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Synthesis of Mono- and Dithiols of Tetraethylene Glycol and Poly(ethylene glycol)s via Enzyme Catalysis

Prajakatta Mulay [1], Gayatri Shrikhande [1] and Judit E. Puskas [1,2,*]

[1] Department of Chemical and Biomolecular Engineering, The University of Akron, Akron, OH 44325, USA; pm62@zips.uakron.edu (P.M.); gss26@zips.uakron.edu (G.S.)

[2] Department of Food, Agricultural and Biological Engineering, The Ohio State University, Wooster, OH 44691, USA

* Correspondence: puskas.19@osu.edu; Tel.: +1-330-263-3861

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Abstract: This paper investigates the transesterification of methyl 3-mercaptopropionate (MP-SH) with tetraethylene glycol (TEG) and poly(ethylene glycol)s (PEG)s catalyzed by *Candida antarctica* Lipase B (CALB) without the use of solvent (in bulk). The progress of the reactions was monitored by ^{1}H-NMR spectroscopy. We found that the reactions proceeded in a step-wise manner, first producing monothiols. TEG-monothiol was obtained in 15 min, while conversion to dithiol took 8 h. Monothiols from PEGs with M_n = 1000 and 2050 g/mol were obtained in 8 and 16 h, respectively. MALDI-ToF mass spectrometry verified the absence of dithiols. The synthesis of dithiols required additional fresh CALB and MP-SH. The structure of the products was confirmed by ^{1}H-NMR and ^{13}C-NMR spectroscopy. Enzyme catalysis was found to be a powerful tool to effectively synthesize thiol-functionalized TEGs and PEGs.

Keywords: *Candida antarctica* Lipase B; transesterification; polymer functionalization; tetraethylene glycol; poly(ethylene glycol)

1. Introduction

Poly(ethylene glycol) (PEG) is the most frequently used polymer for biomedical research and applications because it is soluble in organic as well as aqueous media [1], is not cytotoxic and immunogenic [2], and is easily excreted from living organisms [3]. Click chemistries and Michael addition reactions are often used for PEGylation of drugs to make them more water soluble [4]. Thiol-functionalized PEGs have an important role in these reactions [5–10] and can be used as a 'Michael donor' or in thiol-ene click reactions to synthesize conjugates for targeted drug delivery [7]. Other uses include the following: An anti-fouling biosensor coating [8], to stabilize gold nanorods used to test water for chemical pollutants [9], and to stabilize gold nanoparticles used as drug delivery vehicles [10]. Thiol-functionalized PEG is also a favorite to make self-assembling monolayers on gold surfaces [8]. Mahou et al. [11] reported the single synthetic strategy to obtain PEG-monothiol. They tosylated one hydroxyl end-group of the PEG-diol using *p*-toluenesulfonyl chloride in the presence of silver oxide and potassium iodide catalyst and toluene solvent. The tosylated PEG was then reduced with sodium hydrosulfide at 60 °C to yield PEG-monothiol with 84% yield. PEG-dithiols have been synthesized by various methods. In one method, the hydroxyl end groups were reacted with allyl bromide at 120 °C, followed by a radical-mediated addition of thioacetic acid and subsequent reduction to thiol using sodium hydroxide/sodium thiomethoxide, with a 56% yield [12–14]. Another route reported tosylation of the hydroxyl end groups, followed by a reaction with a xanthate and de-protection with an alkyl amine that gave 98% yield [15,16]. The simplest method used esterification of mercapto-acids

in toluene at 120 °C using *p*-toluenesulfonic acid or sulfuric acid as catalysts: An example is shown in Figure 1 [17–25].

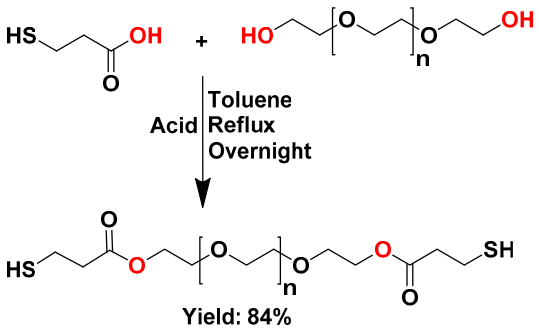

Figure 1. Synthesis of poly(ethylene glycol) (PEG)-dithiol [22].

These methods employ acid catalysts; and hence are not "green". Against this background, we investigated the synthesis of thiol-functionalized tetraethylene glycol (TEG) and PEGs by transesterification of methyl 3-mercaptopropionate (MP-SH) under solvent-less conditions using a heterogeneous catalyst, namely, *Candida antarctica* Lipase B (CALB). CALB-catalyzed functionalization of low molecular weight polymers was first reported by our research group yielding pure products with high efficiency [26–32]. For example, halogen-functionalized PEGs were made by the transesterification of halo-esters with PEG monomethyl ether under solvent-less conditions at 65 °C for 4 h under vacuum (70 milliTorr) [31]. Methacrylate, acrylate, and crotonate functionalization of PEGs was also achieved under solvent-less conditions within 4 h at 50 °C by reacting PEG with the corresponding vinyl esters (vinyl methacrylate, vinyl acrylate, and vinyl crotonate) in the presence of immobilized CALB [32].

