

End-Functionalized ROMP Polymers for Biomedical Applications

Ahmad E. Madkour, Amelie H. R. Koch, Karen Lienkamp,* and Gregory N. Tew

Department of Polymer Science & Engineering, University of Massachusetts, Amherst, Massachusetts 01003

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ABSTRACT: We present two novel allyl-based terminating agents that can be used to end-functionalize living polymer chains obtained by ring-opening metathesis polymerization (ROMP) using Grubbs' third generation catalyst. Both terminating agents can be easily synthesized and yield ROMP polymers with stable, storable activated ester groups at the chain end. These end-functionalized ROMP polymers are attractive building blocks for advanced polymeric materials, especially in the biomedical field. Dye labeling and surface coupling of antimicrobially active polymers using these end-groups were demonstrated.

Introduction

Polymers with defined reactive end-groups are of great interest for a variety of applications, such as attaching fluorescent or radioactive labels, building polymers with defined molecular architecture, or functionalizing surfaces and nanoparticles.^{1–5} Reactive end-groups allow precise incorporation of one entity at a defined position on the macromolecule and thus provide superior control and specificity compared to other methods, such as using partially functionalized macromolecules or copolymers of functionalized and nonfunctionalized repeat units. End-group incorporation techniques are well established for polymerization methods such as anionic and controlled radical polymerizations. However, there have been few efficient methods to obtain end-functionalized polymers by ring-opening metathesis polymerization (ROMP) using Grubbs' catalyst. This is due to the nature of the Grubbs' catalyst – it is extremely tolerant of many functional groups including alcohols, aldehydes, esters, amides, ketones, carboxylic acids, and even water. Thus, it can yield polymers with extremely rich functionalities imparting water solubility,^{6–8} antimicrobial^{9–13} and antibiofouling¹⁴ activity, and even metal-containing functional groups.¹⁵ However, with this high functional group tolerance, finding a suitable terminating agent that efficiently removes the Grubbs' catalyst at the chain end is not trivial. While the less functional-group-tolerant metathesis catalysts containing titanium, tungsten, and molybdenum can be quenched by carboxylic acids, alcohols, and aldehydes, the ruthenium-containing Grubbs' catalysts can only undergo metathesis with olefins and molecular oxygen. The latter yields aldehyde end-groups; however, this reaction is rather slow.¹⁶

The end-functionalization methods for ROMP polymers obtained from titanium-, tungsten-, molybdenum-, and ruthenium-containing metathesis catalysts have been summarized recently in an excellent review.¹⁷ Several methods for end-functionalizing polymers obtained using Grubbs' catalysts have been reported, which we will quickly summarize. As ruthenium carbenes only react with olefins in a metathesis reaction, the end-functionalization agent must contain an olefinic group and form a Ru=C species that is inactive as a metathesis catalyst. In the “lactone-terminating approach”, this is an unsaturated lactone or cyclic carbonate, which yields a carboxylic acid group or an aldehyde, respectively, at the chain end.¹⁸ In the “sacrificial synthesis route”, block copolymers of the desired monomer and an acid-labile

monomer are prepared. This yields hydroxy end-groups after hydrolysis of the block with the labile repeat units.¹⁹ Heterotelechelic polymers, i.e., polymers with two different functional groups at each chain end, were obtained by combination of the “sacrificial” and the “lactone” approach.²⁰

The most widely used approach to end-functionalize the living ROMP polymer is to terminate the chain with substituted vinyl ethers. These molecules transfer a substituted methylene group onto the polymer and simultaneously remove the catalyst from the polymer chain end.¹⁷ By this “vinyl ether type terminating” approach, a variety of functional groups were appended to the chain end of ROMP polymers, such as trimethylsilyl groups, carboxylate groups, trimethylsilyl esters, masked acids, ketones, masked amines, and azides.^{21–23} However, in some cases, these functionalization reactions were not quantitative. Allyl ethers can also be used to end-functionalize ROMP polymers, yielding acid or amine end-groups.²⁴ By a similar approach using an allyl ether, ester end-groups have been incorporated, which could be hydrolyzed to OH groups.²⁵ Only very recently, Grubbs reported terminating agents that yielded groups able to initiate atom transfer radical polymerization as well as an *N*-hydroxysuccinimide ester end-group.²⁶

