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Journal
Macromolecules, 49(17)

ISSN
0024-9297

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Publication Date
2016-09-13

DOI
10.1021/acs.macromol.6b01512

Peer reviewed

Macromolecules

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"One-Pot" Aminolysis/Thiol–Maleimide End-Group Functionalization of RAFT Polymers: Identifying and Preventing Michael Addition Side Reactions

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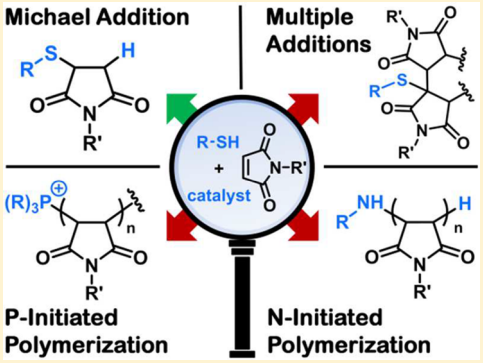
Supporting Information

ABSTRACT: We show that many of the nucleophiles (catalysts, reducing agents, amines, thiols) present during "one-pot" aminolysis/thiol–maleimide end-group functionalization of RAFT polymers can promote side reactions that substantially reduce polymer end-group functionalization efficiencies. The nucleophilic catalyst 1,8-diazabicyclo[5.4.0]undec-7-ene and the reducing agent tributylphosphine were shown to initiate anionic polymerization of *N*-methylmaleimide (NMM) in both polar and nonpolar solvents whereas hexylamine-initiated polymerization of NMM occurred only in high-polarity solvents. Furthermore, triethylamine-catalyzed Michael reactions of the representative thiol ethyl 2-mercaptopropionate (E2MP) and NMM in polar solvents resulted in anionic maleimide polymerization when $[NMM]_0 > [E2MP]_0$. Base-catalyzed enolate formation on the α-carbon of thiol–maleimide adducts was also shown as an alternative initiation pathway for maleimide polymerization in polar solvents. Ultimately, optimal "one-pot" reaction conditions were identified allowing for up to 99% maleimide end-group functionalization of dithiobenzoate-terminated poly(*N*,*N*-dimethylacrylamide). Much of the work described herein can also be used to ensure near-quantitative conversion of small molecule thiol–maleimide reactions while preventing previously unforeseen side reactions.



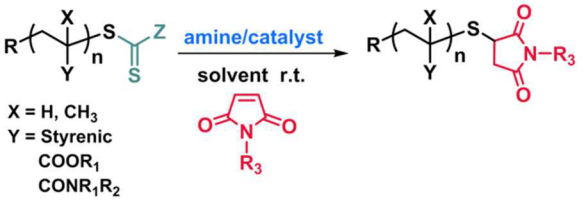
INTRODUCTION

Reversible addition–fragmentation chain transfer (RAFT) polymerization has made possible the synthesis of functionally diverse polymers with predetermined molecular weights and low dispersities ($Đ$) using a wide variety of monomer types and polymerization conditions (e.g., aqueous and organic media).[1−3] The versatility of RAFT in synthesizing tailor-made polymers also stems from the fidelity by which polymer end-functionality can be controlled. Such end-functionalized polymers have been used to prepare advanced macromolecular architectures including block copolymers,[4] star copolymers,[5] molecular brushes,[6,7] and polymer bioconjugates.[8,9] Telechelic RAFT polymers can be synthesized directly by controlling the RAFT agent R- and Z-group functionality[10] or by postpolymerization end-group modification.[11−13] The latter approach often exploits the inherent reactivity of the residual thiocarbonylthio moiety present on RAFT polymers, allowing for facile removal and replacement of the unstable RAFT agent with a benign or functional end-group.