Precise thiol-functionalization of TEG and PEGs by enzyme catalysis has not been reported previously in the literature. This study presents the first examples of precision synthesis of TEG and PEG monothiols and dithiols. In this study, two types of PEGs (M_n = 1000 g/mol and M_n = 2050 g/mol) were used to evaluate the effect of PEG chain length on the kinetics of the CALB-catalyzed transesterification reaction of methyl 3-mercaptopropionate (MP-SH) with PEGs at 50 °C, an optimum temperature for CALB-catalyzed reactions [33]. MP-SH was selected because of its low cost and the convenient removal of the methanol side product by vacuum. CALB supported on various carriers were reported to be more effective than the native enzyme [34–36] and depending on the specific conditions were shown to be recyclable four [37] or twenty times [36]. We have been using the only commercially available CALB (20 wt% immobilized on a macroporous acrylic resin, Novozyme® 435).

2. Results and Discussion

2.1. CALB-Catalyzed Transesterification of MP-SH with TEG

First, MP-SH was transesterified with TEG under solvent-less conditions using CALB as the catalyst. The catalytic triad for transesterification of CALB was shown to consist of serine (Ser105), histidine (His224), and aspartate (Asp187) [38]. Figure 2 illustrates our rendition of the mechanism of transesterification of MP-SH by TEG [33]. The top (dark shaded) portion of the enzyme is the so-called "carbonyl pocket" while the bottom (lighter shaded) is the "hydroxyl pocket". First, the nucleophilic serine (Ser105) in the free enzyme interacts with the carbonyl group of the thioester, forming the first tetrahedral intermediate (THI-1) that is stabilized by the so-called oxyanion hole (three hydrogen bonds: One from glutamine (Gln106) and two from threonine (Thr40)) [38]. In the second step, the ester bond in THI-1 is cleaved to form an acyl-enzyme complex (AEC) that releases the first product, methanol in this case, which is removed due to the applied vacuum (420 Torr), making the reaction irreversible. In the third step, the HO- group of the diol positioned in the hydroxyl pocket interacts with the carbonyl group of the AEC, forming the second tetrahedral intermediate (THI-2), which is also

stabilized by the oxyanion hole. In the last step, the enzyme is deacylated to form a TEG-monothiol that is released from the THI-2 and the enzyme is regenerated.

The second -OH group of the TEG-monothiol will then be converted to thiol in a second cycle in a similar manner as the first cycle as shown in Figure 3. However, the first and second cycle may proceed simultaneously in a competitive reaction between the hydroxyl groups of unreacted TEG and TEG-monothiol. Thus, we first studied the kinetics of CALB-catalyzed transesterification of MP-SH with TEG.

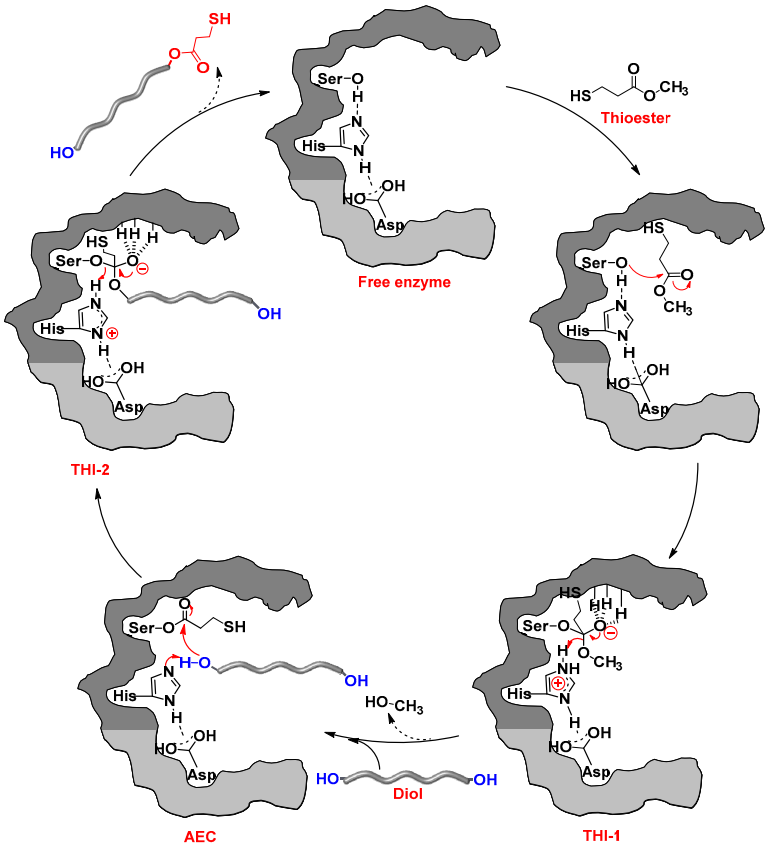

Figure 2. Reaction mechanism of *Candida antarctica* Lipase B (CALB)-catalyzed transesterification of methyl 3-mercaptopropionate (MP-SH) with tetraethylene glycol (TEG)—first cycle.

