Cent. Eur. J. Chem. • 10(1) • 2012 • 113-120
DOI: 10.2478/s11532-011-0113-8

VERSITA

Central European Journal of **Chemistry**

Synthesis and properties of new biotin compounds containing hexyltriethylene glycol chain

Research Article

Algirdas Matijoška[1], Tatjana Charkova[1], Zenonas Kuodis[1], Vladislava Voiciuk[2], Olegas Eicher-Lorka[1*]

[1]*Institute of Chemistry of Center for Physical Sciences and Technology, LT-02300 Vilnius, Lithuania*

[2]*Faculty of Natural Sciences, Vilnius University, LT-03101 Vilnius, Lithuania*

Received 18 May 2011; Accepted 14 September 2011

Abstract: The synthesis of a new halogenide containing hexyltriethylene glycol chain functionalized with biotin is reported. The general possibility of this linker to use as the building block for biotinylated compounds syntheses is demonstrated. Two biotinylated esters with different properties for useful surface modification and as fluorescence probes for proteins marking were synthesized. The properties of mentioned compounds were investigated by using surface plazmon resonance ellypsometry and fluorescence spectroscopy.

Keywords: *Biotinylated esters • Self-assembled monolayers • Stilbazolium • Fluorescent probe*

1. Introduction

Self-assembled monolayers (SAMs) are the popular tool for tailoring the reactive properties of the surface. Ordered molecular assemblies are formed spontaneously by the adsorption of surfactant – often organosulfur derivatives with a specific affinity of its headgroup to a substrate – generally noble metals [1-5]. Due to their often dense and stable structure, SAMs have been investigated for use in electrochemical sensors technology, electronics, nanodevices of any kind, and other areas [6-11]. Besides, the close similarity of the SAMs with biomembranes enables the use of them as model systems to study different cell processes, such as molecular transport through membranes, investigation of complex membrane proteins, and others [12-14].

In our ongoing project of developing novel syntheses of different molecular systems based on tailoring the reactive properties of surfaces, we decided to synthesize a new halogenide containing alkyltriethylene glycol chain functionalized with biotin. We believe that further investigations will help to bring it into laboratory practice as a building block for many syntheses of biotinylated compounds. To prove this, we synthesized a new compound containing hexyltriethylene glycol chain functionalized with biotin and dimethylaminostilbazole – trietyleneoxyhexyldimethylaminostilbazolium biotin (BES) ester (Fig. 1). Stilbazolium derivatives have the ability to transfer electrons as well as strong nonlinear optical properties that could possibility produce self-assembled chromophoric structures such as fluorescence probes for protein marking, monitoring free radical polymerization processes and for construction of potentially useful electro-optic devices of any kind [15-19]. Stilbazolium fluorophore was selected in this work 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 tethered bilayer lipid membranes [20-22]. In this work, we describe a novel synthesis strategy of the trietyleneoxyhexylthiol biotin (BET) ester that can be widely used to produce monolayers for different surface modifications and biological applications.

We decided to synthesize biotinylated esters for several reasons. Biotin is a small molecule with tight and specific binding to various proteins such as avidin and streptavidin. This great binding affinity makes the biotin-

* *E-mail: lorka@ktl.mii.lt*

streptavidin couple one of the strongest non-covalent interactions known in nature. Biotin surfaces are one of the most known tools to immobilize antibodies onto surfaces and are widely used for biological detection and purification that has potential application for construction of sensors [23-25]. 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 polyethylene glycol (PEG) groups. The properties of PEG 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 solution species [26]. Among various biotinylated derivatives reported in literature the most popular are biotin alkyl thiols (BATs) (Fig. 1) [27-28]. We decided to synthesize a similar BET ester (Fig. 1). Like BATs structures, it consists of three main parts: biotin, PEG, and alkyl chain. However, in the BET ester, the ether bond connects the hexyl chain and the triethylene glycol group. In BATs there are two peptide bonds instead of ether and ester bonds. Our purpose in this synthesis was to produce a derivative without peptide bounds to avoid possible hydrogen binding between biotinylated system and protein. We wanted to prove that synthesized biofunctionalized halogenide can be used for the syntheses of biotinylated compounds with different properties for useful surface modifications and biological applications.

2. Experimental procedure

2.1. General procedures

Chemicals of commercial grade were used without further purification, except dry solvents. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD and DMSO-d_6 on Varian Unity Inova NMR spectrometer (δ in ppm, J in Hz) at ^{1}H operating frequencies of 299.75 MHz and 75.37 MHz for ^{13}C; spectra were referenced using the solvent signal as internal standard. The IR spectra were recorded on the Perkin-Elmer Spectrum GX FT-IR spectrometer. Elemental analyses were carried out on a Perkin-Elmer 2400-B microanalyser. APCI mass spectra were recorded on an Agilent 1100 ion trap mass spectrometer in positive mode.

2.2. Synthesis of compounds 1-8

2.2.1. 2-(2-(2-(6-Chlorohexyloxy)ethoxy)ethoxy)ethanol (1)

Commercial triethylene glycol (150 g, 1000 mmol) was dissolved in benzene (250 mL) and refluxed in a three-neck round bottom flask equipped with a Dean-Stark receiver for 2 hours. The sodium (4.6 g, 200 mmol) was added and refluxed with a Dean-Stark receiver until the reaction finished. Next, 1,6-dichlorohexane (62 g, 400 mmol) was added and the reaction mixture refluxed for 4 hours. After standing overnight at room temperature, it was taken up in hexane (500 mL) and decanted. The residue was dissolved in benzene (600 mL) and washed with water (2×200 mL). The combined benzene extracts were dried ($MgSO_4$), filtered, and evaporated to give 36.6 g (68%) of the corresponding compound **1**.