In recent years, reduction or aminolysis of thiocarbonylthio-terminated RAFT polymers to the corresponding polymeric thiol has afforded a myriad of thiol "click" end-group functionalization routes including thiol–isocyanate,[14] thiol–epoxy,[15] thiol–halogen,[16] thiol–disulfide,[17,18] and thiol–ene reactions.[19−24] Particularly advantageous is the thiol–maleimide Michael reaction which proceeds to near-quantitative conversion at room temperature in the presence of oxygen and water and typically occurs much more rapidly than analogous thiol–acrylate or thiol–acrylamide reactions.[25−31] Furthermore, thiol–maleimide end-group modification of RAFT polymers can be performed as "one-pot" reactions without isolation of the intermediate polymeric thiol (Scheme 1).[21]

To ensure quantitative polymer end-group functionalization, a molar excess of maleimide relative to polymeric thiol is required. It is therefore desirable to optimize the reaction conditions to favor rapid and efficient polymer conjugation with minimal excess of functional maleimide, especially when

Scheme 1. "One-Pot" Aminolysis/Thiol–Maleimide End-Group Functionalization of RAFT Polymers

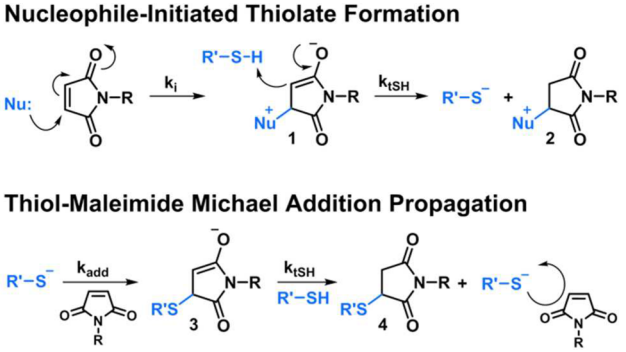


Received: July 13, 2016
Revised: August 11, 2016
Published: August 22, 2016

DOI: 10.1021/acs.macromol.6b01512
Macromolecules 2016, 49, 6193−6202

using costly biologic, therapeutic, or diagnostic agents. Typically, thiol−Michael addition reactions employ a base catalyst (e.g., tertiary amine) to generate the nucleophilic thiolate species.[31] However, nucleophilic catalysts such as amidines, phosphines, and amines have been used to increase the rates Michael addition reactions by several orders of magnitude.[25,32−38] The proposed mechanism of nucleophile "catalyzed" thiol−maleimide Michael addition is illustrated in Scheme 2.[38] Rather than direct deprotonation of thiol,

Scheme 2. Mechanism of Nucleophile-Initiated Thiol−Maleimide Michael Addition



conjugate addition of the nucleophile to the maleimide double bond forms the zwitterionic enolate **1**, which in turn functions as a strong base ($pK_a \approx 25$) capable of generating the nucleophilic thiolate species while also forming a nucleophile−succinimide byproduct **2**. In this regard, the nucleophile does not function as a catalyst which is regenerated during each catalytic cycle, but rather serves as an initiator that generates the steady state enolate/thiolate concentration necessary for the thiol−ene chain transfer mechanism to operate. Subsequent propagation occurs by thiolate addition to maleimide forming the corresponding enolate **3**, which abstracts a proton from thiol, regenerating the thiolate along with the desired thiol−maleimide Michael addition product **4**.

Recently, while investigating the potential of nucleophilic catalysts to improve the efficiency of RAFT polymer end-group functionalization with N-substituted maleimides, we discovered that in certain instances these catalysts *reduce* the extent of end-group functionalization compared to reactions performed using only a base catalyst. Reagent order of addition and solvent polarity were also determined to have marked effects on end-group functionalization efficiency (vide infra). These observations have prompted this study aimed at understanding the influences of nucleophile type, solvent, and reaction conditions on the efficacy of "one-pot" aminolysis/thiol−maleimide end-group functionalization of RAFT polymers. Furthermore, the results discussed herein offer new mechanistic insights into potentially detrimental side reactions that can occur during thiol−maleimide Michael addition reactions.