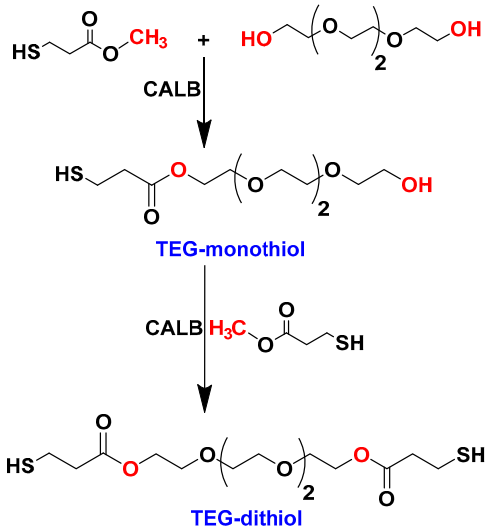

Figure 3. CALB-catalyzed transesterification of MP-SH with TEG.

2.1.1. Kinetics of CALB-Catalyzed Transesterification of MP-SH with TEG

The progress of the reaction was monitored by ^{1}H-NMR spectroscopy. At time 0, the protons from MP-SH (thiol proton triplet at 1.60 ppm (a), methylene protons—quartet at δ = 2.73 ppm (b) and triplet at δ = 2.61 ppm (c)) can be seen together with the proton signals of TEG (CH_2 protons next to the -OH end group at δ = 3.57 ppm (e) and at δ = 3.64 ppm (f) and the internal CH_2 protons of TEG at δ = 3.63 ppm (g)). The methyl protons of MP-SH (h) also appear in this region at δ = 3.66 ppm, overlapping with the methylene proton signals (f) of TEG. It can be observed from Figure 4 that the intensity of the signal at δ = 3.57 ppm (e) gradually decreases as the reaction time increases. The formation of the ester bond is demonstrated by the appearance of a new signal at δ = 4.23 ppm, corresponding to the methylene protons next to the carbonyl group in the product (e', Figure 4). The proton signals (b) and (c) slightly shift to 2.75 ppm (b') and δ = 2.64 ppm (c'). After 15 min of reaction time, the ratio of the internal CH_2 protons of TEG at δ = 3.61 ppm (g) to (e') in the product was 8:1.98, indicating the formation of TEG-monothiol. After the formation of TEG-monothiol, the reaction slowed down considerably. Complete conversion to dithiol took 450 min, and the relative integrals of (g): (e') at 8:3.88 indicated the formation of TEG-dithiol (Figure 4).

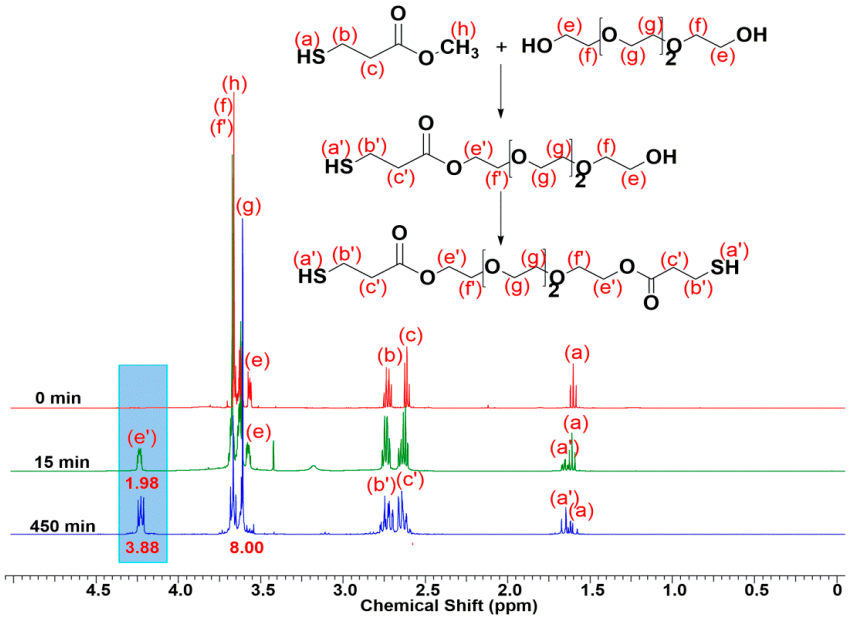

Figure 4. ^{1}H-NMR monitoring of the kinetics of the transesterification of MP-SH with TEG [15 min: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.23 (2H) 3.61(8H); 450 min: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.23 (4H) 3.61 (8H)].

We theorize that in the second cycle the carbonyl group of the free MP-SH competes with the carbonyl group of the TEG-monothiol for complexation in the carbonyl pocket of CALB, thereby slowing down the second cycle of the reaction. Another reason might be the deactivation of CALB by the methanol released in the reactions that is not completely removed in the vacuum. Thus, the reaction proceeds sequentially in a consecutive manner.

