SYNTHESIS OF POLYLACTIDE WITH THIOL END GROUPS

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Four synthetic routes to poly(L-lactide) with thiol end groups based on ring-opening polymerization of L-lactide (LA) catalysed with tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) are reported. The following alcohols were used as co-initiators of polymerization: 2-sulfanylethan-1-ol, 2-[(2,4-dinitrophenyl)sulfanyl]ethan-1-ol, 2-(tritylsulfanyl)ethan-1-ol and allyl alcohol. End groups introduced into polymers by co-initiators were transformed to thiol groups by a subsequent modification reaction. The efficiencies of the used synthetic methods were evaluated and discussed. The best results were obtained with co-initiator 2-(tritylsulfanyl)ethan-1-ol.
Keywords: Polylactides; Thiol-functionalized polymers; End group modification; Thiols; Ring-opening polymerizations; Adsorption on gold.

Polylactide (PLA) is widely used as a biomaterial. For application such as tissue engineering, knowledge of interaction of PLA surfaces with components of biological fluids is very important. The surface plasmon resonance (SPR) technique[1] is an efficient method for studies of protein adsorption on surfaces exposed to protein solutions or complex biological fluids, such as blood plasma or lymph. The SPR technique uses gold-plated optical substrate, the gold surface of which has to be first coated with a very thin layer of the polymer under study. Using SPR, the changes at the polymer surface due to adsorption of molecules from solution can be followed in real time and the dynamics of adsorption process can be evaluated in dependence on the material properties. To use SPR for evaluation of PLA-based materials, a contiguous thin layer of PLA well-adherent to the gold surface has to be prepared.

To prepare a PLA layer strongly attached to the gold surface we decided to use thiol-monofunctionalized PLA. Low-molecular-weight thiols are well known to form highly regular and stable self-assembled monolayers on

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gold[2] due to the formation of thiolate bonds to the metal surface. Physico-chemical properties of the monolayer depend on the structure of the rest of the thiol molecule. The preparation of a thin polymer film on gold from polystyrene with thiol end groups has been described in literature[3]. In the same way thiol-functionalized polylactides should form either a contiguous coating or, at least, an anchoring layer on gold substrates, onto which an ultrathin film of PLA can be deposited by spin casting.

Poly(lactic acid) as well as other aliphatic poly(hydroxy acids) are most commonly prepared by ring-opening polymerization of cyclic esters of these acids. Tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) as catalyst and an alcohol as co-initiator are the often used initiating system of these polymerizations. Penczek's group[4,5] thoroughly investigated the mechanism and kinetics of the polymerization and proven the process to be "living". The molecular weight of polymer products can be varied in a wide range by adjusting the initial monomer-to-ROH molar ratio. The kinetic data and MALDI-TOF mass spectra indicate[6] that the actual initiator of the polymerization is tin(II) alkoxide (Oct)SnOR formed from $Sn(Oct)_2$ and the co-initiating alcohol ROH in the reaction: $Sn(Oct)_2 + ROH \leftrightarrow (Oct)Sn–OR + OctOH$. The propagation proceeds by a monomer insertion into the Sn–OR bond of the tin(II)-alkoxide active centres reversibly formed from polyester hydroxy end groups and $Sn(Oct)_2$. Since the co-initiating compound ROH is incorporated into the polymer as the other chain end group, the polymerization can be efficiently used for the preparation of functionalized polylactides and block copolymers with polylactide blocks[7,8].

So far very few papers on thiol-functionalized aliphatic poly(hydroxy acid)s have been published. Recently, the preparation of poly(ε-caprolactone) (*i.e.* poly(hexano-6-lactone)) with thiol groups has been described using an alcohol with a 2,4-dinitrophenyl-protected thiol groups as co-initiator[9]. The thiol groups were recovered after cleaving off the 2,4-dinitrophenyl groups by excess of a low-molecular-weight thiol.

In this paper we test and compare four synthetic routes to thiol-functionalized polylactide. Three of them introduce thiol groups through a co-initiator (functionalization by initiatiator). As co-initiator we tested 2-sulfanylethan-1-ol with the thiol groups protected by 2,4-dinitrophenyl and trityl groups and also with free thiol groups to check the necessity of protection. The fourth route tested makes use of a polymer precursor with allyl end groups which are subsequently modified by a sulfur reagent (polymer functionalization). The suitability of each route for the preparation of well-defined polymer thiols is evaluated on the basis of polymer parameters in particular the degree of functionalization.