For our research on biomedical polymers, we desired a stable activated ester end-group for dye labeling and surface modification. This group should be reactive in solution but inactive in bulk so that the functionalized polymer would have a reasonably long shelf life and a number of materials could be synthesized from one master batch. For this purpose, we chose a pentafluorophenol ester as the activated ester group, which had been previously employed in combination with other polymerization techniques,²⁷ and designed two allyl-type terminating agents containing the pentafluorophenol group (Figure 1). These terminating agents should be easy to synthesize, yield quantitatively end-functionalized polymers, and allow coupling of the end-functionalized polymers to small molecules, fluorescent dyes, and polymer surfaces. Our novel pentafluorophenol ester-based terminating agents **1** and **2** were shown to fulfill all of these criteria, as will be detailed below, and thus were valuable coupling tools for a variety of new materials.

Results and Discussion

The terminating agent synthesis is illustrated in Figure 1. The first terminating agent molecule (**1**) was obtained from 2-butene-1,4-diol and succinic anhydride, followed by esterification of the

*Corresponding author. E-mail: karen.lienkamp@googlemail.com.

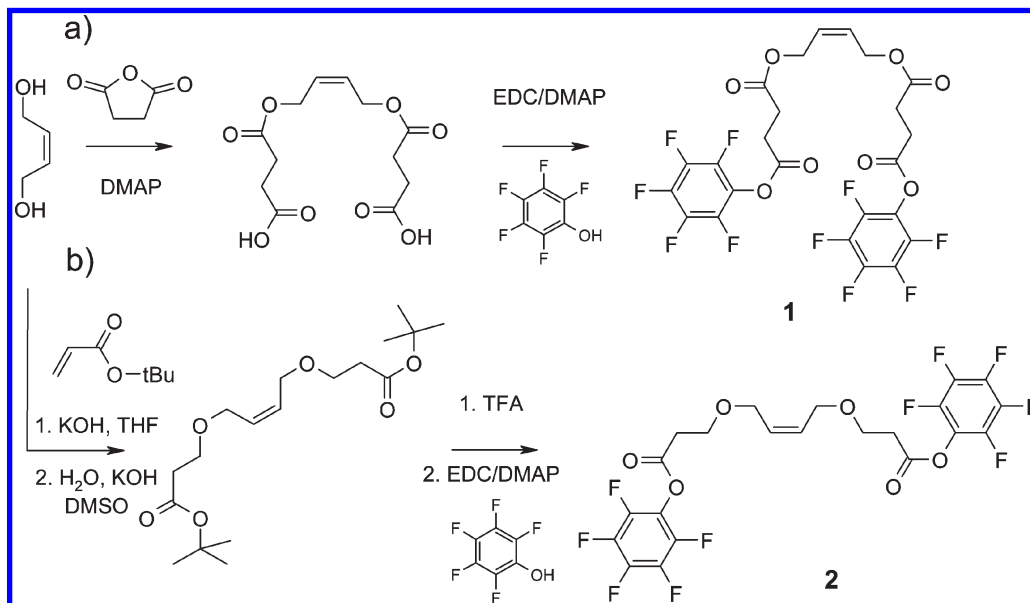


Figure 1. Synthesis of terminating agent molecules **1** and **2**.

