Addition of Thiol-Containing Ligands to a Surface-Active Michael Acceptor

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ABSTRACT: This paper introduces the synthesis, characterization, surface properties, and reactions of maleic acid monoester end group functionalized polymers. Acylation using maleic acid anhydride of hydroxyl end groups present in either poly(ethylene glycol) (PEG) or poly(trimethylene carbonate) (PTMC) afforded the corresponding maleic acid monoester salts (PEGDM) or (PTMCM). Michael additions of ethanethiol or cysteine to the conjugated double bonds were performed in solution or at a polymer film−water interface. To further evaluate the reactivity and regioselectivity of the thiol addition to maleic acid monoester, a model compound, benzyl 6-hydroxyhexanoate, was used to aid a spectroscopic characterization. Film properties, evaluated with contact angle measurements, of PTMCM revealed surface-active properties of ionic maleic acid monoester salt. Further contact angle measurements showed that maleic acid monoesters present at the polymer−water interface could be reacted with the mercapto group in cysteine to give an amino acid decorated surface.

1. Introduction

In this paper we present a flexible protocol for the surface functionalization of hydrophobic polymeric materials. The protocol involves the Michael addition of thiol-containing compounds to a surface-active maleic acid monoester or maleinate monoester.

Biomaterial surface properties are of major importance in the initial contact with the biological environment. For example, the favorable addition of specific proteins compared to the nonspecific interaction of the same could ultimately decide the fate of a biomaterial in vivo. Novel biomaterials that achieve biological activity through their surface chemistry are therefore important, and much effort is given to the development of such.[1−4] Success in this may affect applications such as medical implants, tissue regeneration, and cell targeting of colloidal or particulate carrier systems in drug delivery.

One strategy to provide specific surface chemistry is the covalent attachment of ligands such as peptides, proteins, or oligosaccharides in controlled and selective reactions. Common conjugation strategies make use of carbodiimide-mediated amide bond formation, reductive amination, isothiocyante addition to amines, thiol addition to enones, disulfide formation, or the recognition between biotin and streptavidin.[2] In this respect functional surfaces based on self-assembled monolayers (SAM's) on gold or polymers have successfully been utilized to study surface phenomena such as cell adhesion.[5] Sensor techniques based on the ability of surface functionalization are exemplified by surface plasmon resonance spectroscopy (SPR)[6] and quartz crystal microbalance (QCM).[7] Other techniques to modify biomaterial surfaces involve photoinduced surface grafting[8] or peptide fluoroalkyl additives.[9] Because of its selectivity and mild reaction conditions, the Michael addition of thiols to enones has been a convenient tool for ligand immobilization at the material surface. Enones such as maleimides[10] or acrylates[11] are conventionally used. Since the reaction can be carried out in water at physiological temperature and near physiological pH, the 1−4 Michael-type addition serves as an ideal method for protein addition. In such cases where thiols are not present in the molecule of interest synthetic protocols for their introduction have been presented.[12]

When designing a flexible protocol for surface derivatization, several aspects have to be considered. The most flexible route is the introduction of ligands at a late state in the preparation. This adds the opportunity for multiple uses of fabricated materials. The heterogeneous covalent addition to a bulk polymer offers this advantage but is often inefficient due to the lack of reactive groups present at the material surface. Homogeneous derivatization by grafting to a functional polymer in solution lacks the flexibility and convenience of a heterogeneous approach[11,13] since further material processing is required which might denaturate sensitive ligands. Further, when working with hydrophobic materials, the incompatibility of polar ligands excludes the use of homogeneous conditions for their condensation.

Because of its surface-active and thiol addition selective properties, we have considered the use of maleic acid monoester as a selective Michael acceptor for the covalent attachment of ligands. Maleic acid monoester is synthesized by anhydride acylation of an appropriate alcohol. When attached to a hydrophobic polymer present in an aqueous environment, the surface enrichment of the hydrophilic monoester takes place, thus presenting the Michael acceptor at the interface. We have recently shown that phosphoryl choline end groups functional biodegradable polymers enrich the polar group at the material−water interface, giving biomimetic hemocompatible surfaces.[14−16] Thus, we believe that the maleic acid monoester has the potential to become a flexible tool for late stage tailoring of biomaterial surface properties. The group of Hubbell have previously proposed a strategy based on thiol addition to acrylate functionalized poly(ethylene glycol).[11,13] This homogeneous system cannot be transferred to hydrophobic materials since the nonpolar acrylate functionality would be embedded in the bulk and nonaccessible at the interface.

In this work, reaction times and thiol selectivity of the homogeneous 1−4-Michael addition of cysteine or ethanethiol

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in water to the unsaturated conjugated esters of a difunctionalized poly(ethylene glycol) maleinate sodium salt (PEGDM) were studied by ^{1}H NMR. Additional studies were made on benzyl 6-hydroxyhexanoate, as a model for biodegradable poly(ϵ-caprolactone) (PCL),[14] and poly(trimethylene carbonate) (PTMC) to provide synthetic means when using homogeneous conditions in organic solvents. The addition products of PEGDM with ethanethiol as well as cysteine, functional benzyl hexanoate, and ethanethiol were isolated and characterized by ^{1}H NMR.

We show that maleic acid monoester functions as a surface-active Michael acceptor (SAMA) as judged by contact angle measurements of water droplets on films of functionalized PTMC. Further, the surface is functionalized through the heterogeneous Michaels addition of cysteine performed at the interface of the material and the aqueous reaction media. To fully take advantage of the properties of maleic acid monoester, the use of a polymer that can induce surface enrichment at ambient is necessary, and therefore poly(trimethylene carbonate) was used. Compared to more harsh methods such as plasma treatment, this protocol offers a mild method for the introduction of biological molecules to the surface.

2. Experimental Section

2.1. Materials. Poly(ethylene glycol) (MW 1800−2000 g/mol) was purchased from Fluka and used as received. Cysteine was purchased from Bachem and used as received. Dowex 50W-X8 (standard grade, particle size 0.150−0.30 mm, 50−100 mesh) poly(styrene) beads were purchased from Kebo Lab and used as received. Trimethylene carbonate was purchased from Böehringer Ingelheim, opened in a glovebox (N_2), and used as received. Phosphate buffered saline (PBS) was purchased form Calbiochem. Al_2O_3 gel powder (basic, mesh 150), stannous(II) ethylhexanoate Sn(Oct)$_2$, and ethanethiol were purchased from Aldrich and used as received. Maleic anhydride was purchased from Aldrich and recrystallized from chloroform before use. Dichloromethane was purified trough distillation over CaH_2. Chloroform (VWR) was filtered through an Al_2O_3 column and extracted with three portions of deionized water (100 mL). The organic layer was dried over sodium sulfate (Na_2SO_4) and distilled from calcium hydride (CaH_2). Triethylamine (Aldrich) was distilled from CaH_2. ϵ-Caprolactone (Aldrich) was distilled from CaH_2 and stored in a glovebox prior to use. Benzyl alcohol (Prolabo) was distilled from CaH_2 and stored in a glovebox.

