**Scheme 8**). Cholesteryl tosylate (**39**) upon refluxing in dry dioxane with the appropriate oligoethylene glycol (OEG4 or OEG5) yielded **40-41** compounds in good yields. Esterification of **3a** with 3-bromopropanoic acid in the presence of a catalytic amount of TsOH in toluene afforded the corresponding bromoester **42** in 50% yield.



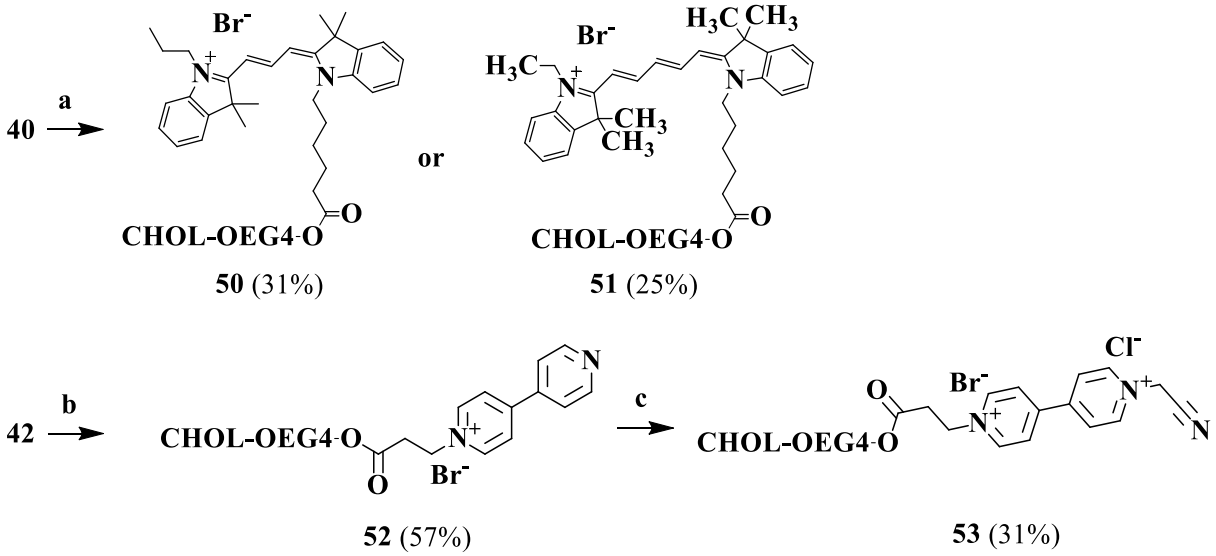
Scheme 8. Synthesis of cholesterol precursors: **a)** MsCl, DCM, reflux, 1 h; **b)** TsCl, C_6H_6, 60-65 °C, 4 h; **c)** NaOH, toluene, reflux, 7 h; **d)** dioxane, reflux, 12 h.

Precursors **40** and **41** were used for synthesis of cholesterol tethers (**Scheme 9**). Derivatives **43** and **44** were synthesized from DTDG and excess of alcohols (**40** or **41**), using TsOH catalyst, which allowed to us to obtain of suspected products in high yields (92% and 88% respectively). Next, we choose a well-tried way and synthesized compounds **47** and **49** through isothiouronium salts, which was subsequently converted by mild basic ($Na_2S_2O_5$) hydrolysis into a desired thiols in good yields (74% and 65% respectively).



Scheme 9. Synthesis of cholesterol tethers: **a)** TsOH, toluene, reflux, 19 h; **b)** MsCl, TEA, TMA·HCl, DCM, reflux, 2 h; **c)** $(NH_2)_2CS$, 2-butanol, reflux, 10 h; **d)** $Na_2S_2O_5$, $CHCl_3/H_2O$, Ar, reflux, 5 h.

Cholesterol markers were formed from precursors **40** and **42** (**Scheme 10**). Both cyanine labeled cholesterols **50** (yield 31%) and **51** (yield 25%) were synthesized from alcohol **40** and commercial Cy3 and Cy5 respectively, using EDC and DMAP system in acetonitrile at room temperature. Synthesis that is more complicated was made for producing cholesterol redox marker **53**. We synthesized 4,4'-bipyridil salt **52** (yield 57%) by the refluxing procedure under MW irradiation. The next step − viologen **53** preparation was made using *N*,*N*,*N*-triethylethanaminium iodide (TEAI) catalyst. The reaction was finished after 25 days under argon at room temperature (yield 31%).