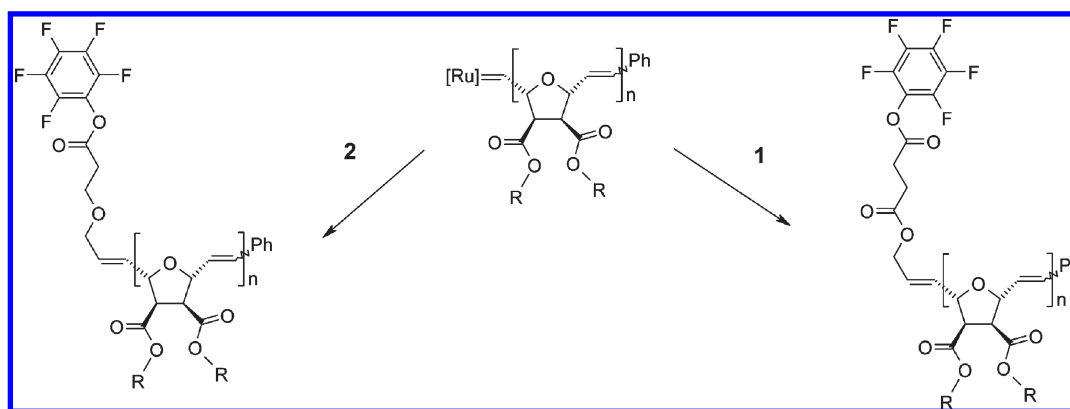


Figure 2. End-functionalization of living ROMP polymers with terminating agents **1** (pentafluoroallyl ester) and **2** (pentafluoroallyl ether).

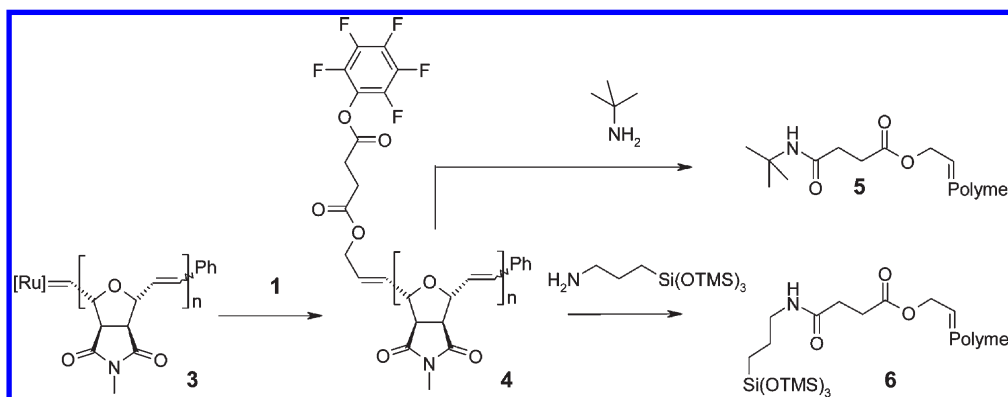


Figure 3. End-functionalization and polymer-analogous reactions of model polymers **3** and **4**.

acid groups with pentafluorophenol, using EDC as a coupling agent (Figure 1a).

The synthesis of the second terminating agent (**2**) is shown in Figure 1b. It is obtained from the Michael addition of 2-butene-1,4-diol to *tert*-butyl acrylate, followed by deprotection using trifluoroacetic acid, and esterification of the resulting acid groups with pentafluorophenol. Both terminating agents are symmetric molecules and can undergo metathesis with a living polymer chain end carrying Grubbs' catalyst (Figure 2), analogous to the

traditional terminating agent, ethyl vinyl ether, which is commonly used to obtain nonfunctionalized ROMP polymers.

In order to investigate the efficiency of the termination reaction using MALDI-TOF mass spectrometry, we used the nonpolar polymer **3** (Figure 3) with a molecular weight of 5000 g mol^{-1} as a model system. Because of its nonpolar nature, this polymer can be more easily investigated by MALDI-TOF MS than our more polar biologically active polymers. The living polymer chain **3** was quenched with 2 equiv of terminating agent **1**, which yielded