2.2. Instrumentation. ^{1}H and ^{13}C NMR spectra were recorded using a JEOL-ECP 400 MHz spectrometer with the residual solvent signal as internal standard.

Static contact angle of distilled water (Milli-Q quality) on the polymer surface was used for evaluation of the surface hydrophilicity. Measurements were performed using a FTÅ200 goniometer. A 10 μL water droplet was dispensed from a syringe onto the polymer surface using a flow of 0.5 μL/s. A camera was used for image capture. The images were analyzed with FTA computer software to calculate the contact angle. Five measurements were performed on each film. The polymer films were immersed into deionized water, and after 30 min the films were removed and dried and the contact angle was measured.

2.3. Synthesis. *Synthesis of PEG Dimaleinate Monoester Sodium Salt (PEGDM).* PEG diol (10 g, ~5 mmol) with MW 1800−2000 g/mol was dissolved in 150 mL of toluene and dried azeotropically at 144 °C (oil) in a predried Dean-Stark setup for 3 h over N_2. The dried toluene−PEG solution was cooled to 0 °C using an ice bath. Maleic anhydride (1.53 g, 16 mmol) was dissolved in 60 mL of dichloromethane and added to the reaction mixture. Triethylamine (2.8 mL, 20 mmol) was dissolved in 30 mL of dichloromethane and added dropwise to the reaction mixture. The mixture was left under stirring for 2 h at room temperature. The resulting whiskey-colored mixture was diluted with ~50 mL of toluene and filtered through a bed of basic Al_2O_3. The filtrate was evaporated to dryness by rotational evaporation. The resulting viscous liquid was further dried at 40 °C under vacuum to yield a white solid material. The material was dissolved in dichloromethane and filtered through a bed of basic Al_2O_3 in order to remove excess maleic anhydride. The solvent was evaporated by rotational evaporation. The resulting liquid was coevaporated once from ethanol. The resulting liquid was dissolved in H_2O, and a white precipitate was formed. The precipitate was removed from the solution by centrifugation, and the resulting solution was lyophilized overnight to yield a white powder.

A column charged with about 20 mL (length of about 20 cm) of Dowex-H^{+}(50W-X80) was set up and washed with saturated NaCl(aq) until neutral pH of the eluent was reached, indicating a total exchange of H^{+} for Na^{+}. The polymer was dissolved in water and filtered through the column exchanging Et$_3$NH^{+} for Na^{+}. The solution was lyophilized to obtain the product in a 64% yield. The product was analyzed with ^{1}H NMR (D_2O) δ: 6.58, 5.9 (d, 1H and d 1H, Na^{+-}OC(O)−*CH*═*CH* OC(O)R), 4.37 (s, 1H, HDO), 4.27 (t 2H, CH$_2$−*CH*$_2$−OC(O)R PEG end group), 3.75 (t, 2H, O−*CH*$_2$−CH$_2$−OC(O)R PEG end group), 3.62 (t, O−*CH*$_2$−*CH*$_2$).

Michael Addition of Cysteine to PEGDM. PEGDM (0.0732 g, 0.03 mmol) and cysteine (0.1656 g, 1.4mmol) were added to a 50 mL round-bottom flask and dissolved in 10 mL of PBS(aq) buffer. The solution was left under stirring in 37 °C water bath for about 87 h. The solution was dialyzed for about 74 h in 2 L of deionized water. The water was changed after 14 h. The dialyzed solution was lyophilized over night. The product was analyzed with ^{1}H NMR (D_2O). δ: 4.32 (t, 2H, CH$_2$−*CH*$_2$−OC(O)R PEG end group starting material), 4.25 (t, 2H, CH$_2$−*CH*$_2$−OC(O)R PEG end group addition product), 3.73 (t, 2H O−*CH*$_2$−CH$_2$−OC(O)R PEG end group), 3.62 (t, 4H × *n*, (O−*CH*$_2$−*CH*$_2$)$_n$).

Michael Addition of Ethanethiol to PEGDM. PEGDM (0.071 g, 0.03mmol) was added to a 50 mL round-bottom flask along with ethanethiol (0.1 mL, 1.35 mmol), 10 mL of PBS buffer, and a magnetic stirring bar. The flask was left in 37 °C water bath for about 65 h while stirring The solvent was evaporated, the reaction product was dissolved in deionized water, and finally the solution was lyophilized overnight to yield 80%. The product was analyzed with ^{1}H NMR (D_2O) δ: 4.27 (t, 2H, CH$_2$−OC(O)R PEG end group), 4.23 (t, 2H, CH$_2$−*CH*$_2$−OC(O)R PEG end group addition product), 3.75 (t, 2H, O−*CH*$_2$−CH$_2$−OC(O)R PEG end group), 3.62 (t, 4H × *n*, (O−CH$_2$−CH$_2$)$_n$), 3.55 (t, 1H, Na^{+-}OC(O)−*HC*(SEt)−CH−OC(O)R maleic acid monoester), 2.9 (d, 2H, Na^{+-}OC(O)−HC(S)−*CH*$_2$−OC(O)R maleic acid monoester addition product), 2.7 (d, 2H, Na^{+-}OC(O)−HC(S)−*CH*$_2$−OC(O)R maleic acid monoester), 2.57 (q, 2H, S−*CH*$_2$−CH$_3$), 1.18 (t, 3H, S−CH$_2$−*CH*$_3$).

Synthesis of Benzyl 6-Hydroxyhexanoate. ϵ-Caprolactone (4.00 g, 35 mmol), benzyl alcohol (4.17 g, 36 mmol), and the catalyst Sn(Oct)$_2$ (0.2 g, 0.50 mmol) were added to a round-bottom flask equipped with a stir bar. NMR was used to monitor the reaction. The reaction mixture was heated to 110 °C and stirred for 5 h. The product was purified with flash chromatography using a silica column and a gradient solvent phase starting from hexane and ending with hexane/ethyl acetate. The nonreacted benzyl alcohol was collected at 2−4% ethyl acetate and the product at 6% ethyl acetate. Fractions containing the benzyl 6-hydroxyhexanoate were pooled, and the excess of solvent was evaporated. The product was distilled, and fractions were collected at reduced pressure (0.028 Torr). The pure product was collected at 105−112 °C. Yield: 20%. ^{1}H NMR ($CDCl_3$) δ: 1.32−1.42 (m, 2H, CH$_2$), 1.51−1.60 (m, 2H, CH$_2$), 1.62−1.80 (m, 2H, CH$_2$), 2.35 (t, 2H, O(O)CCH$_2$), 3.63 (t, 2H, CH$_2$OH), 5.1 (s, 2H, PhCH$_2$), 7.30−7.368 (m, 5H, ArH). ^{13}C NMR ($CDCl_3$) δ: 24.7 (CH$_2$), 25.4 (CH$_2$), 32.3 (CH$_2$), 34.3 (CH$_2$), 63.0 (CH$_2$OH), 66.2 (PhCH$_2$), 128.3 (ArCH), 128.6 (ArCH), 136.1 (ArC), 174 (C═O).