EXPERIMENTAL SECTION

Materials. 2-Cyano-2-propyl benzodithioate was synthesized according to a literature procedure.[39] Azobis(isobutyronitrile) (AIBN) (Aldrich, 98%) was recrystallized from anhydrous methanol and stored at −10 °C. *N*,*N*-Dimethylacrylamide (Aldrich, 99%) and benzylamine (Aldrich, 99%) were vacuum distilled immediately prior to use. Maleic anhydride (Aldrich, 99%), acetic anhydride (Fisher, 99.2%), sodium acetate (Fisher, anhydrous), *N*-methylmaleimide (Aldrich, 97%), ethyl 2-mercaptopropionate (Aldrich, >95%), benzyl mercaptan (Fluka, >99%) hexylamine (Aldrich, 99%), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Aldrich, >99.0%), triethylamine (Aldrich, >99.5%), tributylphosphine (Aldrich, 97%), trimethyl phosphite (Aldrich, >99%), dimethyl sulfoxide-d_6 (Cambridge Isotopes, 99.9%), acetonitrile-d_3 (Cambridge Isotopes, 99.8%), methylene chloride-d_2 (Cambridge Isotopes, 99.8%), ethanol-D (Cambridge Isotopes, D 99%, <6% D_2O), and deuterium oxide (Aldrich, 99.9%) were used as received.

Characterization. NMR spectra for structural analysis and kinetic studies were obtained using a Varian INOVA 300 MHz NMR spectrometer. Polymer molecular weights and molecular weight distributions (M_w/M_n) were determined by size exclusion chromatography (SEC) using DMF with 20 mM LiBr as the eluent at a flow rate of 1.0 mL/min in combination with two Agilent PolarGel-M columns heated to 50 °C and connected in series with a Wyatt Optilab DSP interferometric refractometer and Wyatt DAWN EOS multiangle laser light scattering (MALLS) detector (λ = 633 nm). Absolute molecular weights and M_w/M_n were calculated using a Wyatt ASTRA SEC/LS software package. Polymer dn/dc in the above eluent at 35 °C was determined offline using a Wyatt Optilab DSP interferometric refractometer and Wyatt ASTRA dn/dc software.

Reactions of N- and P-Based Nucleophiles with *N*-Methylmaleimide. A solution of *N*-methylmaleimide (25.0 mg, 2.23×10^{-4} mol, 10 equiv) and CH_2Cl_2 (10 μL, ^{1}H NMR internal standard) in DMSO-d_6 (1.00 mL) was prepared in an NMR tube in the presence of air. An initial ^{1}H NMR spectrum was taken (t = 0 min) followed by direct addition of the appropriate nucleophile (2.23×10^{-5} mol, 1 equiv) to the NMR tube, and the solution was mixed by inverting three times. Subsequent spectra were taken at timed intervals and the fractional change in maleimide concentration ($[Mal]/[Mal]_0$) measured by comparing the relative integrated peak areas of the maleimide olefin protons (DMSO-d_6, 7.02 ppm, 2H) to the protons of CH_2Cl_2 (DMSO-d_6, 5.76 ppm, 2H).

Reaction of Ethyl 2-Mercaptopropionate with *N*-Methylmaleimide. A solution of *N*-methylmaleimide (25.0 mg, 2.23×10^{-4} mol, 10 equiv), triethylamine (3.13 μL, 2.23×10^{-5} mol 1.0 equiv), and CH_2Cl_2 (10 μL, ^{1}H NMR internal standard) in DMSO-d_6 (1.00 mL) was prepared in an NMR tube in the presence of air. An initial ^{1}H NMR spectrum was taken (t = 0 min) upon which ethyl 2-mercaptopropionate (2.90 μL, 2.23×10^{-5} mol 1.0 equiv) was added to the NMR tube, and the solution was mixed by inverting three times. Subsequent spectra were taken at timed invervals and the fractional change in maleimide concentration measured by comparing the relative integrated peak areas of the maleimide olefin protons (DMSO-d_6, 7.02 ppm, 2H) to the protons of CH_2Cl_2 (DMSO-d_6, 5.76 ppm, 2H).