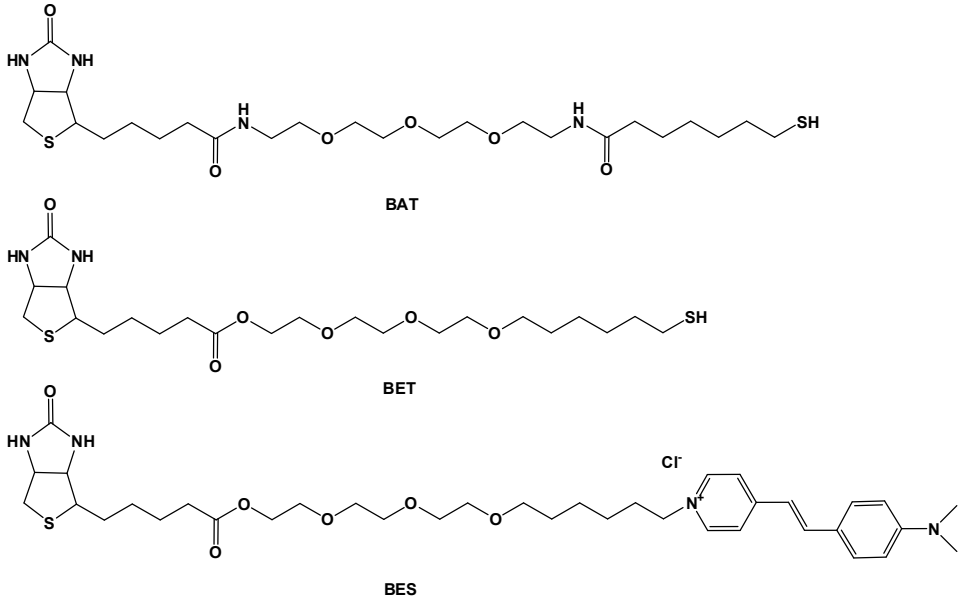


Figure 1. Biotinylated structures.

^{1}H NMR (CD_3OD, δ ppm): 1.33 – 1.51 (m, 4H, $ClCH_2CH_2\underline{CH_2CH_2}$); 1.59 (q, *J* = 6.72; 2H, $OCH_2\underline{CH_2}$); 1.77 (q, *J* = 6.61; 2H, $ClCH_2\underline{CH_2}$); 3.47 (t, *J* = 6.51; 2H, $O\underline{CH_2}CH_2$); 3.53 – 3.67 (m, 14H, $HO(\underline{CH_2CH_2}O)_3$ and $Cl\underline{CH_2}$). ^{13}C (CD_3OD, δ ppm): 26.47 ($OCH_2CH_2\underline{C}H_2$), 27.71 ($ClCH_2CH_2\underline{C}H_2$), 30.53 ($OCH_2\underline{C}H_2$), 33.74 ($ClCH_2\underline{C}H_2$), 45.69 ($Cl\underline{C}H_2$), 62.20 ($HO\underline{C}H_2$), 71.10 ($OCH_2\underline{C}H_2OCH_2CH_2$), 71.37 ($O\underline{C}H_2CH_2OCH_2CH_2$), 71.54 ($HOCH_2CH_2OCH_2\underline{C}H_2$), 71.59 ($HOCH_2CH_2O\underline{C}H_2$), 72.12 ($O\underline{C}H_2CH_2CH_2$), 73.65 ($HOCH_2\underline{C}H_2$). Found, %: C, 53.45; H, 9.45; Cl, 13.28. Calculated for $C_{12}H_{25}ClO_4$ (268.78): C, 53.62; H, 9.37; Cl, 13.19.

2.2.2. 21-Chloro-3,6,9,12,15-pentaoxahenicosan-1-ol(2)

The compound **2** was prepared by the same way as compound **1** from commercial pentaethylene glycol (119 g, 500 mmol), 1,6-dichlorohexane (31 g, 200 mmol), and sodium (2.3 g, 100 mmol). Yield 19 g (53%).

^{1}H NMR (CD_3OD, δ ppm): 1.33 – 1.51 (m, 4H, $ClCH_2CH_2\underline{CH_2CH_2}$); 1.58 (q, *J* = 6.78; 2H, $OCH_2\underline{CH_2}$); 1.76 (q, *J* = 6.68; 2H, $ClCH_2\underline{CH_2}$); 3.47 (t, *J* = 6.51; 2H, $O\underline{CH_2}CH_2$); 3.53 – 3.67 (m, 22H, $HO(\underline{CH_2CH_2}O)_5$ and $Cl\underline{CH_2}$). ^{13}C (CD_3OD, δ ppm): 26.43 ($OCH_2CH_2\underline{C}H_2$), 27.67 ($ClCH_2CH_2\underline{C}H_2$), 30.47 ($OCH_2\underline{C}H_2$), 33.68 ($ClCH_2\underline{C}H_2$), 45.74 ($Cl\underline{C}H_2$), 62.14 ($HO\underline{C}H_2$), 71.08 ($OCH_2\underline{C}H_2OCH_2CH_2$), 71.30 ($O\underline{C}H_2CH_2OCH_2CH_2$), 71.46 ($\underline{C}H_2\underline{C}H_2O)_3$, 72.09 ($O\underline{C}H_2CH_2CH_2$), 73.59 ($HOCH_2\underline{C}H_2$). Found, %: C, 53.75; H, 9.41; Cl, 9.98. Calculated for $C_{16}H_{33}ClO_6$ (356.88): C, 53.84; H, 9.31; Cl, 9.93.

2.2.3. 2-(1-Hydroxy-3,6,9,12,15-pentaoxahenicosan-21-yl)isothiuronium chloride (3)

The compound **2** (17.8 g, 500 mmol) and thiourea (3.8 g, 50 mmol) were dissolved in *n*-butanol (100 mL). The mixture was refluxed using an oil bath for 5 hours. After standing overnight at room temperature, the precipitate was filtered, and solvent was removed under reduced pressure. The residue was washed with acetone (3×50 mL), refluxed in acetonitrile (100 mL) for 10 min, and hot filtered. Next, acetone (100 mL) was added and the solution was allowed to cool in a refrigerator until oil product formed. The product was washed with acetone (3×50 mL), decanted, and allowed to dry at room temperature yielding 11 g (51%) of compound **3**.