EXPERIMENTAL

Materials

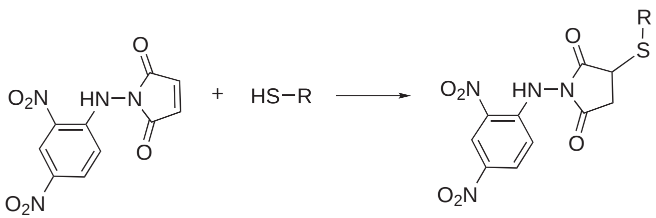
1,4-Dioxane for polymerizations was dried by refluxing over sodium. 2-Sulfanylethan-1-ol (Fluka AG) (co-initiator **1**) was distilled and dried over molecular sieve. Allyl alcohol (co-initiator **2**) was distilled from CaH_2. L-Lactide was synthesized[10] from L-lactic acid and re-crystallized from a mixture of dry toluene and ethyl acetate (1:1 v/v) prior to use. 2,2′-Azobisisobutyronitrile (AIBN) (Aldrich) was recrystallized from methanol. *N*-(2,4-Dinitroanilino)-maleimide (DNAMI) (Sigma) was used as received. Tin(II) 2-ethylhexanoate (Fluka AG) was purified by vacuum distillation. Trifluoroacetic acid (TFA) and triethylamine (TEA) were distilled prior to use. All other reagents were purchased from Aldrich and used as received.

Measurements

NMR spectra were recorded on a Bruker Avance DPX-300 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. Calculation of the number-average molecular weight is based on the ratio of integrated signals of methine protons of the CO**CH**(CH_3)OH end group (4.29 ppm) and of the CO**CH**(CH_3)O main-chain units (5.09 ppm) in the ^{1}H NMR spectra. Size exclusion chromatography (SEC) was performed on a Waters HPLC-SEC modular system using PLgel 10^3 Å, 10 μm (7.5 × 600 mm) column, eluent THF and Waters 410 RI and Waters 484 UV detectors. The columns were calibrated with polystyrene standards and the molecular-weight/elution volume dependence was recalculated for PLLA by using Mark–Houwink coefficients for polystyrene and PLLA [11].

Determination of Thiol Groups

Two parallel methods were used: (i) By NMR spectroscopy, the content of free thiol end groups was determined from ratio of intensities of methylene (2.68 dt, 2 H, HSC**H**$_2$CH$_2$O or 4.17 t, 2 H, HSCH$_2$C**H**$_2$O) and methine end groups (4.29 q, 1 H, COC**H**(CH_3)OH) in ^{1}H NMR spectrum. (ii) By the reaction of thiol groups with *N*-(2,4-dinitroanilino)maleimide[12]. A polymer sample and three equivalents of DNAMI were dissolved in DMF and allowed to react at room temperature for 5 h (Scheme 1). To separate the labeled polymer fraction from an excess of unreacted DNAMI and to quantify it, SEC with UV detection (λ = 323 nm) was used. The molar amount of the transformed thiol groups was calculated from the peak area of the labeled polymer. By comparison of the molar amount of the injected polymer and the measured molar amount of thiol groups, the fraction of thiol-functionalized PLA was calculated.



SCHEME 1

2-[(2,4-Dinitrophenyl)sulfanyl]ethan-1-ol (**3**)

Adopting the procedure described in literature[9], a solution of 2-sulfanylethan-1-ol (30 mmol, 2.1 ml) in 30 ml of $CHCl_3$ was slowly added into a mixture of 1-fluoro-2,4-dinitrobenzene (5.6 g, 30 mmol) and TEA (7 ml). The brownish reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with $CHCl_3$ (200 ml), extracted with 1 M HCl (1 × 20 ml) and water (2 × 20 ml). The chloroform phase was separated, dried over anhydrous $MgSO_4$ and filtered. The yellow product was twice recrystallized from $CHCl_3$. Yield 4.5 g (61%); m.p.: 99.5–101 °C. For $C_8H_8N_2O_5S$ (244.2) calculated: 39.34% C, 3.30% H, 11.47% N, 13.13% S; found: 39.40% C, 3.20% H, 11.30% N, 13.06% S. ^{1}H NMR ($CDCl_3$): 8.95 s, 1 H, J(3,5) = 2.5 (H-3), H-arom.; 8.28 dd, 1 H, J(3,5) = 2.5, J(5,6) = 9.0 (H-5), H-arom.; 7.62 d, 1 H, J(5,6) = 9.0 (H-6), H-arom.; 3.97 t, 2 H, J = 5.9, $SCH_2C\mathbf{H}_2OH$; 3.24 t, 2 H, J = 5.9, $SC\mathbf{H}_2CH_2OH$.