2.1.2. Synthesis of TEG-monothiol and TEG-dithiol

Figure S1 shows the ^{1}H- and ^{13}C-NMR spectra of TEG-monothiol synthesized with a reaction time of 15 min after filtering the enzyme and removing the excess thioester but without further purification (93% reaction yield because some material is lost with the enzyme). In the ^{1}H-NMR spectrum of the monothiol (Figure S1A), the ratio of the integral of the methylene protons next to the SH group in the product at 2.75 ppm (b') and δ = 2.64 ppm (c') to the integral of the methylene protons next to the carbonyl group at δ = 4.23 ppm (e') is 4.00:2.00, indicating the formation of the TEG-monothiol

with 100% conversion. In the ^{13}C-NMR spectrum of the monothiol (Figure S1B), signals corresponding to the carbons in the thiol end group (B, C, D, E′ and F′) and the carbons next to the -OH end group (E and F) appears distinctly, that demonstrates the formation of the TEG-monothiol.

Figure S2 shows the ^{1}H- and ^{13}C-NMR spectra of the TEG-dithiol that was synthesized with a reaction time of 7.5 h (88% reaction yield). The ratio of the integral values of signals (b′) + (c′) to (e′) are 8.00:3.88, indicating the formation of the TEG-dithiol. The ^{13}C-NMR spectrum in Figure S2B shows only the signals corresponding to the thiol end groups, with only traces of signals corresponding to the carbons next to the -OH (E and F) at δ = 72.38 ppm and δ = 61.16 ppm, possibly from traces of residual TEG-monothiol, indicating 100% conversion of the -OH groups to the thiols.

In summary, CALB-catalyzed transesterification of MP-SH with TEG in bulk yielded TEG-monothiol in 15 min with 93% reaction yield, and TEG-dithiol in 7.5 h with 88% reaction yield and 100% conversion without purification, such as column chromatography.

2.2. *CALB-Catalyzed Transesterification of MP-SH with PEGs*

2.2.1. Kinetics of CALB-catalyzed transesterification of MP-SH with PEG

MP-SH was reacted with PEG_{1000} using enzyme catalysis, and the reaction was monitored over 24 h with ^{1}H-NMR spectroscopy (see Figure 5). Because low molecular weight PEGs (<3000 g/mol) are liquid at the reaction temperature and are miscible with MP-SH, no solvent was necessary as a medium for the reaction. The main chain protons (g) and the methylene protons next to the –OH (e and f) and the thioester (f′) appear at δ = 3.61 ppm. For PEG_{1000}-monothiol, the new methylene protons next to the carbonyl group (e′) appear at δ = 4.23 ppm, which makes the integral value of internal protons of PEG_{1000} (g, e, f, and f′): 88 − 2 = 86. Therefore, the integral value of the internal protons was set to 86 for calculating the extent of the reaction. Based on the integral ratio of (g, e, f, and f′): (e′), about 60% of the PEG_{1000} was converted to PEG_{1000}-monothiol in 60 min (Figure 5). Then the reaction slowed down, and it took 8 h to convert all PEG_{1000} into monothiol. Dithiol was not detected even after 24 h. The mechanism presented in Figure 2 for TEG also applies for PEG. Thus, we theorize that in the second cycle the carbonyl group of the free MP-SH competes with the carbonyl group of the PEG-monothiol for complexation in the carbonyl pocket of CALB, thereby slowing down the second cycle of the reaction. In addition, the CALB may be deactivated by the methanol released in the reactions that is not completely removed by the vacuum.

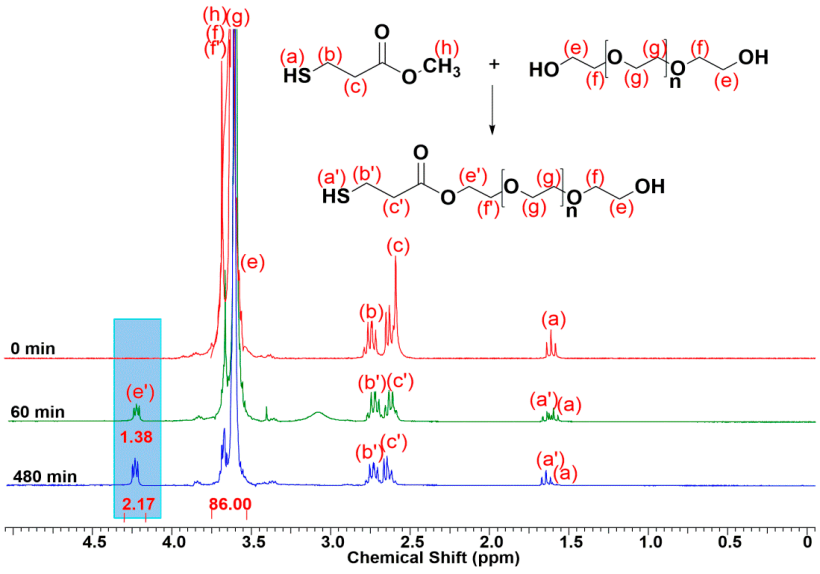

Figure 5. ^{1}H-NMR monitoring of the kinetics of transesterification of MP-SH with PEG_{1000} [480 min: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.23 (2H) 3.61(86H)].