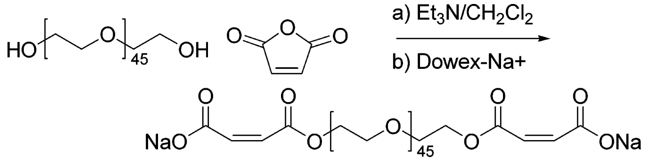
Synthesis of Benzyl-6-maleic Acid Oxyhexanoate Triethylammonium Salt. Benzyl 6-hydroxyhexanoate (0.24 g, 1.08 mmol) and maleic acid anhydride (0.13 g, 1.33 mmol) were dissolved in 5 mL of chloroform and added to a two-necked round-bottom flask equipped with a stir bar and fitted with a dropping funnel. The reaction mixture was cooled to 0 °C using an ice bath. Triethylamine (225 μL, 1.62 mmol) dissolved in 3 mL of chloroform was added dropwise for 30 min. The solution was allowed to reach ambient temperature under continuous stirring for 5 h. NMR was used to monitor the reaction. The product was extracted with three portions of 10 mL of deionized water. The organic phase was dried over Na_2SO_4, and the excess of solvent was evaporated. The product was used without further purifications; yield 81%. ^{1}H NMR ($CDCl_3$) δ: 1.21−1.25 (t, 9H, NCH_2CH_3) 1.32−1.39 (m, 2H, CH_2), 1.58−1.69 (m, 4H, CH_2), 2.32−2.36 (t, 2H, $O(O)CCH_2$), 2.93 (m, 6H, NCH_2CH_3), 4.05 (t, 2H, $CH_2OC(O)$), 5.1 (s, 2H, $PhCH_2$), 5.69−5.72 (d, 1H, RCH═CHR), 6.52−6.56 (d, 1H, RCH═CHR), 7.32−7.35 (m, 5H, ArH−). ^{13}C NMR ($CDCl_3$) δ: 8.6 (NCH_2CH_3) 24.6 (CH_2), 25.6 (CH_2), 28.3 (CH_2), 34.2 (CH_2), 45.2 ($N-CH_2CH_3$), 64.4 ($CH_2OC(O)$), 66.2 ($PhCH_2$), 121.4 (C═C), 128.3 (ArCH), 128.6 (ArCH), 136.1 (ArC), 139.3 (C═C), 166.1 (C═O), 170.8 (C═O), 173.4 (C═O).

Michael Addition of Ethanethiol to Benzyl-6-maleic Acid Oxyhexanoate Triethylammonium Salt. Benzyl-6-maleic acid hexanoate (0.19 g, 0.44 mmol) was dissolved in 2.5 mL of chloroform and added to a two-necked round-bottom flask equipped with a stir bar and fitted with a dropping funnel. Ethanethiol (130 μL, 1.76 mmol) dissolved in 3 mL of chloroform was added dropwise for 30 min. The reaction mixture was kept at ambient temperature for 22 h. The solvent was evaporated to afford the addition product without any further purification; yield 75%. ^{1}H NMR ($CDCl_3$) δ: 1.22−1.27 (m, 9H, NCH_2CH_3), 1.22−1.27 (m, 3H, SCH_2CH_3), 1.29−1.40 (m, 2H, CH_2), 1.58−1.69 (m, 4H, CH_2), 2.33−2.38 (t, 2H, $OC(O)CH_2$), 2.60−2.64 (d, 1H, $OC(O)CH_2CH$), 2.64−2.74 (m, 2H, SCH_2CH_3), 2.94−2.99 (d, 1H, $OC(O)CH_2CH$), 2.99−3.07 (q, 6H, NCH_2CH_3), 3.63−3.66 (m, 1H, CHC(O)O), 3.99−4.10 (m, 2H, $CH_2OC(O)$), 5.1 (s, 2H, $PhCH_2$), 7.28−7.38 (m, 5H, ArH). ^{13}C NMR ($CDCl_3$) δ: 8.5 ($N-CH_2CH_3$), 14.6 ($S-CH_2CH_3$), 24.6 (CH_2), 25.5 ($S-CH_2CH_3$), 25.6 (CH_2), 28.4 (CH_2), 34.2 (CH_2), 37.6 ($S-CH-CH_2$), 43.7 (S−CH), 45.0 ($N-CH_2CH_3$), 64.4 ($CH_2OC(O)$), 66.3 ($PhCH_2$), 128.3 (ArCH), 128.6 (ArCH), 136.1 (ArC), 171.6 (C═O), 173.6 (C═O), 175.8 (C═O).

General Procedure for Ring-Opening Polymerization of Trimethylene Carbonate (PTMC). All glassware was dried prior to use. Trimethylene carbonate (TMC) (5.1 g, 50 mmol), benzyl alcohol (90 mg, 0.83 mmol for a degree of polymerization (DP) of 60), and $Sn(Oct)_2$ (0.05 g, 0.12 mmol) were added into a two-necked round-bottom flask equipped with a stir bar under a nitrogen atmosphere inside a glovebox. The flask was sealed using a septum. The reaction mixture was stirred and heated using an oil bath (110 °C) for 2.5 h. A colorless, viscous liquid was formed. The reaction was monitored using ^{1}H NMR analysis. The product was dissolved in chloroform, and the solution was poured into an excess amount of methanol to precipitate the polymer. The excess of methanol was decanted, and the precipitated polymer was dried under vacuum until a constant weight was reached. A colorless rubbery mass was obtained in a yield of 86%. ^{1}H NMR ($CDCl_3$) δ: 2.01−2.10 (m, 2H, CH_2, poly), 3.71−3.75 (t, 2H, CH_2OH, ω-end), 4.17−4.31 (t, 4H, CH_2, poly), 5.14 (s, 2H, $PhCH_2$, α-end), 7.35−7.37 (m, 5H, ArH, ω-end).