^{1}H NMR (CD_3OD, δ ppm): 1.35 – 1.53 (m, 4H, $SCH_2CH_2\underline{CH_2CH_2}$); 1.58 (q, *J* = 6.75; 2H, $OCH_2\underline{CH_2}CH_2$); 1.72 (q, *J* = 7.16; 2H, $SCH_2\underline{CH_2}CH_2$); 3.17 (t, *J* = 7.16; 2H, $S\underline{CH_2}$); 3.47 (t, *J* = 6.40; 2H, $O\underline{CH_2}CH_2$); 3.54 – 3.68 (m, 22H, $HO(\underline{CH_2CH_2}O)_5$). ^{13}C ($CD_3OD$, δ ppm): 26.53 ($OCH_2CH_2\underline{C}H_2$), 29.14 ($S\underline{C}H_2$), 29.57 ($SCH_2CH_2\underline{C}H_2$), 30.35 ($OCH_2\underline{C}H_2$), 31.78 ($SCH_2\underline{C}H_2$), 62.10 ($HO\underline{C}H_2$), 71.02 ($OCH_2\underline{C}H_2OCH_2CH_2$), 71.28 ($O\underline{C}H_2CH_2OCH_2CH_2$), 71.44 ($\underline{C}H_2\underline{C}H_2O)_3$, 72.02 ($O\underline{C}H_2CH_2CH_2$), 73.57 ($HOCH_2\underline{C}H_2$). Found, %: C, 47.06; H, 8.65; Cl, 8.27; N, 6.35; S, 7.49. Calculated for $C_{17}H_{37}ClN_2O_6S$ (433.00): C, 47.15; H, 8.61; Cl, 8.18; N, 6.47; S, 7.40.

2.2.4. 21-Mercapto-3,6,9,12,15-pentaoxahenicosan-1-ol (4)

The compound **3** (6 g, 14 mmol) was dissolved in water (50 mL). The sodium hydroxide (0.64 g, 16 mmol) water (15 mL) solution was added and the reaction mixture was stirred for 5 hours at 60ºC under argon. After the mixture was allowed to cool to room temperature, the chloroform (150 mL) and hydrochloric acid solution (0.5 ml HCl/2.5 ml H_2O) were added. The combined chloroform extracts were washed with water (3×50 mL), dried ($MgSO_4$), and solvents were evaporated. The residue was dried under reduced pressure, yielding 2.8 g (56%) of compound **4**.

^{1}H NMR ($CDCl_3$, δ ppm): 1.34 (t, *J* = 7.74; 1H, SH); 1.33 – 1.45 (m, 4H, $HSCH_2CH_2\underline{CH_2CH_2}$); 1.54 – 1.70 (m, 4H, $HSCH_2\underline{CH_2}CH_2CH_2\underline{CH_2}$); 2.48 – 2.56 (td, *J* = 7.29 and 7.07; 2H, $HS\underline{CH_2}$); 3.45 (t, *J* = 6.63; 2H, $O\underline{CH_2}CH_2CH_2$); 3.52 – 3.73 (m, 10H, $(\underline{CH_2CH_2}O)_5$. ^{13}C ($CDCl_3$, δ ppm): 24.33 ($\underline{C}H_2SH$), 25.35 ($OCH_2CH_2\underline{C}H_2$), 27.94 ($HSCH_2CH_2\underline{C}H_2$), 29.26 ($OCH_2\underline{C}H_2$), 33.72 ($HSCH_2\underline{C}H_2$), 61.42 ($HO\underline{C}H_2$), 69.83 ($OCH_2\underline{C}H_2OCH_2CH_2$), 70.09 ($O\underline{C}H_2CH_2OCH_2CH_2$), 70.36 ($\underline{C}H_2\underline{C}H_2O)_3$, 71.06 ($O\underline{C}H_2CH_2CH_2$), 72.37 ($HOCH_2\underline{C}H_2$). Found, %: C, 54.10; H, 8.05; Cl, 9.78; S, 9.19. Calculated for $C_{16}H_{34}O_6S$ (354.50): C, 54.21; H, 7.93; Cl, 9.66; S, 9.04.

2.2.5. (2-(2-(6-Chlorohexyloxy)ethoxy)ethoxy)ethyl 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (5)

Biotin (1 g, 4 mmol) was dissolved in dimethylformamide (20 mL) at 65ºC under argon. After the temperature was decreased to 40ºC, 3-((ethylimino)methyleneamino)-N,N-dimethylpropan-1-aminium chloride – EDC HCl (1.12 g, 5.8 mmol) in dry dichloromethane (50 mL), N,N-dimethylpyridin-4-amine – DMAP (0.165 g, 0.353 mmol) and compound **1** (1.07 g, 4 mmol) were added. The mixture was stirred for 20 hours at 60ºC, and the solvent was evaporated. The residue was infused with $NaHCO_3$ (25 mL) and the aqueous layer was extracted with dichloromethane (3×25 mL). The organic phase was washed with water (3×50 mL), dried ($MgSO_4$), and evaporated. The residue was recrystallized from dichloromethane and hexane mixture (1:20), washed with hexane (50 mL), and dried under reduced pressure, yielding 1.2 g (60.5%) of compound **5**.

^{1}H NMR ($CDCl_3$, δ ppm): 1.34 – 1.82 (m, 14H, $ClCH_2(\underline{CH_2})_4CH_2O$ and $\underline{CH_2CH_2CH_2}CH_2COO$); 2.38 (t, *J* = 7.40; 2H, $CH_2\underline{CH_2}COO$); 2.73 – 2.94 (m, 2H, $CH\underline{CH_2}S$); 3.12 – 3.18 (m, 1H, $CH\underline{CH}S$); 3.46 (t, *J* = 6.53; 2H, $O\underline{CH_2}CH_2CH_2$); 3.54 (t, *J* = 6.78; 2H, $Cl\underline{CH_2}CH_2$); 3.58 – 3.66 (m, 8H, $COOCH_2CH_2O\underline{CH_2CH_2}O\underline{CH_2CH_2}O$); 3.70 (t, *J* = 4.91; 2H, $COOCH_2\underline{CH_2}O$); 4.21 – 4.24 (m, 2H, $COO\underline{CH_2}CH_2O$); 4.28 – 4.33 (m, 1H, $NH\underline{CH}CHS$); 4.48 – 4.52 (m, 1H, $NH\underline{CH}CH_2S$); 5.61 (m, 1H, $\underline{NH}CHCHS$); 6.18 (m, 1H, $\underline{NH}CHCH_2S$). ^{13}C ($CDCl_3$, δ ppm): 24.65 ($CH\underline{C}H_2CH_2$), 25.33 ($OCH_2CH_2\underline{C}H_2$), 26.60 ($ClCH_2CH_2\underline{C}H_2$), 28.14 ($CHCH_2\underline{C}H_2$), 28.24 ($CHCH_2CH_2\underline{C}H_2$), 29.36 ($OCH_2\underline{C}H_2$), 32.45 ($ClCH_2\underline{C}H_2$), 33.68 ($CH_2\underline{C}H_2COO$), 40.46 ($S\underline{C}H_2$), 44.90 ($Cl\underline{C}H_2$), 55.49 ($S\underline{C}HCH_2$), 60.03 ($NH\underline{C}HCH_2S$), 61.85 ($NH\underline{C}HCHS$), 63.33 ($COO\underline{C}H_2CH_2O$), 69.06 ($COOCH_2\underline{C}H_2O$), 69.99 ($COOCH_2CH_2O\underline{C}H_2$), 70.43 ($COOCH_2CH_2OCH_2\underline{C}H_2O$), 70.48 ($O\underline{C}H_2CH_2OCH_2CH_2$), 70.55 ($OCH_2\underline{C}H_2OCH_2CH_2$), 71.14 ($OCH_2CH_2O\underline{C}H_2CH_2$), 163.75 ($NH\underline{C}ONH$), 173.59 ($\underline{C}OO$). MS m/z (relative intensity): 492.5 (100), 224.8 (15). IR (film) 3247, 2937, 2863, 1730, 1702, 1459, 1118 cm^{-1}. Found, %: C, 53.26; H, 8.05; Cl, 7.27; N, 5.71; S, 6.39. Calculated for $C_{22}H_{39}ClN_2O_6S$ (495.07): C, 53.37; H, 7.93; Cl, 7.16; N, 5.65; S, 6.47.