Scheme 10. Synthesis of cholesterol markers: **a)** Cy3 or Cy5, EDC, DMAP, CH_3CN, r. t., 16 h.; **b)** 4,4'-bipyridine, CH_3CN, MW, 8 h.; **c)** TEAI, $ClCH_2CN$, Ar, r. t., 25 days.

All synthesized derivatives were isolated by simple extraction, decantation and recrystallization methods, except compounds **43**, **44**, **50** and **51**, which were purified by column chromatography. As expected, both synthesized thiols **47** and **49** were self-oxidized to corresponding disulphides and sulphone derivatives after three weeks standing at freezer (-12 °C). Synthesized cholesterol cyclic disulphides **43** and **44** were kept under the same conditions without changes during the several months, but it resolve into primary structures standing per month at room temperature. We recommend farther keeping of synthesized cholesterol tethers under inert atmosphere of argon or nitrogen at low temperatures. Synthetic cholesterol markers **50**, **51** and **53** were successfully kept under mention conditions too.

3. Conclusions

1) Glycerol monoprotection by PMBOH was optimized (20% A-15, 40 °C, DCM, 10 h), allowing to isolate mixture of primary and secondary PMB glycerols with the best ratio of products (9.4:1).
2) It was found, that selective protection of monoPMB glycerols by cyclohexanone and followed acid hydrolysis in the presence of anh. $CaCl_2$, allows separating compounds by simple extraction methods.
3) An effective synthesis route of several steps and isolation method without column chromatography for the synthesis of thiolipids were discovered.
4) New glycerol derivative, with PMB and TBS groups, convenient for the synthesis of unsymmetrical thiolipids, was synthesized.
5) New (chlorohexyl)triethylene glycol functionalized with biotin was synthesized and used for the synthesis of biotinylated esters, which binds to streptavidine; it allows using them for tethering of biological objects.
6) Vibrational bands of new 4,4′-disubstituted-1,2-ditholanes were assigned by theoretical calculation and analysis of potential energy distribution. It was found, that adsorption of 1,2-dithiolane ring on the gold surface leads to decomposition of S-S (508 cm^{-1}) bond and formation of Au-S (255 cm^{-1}) bond.
7) New OEG cholesterol cyclic disulphides – stable tethers for model membranes, were synthesized.
8) New OEG cholesterol markers with cyanine and viologen groups were synthesized as perspective research tools for artificial membranes.

4. List of publications

Publication in the journals

1) A. Matijoška, T. Charkova, Z. Kuodis, V. Voiciuk, O. Eicher-Lorka. *Synthesis and properties of new biotin compounds containing (hexyl)triethylene glycol chain.* Central European Journal of Chemistry 10,1 (2012) 113-120.
2) T. Charkova, Z. Kuodis, A. Matijoška, O. Eicher-Lorka. *Glycerol-based thiolipids for model membranes.* Chemija 25,4 (2014) 224-228.
3) O. Eicher-Lorka, T. Charkova, A. Matijoška, Z. Kuodis, G. Urbelis, T. Penkauskas, M. Mickevičius, A. Bulovas, G. Valinčius. *Cholesterol-based tethers and markers for model membranes investigation.* Chemistry and Physics of Lipids (under review).

Conference materials

1) O. Eicher-Lorka, A. Matijoška, Z. Kuodis, A. Rutavičius, T. Charkova. *Mikrobanginė merkaptoalkil-oligo(metileno oksidų), skirtų metalų paviršiaus funkcionalizavimui sintezė.* 9th International conference of Lithuanian chemists „Chemistry 2009“, ChI, Vilnius, Lithuania.
2) A. Matijoška, T. Charkova, Z. Kuodis, A. Rutavičius, O. Eicher-Lorka. *Merkaptoheksiltrietilenoksibiotino sintezė.* Conference „Chemistry and Chemical Technology 2010“, KTU, Kaunas, Lithuania.
3) T. Charkova, O. Eicher-Lorka. *Tiolipidų sintezė dirbtinių dvisluoksnių membranų konstravimui*. Conference for students „FIZTECH 2010“, FTMC ChI, Vilnius, Lithuania.
4) T. Charkova, O. Eicher-Lorka, A. Matijoška, Z. Kuodis, A. Rutavičius, L. Labanauskas, G. Urbelis, R. Striela. *Thiolipids for modelling tethered bilayer lipid membranes*. 10th International conference of Lithuanian chemists „Chemistry 2011“, FTMC ChI, Vilnius, Lithuania.
5) T. Charkova, A. Matijoška, Z. Kuodis, O. Eicher-Lorka. *Daugiafunkcinių cholesterolio darinių sintezė prikabintų dvisluoksnių membranų kūrimui*. 3th Conference for young scientists „Tarpdalykiniai tyrimai fiziniuose ir technologijos moksluose – 2012“, LMA, Vilnius, Lithuania.