MP-SH was also transesterified with PEG_{2050} and the reaction was monitored over 24 h by ^{1}H-NMR spectroscopy (not shown). Similarly to the PEG_{1000}, only monothiol was obtained. In addition, complete conversion to monothiol was achieved in 16 h, which suggests higher molecular weight required longer reaction time.

2.2.2. Synthesis of PEG_{1000}-monothiol

The ^{1}H-NMR of the PEG_{1000}-monothiol is shown in Figure 6A. The integral ratio of (b′) + (c′) to the methylene protons in the new ester bond at δ = 4.23 ppm is 4:00:1.86, indicating the formation of PEG_{1000}-monothiol. Figure 6B shows the ^{13}C-NMR spectrum of PEG_{1000}-monothiol. Signals corresponding to the carbons next to the thioester (E′ and F′) and –OH end groups (E and F) appear simultaneously, indicating the formation of monothiol.

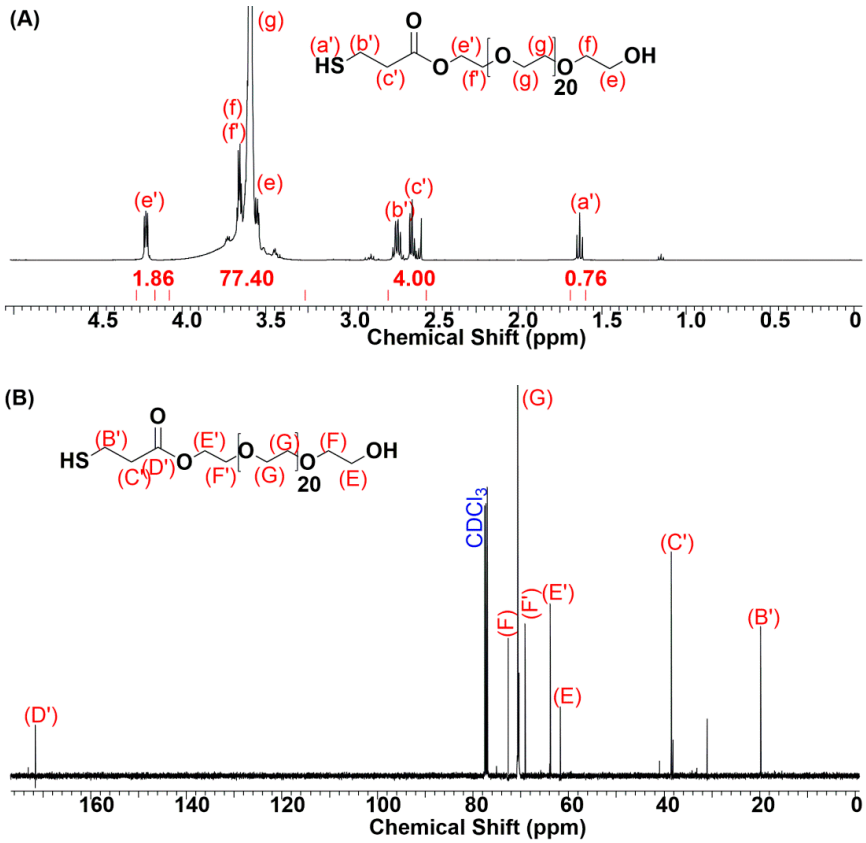

Figure 6. (**A**) ^{1}H-NMR and (**B**) ^{13}C-NMR of PEG_{1000}-monothiol [^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.21 (2H) 3.61(86H) 2.72 (2H) 2.63 (2H) 1.65 (1H); ^{13}CNMR (500 MHz, $CDCl_3$): δ 171.5, 72.66, 70.6, 69.1, 63.8, 61.72, 38.5, 19.7].

The product was further analyzed by MALDI-ToF mass spectrometry and Figure 7 shows the spectrum. There are two major distributions of peaks (Figure 7A), each separated by 44 m/z units (Figure 7B). The peak at m/z 1097.63 corresponds to the Na complex of the 22-mer fraction of PEG_{1000} monothiol [1097.63 = 22 × 44.03 (C_2H_4O repeat unit) + 89.14 (HSC_2H_4CO- end group) + 17 (HO- end group) + 22.99 (Na^+)]. The peak at m/z 560.31 corresponds to the doubly charged Na complex of the 22-mer fraction of PEG_{1000} monothiol [560.31 = [(1097.63 ([M + Na]$^+$) + 22.99 (Na^+)]/2]. The small distribution of peaks appearing under the doubly charged Na complex distribution corresponds to traces of unreacted PEG_{1000} from the reaction mixture (<5%) that could not be detected by NMR. Thus, based on the MALDI mass spectrometry data, over 95% conversion of one of the OH groups to thiol was achieved in 24 h. No traces of PEG-dithiol were found. Therefore, it can be concluded that the product was exclusively PEG_{1000}-monothiol with no traces of dithiol, with 100% yield.