2.2.6. 2-(5-Oxo-1-(2-oxohexahydro-1H-thieno[3,4-d] imidazol-4-yl)-6,9,12,15-tetraoxahenicosan-21-yl)isothiuronium chloride (6)

The compound **5** (0.4 g, 0.81 mmol) and thiourea (0.074 g, 0.97 mmol) were dissolved in *n*-butanol (20 mL). The mixture was refluxed under argon for 8 hours. Then it was evaporated, residue was dissolved in acetone and methanol mixture (2:1) and taken up in dry diethyl ether (100 mL). The mixture was allowed to cool in refrigerator until the oil product was formed, and solvents were decanted. The residue was washed with dry diethyl ether (50 mL) and dried under reduced pressure to give 0.27 g (58.5%) of compound **6**.

^{1}H NMR (DMSO-d_6, δ ppm): 1.30 – 1.61 (m, 14H, $ClCH_2(\underline{CH_2})_4CH_2O$ and $\underline{CH_2CH_2CH_2}CH_2COO$); 2.31 (t, *J* = 7.34; 2H, $CH_2\underline{CH_2}COO$); 2.57 – 2.86 (m, 2H, $CH\underline{CH_2}S$); 3.07 – 3.11 (m, 1H, $CH\underline{CH}S$); 3.15 (t, *J* = 7.19; 2H, $NH_2S\underline{CH_2}CH_2$); 3.37 (t, *J* = 6.52; 2H, $O\underline{CH_2}CH_2CH_2$); 3.40 – 3.52 (m, 8H, $COOCH_2CH_2O\underline{CH_2CH_2}O\underline{CH_2CH_2}O$); 3.60 (t, *J* = 4.72; 2H, $COOCH_2\underline{CH_2}O$); 4.11 – 4.16 (m; 2H; $COO\underline{CH_2}CH_2O$ and 1H; $NH\underline{CH}CHS$); 4.29 – 4.34 (m, 1H, $NH\underline{CH}CH_2S$); 6.41 (m, 1H, $\underline{NH}CHCHS$); 6.47 (m, 1H, $\underline{NH}CHCH_2S$). ^{13}C (DMSO-d_6, δ ppm): 24.52 ($CH\underline{C}H_2CH_2$), 25.10 ($OCH_2CH_2\underline{C}H_2$), 27.67 ($S\underline{C}H_2$), 28.01 ($CHCH_2\underline{C}H_2\underline{C}H_2$), 28.40 ($SCH_2CH_2\underline{C}H_2$), 29.04 ($OCH_2\underline{C}H_2$), 29.98 ($SCH_2\underline{C}H_2$), 33.27 ($CH_2\underline{C}H_2COO$), 39.88 ($S\underline{C}H_2CH$), 55.39 ($S\underline{C}HCH_2$), 59.22 ($NH\underline{C}HCH_2S$), 61.06 ($NH\underline{C}HCHS$), 63.09 ($COO\underline{C}H_2CH_2O$), 68.33 ($COOCH_2\underline{C}H_2O$), 69.50 ($COOCH_2CH_2O\underline{C}H_2$), 69.78 ($OCH_2\underline{C}H_2O\underline{C}H_2CH_2OCH_2CH_2$), 69.82 ($\underline{C}H_2OCH_2CH_2$), 70.21 ($O\underline{C}H_2CH_2$), 162.76 ($NH\underline{C}ONH$), 169.96 ($S\underline{C}NH_2$), 172.86 ($\underline{C}OO$). Found, %: C, 48.26; H, 7.65; Cl, 5.67; N, 9.71; S, 11.29. Calculated for $C_{23}H_{43}ClN_4O_6S_2$ (571.19): C, 48.36; H, 7.58; Cl, 5.61; N, 9.80; S, 11.22.

2.2.7. 2-(2-(2-(6-Mercaptohexyloxy)ethoxy)ethoxy) ethyl 5-(2-oxohexahydro-1H-thieno[3,4-d] imidazol-4-yl)pentanoate (7)

The compound **6** (0.27 g, 0.473 mmol) was dissolved in water (10 mL) and chloroform (30 mL) mixture. The sodium metabisulfite – $Na_2S_2O_5$ (0.162 g, 0.851 mmol) was added and reaction mixture was refluxed under argon for 5 hours. Then it was allowed to cool to room temperature and extracted with chloroform (30 mL). The combined chloroform extracts were washed with water (3×50 mL), dried ($MgSO_4$), and solvents removed under reduced pressure. The residue was dried under reduced pressure, yielding 0.11 g (47%) of compound **7**.