2-(Tritylsulfanyl)ethan-1-ol (**4**)

A procedure described in literature was followed[13]. Trityl chloride (4 g, 14 mmol) was dissolved in 60 ml of petroleum ether and 1 ml (14 mmol) of 2-sulfanylethan-1-ol was added. The mixture was refluxed for 15 min. White crystals that precipitated in reaction mixture were collected by filtration and the product was crystallized from ethanol. Final recrystallization was from a mixture of toluene and petroleum ether (1:1 v/v). Yield 3.2 g (71%); m.p.: 115–116 °C. For $C_{21}H_{20}OS$ (320.5) calculated: 78.71% C, 6.29% H, 10.01% S; found: 78.6% C, 6.3% H, 9.9% S. ^{1}H NMR ($CDCl_3$): 7.14–7.38 m, 15 H, H-arom.; 3.31 t, 2 H, J = 6.0, $SCH_2C\mathbf{H}_2OH$; 2.41 t, 2 H, J = 6.0, $SC\mathbf{H}_2CH_2OH$.

Polymers

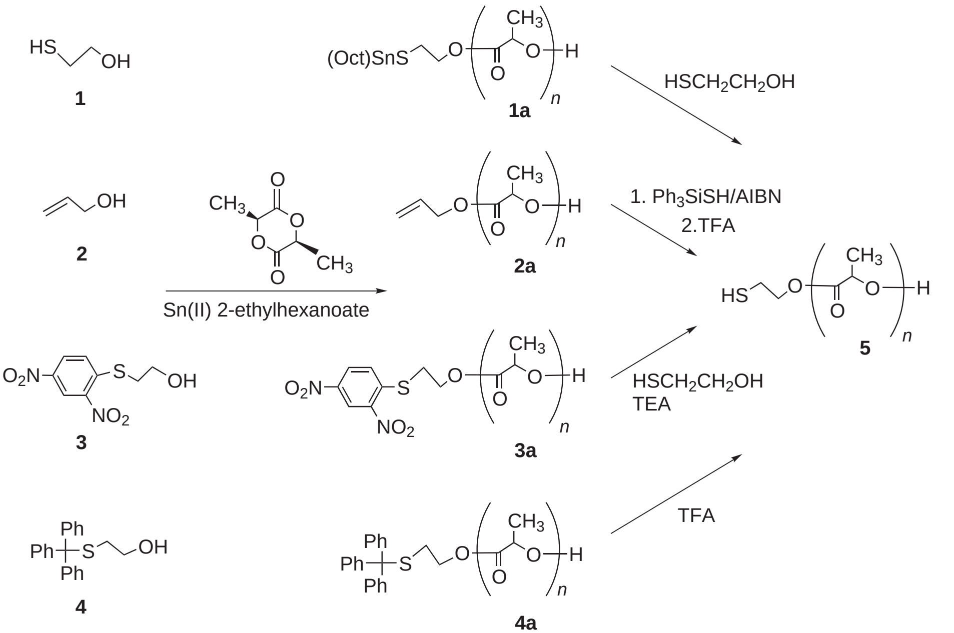
Polymers were prepared by ring-opening polymerization of L-lactide in dioxane initiated with tin(II) 2-ethylhexanoate and co-initiated with one of the four different co-initiators possessing OH groups (Scheme 2). The monomer-to-$Sn(Oct)_2$ molar ratio was 50:1 except the case of polymerization with co-initiator **1** when the 15:1 ratio was used. The monomer-to-co-initiator ratio was varied as shown in Table I. The polymerization was carried out under nitrogen atmosphere in sealed ampoules at 60 °C for 40 h. The reaction mixture was finally poured into methanol and the precipitated polymer was isolated by filtration or centrifugation.