Synthesis of Maleic Acid Monoester Functionalized Poly(trimethylene carbonate) (PTMCM). For the synthesis PTMC (DP of 60) (2.3 g, 0.11 mmol) and maleic acid anhydride (61 mg, 0.62 mmol) were dissolved in 35 mL of chloroform added to a two-necked round-bottom flask equipped with a stir bar and fitted with a dropping funnel. Triethylamine (110 μL, 0.68 mmol) mixed with 3 mL of chloroform was added dropwise at 0 °C. The solution was allowed to reach ambient temperature, and the reaction mixture was kept for 77 h under continuous stirring. The color of the reaction mixture was light yellow and darkened as the reaction progressed. The product was extracted with three portions of 20 mL of 1 M $NaHCO_3$. The organic phase was poured into an excess amount of methanol to precipitate the polymer. The precipitate was collected and dried under vacuum until constant weight. A colorless rubbery mass was obtained in a yield of 75%. ^{1}H NMR ($CDCl_3$) δ: 2.01−2.07 (m, 2H, CH_2, poly), 4.19−4.24 (t, 4H, CH_2, poly), 5.15 (s, 2H, $PhCH_2$, α-end), 6.33−6.37 (d, 1H, RCH═CHR, ω-end), 6.45−6.48 (d, 1H, RCH═CHR, ω-end), 7.37−7.38 (m, 5H, ArH, ω-end).

Scheme 1. Synthesis of Maleic Acid Monoester Functionalized PEG (PEGDM)



3. Results and Discussion

The main novelty of this work is the introduction of a surface-active Michael acceptor (SAMA) that can be used to further functionalize hydrophobic material surfaces. The performance of the SAMA group was analyzed in both homogeneous and heterogeneous conditions. Michael addition selectivity and reaction rates were studied in a series of model reactions at homogeneous conditions. Further, heterogeneous conditions were used to study the interfacial addition of cysteine to a polymer film.

3.1. Model Reactions Using Homogeneous Conditions. The high selectivity and general mildness of the Michael addition of thiols to conjugated enones make this reaction attractive.[17] The concept of combining this reaction with a surface-active end group, as means for tailoring of biomaterials, is however new to surface functionalization.

Previous studies have been made on the Michael addition of thiols to acrylate functionalized PEG using homogeneous aqueous conditions to produce functional PEG or hydrogels.[11,13] The acrylate group is however hydrophobic by nature and lack surface-active properties in water ruling out the use of this group for direct interfacial functionalization reactions.

Because of its surface activity and thiol selective features, α,β-unsaturated monoester salts of maleic acid are candidates for selective Michael addition of thiols to material surfaces at heterogeneous conditions. The heterogeneous system of a maleic acid monoester functionalized PTMC in an aqueous thiol reaction mixture was employed in order to prove the surface enrichment behavior of the hydrophilic end group and to demonstrate that the reaction can be carried out at the interface of the hydrophobic material and an aqueous reaction environment. Functionalized PTMC has been successfully used in recent surface enrichment experiments of zwitterionic phosphoryl choline.[15,16] The biodegradable PTMC was chosen due to its elastic properties at ambient temperature resulting in dynamic bulk properties suggested to favor the surface enrichment of the Michael acceptor.

In order to characterize the selectivity and reaction rate of the Michael-type addition of cysteine to the maleic double bond, a model ^{1}H NMR study of the reaction was conducted in an aqueous homogeneous system using bis-functionalized poly(ethylene glycol), PEG-bis maleinate (PEGDM), as a model substance. Because of the prochirality of the maleic acid monoester and the chirality of cysteine resulting in diastereomeric addition products, the careful product characterization was difficult. To overcome this, ethanethiol was used as a thiol model to simplify the analysis. Because ethanethiol is nonchiral, addition products on the same carbon atom but from different faces would only result in enantiomers. In addition, ethanethiol was also used on the benzyl 6-hydroxyhexanoate model for poly(ϵ-caprolactone) to provide a synthetic model for homogeneous coupling in organic solvents as well.

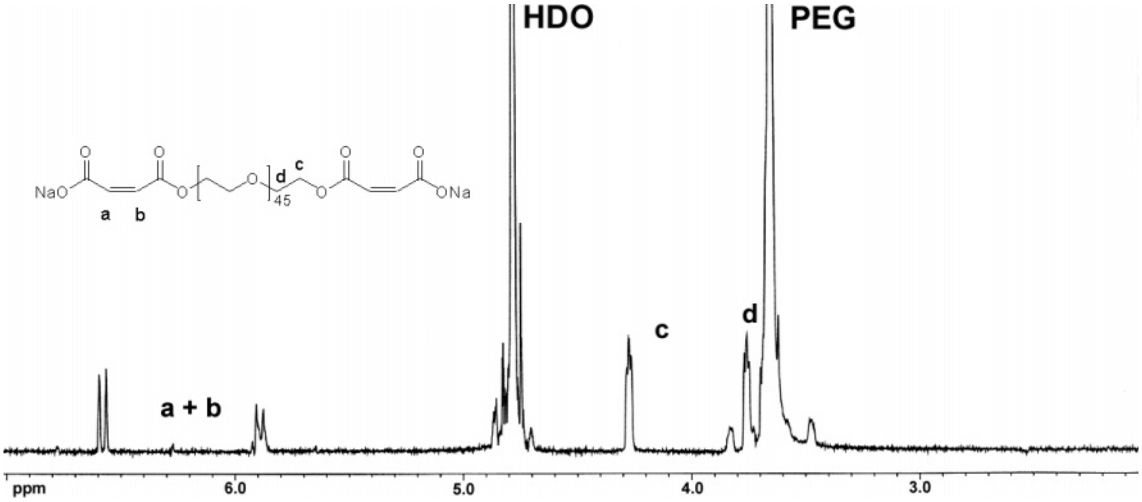


Figure 1. ^{1}H NMR of PEGDM.

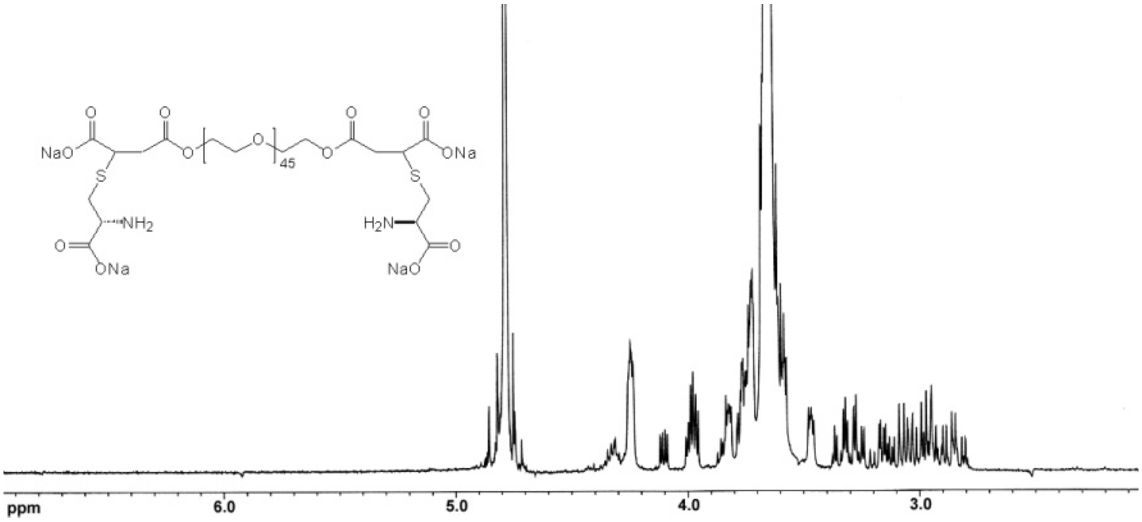


Figure 2. ^{1}H NMR showing the Michael addition product from the cysteine reaction with maleinate monoester.