6) A. Matijoška, T. Charkova, Z. Kuodis, O. Eicher-Lorka. *Viologeno dariniai modelinių membranų tyrimams*. Conference „Chemistry and Chemical Technology 2013", KTU, Kaunas, Lithuania.
7) A. Matijoška, T. Charkova, Z. Kuodis, O. Eicher-Lorka, G. Urbelis, L. Labanauskas. *Dyes for investigation of model membranes*. 11th International conference of Lithuanian chemists „Chemistry 2013", FTMC ChI, Vilnius, Lithuania.
8) T. Charkova, A. Matijoška, Z. Kuodis, T. Penkauskas, O. Eicher-Lorka. *Cholesterol-based compounds for model membranes*. 8th Biennial international conference on organic synthesis „Balticum Organicum Syntheticum 2014", Congress hall, Vilnius, Lithuania.

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Reziumė

Savitvarkiai monosluoksniai suteikia unikalią molekulinio lygio tyrimų galimybę, kuri lemia šios daugiamokslinės srities nuolatinį vystymą bei platų pritaikymą (biosensorių kūrimas, molekulinė elektronika ir nanotechnologijos). Specifinė savitvarka leidžia sėkmingai naudoti šias struktūras modelinių membranų kūrimui, kurių konstravimas atveria naujus gyvoje ląstelėje vykstančių procesų (fotosintezės, kvėpavimo, baltymų sąveikos ir kt.) tyrimo kelius.

Perspektyviausi membraniniai modeliai – prikabintos dvisluoksnės membranos. Jų tarpinės oligoetilenglikolių grandinės saugo įterptas į dvisluoksnį biomedžiagas nuo nepageidaujamos sąveikos su metalo substratu. Todėl modelinė membrana išlieka skysta, lanksti, stabili ir tinkama įvairių rūšių manipuliavimui. Oligoetilenoksidų tioliai ir sulfidai yra pagrindiniai minėtos molekulinės savitvarkos statybiniai blokai, o jų lipidų, biotino ir cholesterolio dariniai – vieni populiariausių membraninių modelių tyrimo įrankiai. Todėl buvo suplanuota kurti daugiafunkcines medžiagas, turinčias oligoetilenoksidinę grandinę, tinkamas prikabintų dvisluoksnių membranų formavimui ir jų savybių tyrimams atlikti.

Šio darbo metu buvo optimizuotas glicerolio monoblokavimas *p*-metoksibenzilalkoholiu geriausiam pirminio ir antrinio monoblokuotų glicerolių santykiui (9,4:1) susidaryti. Pavyko surasti selektyvų monoblokuotų glicerolių atskyrimo būdą (blokavimas cikloheksanonu su tolimesne rūgštine hidrolize), leidžiantį atskirti junginius paprastais ekstrakcijos metodais. Taip pat buvo susintetinti perspektyvūs diblokuoti skirtingomis grupėmis (PMB ir TBS) glicerolio junginiai, tinkami daugiafunkcinių, turinčių skirtingus pakaitus, darinių sintezei. Pavyko surasti daugiastadijinį tiolipidų sintezės kelią, leidžiantį išskirti produktus nenaudojant kolonėlinės chromatografijos metodo. Buvo susintetinti nauji daugiafunkciniai biotino junginiai su heksiltrietilenoksidine grandine ir su tiolio arba stilbazolio grupėmis. Įsitikinta, kad jie sudaro kompleksus su streptavidinu, todėl yra tinkami biologinių objektų tyrimams. Remiantis kvantų cheminiais skaičiavimais ir potencinės energijos pasiskirstymo analize buvo atlikti tikslūs naujų 4,4′-dipakeistų-1,2-ditiolano darinių virpesių juostų priskyrimai. Jų pagrindu buvo sukurti nauji cholesterolio inkariniai (tioliai ir cikliniai disulfidai) dariniai, sėkmingai patikrinti prikabintų dvisluoksnių membranų formavimui. Taip pat buvo susintetinti nauji žymėti (fluorescenciniai ir redokso) cholesteroliai bei išbandytas jų funkcionalumas.

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