Modification of End Groups

Isolated polymer precursors **1a**–**4a** were subjected to the following treatment, which converted the initiator end groups into thiol groups.

To regenerate thiol end groups from precursor **1a**, an excess of low-molecular-weight thiol was used. An amount of 1.5 g of polymer **1a** was dissolved in dichloromethane (16 ml) and 4 ml of 2-sulfanylethan-1-ol was dropwise added to the solution. Upon stirring for 30 min, dark-red precipitation of low-molecular-weight tin(II) thiolate was formed. It was removed by filtration and the polymer solution was precipitated into methanol.

Allyl end groups of the polymer **2a** were modified by radical addition of triphenylsilanethiol to the double bond using the procedure described for low-molecular-weight alkenes[14]. Polymer **2a**, corresponding to 146 μmol of chain ends, triphenylsilanethiol (121 mg,



Scheme 2

414 µmol) and AIBN (17 mg, 104 µmol) were dissolved in 1.3 ml of benzene, heated to 95 °C and stirred for 5 h. The reaction mixture containing the polymer-thiol adduct was cooled and treated with 5 equivalents of TFA and stirred for 0.5 h to remove the triphenylsilyl protecting groups. The reaction mixture was precipitated into methanol.

2,4-Dinitrophenyl protecting groups of polymer **3a** were cleaved with an excess of 2-sulfanylethan-1-ol. An amount of 1.5 g of polymer **3a** was dissolved in 15 ml of mixture $CHCl_3$/2-sulfanylethan-1-ol (2:1 v/v) containing TEA in 1% (w/w) concentration. The solution was stirred at room temperature for 15 h and then precipitated into methanol.

The trityl protecting groups of polymer **4a** were removed by trifluoroacetic acid. An amount of 1.5 g of polymer **4a** was dissolved in 10 ml of TFA. Yellow colour of the triphenylmethyl cation immediately appeared. After 1 h of stirring at room temperature, the solution was precipitated into methanol.

RESULTS AND DISCUSSION

The thiol end groups content is a key parameter for comparison of thiol-functionalized polymers prepared by different routes. The determination based on the intensity of peaks in ^{1}H NMR spectrum (2.68 dt, 2 H, $HSC\mathbf{H}_2CH_2O$ and 4.17 t, 2 H, $HSCH_2C\mathbf{H}_2O$) is not quite reliable due to a partial overlap of these signals with those of the other end groups of PLA chain (4.29 q, 1 H, $COC\mathbf{H}(CH_3)OH$ and 2.62 s, 1 H, $COCH(CH_3)O\mathbf{H}$). Therefore, we have developed an alternative analytical method derived from the method commonly used in protein chemistry for quantification of cysteine residues[15]. It is based on the SH groups labeling with a chromophore and its subsequent spectrophotometric determination. Both methods gave SH-contents, which are in a reasonable agreement, thus confirming that they represent the actual thiol content. They are given together with the other parameters of the prepared polymers in Table I.

The polymerization co-initiated with 2-sulfanylethan-1-ol (**1**) could be the simplest way to obtain polymer thiol without the necessity of preparation of a commercially unavailable protected co-initiator. However, the formation of tin(II) thiolate as a product of reaction of $Sn(Oct)_2$ with the thiol groups and, consequently, an influence on the course of polymerization is to be expected. To compensate the loss of catalytic activity of $Sn(Oct)_2$, the amount of the catalyst in the reaction mixture was increased up to equimolar to the co-initiator. Tin(II) thiolate apeared as a brownish turbidity in the reaction mixture in the course of polymerization. The influence of side reaction between co-initiator and catalyst was markedly reflected in a low degree of functionalization and in lower molecular weight of the polymers (polylactides 1-1 and 1-2) than expected from the composition of the starting reaction mixture. To get a higher degree of thiol functionalization, either protection of the thiol group in 2-sulfanylethan-1-ol or another chemical strategy has to be used.