Maleic anhydride was reacted with poly(ethylene glycol) in chloroform, catalyzed with triethylamine, yielding a pure 100% bis-functionalized PEG maleinate. The triethylammonium counterion was exchanged for sodium using Dowex-Na. The compound was used as a Michael acceptor for a thiol-containing nucleophile; the overall synthetic route is seen in Scheme 1.

The cysteine addition to the monoester in the aqueous system was monitored by ^{1}H NMR, showing complete conversion of the maleic acid monoester double bond in 24 h (Figure 2).

By comparing the relative intensities of the olefinic protons and the protons corresponding to the ethylene glycol carbon adjacent to the ester in the starting material and in the addition products (4.2−4.4 ppm), the relative decrease of the double bond could be linked to the kinetics of the addition reaction.

It is suggested that the active nucleophile in the Michael-type addition of cysteine is the thiolate form of cysteine.[11] As the cysteine addition reaction in PBS buffer was found to be significantly slower than expected, it was assumed that a raise in pH might increase the reaction rate. In order to provide a slightly more basic environment, the pH of the aqueous cysteine solution was adjusted to 8.6 with $NaHCO_3$. The new reaction was monitored identically to the PBS system using ^{1}H NMR. A 90% complete reaction to the maleic acid monoester double bonds was obtained within 3 h.

Because of the prochirality of the starting material, there are four different possible sites of addition. This in combination with the chirality of the cysteine was found to complicate the characterization of the reaction product. The signals at 4.10 ppm, 3.98 ppm, and the region 3.4−2.7 ppm was suggested to correspond to cysteine. The signals were found to be too complex to be fully characterized. However, the distinct patterns of these signals indicate only a few addition products, which in turn suggest a selective reaction.

The complicated spectra of the diastereomeric addition products led to the idea of experiments with ethanethiol as nucleophile in order to obtain a less complex spectra (Figure 3). The triplet 4.27 ppm corresponds to the two hydrogens on the sp^3 carbon adjacent to the monoester in the starting material and the triplet at 4.23 ppm to the same two hydrogens at their new shifts in the reaction product. The doublets at 2.7 and 2.9 ppm correspond to the formed diastereotopic methylene protons of the monoester. The triplet at 3.6 ppm corresponds to the proton of the methine carbon at the site of nucleophilic attack. Further, the ^{13}C NMR shows that only one diastereomer is formed.

The resulting ^{1}H NMR spectra for the isolated thiol addition products of the homogeneous system showed a complete conversion for the cysteine addition to maleic acid monoester (Figure 2). The corresponding ethanethiol experiment yielded 80% addition product (Scheme 2). Because of the heterotopic faces of maleic acid monoester, the ethanethiol addition product formation is suggested to be racemic. In the case of ethanethiol,