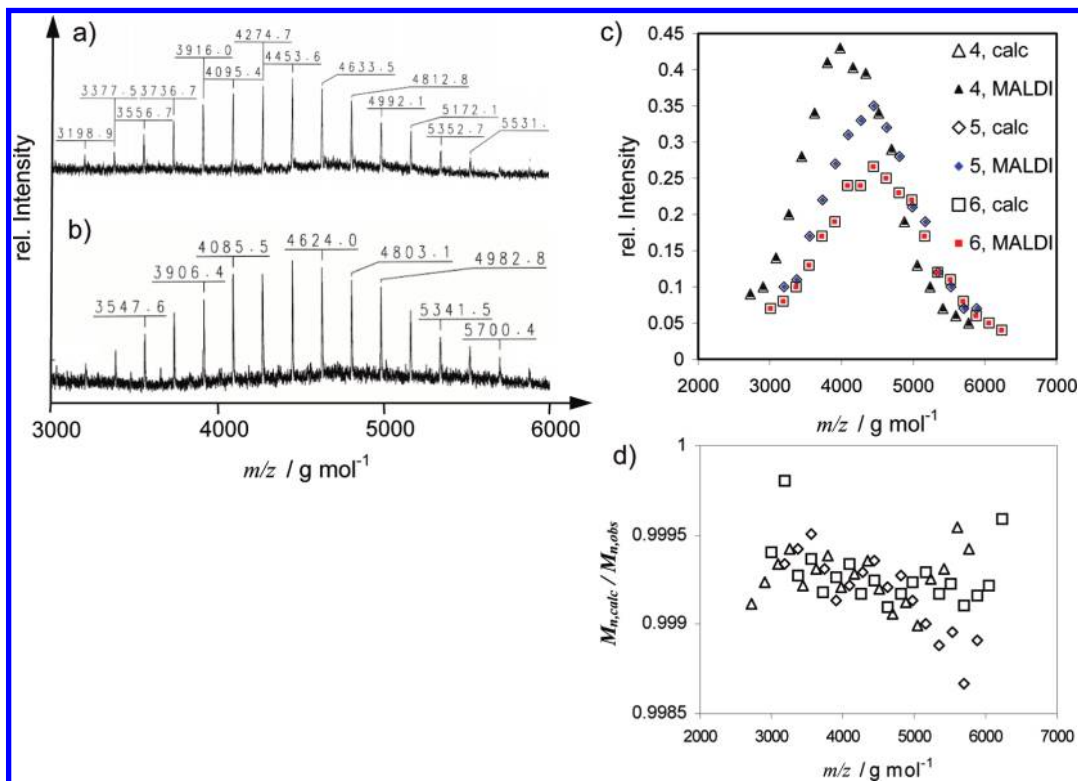


Figure 4. (a) MALDI-TOF mass spectrum of polymer 4, (b) MALDI-TOF mass spectrum of polymer 6, and (c) overlay of the calculated and observed MALDI-TOF MS peaks. Open symbols refer to the calculated and solid symbols to the observed peak positions: triangles, polymer 4; diamonds, polymer 5; squares, polymer 6. (d) $M_{n,calc}/M_{n,obs}$ vs m/z for polymers 4, 5, and 6.

the end-functionalized polymer 4. As can be seen from its MALDI spectrum in Figure 4a, the interpeak distance corresponds to the mass of the repeat unit, and only one molar mass distribution is present, which indicates that the quenching of polymer 3 with 1 was quantitative within the accuracy of the method. Polymer 4 was subsequently reacted with an amine-bearing siloxane and with *tert*-butylamine to demonstrate the reactivity of the new end-group, yielding polymers 6 and 5, respectively (Figure 3). The siloxane was chosen because of its distinct MALDI shift as compared to the parent polymer, while *tert*-butylamine was used to demonstrate that the activated ester was also capable of reacting with bulkier primary amine groups.

Representative MALDI spectra of polymers 4 and 6 are shown in Figure 4a,b, together with an overlay of the calculated and observed MALDI peaks for polymers 4, 5, and 6 in Figure 4c. The calculated and measured peak positions coincide for all polymers. Like polymer 4, the spectra for both polymer 5 (MALDI not shown) and 6 (Figure 4b) also contain only one molar mass distribution, indicating a unique and well-defined end-group. The ratio of the calculated molecular weight over the molecular weight observed by MALDI ($M_{n,calc}/M_{n,obs}$) is shown in Figure 4d. This data demonstrates that these polymer-analogous reactions proceed quantitatively and emphasizes the versatility of terminating agent 1.