^{1}H NMR (CD_3OD, δ ppm): 1.36 – 1.73 (m, 14H, $ClCH_2(\underline{CH_2})_4CH_2O$ and $\underline{CH_2CH_2CH_2}CH_2COO$); 2.37 (t, *J* = 7.22; 2H, $CH_2\underline{CH_2}COO$); 2.47 – 2.52 (dt, *J* = 7.11; 2H, $HS\underline{CH_2}$); 2.68 – 2.96 (m, 2H, $CH\underline{CH_2}S$); 3.17 – 3.24 (m, 1H, $CH_2\underline{CH}S$); 3.47 (t, *J* = 6.47; 2H, $O\underline{CH_2}CH_2CH_2$); 3.56 – 3.64 (m, 8H, $COCH_2CH_2O\underline{CH_2CH_2}O\underline{CH_2CH_2}O$); 3.70 (t, *J* = 4.77; 2H, $COOCH_2\underline{CH_2}O$); 4.21 (t, 2H, *J* = 4.49; $COO\underline{CH_2}CH_2O$); 4.28 – 4.33 (dd, *J* = 4.45 and 3.70; 1H, $NH\underline{CH}CHS$); 4.47 – 4.41 (m, 1H, $NH\underline{CH}CH_2S$); 4.87 (m, 2H, $\underline{NH}CO\underline{NH}$). ^{13}C ($CD_3OD$, δ ppm): 24.88 ($HS\underline{C}H_2$), 24.92 ($CH\underline{C}H_2CH_2$), 25.92 ($OCH_2CH_2\underline{C}H_2$), 29.19 ($HSCH_2CH_2\underline{C}H_2$), 29.48 ($CHCH_2\underline{C}H_2\underline{C}H_2$), 30.62 ($OCH_2\underline{C}H_2$), 34.57 ($CH_2\underline{C}H_2COO$), 35.17 ($HSCH_2\underline{C}H_2$), 41.06 ($S\underline{C}H_2CH$), 56.99 ($S\underline{C}HCH_2$), 61.62 ($NH\underline{C}HCH_2S$), 63.37 ($NH\underline{C}HCHS$), 64.62 ($COO\underline{C}H_2CH_2O$), 70.15 ($COOCH_2\underline{C}H_2O$), 71.18 ($COOCH_2CH_2O\underline{C}H_2CH_2O$), 71.55 ($COOCH_2\underline{C}H_2OCH_2\underline{C}H_2O$), 71.59 ($O\underline{C}H_2CH_2OCH_2CH_2$), 71.61 ($\underline{C}H_2OCH_2CH_2$), 72.24 ($O\underline{C}H_2CH_2$), 166.71 ($NH\underline{C}ONH$), 175.24 ($\underline{C}OO$). MS m/z (relative intensity): 491.2 (100), 447.3 (23), 359.3 (60), 225.5 (80). IR (film) 3247, 2929, 2859, 2560, 1732, 1704, 1460, 1118 cm^{-1}. Found, %: C, 53.56; H, 8.25; N, 5.61; S, 13.12. Calculated for $C_{22}H_{40}N_2O_6S_2$ (492.69): C, 53.63; H, 8.18; N, 5.68; S, 13.01.

2.2.8. (E)-4-(4-(dimethylamino)styryl)-1-(5-oxo-1-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-6,9,12,15-tetraoxahenicosan-21-yl)pyridinium chloride (8)

(E)-N,N-dimethyl-4-(2-(pyridin-4-yl)vinyl)aniline (0.2 g, 0.892 mmol), compound **5** (0.44 g, 0.892 mmol) and

tetraethylammonium iodide (0.002 g, 0.00781 mmol) were dissolved in toluene (10 mL). The mixture was refluxed in a domestic microwave for 2.5 hours. Then, it was cooled to room temperature, decanted, and washed with benzene (3×50 mL). The residue was dissolved in dry methanol (3 mL), taken up in dry diethyl ether (100 mL), decanted, and dried under reduced pressure. The product was chromatographed on a column of silica gel using dichloromethane and methanol (5:1) solvents as eluent. Yield 0.11 g (47%).

^{1}H NMR ($CDCl_3$, δ ppm): 1.33 – 1.78 (m, 12H, $ClCH_2\underline{CH_2CH_2CH_2}CH_2CH_2N$ and $\underline{CH_2CH_2CH_2}CH_2COO$); 1.91 -2.02 (m, 2H, $NCH_2\underline{CH_2}$); 2.35 (t, *J* = 7.05; 2H, $CH_2\underline{CH_2}COO$); 2.83 – 2.94 (m, 2H, $CH\underline{CH_2}S$); 3.06 (s, 6H, $N(\underline{CH_3})_2$); 3.11 – 3.18 (m, 1H, $CH\underline{CH}S$); 3.43 (t, *J* = 6.65; 2H, $O\underline{CH_2}CH_2CH_2$); 3.55 – 3.65 (m, 8H, $O\underline{CH_2CH_2}O\underline{CH_2CH_2}O$); 3.70 (t, *J* = 4.59; 2H, $COOCH_2\underline{CH_2}O$); 4.21 – 4.24 (m, $COO\underline{CH_2}CH_2O$); 4.29 – 4.33 (m, 1H, $NH\underline{CH}CHS$); 4.52 – 4.56 (m, 1H, $NH\underline{CH}CH_2S$); 4.63 (t, *J* = 6.95; 2H, $N\underline{CH_2}CH_2$); 5.81 (m, 1H, $\underline{NH}CHCHS$); 5.88 (m, 1H, $\underline{NH}CHCH_2S$); 6.67 – 6.70 (m, 2H, Ph-3,5); 6.82 – 6.88 (d, *J* = 15.95; 1H, CH=$\underline{CH}$Ph); 7.51 – 7.53 (m, 2H, Ph-2,6); 7.60 – 7.65 (d, *J* = 15.96; 1H, CH=$\underline{CH}$Py); 7.87 – 7.89 (m, 2H, Py-2,6); 8.89 – 8.90 (m, 2H, Py-3,5). ^{13}C ($CDCl_3$, δ ppm): 24.64 ($CH\underline{C}H_2CH_2$), 25.46 ($OCH_2CH_2\underline{C}H_2$), 25.74 ($NCH_2CH_2\underline{C}H_2$), 28.20 ($CHCH_2\underline{C}H_2CH_2$), 28.29 ($CHCH_2CH_2\underline{C}H_2$), 29.17 ($OCH_2\underline{C}H_2CH_2$), 33.72 ($CH_2\underline{C}H_2COO$), 40.04 ($\underline{C}H_3)_2$, 40.34 ($S\underline{C}H_2CH$), 40.60 ($NCH_2\underline{C}H_2$), 55.64 ($S\underline{C}HCH_2$), 59.99 ($N\underline{C}H_2$), 60.14 ($NH\underline{C}HCH_2S$), 61.75 ($NH\underline{C}HCHS$), 63.32 ($COO\underline{C}H_2CH_2O$), 68.97 ($COOCH_2\underline{C}H_2O$), 69.92 ($COOCH_2CH_2O\underline{C}H_2$), 70.40 ($OCH_2\underline{C}H_2OCH_2CH_2O$), 70.44 ($O\underline{C}H_2CH_2OCH_2CH_2CH_2$), 70.49 ($\underline{C}H_2OCH_2CH_2$), 70.87 ($O\underline{C}H_2CH_2$), 111.83 (Ph-3,5); 116.55 (Ph-1); 122.34 (Ph-$\underline{C}$H=); 122.79 (Py-3,5); 130.48 (Ph-2,6); 142.61 (Py-$\underline{C}$H=); 143.52 (Py-2,6); 152.10 (Ph-4); 153.87 (Py-4); 164.09 ($NH\underline{C}ONH$), 173.51 ($\underline{C}OO$). MS m/z (relative intensity): 682.7 (50), 223.1 (100). IR (film) 3249, 2922, 2860, 1724, 1693, 1642, 1582, 1527, 1162 cm^{-1}. Found, %: C, 61.68; H, 7.75; Cl, 4.51; N, 7.71; S, 4.49. Calculated for $C_{37}H_{55}ClN_4O_6S$ (719.37): C, 61.77; H, 7.70; Cl, 4.45; N, 7.78; S, 4.45.