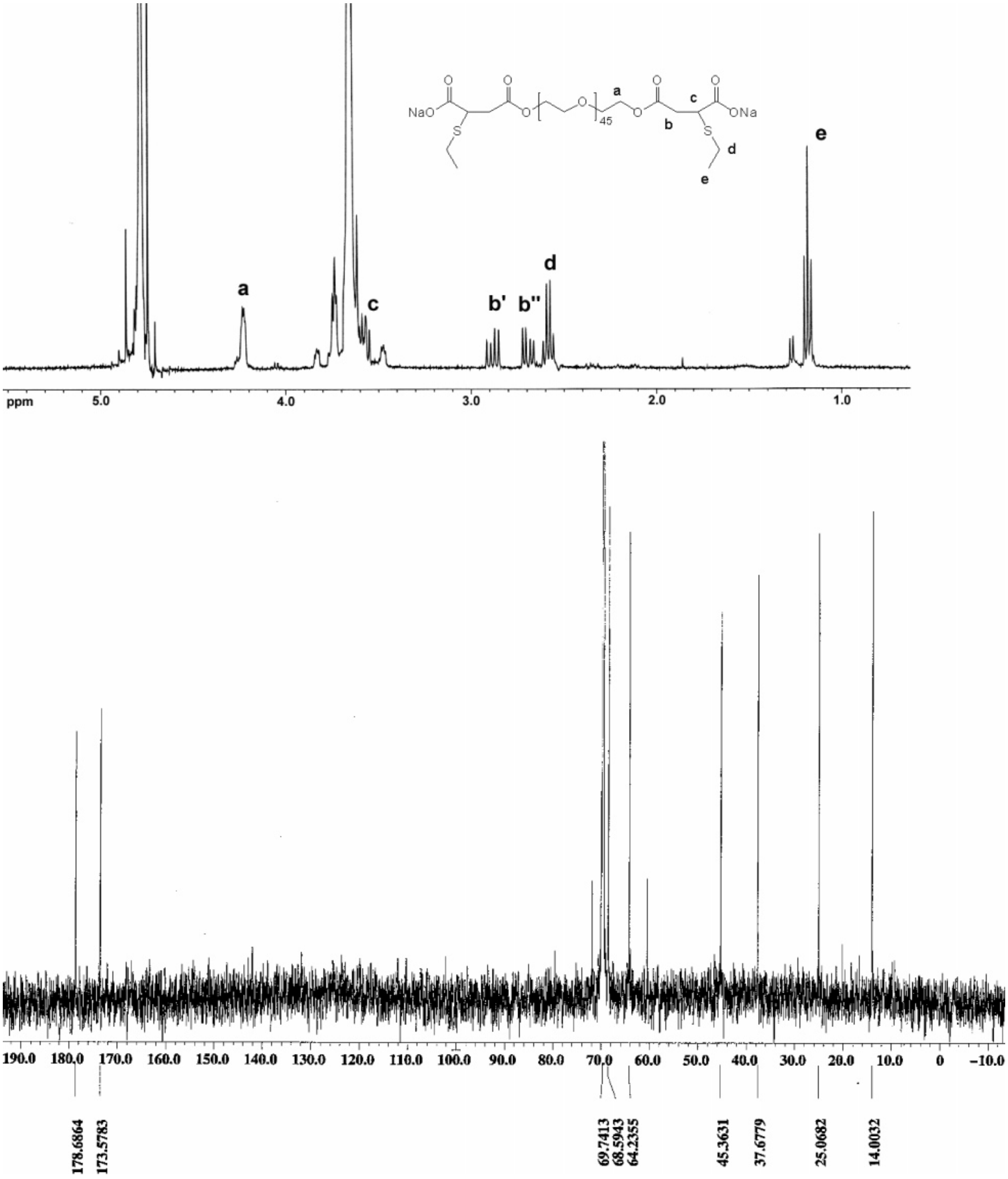


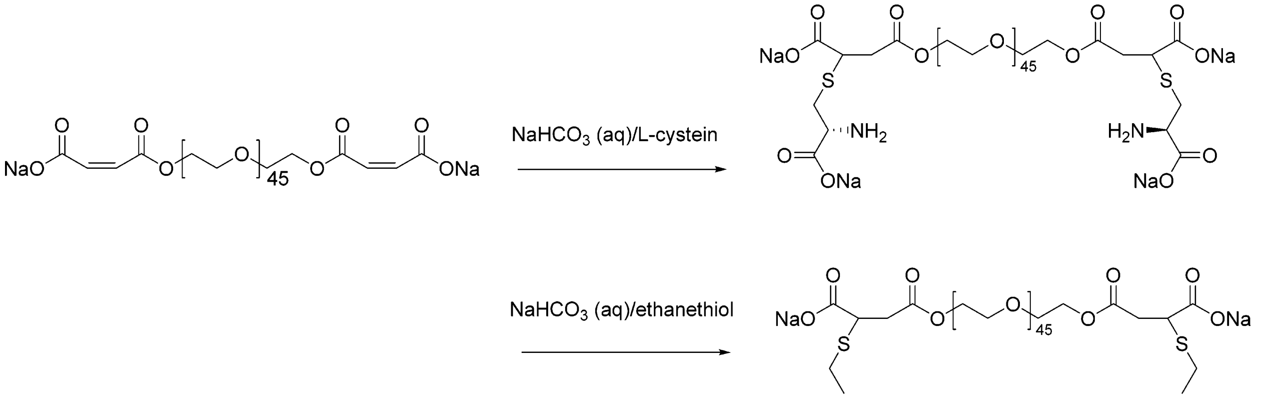
Figure 3. ^{1}H and ^{13}C NMR showing the Michael addition product from the ethanethiol reaction with maleinate monoester.

highly regiospecific product formation was observed with suggested addition to the sterically and electronically more favorable carbon closest to the carboxylate group in the monoester (Figure 2).

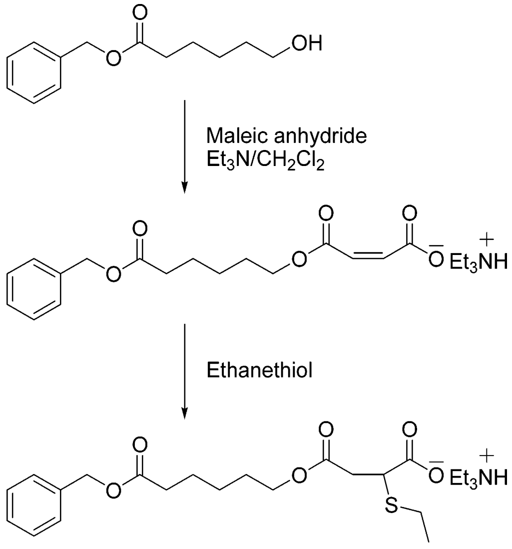
Because of the difficulty of the ^{1}H NMR characterization cysteine addition products, a possible thiol attack at the sterically less favorable carbon of the maleic acid monoester double bond cannot be neglected. One can however argue that since the ethanethiol is a smaller and less sterically hindered molecule than cysteine, a possible formation of a less sterically favorable addition product would be more likely to occur using the former molecule than latter. Since no indication of a diastereomeric product formation was found, using ethanethiol, it is reasonable to believe that a no product formation from thiol addition at the sterically more demanding carbon takes place in irrespective of nucleophile.

It can be concluded from these experiments that the homogeneous Michael-type addition of ethanethiol and cysteine to PEG-dimaleinate is regiospecific and can be carried out in buffered aqueous solutions (pH 8.6) up to 24 h. No hydrolysis of the maleic acid monoester group was observed during the 24 h experiments according to NMR analysis. Similar acrylate systems have shown hydrolytic instability of the coupling product and an ester hydrolysis rate related to the linker.[18] Even though this was not observed for the malienates in our study, we believe that these linkers show degradability over extended

Scheme 2. Michael Addition of Cysteine or Ethanethiol to Maleic Acid Monoester



Scheme 3



time. Such a chemical behavior could be an advantage when designing fully biodegradable systems.

The model study in organic solvents was conducted in a three-step process as shown in Scheme 3. First, benzyl 6-hydroxyhexanoate was synthesized through ring-opening of ϵ-caprolactone in the presence of a catalytic amount of $Sn(Oct)_2$.[14] In a second step benzyl 6-hydroxyhexanoate was reacted with maleic acid anhydride to obtain a model compound for the polymer system with a polar end group. Finally, the intermediate was reacted with ethanethiol in a Michael addition step.

The transformation of the hydroxyl group into a maleic acid monoester could be monitored using ^1H NMR analysis. The signal from the protons adjacent to the hydroxyl group at 3.6 ppm was shifted to 4.1 ppm when the ester functionality was introduced. In addition, doublets from the maleic acid monoester end group could be detected at 5.7 and 6.5 ppm. The Michael addition of ethanethiol to the intermediate was confirmed by vanishing signals from the unsaturated double bond at 5.7 and 6.5 ppm and the appearance of peaks arising from the three protons in the maleic acid monoester end group at 2.6, 2.9, and 3.6 ppm. A signal from the methylene group in ethanethiol was detected at 2.7 ppm; the signal arising from the methyl group in ethanethiol is difficult to detect due to overlap with the peak of the methyl group in triethylamine. It can also be concluded that only one of the two possible diastereomers is formed through the Michael addition (Figure 4).

The assignment of the Michael addition product was confirmed by homonuclear correlation spectroscopy (COSY) that provides information about $^1H-^1H$ couplings. From the signal at 3.6 ppm to both the signal at 2.6 ppm and the signal at 2.9 ppm, cross-peaks were observed, confirming the above assignment.