According to literature[9], 2-[(2,4-dinitropheny)sulfanyl]ethan-1-ol (**3**) was used as a protected form of 2-sulfanylethan-1-ol in the synthesis of thiol-functionalized poly(ε-caprolactone), giving high yields of functionalization. Although our polymerization conditions were comparable to those described in literature, we did not succeed in obtaining high degree of functionalization, as shown in Table I (polylactides 3-1 and 3-2). Similar indications of a side reaction between the catalyst and co-initiator (probably its NO_2 groups) were observed to those in the case of the polymerization with co-initiator **1**: low functionalization, lower than theoretical weight and a broad MWD. Moreover, the conditions used for deprotection caused partial degradation of PLA, thus decreasing the content of thiol end groups.

Trityl was tested as another thiol protecting group for 2-sulfanylethan-1-ol. The products of polymerization initiated with **4** (polylactide 4-1 to 4-6) were quantitatively functionalized as follows from the ratio of peak integrals of PLA end groups: quartet 4.29 ppm in OC**H**(CH_3)OH, triplets 3.80 and 2.38 ppm in trityl-SC$\mathbf{H}_2$C$\mathbf{H}_2$O-PLA (Fig. 1). Characteristics of the precursor polymers **4a** indicate that the polymerization was controlled. After deprotection the content of thiol end groups remained high. The

TABLE I
Characteristics of prepared thiol-functionalized polylactides and theirs precursors

Code	Co-initiator	Polymer precursor							Polymer product				
		monomer/co-initiator mole ratio	M_n			M_w/M_n	co-initiator content, % NMR	Yield %	M_n		M_w/M_n	SH content, %	
			theory	SEC	NMR				SEC	NMR		SEC	NMR
1-1	**1**	16	2300	1100[a]	–	2.7	–	34	3600	2300	1.3	24	27
1-2	**1**	30	4320	2800[a]	–	2.0	–	65	5000	3300	1.4	23	32
2-1	**2**	11.5	1660	1800[a] 2600[b]	2500[b]	1.2[b]	99	70	2800	2600	1.2	0	0
2-2	**2**	23.2	3340	4600	3500	1.3	99	92	5000	4100	1.3	0	0
3-1	**3**	56.3	8100	2900	2100	1.2	42	30	3000	1800	1.3	16	22
3-2	**3**	277.8	40000	11000	18000	2.8	<5	73	6500	4000	3.0	0	0
4-1	**4**	27.1	3900	5200	4400	1.3	99	92	5200	4300	1.3	87	77
4-2	**4**	27.4	4000	5500	5000	1.3	99	99	5300	4700	1.4	55	60
4-3	**4**	86.8	12500	14600	12400	1.4	99	99	14100	10400	1.5	49	63
4-4	**4**	138.9	20000	15300	19000	1.9	78	96	10900	18400	2.3	42	45
4-5	**4**	13.2	1900	2370[a] 3000[b]	2700[b]	1.4[b]	99	99	–	–	–	–	–
4-6	**4**	53.5	7700	10100	8200	1.3	96	97	–	–	–	–	–

[a] Not isolated. [b] Isolated.

deprotected polymer, however, still contains a small fraction of the protected species detected as triplets 3.80 and 2.38 ppm (Fig. 2). If the low-molecular-weight (M_n = 2500) polymer precursor was deprotected, originally unimodal MWD changed to bimodal. Molecular weight of the new polymer fraction in the polymer was twice higher than the original one. This indicates partial oxidation of the polymer thiol to polymer disulfide, which is a typical reaction of low-molecular-weight thiols but was also observed with polymer thiols[16]. The triplet 2.86 ppm in ^{1}H NMR spectrum was assigned to CH_2 group in the substructure $OCH_2\mathbf{CH_2}S–SCH_2CH_2O$. The oxidation of polymer thiol of higher molecular weight than 2500 was not observed. The triplet at 3.05 ppm in the ^{1}H NMR spectrum (Fig. 2) of deprotected polymer **4a** indicates the presence of a substructure $S\mathbf{CH_2}CH_2O$ different from the $HS\mathbf{CH_2}CH_2O$, trityl-$S\mathbf{CH_2}CH_2O$ and $OCH_2\mathbf{CH_2}S–SCH_2CH_2O$ groups. This triplet was also observed in ^{1}H NMR spectra of polymers prepared by deprotection of **1a** and **3a**. We assume that this substructure (see Scheme 3) may originate from the reesterification of polymer ester with free thiol. The estimated value (CS ChemDraw Ultra 5.0) of chemical shift is 3.15 ppm for $OCOSC\mathbf{H_2}CH_2O$, which correspods to the experimental value.