2.3. Measurements

2.3.1. Surface plasmon resonance ellipsometry (SPRE) measurements

The experimental set-up consisted of a spectral ellipsometer, SOPRA GES-5 with rotating analyzer and 45° BK7 glass prism connected with gold coated glass slide by the refraction index matching fluid. The glass prism was mounted on the cell; the gold sensor surface was placed in contact with solutions injected in the cell. The experimental ellipsometric data was analyzed using the SOPRA program Winelli. Mixed SAM was prepared by injecting into the cell the 0.2 mM ethanolic solution of 5:95 mol% ratio mixtures of compounds **7** and **4**. After incubation for 24 hours, the cell was washed with ethanol, and water was injected. 1 μM streptavidin (ProSpec-Tany TechnoGene, Israel) water solution was injected to initiate the adsorption to biotin exposed at the surface of the SAM. After the steady signal was observed (~ 10 min), the cell was washed with water.

2.3.2. Fluorescence measurements

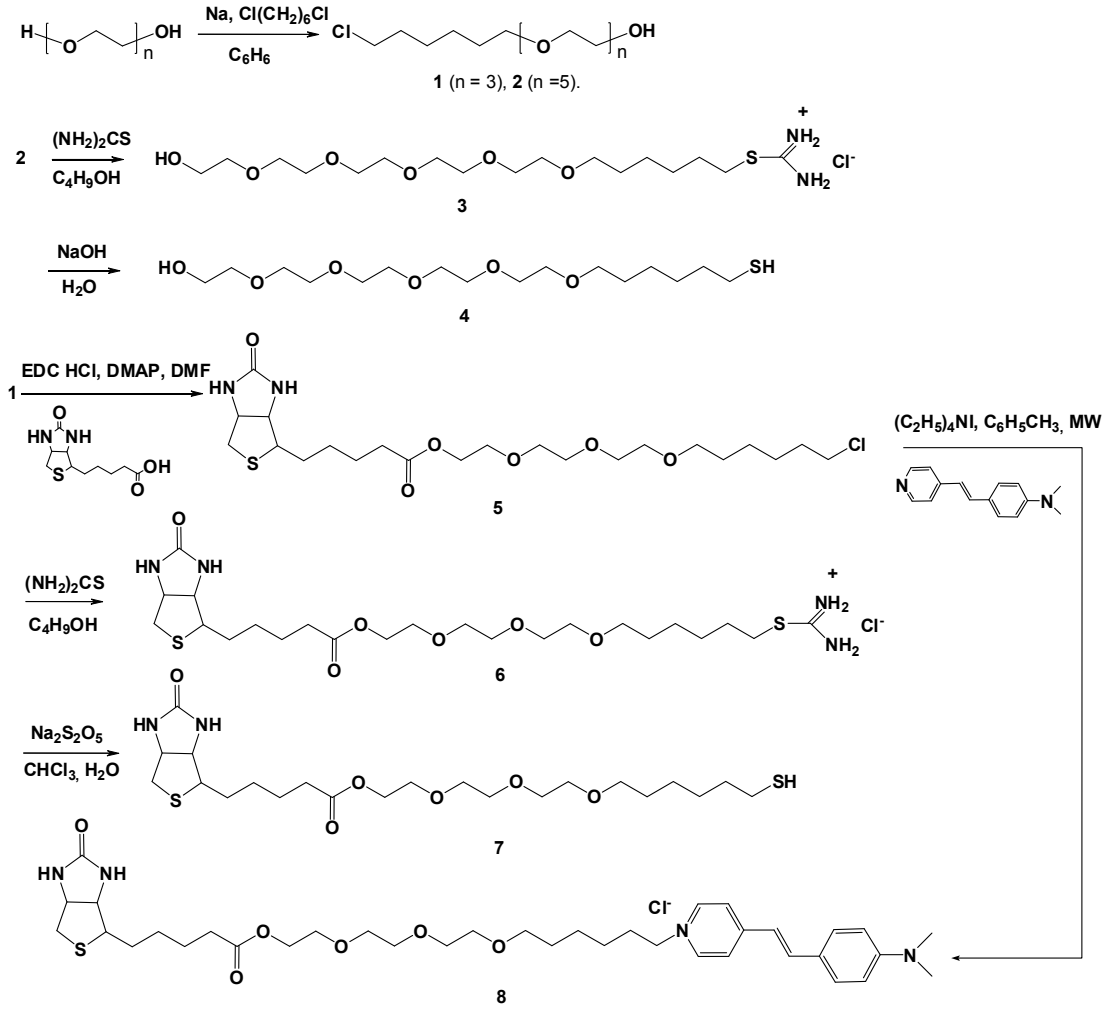
Fluorescence spectra were recorded using the Fluorescence Spectrometer (LS 55, PerkinElmer, USA). 15 μM streptavidin and 200 μM compound **8** water solution was dialyzed using the 12.000-14.000 MWCO dialysis membranes (ROTH ZelluTrans, Germany) for 7 days. The size of membrane's pores and dialysis duration were tested to ensure complete elimination of free chromophore molecules from the solution.