Synthesis of 3-Benzylsulfanyl-1-methylmaleimide (7). An initially colorless solution of benzyl mercaptan (2.64 g, 21.2 mmol) and *N*-methylmaleimide (2.36 g, 21.2 mmol) in MeCN (50 mL) was first prepared at room temperature followed by the addition of TEA (0.281 mL, 2.12 mmol) via syringe. The resulting red solution was stirred at room temperature for 30 min and quenched with acetic acid (1.0 mL) to give a colorless solution. The solvent was then removed by rotary evaporation, and the crude reaction mixture was redissolved in diethyl ether (100 mL) and washed with 0.1 M HCl (100 mL), H_2O (100 mL), and saturated NaCl (100 mL). The product was further purified by column chromatography (65:35 hexanes:EtOAc, R_f = 0.35), yielding **7** (4.55 g, 91%) as a viscous oil that solidified into a waxy solid after 7 days; mp 47−52 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.31 (m, 4H), 7.24 (m, 1H), 3.93 (dd, J = 62.3, 13.1 Hz, 2H), 3.76 (dd, J = 9.0, 3.9 Hz, 1H), 3.06 (dd, J = 18.5, 9.0 Hz, 1H), 2.78 (s, 3H), 2.45 (dd, J = 18.5, 3.9 Hz, 1H).

Reaction of 7 with *N*-Methylmaleimide. A solution of *N*-methylmaleimide (25.0 mg, 2.23×10^{-4} mol, 10 equiv), **7** (5.29 mg, 2.23×10^{-5} mol, 1.0 equiv), and CH_2Cl_2 (10 μL, ^{1}H NMR internal standard) in DMSO-d_6 (1.00 mL) was prepared in an NMR tube in the presence of air. An initial ^{1}H NMR spectrum was taken upon which TEA (3.13 μL, 2.23×10^{-5} mol, 1 equiv) was added directly to the NMR tube, and the solution was mixed by inverting three times. Subsequent spectra were acquired at timed intervals, and the fractional