While a slight excess of terminating agent 1 yielded completely end-functionalized polymer (after stirring overnight at room temperature), similar conditions did not yield end-functionalized polymer with terminating agent 2. Therefore, several functionalization conditions were studied for this molecule. It was found that 10 equiv (relative to Grubbs' catalyst) was needed to get complete conversion, while 1, 2, and even 5 equiv did not shift the equilibrium to quantitative termination (23%, 47%, and 70% conversion, respectively, were obtained). Longer reaction times (48 and 72 h at room temperature) and higher temperatures (40 and 60 °C) did not improve the conversion. Since terminating

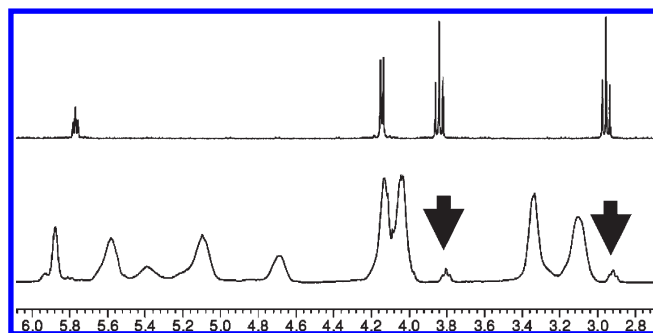


Figure 5. ¹H NMR spectra of terminating agent 2 (top) and SMAMP 7 (bottom).

agent 2 is easy to synthesize and the excess is removable by simple filtration through silica gel, the higher excess needed is not a big disadvantage. The classical terminating agent, ethyl vinyl ether, is typically added in much larger excess. Also, the advantage of terminating agent 2 over terminating agent 1 is that it connects the polymer to the functional group by an ether bond, rather than an ester bond, resulting in polymers with better long-term stability, as the ether bond is not cleavable by simple hydrolysis. However, there might be cases where hydrolyzability is a desired property, in which cases terminating agent 1 may be preferable. Like terminating agent 2, its excess is also removable by filtration through silica gel (see Supporting Information).

The above-described chemistry was developed for biomedical applications including the dye labeling of ROMP polymers as well as their attachment to surfaces. We therefore will now focus on terminating agent 2 when discussing these applications, as they require a nonhydrolyzable linkage. After our successful studies with model polymer 4, we used this approach both for dye incorporation onto and surface functionalization of bioactive polymers, as will be detailed below.