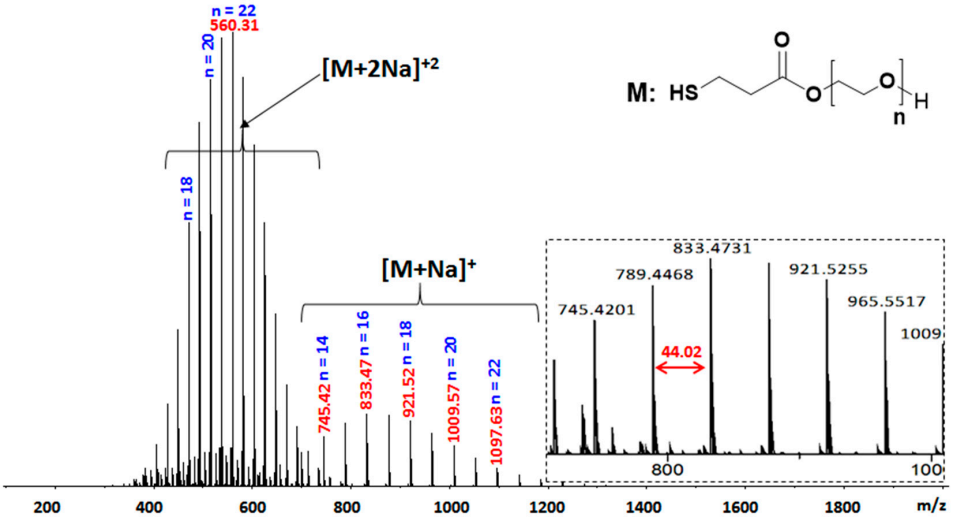

Figure 7. MALDI-ToF mass spectra of PEG_{1000} monothiol. Inset: The zoomed version of the 14- to 20-mer fractions, 44 m/z = PEG repeat unit.

2.2.3. Synthesis of PEG_{1000}-dithiol

PEG_{1000}-dithiol was obtained by reacting PEG_{1000}-monothiol with fresh MP-SH and CALB for 24 h under solvent-less conditions. Figure 8 shows the ^{13}CNMR spectrum of the PEG_{1000}-dithiol. The disappearance of the signals (F and E, Figure 6) at δ = 72.66 ppm and δ = 61.72 ppm, corresponding to the methylene protons next to the hydroxyl end-groups from the PEG_{1000}-monothiol indicates full conversion to PEG_{1000}-dithiol in 24 h with 85% reaction yield.

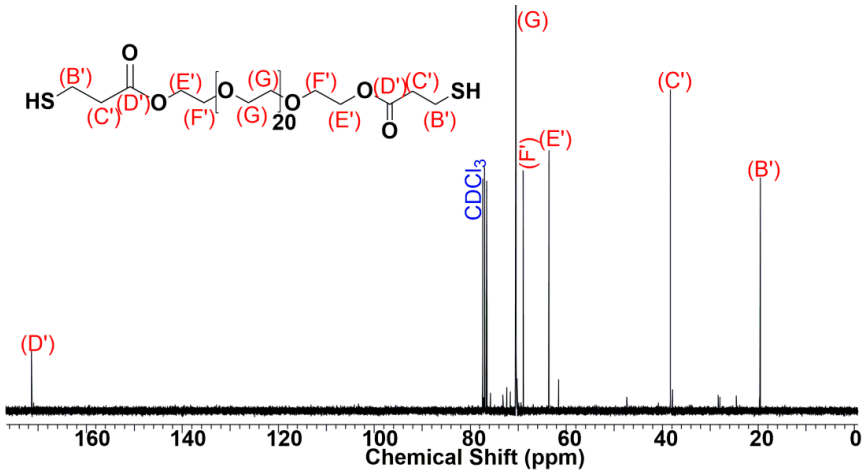

Figure 8. ^{13}C-NMR of PEG_{1000} dithiol [^{13}C-NMR (500 MHz, $CDCl_3$): δ 171.3, 70.4, 68.9, 63.6, 38.3, 19.6].

PEG_{2050} mono- and di-thiols were also synthesized as described in the Experimental section. The ^{1}H-NMR spectra shown in Figure S3 verified the structure of the PEG_{2050} mono- and di-thiols that were obtained with 100% and 94% reaction yield.

3. Materials and Methods

3.1. Materials

Candida antarctica Lipase B (CALB, 33273 Da, 20 wt% immobilized on a macroporous acrylic resin Novozyme® 435) was obtained from Sigma Chemicals (St. Louis, MO, USA). Poly(ethylene glycol)s (PEG_{1000}, $\overline{M_n}$ = 1000 g/mol, Đ = 1.14; and PEG_{2050}, $\overline{M_n}$ = 2050 g/mol, Đ = 1.09), and

methyl-3-mercaptopropionate (MP-SH, 98%) were obtained from Aldrich Chemicals (St. Louis, MO, USA). Tetraethylene glycol (TEG) and tetrahydrofuran (THF, ≥99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Diethyl ether (95.8%) was obtained from Fisher Chemicals (Hampton, NH, USA). Deuterated chloroform ($CDCl_3$, D 99.8%) was obtained from Cambridge Isotope Laboratories Inc.