DOI: 10.1021/acs.macromol.6b01512
Macromolecules 2016, 49, 6193−6202

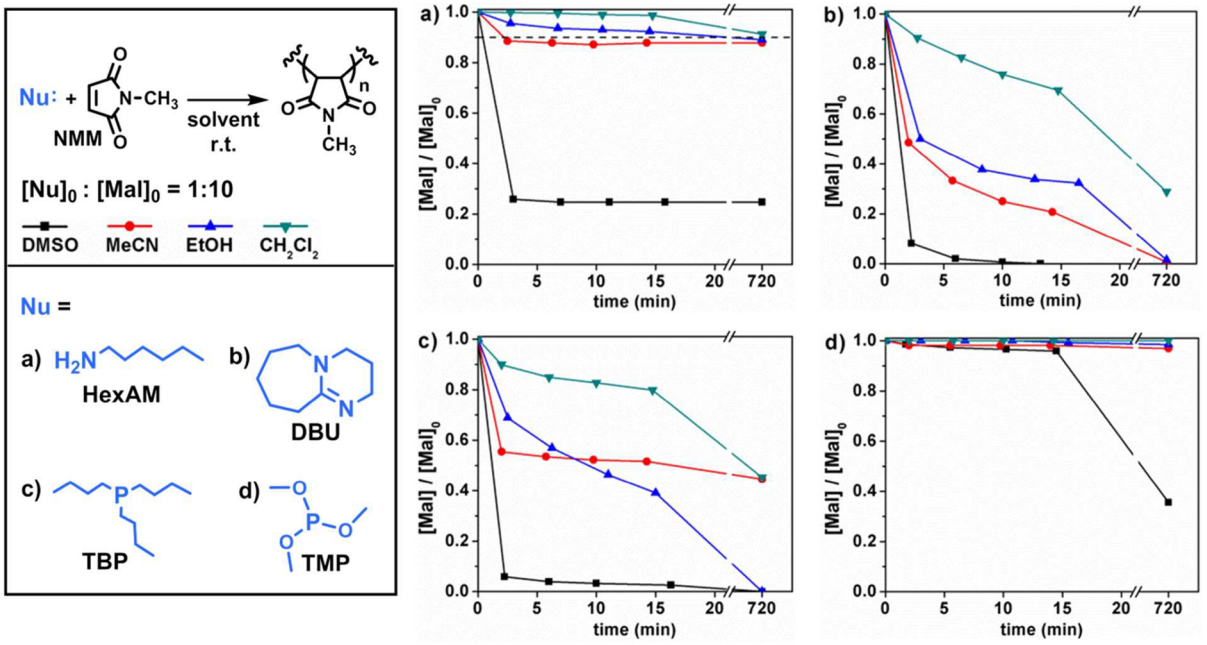


Figure 1. Effect of solvent on the time-dependent fractional change in $[Mal]/[Mal]_0$ upon reaction of NMM with representative nucleophiles (a) hexylamine (HexAM), (b) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), (c) tributylphosphine (TBP), and (d) trimethyl phosphite (TMP) as measured by *in situ* ^{1}H NMR analysis.

change in maleimide concentration was measured by comparing the relative integrated peak areas of the maleimide olefin protons (DMSO-d_6, 7.02 ppm, 2H) to the protons of CH_2Cl_2 (DMSO-d_6, 5.76 ppm, 2H).

Hydrogen−Deuterium Exchange Kinetics of 7. ^{1}H NMR spectra were recorded with a Bruker Ascend 600 MHz spectrometer. Briefly, a solution of **7** (10.0 mg, 4.25×10^{-5} mol, 1 equiv) and D_2O (100 μL, 5.54 mmol, 130 equiv) in DMSO-d_6 (0.600 mL) was prepared in an NMR tube in the presence of air, and an initial ^{1}H NMR spectrum was acquired (t = 0 min). TEA (5.93 μL, 4.25×10^{-5} mol, 1 equiv) was then added to the NMR tube, and the solution was mixed by inverting three times. Subsequent spectra were acquired at timed intervals and the fractional change in peak area (A_t/A_0) of protons H_a (3.80−3.75 ppm, 1H), H_b (3.10−3.00 ppm, 1H), and H_c (2.50−2.40 ppm, 1H) were measured relative to the peak area of the benzylsulfanyl aromatic protons (7.30−7.20 ppm, 5H).

Synthesis of PDMA-CPDB. *N*,*N*-Dimethylacrylamide (28.0 g, 282 mmol), 2-cyano-2-propyl benzodithioate (298.0 mg, 1.34 mmol), AIBN (44.1 mg, 0.27 mmol), and benzene (100 mL) were combined in a 250 mL round-bottomed flask equipped with magnetic stir bar and sealed with a rubber septum before purging with N_2 for 45 min. The reaction vessel was then heated in an oil bath at 60 °C for 5 h, upon which the reaction was quenched via exposure to air and freezing in liquid nitrogen. The solvent was removed by rotary evaporation, and the polymer precipitated four times into pentane, redissolving in a minimal amount of CH_2Cl_2 between precipitations. The final product was dried overnight *in vacuo* before characterizing via ^{1}H NMR (D_2O) and SEC-MALLS (DMF 20 mM LiBr). M_n(NMR) = 3220 g/mol, M_n(SEC) = 3360 g/mol, M_w/M_n = 1.06.

***N*-Benzylmaleimide.** A solution of maleic anhydride (20.00 g, 204 mmoL) in anhydrous diethyl ether (250 mL) was first prepared at room temperature in a three-necked 1 L round-bottom flask equipped with magnetic stir bar, condenser, and addition funnel. A solution of benzylamine (21.86 g, 204 mmol) in anhydrous diethyl ether (100 mL) was added dropwise via addition funnel over 30 min such that the exothermic reaction produced a mild reflux of the solvent. The reaction mixture was stirred for 1 h at room temperature before isolating the resulting solids by vacuum filtration followed by washing with anhydrous diethyl ether (100 mL). The isolated *N*-benzylmaleamic acid intermediate was dried *in vacuo* and used without further purification (40.70 g, 97%).