The summarized results from the two homogeneous model systems in aqueous and organic solvents strongly support that the Michael-type addition of nucleophiles to the unsaturated double bond of polar maleic acid monoester takes place.

3.2. Heterogeneous Michael Addition. The next focus was to verify whether this reaction also could be performed using heterogeneous conditions and at the interface between a cast film and an aqueous medium. Initially, semicrystalline poly(caprolactone) (PCL) was used; however, since the surface enrichment did not occur spontaneously for temperatures below the melt transition, $T_m \sim 55$ °C, amorphous poly(trimethylene carbonate) (PTMC) with a T_g of ~ -20 °C was used instead.[11−13] PTMC is an amorphous biodegradable polymer with elastic properties at ambient temperature. The mechanical stability and elasticity originate from molecular mobility and entanglements since the material is positioned within the rubbery plateau. Cast films of maleic acid monoester functionalized PTMC, synthesized according to the route developed for the model compounds, were produced by a solvent evaporation technique. Three different PTMC of different molecular weight (PTMC DP = 40, 60, and 200) were synthesized and functionalized. A high molecular weight PTMC was used as a reference material. The surface enrichment properties were studied with the help of static contact angle measurements on both functionalized materials and surfaces treated under Michael addition conditions. Static contact angle measurements were chosen as a rapid screening technique for establishing surface polarity trends. No attempts to evaluate hysteresis with dynamic contact angle measurements were made.[19]

3.3. Surface Properties of the SAMA Group. Cast films were treated in aqueous solutions prior to contact angle measurements. The rational behind this is that in the dry state the polar groups will be buried in the material bulk and not contribute to the surface polarity.[15,16] All films containing the SAMA group showed a lower contact angle than an unfunctionalized reference film. When the film is in a hydrophilic environment, the polar groups will enrich at the surface as a result of minimizing the interfacial free energy. To ascertain that rapid redistribution of charged groups back into the bulk did not occur during measurements, a screening time study was conducted. It was observed that if the contact angle of the film was measured within minutes from its removal from the aqueous solution, no substantial redistribution had occurred. If the films were left to dry for several minutes, an increased contact angle was observed as a result of redistribution of polar groups. The

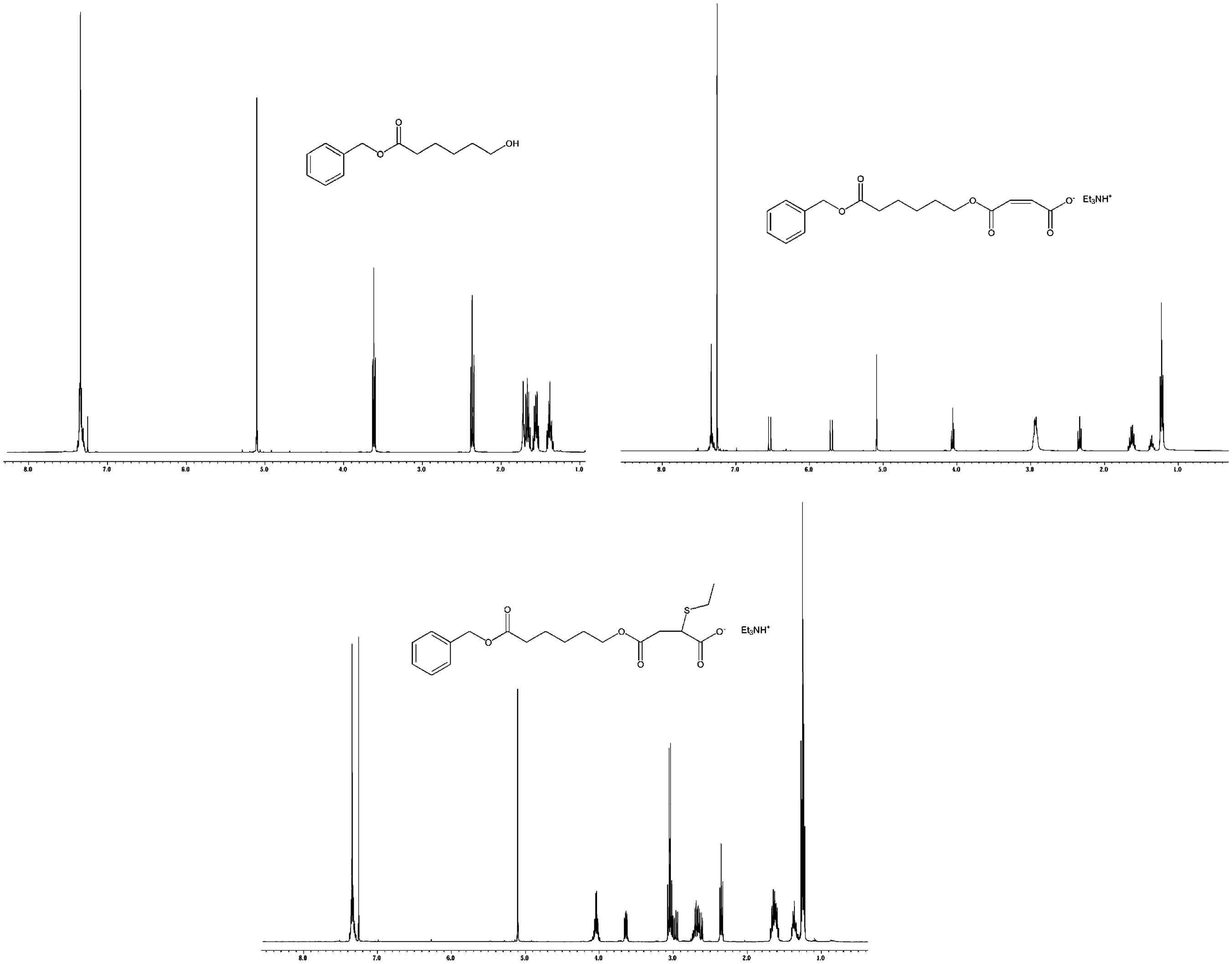
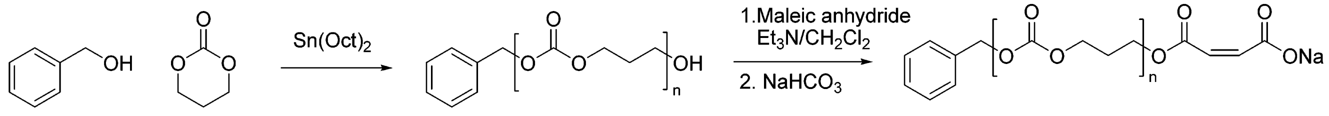


Figure 4. Model study ^{1}H NMR.