3. Results and discussion

3.1. Synthesis

It was found in literature [29], that compound **1** can be made by the addition of *n*-ethylene glycol to dihaloalkane or terminal haloalkene. Although this alkylation by means of haloalkene is usually more effective, we decided to achieve monoalkylated product **1** with dihaloalkane for further addition of biotin. The route for the main synthesis started with the commercially available triethylene and pentaethylene glycols as shown in Scheme 1. In the first step, triethylene glycol was deprotonated in anhydrous benzene by sodium, then reacted with 1,6-dichlorohexane to yield 6-(chlorohexyl)triethylene glycol (**1**) (68% yield). Inorganic components were removed by extraction with water and benzene. The compound **2** was prepared similarly to give 53% yield. Diluent compound **4** for SPRE measurements was prepared by method of two steps. Firstly, thiuronium chloride **3** was synthesized from compound **2** and thiourea (51% yield). Then, it was hydrolyzed with sodium hydroxide yielding (56%) 6-(mercaptohexyl)pentaethylene glycol (**4**).

Next, we decided to choose the same strategy of biotinylated linker's **5** synthesis through halogenides like Hansen [30] and made it coupling 6-(chlorohexyl) triethylene glycol (**1**) with the carbodiimide activated biotin. The resulting compound **5** was recrystallized from dichloromethane and hexane mixture in good yield (60%); next it was refluxed with excess thiourea in *n*-butanol under argon. Thiuronium salt **6** was isolated from methanol, acetone, and ether mixture (58.5% yield). The usual procedures for conversion



Scheme 1. Synthesis of compounds 1-8.

of the mercapto group from thiuronium salts employ potassium hydroxide in ethanol. In our synthesis, these reaction conditions cannot be used because of the presence of ester groups. So, we choose mild hydrolysis by sodium metabisulfite solution in chloroform to provide the corresponding thiol **7** (47% yield) [31]. Stilbazolium salt **8** production was attempted by conventional heating. The week-long synthesis was unsuccessful and finished with only traces of product. Finally, stilbazolium salt **8** was prepared (47% yield) from compound **5** and dimethylaminostilbazole by microwave irradiation with toluene as the solvent.

We have designed and synthesized biofunctionalized halogenide **5** and used it for the syntheses of two biotinylated esters. The synthesis of BET ester (**7**) was performed under an atmosphere of argon by an efficient method of three steps. BES ester (**8**) was made by microwave irradiation in two steps. The reactive surface properties of compound **7** and chromophoric properties for protein marking of compound **8** were proved with streptavidin using SPRE and fluorescence experiments described below.

All products, except compound **8**, were purified without column chromatography.

3.2. Surface plasmon resonance ellipsometry (SPRE)

Fig. 2 shows the results of SAM formation on the gold surface and subsequent streptavidin adsorption to biotin groups of compound **7**. Refractive index dispersion of compact octadecanethiol monolayer was used for SAM's modeling, thus obtaining the resulting SAM thickness of 2.6 nm which is consistent with the length (2.7 nm) of molecule **4**, the major constituent of the SAM. This result indicates the high fill factor and the quality of the monolayer. The estimated thickness of adsorbed streptavidin layer assuming the homogeneous layer at the SAM's surface is 2.6 nm.

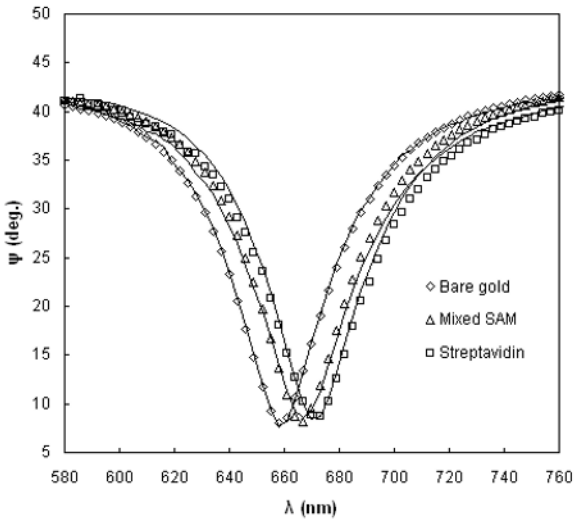


Figure 2. SPR ellipsometry spectra of parameter ψ for bare Au substrate; Au is coated with mixed compound 7/compound **4** SAM; streptavidin is adsorbed onto the monolayer's surface (all spectra recorded in water). Experimental data points are shown with markers, smooth lines represent data fits.

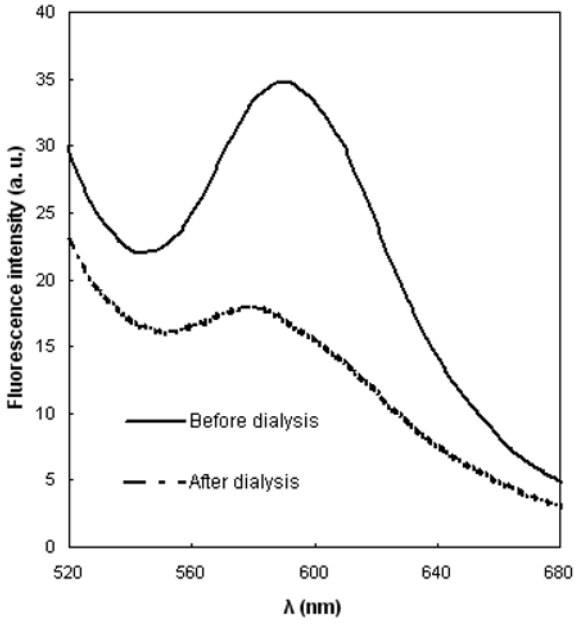


Figure 3. Fluorescence spectra of 15 μM streptavidin and compound **8** water solution before and after the dialysis. Concentration of the chromophore before dialysis was 200 μM, after dialysis, considering binding to tetrameric protein – 60 μM. Excitation wavelength is 479 nm.