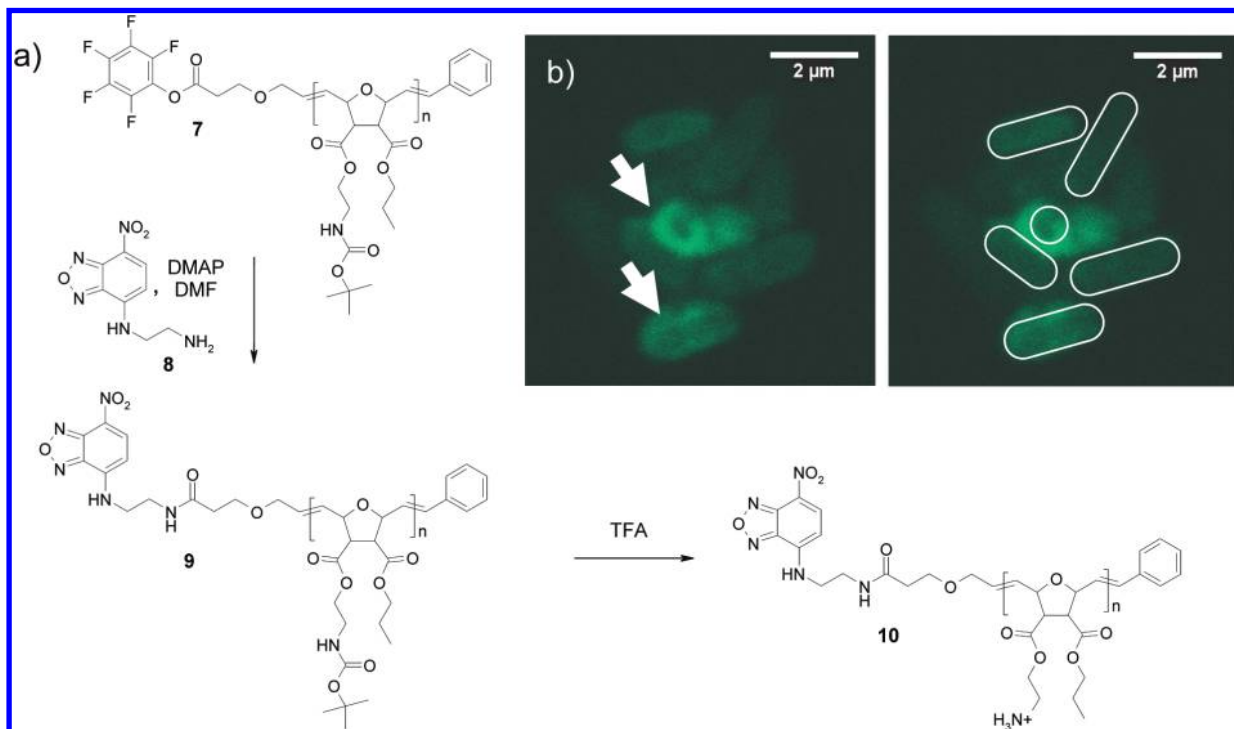


Figure 6. Dye incorporation: (a) reaction scheme; (b) confocal micrograph of *E. coli* bacteria incubated with the SMAMP **10**. The right image illustrates the position of the bacteria cells, which are mostly viewed side-on, except for the central one, which is viewed end-on.

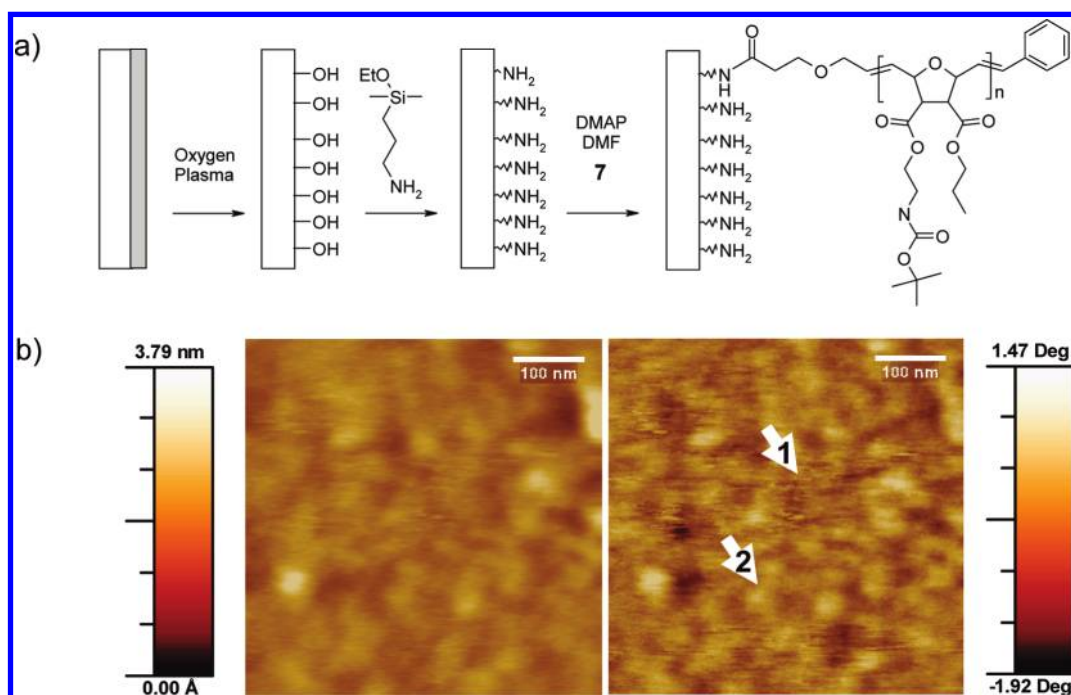


Figure 7. (a) Surface functionalization steps. (b) AFM images of functionalized surface (left: height image; right: phase image). Arrow 1 indicates a zone on the Si wafer that is not covered by polymer, and arrow 2 points to a diffuse surface feature that represents a polymer coil.