Scheme 4. Synthesis of Maleic Acid Monoester Functionalized PTMC



reported contact angles represent an average of 5 drops as measured on different spots on each film. A lower contact angle indicating a more polar surface for the functional polymers was observed (Figure 5). This is the result of enrichment of the carboxylate anion at the interface.

It was further observed that a progressively larger decrease in contact angle was obtained when the molecular weight of polymer was decreased. These differences can be explained by a relatively higher abundance of the SAMA group at the interface for low molecular weight PTMC as a result of increased close packing of end groups in a material of lower molecular weight.

3.4. Michael Addition of Cysteine to SAMA at the Interface. The same reaction conditions used in the homogeneous water reaction were employed for the heterogeneous study. Films immersed in buffer were incubated with cysteine at room temperature for 24 h. It was observed that the contact angle decreased upon this treatment (Figure 5). However, a reference film of pure nonfunctionalized PTMC showed a constant contact angle before and after cysteine treatment. These results give qualitative proof that the Michael addition takes place at the interface. To understand why the contact angle decreases when cysteine is present on the surface, one has to analyze the charge differences between an unreacted and a reacted surface at pH 8.6. The unreacted surface carries a formal negative charge in the form of a carboxylate ion whereas the cysteine-decorated surface has two negative charges due to an extra carboxylate ion. Since both donor and acceptor carry a negative charge, a noncovalent addition of cysteine can be ruled out due to charge−charge repulsion. A molecular weight dependence on the contact angle was observed in analogy with the previous results concerning the surface enrichment of the SAMA group (vide supra). Analysis of functionalized surfaces using XPS, probing sulfur, was unsuccessful since no signal corresponding to cysteine could be observed. This is expected due to the low total concentration of cysteine and the rearrangement and distribution of polar groups into the bulk that takes place in vacuum.[14]

Adding cysteine to the surface has only a minor effect on the surface polarity. A larger effect on the polarity is expected

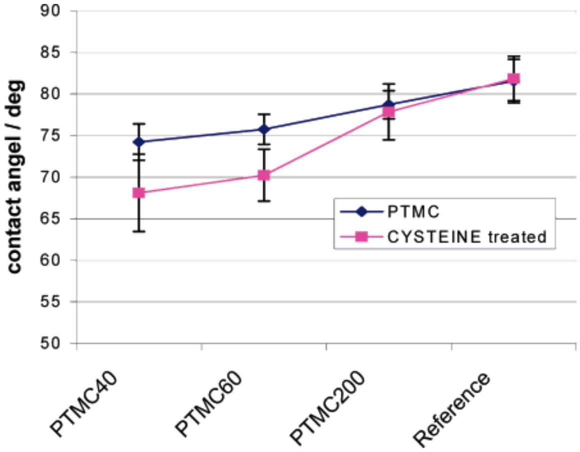


Figure 5. Upper trace displays the contact angle measurements for maleic acid monoester functionalized PTMC films of different molecular weight (PTMC DP = 40, 60, 200) and a nonfunctionalized high molecular weight PTMC reference. Lower trace displays the contact angle measured on films of maleic acid functionalized PTMC incubated with cysteine.

when macromolecules, such as proteins or polysaccharides, are coupled to the surface as a consequence of increased functional group density.

4. Summary

This paper has presented a new flexible protocol for the surface functionalization of hydrophobic macroscopic materials. The strength of this method lies in its simplicity of incubating the materials in water solutions with thiol-containing compounds. Focus has been given to present maleic acid monoester as a SAMA group. It was shown that cysteine could be selectively added to the double bond of the maleinate homogeneously in solution or heterogeneously at the interface of a thin film and an aqueous surrounding. Future developments will deal with the addition of biomolecules such as peptides, proteins, or oligosaccharides.

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References and Notes

(1) Hubbell, J. A. *Curr. Opin. Biotechnol.* **1999**, *10*, 123−129.
(2) Drotleff, S.; Lungwitz, U.; Breunig, M.; Dennis, A.; Blunk, T.; Tessmar, J.; Göpferich, A. *Eur. J. Pharmacol. Biopharm.* **2004**, *58*, 385−407.
(3) Tirrell, M.; Kokkoli, E.; Biesalski, M. *Surf. Sci.* **2002**, *500*, 61−83.
(4) Kasemo, B. *Curr. Opin. Solid State Mater. Sci.* **1998**, *3*, 451−459.
(5) Mrksich, M. *Chem. Soc. Rev.* **2000**, *29*, 267−273.
(6) Cooper, M. A. *Nat. Rev. Drug Discovery* **2002**, *1*, 515−528.
(7) Höök, F.; Rodahl, M.; Brzezinski, P.; Kasemo, B. *Langmuir* **1998**, *14*, 729−734.
(8) Edlund, U.; Källrot, M.; Albertsson, A.-C. *J. Am. Chem. Soc.* **2005**, *127*, 8865−8871.
(9) Ernsting, M. J.; Bonin, G. C.; Yang, M.; Labow, R. S.; Santerre, J. P. *Biomaterials* **2005**, *26*, 6536−6546.
(10) Houseman, B. T.; Gawalt, E. S.; Mrksich, M. *Langmuir* **2003**, *19*, 1522−1531.
(11) Lutolf, N. P.; Tirelli, N.; Cerritelli, S.; Cavalli, L.; Hubbell, J. A. *Bioconjugate Chem.* **2001**, *12*, 1051−1056.
(12) (a) Buskas, T.; Soderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000,** *65*, 958−963. (b) Tam, J. P.; Lu, Y.-A.; Liu, C.-F.; Shao, J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12485−12489. (c) Muir, T. W. *Annu. Rev. Biochem.* **2003**, *72*, 249−289.
(13) Heggli, M.; Tirelli, N.; Zisch, A.; Hubbell, A. J. *Bioconjugate Chem.* **2003**, *14*, 967−963.
(14) Nederberg, F.; Bowden, T.; Hilborn, J. *Macromolecules* **2003**, *37*, 954−965.
(15) Nederberg, F.; Bowden, T.; Hilborn, J. *Polym. Adv. Technol.* **2005**, *16*, 108−112.
(16) Nederberg, F.; Bowden, T.; Hong, J.; Nilsson B.; Hilborn, J. *J. Am. Chem. Soc.* **2004**, *126*, 15350−15351.
(17) Friedman, M.; Cavins, J. F.; Wall, J. S. *J. Am. Chem. Soc.* **1965**, *87*, 3672−3682.
(18) Schoenmakers, R. G.; van de Wetering, P.; Elbert, D.; Hubbell, J. A. *J. Controlled Release* **2004**, *95*, 291−300.
(19) Garbassi, F.; Morra, M.; Occiello, E. In *Polymer Surfaces, from Physics to Technology*; John Wiley: Chichester, 2000; Chapter 4, pp 169−210.

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