The dimensions of streptavidin tetramer molecule are 4.5×4.5×5 nm, wherefore the protein layer was filled incompletely, resulting in about 50% of surface fill factor. This can be explained due to inhomogeneous distribution of compound **7** in the monolayer, resulting in close packing of biotin groups that hinder molecular recognition with the biotin-binding pockets of streptavidin at the surface. Alternatively, the 5:95 solution alkylthiolate molar ratio employed in this experiment may have resulted in SAMs with insufficient portion of compound **7** molecules adsorbed at the gold surface. Additionally, the used of diluent compound **4** could be unfavorable due to its length, approximate to compound **7** molecule's length, as well as resulting in reduced availability of the biotin terminus at the surface.

3.3. Fluorscence

The results of streptavidin labeling with chromophore via biotin group are shown in Fig. 3. The fluorescence spectra of compound **8** and streptavidin solution were recorded before and after dialysis. The effective binding of the chromophore is evidenced by the ratio of fluorescence integral intensity before and after the dialysis. Initial 200 μM chromophore concentration is expected to decrease by 3.3 fold to 60 μM considering the 15 μM streptavidin × 4 binding sites per tetrameric molecule. The ratio of before/after dialysis integral intensity is 2.9; the slight variance can be explained by unfolding of some protein structures, resulting in fewer biotin binding sites and/or imprecise baseline subtraction.

4. Conclusions

In conclusion, we have developed an efficient synthesis of halogenide containing hexyltriethylene glycol chain functionalized with biotin, which is a building block for the synthesis of biotinylated compounds. Two biotinylated esters were successful synthesized in several steps and their useful properties for surface modifications and protein marking were investigate using the surface plasmon resonance ellypsometry and fluorescence spectroscopy.

Acknowledgements

The authors gratefully acknowledge the Lithuanian State Science and Studies Foundation (Project B-38/2008) for financial support. We thank Aušra Linkevičiūtė and dr. Zigmas Balevičius from Center For Physical Sciences and Technology for MS and SPRE spectrum recording. The authors gratefully acknowledge the Department of Bioelectrochemistry and Biospectroscopy at the Institute of Biochemistry of Vilnius University for possibility to use the FT-IR spectrometer.

References

[1] D. Chen, J. Li, Surf. Sci. Reports 61, 445 (2006)
[2] F. Schreiber, Prog. Surf. Sci. 65, 151 (2000)
[3] J. Noh, M. Hara, RIKEN Review 38, 49 (2001)
[4] A.B. Sieval, R. Linke, H. Zuilhof, E.J.R. Sudhölter, Adv. Mater. 12(19), 1457 (2000)
[5] H.-Y. Nie, N.S. McIntyre, W.M. Lau, J.M. Feng, Thin Solid Films 517, 814 (2008)
[6] J.J. Gooding, F. Mearns, W. Yang, J. Liu, Electroanal. 15(2), 81 (2003)
[7] N.K. Chaki, K. Vijayamohanan, Biosens. Bioelectron. 17, 1 (2002)
[8] J.H. Fendler, Chem. Mater. 13, 3196 (2001)
[9] M. Aslam, N.K. Chaki, J. Sharma, K. Vijayamohanan, Curr. Appl. Phys. 3, 115 (2003)
[10] J. Xu, J. Xia, Z. Lin, Angew. Chem. Int. Ed. 46, 1860 (2007)
[11] L. Wang, A. Rastelli, S. Kiravittaya, R. Songmuang, O.G. Schmidt, B. Krause, T.H. Metzger, Nanoscale Res. Lett. 1, 74 (2006)
[12] Y.-H.M. Chan, S.G. Boxer, Curr. Opin. Chem. Biol. 11, 1 (2007)
[13] W. Knoll, I. Köper, R. Naumann, E.-K. Sinner, Electrochim. Acta 53, 6680 (2008)
[14] I. Köper, Mol. BioSyst. 3, 651 (2007)
[15] W. Huang, M. Helvenston, Langmuir 15, 6510 (1999)
[16] A. Bajorek, K. Trzebiatowska, B. Jędrzejewska, M. Pietrzak, R. Gawinecki, J. Pączkowski, J. Fluoresc. 14(3), 295 (2004)
[17] M.F. Pepitone, G.G. Jernigan, J.S. Melinger, O.-K. Kim, Org. Lett. 9(5), 801 (2007)
[18] P.G. Lacroix, J.C. Daran, Chem. Mater. 10, 1109 (1998)
[19] B.Rudat, E. Birtalan, I. Thomé, D.K. Kölmel, V.L. Horhoiu, M.D. Wissert, U. Lemmer, H.-J. Eisler, T.S. Balaban, S. Bräse, J. Phys. Chem. B 114, 13473 (2010)
[20] T. H. Nguyen Pham, R.J. Clarke, J. Phys. Chem. B, 112, 6513 (2008)
[21] D.N. Ng, P. Fromherz, ACS Chem. Biol. 6, 444 (2011)
[22] B. Kuhn, P. Fromherz, J. Phys. Chem. B, 107, 7903 (2003)
[23] E. Gizeli, M. Liley, C.R. Lowe, H. Vogel, Anal. Chem. 69, 4808 (1997)
[24] S. Hleli, C. Martelet, A. Abdelghani, N. Burais, N. Jaffrezic-Renault, Sensor. Actuator. B113, 711 (2006)
[25] P.A. Millner, H.C.W. Hays, A. Vakurov, N.A. Pchelintse, M.M. Billah, M.A. Rodger, Semin. Cell. Dev. Biol. 20, 34 (2009)
[26] J.M. Harris, S. Zalipsky (Eds.), Poly(ethylene glycol) Chemistry and Biological Applications (American Chemical Society, San Francisco, 1997)
[27] E. Prats-Alfonso, F. Garcia-Martin, N. Bayo, L.J. Cruz, M. Pla-Roca, J. Samitier, A. Errachid, F. Albericio, Tetrahedron 62, 6876 (2006)
[28] C.A. Canaria, J.O. Smith, C.J. Yu, S.E. Fraser, R. Lansford, Tetrahedron Lett. 46, 4813 (2005)
[29] D. Witt, R. Klajn, P. Barski, B.A. Grzybowski, Curr. Org. Chem. 8, 1763 (2004)
[30] T.M. Hansen, M.M. Engler, C.J. Fortsyth, Bioorg. Med. Chem. Lett. 13, 2127 (2003)
[31] M. Kažemėkaitė, A. Bulovas, V. Smirnovas, G. Niaura, E. Butkus, V. Razumas, Tetrahedron Lett. 42, 7691 (2001)