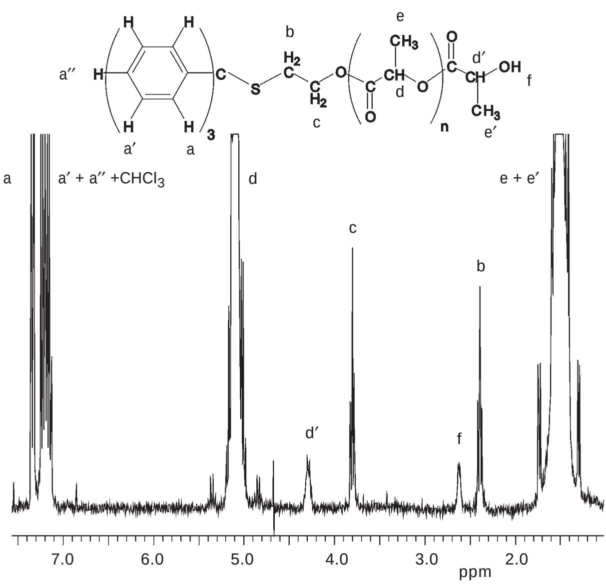
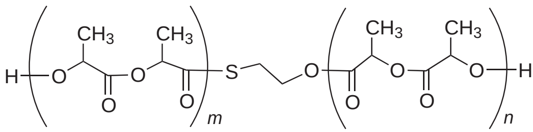


FIG. 1
^{1}H NMR spectrum of poly(L-lactide) with 2-(tritylsulfanyl)ethoxy end groups (4-1, before deprotection)

The content of this substructure in the product never exceeded 10% of all SCH_2 structures.



SCHEME 3

Functionalization of a well-defined polymer precursor with a sulfur reagent is an alternative to functionalization by initiator. Triphenylsilanethiol and allyl-terminated PLA were chosen for the fourth synthetic route, since triphenylsilanethiol should not react with monomer units in radical addition of SH on the terminal allyl double bond. Although, allyl end groups were introduced with high efficiency into the PLA by polymerization co-initiated with allyl alcohol (**2**), the subsequent radical addition of thiol did not proceed as efficiently as described for low-molecular-weight analogues[16]. The ^1H NMR spectra did not indicate any significant modification of the allyl groups under the described conditions.

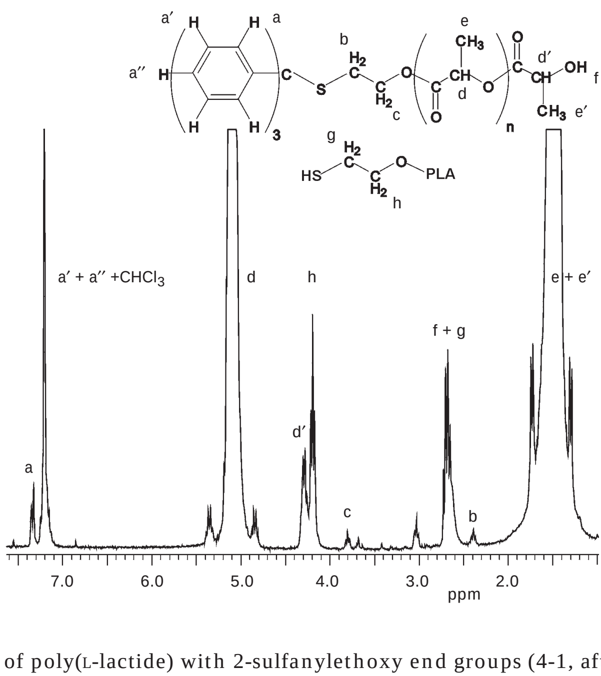


FIG. 2
^1H NMR spectrum of poly(L-lactide) with 2-sulfanylethoxy end groups (4-1, after deprotection)

CONCLUSIONS

2-(Tritylsulfanyl)ethan-1-ol (**4**) was found to be the best initiator out of those tested for preparation of thiol-monofunctionalized polylactide. Polymers prepared in this way show thiol contents suitable for future adsorption studies on gold substrates. Molecular parameters of the functionalized polymers can be reasonably controlled by the reaction conditions.

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