3.2. Methods

3.2.1. CALB-catalyzed transesterification of MP-SH with TEG

1. Kinetic study

TEG (1.9782 g, 10.2 mmol) was dried under vacuum (Schlenk line) at 65 °C and 0.2 Torr until bubble formation ceased. It was then mixed with MP-SH (3.6204 g, 30.1 mmol) at 50 °C and 420 Torr in the presence of CALB (0.2549 g resin @ 20 wt% enzyme, 0.0015 mmol). After 1 min, the vacuum was removed, N_2 gas was passed through the system and an aliquot was collected. The vacuum was reinstated, and the procedure was repeated to collect aliquots at 3, 5, 10, 15, 30, 60, 120, 240, 300, 390, and 450 min. ^{1}H-NMR spectroscopy was used to check the extent of the reaction.

2. Synthesis of TEG-monothiol

TEG (3.8805 g, 20 mmol) was dried under vacuum (Schlenk line) at 65 °C and 0.2 Torr until bubble formation ceased. It was then mixed with MP-SH (7.4007 g, 61.6 mmol) at 50 °C and 420 Torr in the presence of CALB (0.4912 g resin @ 20 wt% enzyme, 0.0029 mmol). After 15 min, the reaction mixture was diluted with 3 mL of dried THF, filtered over a Q5 filter paper and then dried under vacuum (Schlenk line) at 50 °C for two hours. The product was then dried in a vacuum oven for further analysis (4.1685 g, 93% reaction yield).

3. Synthesis of TEG-dithiol

TEG (1.9782 g, 10.2 mmol) was dried under vacuum (Schlenk line) at 65 °C and 0.2 Torr until bubble formation ceased. It was then mixed with MP-SH (3.6204 g, 30.1 mmol) at 50 °C and 420 Torr in presence of CALB (0.2549 g resin @ 20 wt% enzyme, 0.0015 mmol). After 450 min, the reaction mixture was diluted with 3 mL of dried THF, filtered over a Q5 filter paper and then dried under vacuum (Schlenk line) at 50 °C for two hours. The product was then dried in a vacuum oven for further analysis (3.5327 g, 88% reaction yield).

3.2.2. CALB-catalyzed transesterification of MP-SH with PEG

1. Kinetic study

- PEG_{1000}

PEG_{1000} (3.9893 g, 4.04 mmol) was dried under vacuum (Schlenk line) at 65 °C and 0.2 Torr for 16 h. It was then mixed with MP-SH (1.4563 g, 12.11 mmol) and reacted at 50 °C and 420 Torr in the presence of CALB (0.0977 g resin @ 20 wt% enzyme, 0.00058 mmol). After 1 min, the vacuum was removed and N_2 gas was passed through the system and an aliquot was collected. The vacuum was reinstated, and the procedure was repeated to collect aliquots at 3, 5, 10, 15, 30, 60, 120, 240, 360, 480, 600, 720, 960, and 1440 min. ^{1}H-NMR spectroscopy was used to check the extent of the reaction.

- PEG_{2050}

PEG_{2050} (4.6117 g, 2.25 mmol) was dried under vacuum (Schlenk line) at 65 °C and 0.2 Torr for 16 h. It was then mixed with MP-SH (1.6550 g, 13.7 mmol) and reacted at 50 °C and 420 Torr in the presence of CALB (0.1134 g resin @ 20 wt% enzyme, 0.00068 mmol). After 1 min, the vacuum was removed and N_2 gas was passed through the system and an aliquot was collected. The vacuum was reinstated, and the procedure was repeated to collect aliquots at 3, 5, 10, 15, 30, 60, 120, 240, 360, 480, 600, 720, 960, and 1440 min. ^{1}H-NMR spectroscopy was used to check the extent of the reaction.

2. Synthesis of PEG-monothiols

- PEG_{1000}-monothiol

PEG_{1000} (6.0882 g, 6.16 mmol) was dried under vacuum (Schlenk line) at 65 °C and 0.2 Torr for 16 h. It was then mixed with MP-SH (2.1793 g, 18.13 mmol) and reacted at 50 °C and 420 Torr in the presence of CALB (0.1501 g resin @ 20 wt% enzyme, 0.00090 mmol). After 24 h, the reaction mixture was diluted with 3 mL of dried THF, filtered over a Q5 filter paper and then dried under vacuum (Schlenk line) at 50 °C for 16 h. The product was then dried in a vacuum oven for further analysis (6.0967 g, ~100% reaction yield).

- PEG_{2050}-monothiol

PEG_{2050} (4.6117 g, 2.25 mmol) was dried under vacuum (Schlenk line) at 65 °C and 0.2 Torr for 16 h. It was then mixed with MP-SH (1.6550 g, 13.7 mmol) and reacted at 50 °C and 420 Torr in the presence of CALB (0.1134 g resin @ 20 wt% enzyme, 0.00068 mmol). After 24 h, the reaction mixture was diluted with 3 mL of dried THF, filtered over a Q5 filter paper and precipitated in 100 mL of diethyl ether. The precipitate was then dried in a vacuum oven for further analysis (4.2034 g, ~100% reaction yield).