Dye Incorporation. Dye incorporation onto polymers is an important tool for tracking them in an organism. We were interested in studying the interaction of previously published synthetic mimics of antimicrobial peptides (SMAMPs) with *E. coli* cell membranes.¹² With our end-functionalization approach, it was possible to position exactly one dye molecule per polymer chain at the chain end. Following the above-described procedure, but this time using a monomer that imparts antimicrobial activity, the terminating agent **2**

was used to obtain the end-functionalized SMAMP precursor **7**. Unfortunately, as **7** did not give meaningful MALDI-TOF MS data, we had to demonstrate the successful incorporation of the end-group into the polymer by ^1H NMR spectroscopy (Figure 5). Figure 5 is an overlay of the ^1H NMR spectrum of the terminating agent **2** (Figure 5, top) and the SMAMP precursor **7** (Figure 5, bottom). The SMAMP precursor **7** had been fractionated previously by preparative GPC to remove any terminating agent. As

shown in Figure 5, the signals of the two methylene groups in the new end-group became broad triplets when attached to the polymer chain (black arrows). The integration of these signals versus peaks from the polymer indicates, within the accuracy of the method, near-quantitative end-group incorporation.

When reacted with the fluorescent nitrobenzoxadiazole derived dye **8** that had been synthesized as described in the literature²⁸ (Figure 6a), the fluorescently labeled polymer **9** was obtained. Polymer **9** was purified by column chromatography to remove excess dye. After removal of the Boc protective group with trifluoroacetic acid, followed by dialysis, the active SMAMP **10** was obtained (Figure 6a). This molecule was then incubated with *E. coli* cells and imaged using confocal microscopy. The results are shown in Figure 6b. As seen, especially for the two bacteria cells highlighted by arrows, the fluorescent SMAMP tends to accumulate in the cell membrane region rather than inside the cytosol. This is in line with current theories on SMAMP–cell interaction, which state that the antimicrobial activity of the SMAMP is due to interaction with the cell membrane, leading either to fatal membrane pore formation or membrane disruption.^{9,29}

Surface Functionalization. The SMAMP precursor **7** was further used to functionalize a silica surface with a thin layer of antimicrobial polymers. This was achieved by an amine-functionalized silicon wafer to which the SMAMP precursor **7**, with a molecular weight of a 10 000 g mol⁻¹, was grafted. Preparation details are given in Figure 7a and the Supporting Information. The resulting surface was characterized by ellipsometry and atomic force microscopy (AFM). According to ellipsometry data, a 5–7 nm thick layer of polymer was grafted to the silicon wafer ($\Delta = 155.2/\Psi = 11.00$). This was confirmed by AFM images (Figure 7b). Both the height and the phase image indicate the presence of a thin, roughly 4 nm thick, diffuse polymer layer on the silica wafer surface. The finding that our polymer with an activated ester end-group can be attached to a surface is consistent with literature, in which an *in situ* created activated ester was used to attach a polymer to a surface.³⁰ The antimicrobial activity of these surfaces is currently under investigation and will be reported in due course.

Conclusions

In this paper, we presented the synthesis of two terminating agents and their possible uses to end-functionalize living ROMP polymers. By subsequent polymer analogous chemistry, it was shown that small molecules, such as a fluorescent dye and amine-substituted surfaces, could be easily coupled to the end-functionalized polymer. We will take advantage of this method in future research, including surface functionalization studies and dye labeling of antimicrobial and cell-penetrating peptides. Additionally, due to the ease of synthesis of the terminating molecules, as well as the stable yet reactive nature of the resulting activated ester end-group, we expect that this approach will find application in areas such as polymer–polymer coupling and bioconjugation reactions and prove to be a versatile nanoparticle functionalization technique.

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Supporting Information Available: Experimental details and more characterization data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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