3. Synthesis of PEG-dithiols

- PEG_{1000}-dithiol

PEG_{1000}-monothiol (2.1005 g, 1.95 mmol) was dried under vacuum (Schlenk line) at 65 °C and 0.2 Torr for 16 h. It was then mixed with MP-SH (3.1031 g, 25.8 mmol) at 50 °C and 420 Torr in presence of CALB (0.0940 g resin @ 20 wt% enzyme, 0.00056 mmol) for 24 h. After 24 h of reaction time, the reaction mixture was diluted with 3 mL of dried THF, filtered over a Q5 filter paper and then dried under vacuum (Schlenk line) at 50 °C for 16 h. The product was then dried in a vacuum oven for further analysis. (2.1461 g, 85% reaction yield).

- PEG_{2050}-dithiol

PEG_{2050}-monothiol (4.000 g, 1.87 mmol) was dried under vacuum (Schlenk line) at 65 °C and 0.2 Torr for 16 h. It was then mixed with MP-SH (2.7125 g, 22.5 mmol) at 50 °C and 420 Torr in the presence of CALB (0.2274 g resin @ 20 wt% enzyme, 0.0013 mmol) for 24 h. After 24 h, the reaction mixture was diluted with 3 mL of dried THF, filtered over a Q5 filter paper and precipitated in 100 mL of diethyl ether. The precipitate was then dried in a vacuum oven for further analysis (3.7791 g, 94% reaction yield).

3.3. Characterization

3.3.1. Nuclear Magnetic Resonance (NMR) Spectroscopy

Varian Mercury 300 MHz and 500 MHz spectrometer (Palo Alto, CA, USA) was used to record the ^{1}H-NMR and ^{13}C-NMR spectra in $CDCl_3$ at 20 mg/ml and 60 mg/ml respectively with the following parameters: 10 second relaxation time, 128 scans (5000 scans for ^{13}C-NMR) and 90° angle. The internal reference for chloroform was δ = 7.26 ppm (^{1}H-NMR) and δ = 77 ppm (^{13}C-NMR).

3.3.2. Mass Spectrometry

Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) experiments were performed on a Bruker UltraFlex III MALDI tandem time-of-flight (ToF/ToF) mass spectrometer (Bruker Daltonics, Billerica, MA, USA) equipped with a Nd:YAG laser emitting at 355 nm. *Trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (98%; Sigma-Aldrich, St.

Louis, MO, USA) and sodium trifluoroacetic acid (98%; Sigma-Aldrich, St. Louis, MO, USA) (NaTFA) served as a matrix and cationizing salt, respectively. Solutions of the matrix (20 mg/mL), cationizing salt (10 mg/mL), and the sample (10 mg/mL) were prepared in THF (Fisher, Fair Lawn, NJ, USA). The matrix/sample/cationizing agent solutions were mixed in the ratio 10:2:1 (vol/vol/vol), and 0.5–1.0 μL of the final mixture were applied to the MALDI sample target and allowed to dry at ambient conditions before spectral acquisition. This sample preparation protocol led to the formation of $[M + Na]^+$ ions. Spectral acquisition was carried out at reflectron mode and ion source 1 (IS 1), ion source 2 (IS 2), source lens, reflectron 1, and reflectron 2 potentials were set at 25.03 kV, 21.72 kV, 9.65 kV, 26.32, and 13.73 kV, respectively.

4. Conclusions

In conclusion, we successfully prepared thiol-functionalized TEGs and PEGs via enzyme catalyzed transesterification of methyl 3-mercaptopropionate with TEG, and PEGs having M_n = 1000, and 2050 g/mol. These reactions were performed without using solvents (in bulk) and using *Candida antartica* Lipase B (CALB) as an enzyme catalyst. The progress of the reactions was monitored using ^{1}H-NMR spectroscopy. The transesterification was found to be a step-wise consecutive reaction. TEG-monothiol was exclusively formed in 15 min, followed by a slower second cycle yielding TEG-dithiol in 7.5 h. PEG_{1000}-monothiol was obtained within 8 h; however, dithiol formation was not observed even after 24 h of reaction. PEG_{1000}-dithiol was obtained by reacting PEG_{1000}-monothiol with fresh CALB and MP-SH for 24 h. PEG_{2050}-monothiol was formed in 16 h, and dithiol formation required additional CALB and MP-SH. Based on our data, it can be concluded that enzyme catalyzed transesterification is a convenient and green method to effectively synthesize PEG mono- and di-thiols that are suitable candidates for thiol-ene click reactions and Michael addition type of reactions [4].

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4344/9/3/228/s1, Figure S1: (A) ^{1}H-NMR and (B) ^{13}C-NMR spectra of TEG-monothiol, Figure S2: (A) ^{1}H-NMR and (B) ^{13}C-NMR of TEG-dithiol, Figure S3: ^{1}H-NMR spectrum of (A) PEG_{2050} monothiol and (B) PEG_{2050} dithiol.

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Conflicts of Interest: Puskas is the Chief Scientific Officer of Enzyme Catalyzed Polymers LLC, a start-up company that has exclusive license from the University of Akron for US Patents 8,710,156 (2014) and 9,885,070 (2018). The other authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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