N-Benzylmaleamic acid (40.70 g, 198 mmol) was added as a solid to a stirred solution of acetic anhydride (90.00 g, 881 mmol) and anhydrous sodium acetate (13.00 g, 158 mmol), and the reaction was heated at 100 °C for 30 min, resulting in the formation of a dark brown homogeneous solution. The reaction mixture was then poured into a vigorously stirred solution of ice cold water (600 mL) followed by stirring for 30 min. The resulting brown precipitate was isolated by vacuum filtration and washed with water (3 × 100 mL). The solids were resuspended in water (500 mL) and stirred vigorously for 30 min before isolation again by vacuum filtration. The crude compound was further purified by recrystallization from ethanol:H_2O (2:1, v:v) to afford *N*-benzylmaleimide (27.02 g, 73%) as fine beige crystals; mp 67−69 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22 (b, 5H), 6.64 (s, 2H), 4.61 (s, 2H). ^{13}C NMR ($CDCl_3$): δ 170.41, 136.17, 134.19, 128.69, 128.38, 127.86, 41.42.

Simultaneous "One-Pot" Aminolysis/Thiol−Maleimide End-Group Modification of PDMA-CPDB (Method 1). A representative procedure is as follows: pDMA-CPDB (100.0 mg, 3.10×10^{-5} mol, 1 equiv) and *N*-benzylmaleimide (29.0 mg, 1.55×10^{-4} mol, 5 equiv) were measured into a 5 mL test tube equipped with rubber septum and dissolved in 1.00 mL of DMSO. The reaction mixture was degassed via three freeze−pump−thaw cycles and backfilled with argon. 100 μL of a solution of hexylamine in DMSO (102 μL/mL, 7.75×10^{-5} mol, 2.5 equiv) and 100 μL of a solution of DBU in DMSO (40.0 μL/mL, 3.10×10^{-5} mol, 1 equiv) were then added sequentially via gastight syringe and the reaction stirred for 12 h at room temperature (23 °C). End-modified pDMA was purified by precipitation three times into diethyl ether (50 mL) and dried overnight *in vacuo*. End-group analysis was performed using ^{1}H NMR (D_2O) by comparing the integrated peak area of the benzyl aromatic protons (7.50−7.15 ppm, 5H) to the integrated peak area of the pDMA *N*,*N*-dimethyl side chain and methyne backbone protons (3.30−2.20 ppm, 213.22H). NMR samples were filtered through a 0.20 μm Millex PTFE filter prior to analysis.

Sequential "One-Pot" Aminolysis/Thiol−Maleimide End-Group Modification of PDMA-CPDB (Method 2). A representative procedure is as follows: pDMA-CPDB (100.0 mg, 3.10×10^{-5} mol, 1 equiv) and trimethyl phosphite (18.3 μL, 1.55×10^{-4} mol, 5 equiv) were measured into a 5 mL test tube equipped with rubber septum

DOI: 10.1021/acs.macromol.6b01512
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and dissolved in 1.00 mL of DMSO. The reaction mixture was degassed via three freeze−pump−thaw cycles and backfilled with argon. 100 μL of a solution of hexylamine in DMSO (102 μL/mL, 7.75×10^{-5} mol, 2.5 equiv) was then added via gastight syringe, and the reaction was stirred for 30 min at room temperature (23 °C) upon which a previously degassed solution of *N*-benzylmaleimide (29.0 mg, 1.55×10^{-4} mol, 5 equiv) in DMSO (0.5 mL) was added and the reaction stirred for 12 h at room temperature. End-modified pDMA was purified by precipitation three times into diethyl ether (3 × 50 mL) and dried overnight *in vacuo*. End-group analysis was performed using ^{1}H NMR (D_2O) by comparing the integrated peak area of the benzyl aromatic protons (7.50−7.15 ppm, 5H) to the integrated peak area of the pDMA *N*,*N*-dimethyl side chain and methyne backbone protons (3.30−2.20 ppm, 213.22H). NMR samples were filtered through a 0.20 μm Millex PTFE filter prior to analysis.

RESULTS AND DISCUSSION

Nucleophile-Promoted Michael Addition Side Reactions. Preliminary efforts in our lab to catalyze the "one-pot" aminolysis/thiol−maleimide end-group modification of acrylamido RAFT polymers in DMSO using the amidine 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) resulted in (i) low end-group functionalization efficiencies as compared to reactions performed in the absence of DBU, (ii) quantitative maleimide consumption, and (iii) the formation of high molecular weight impurities observable by SEC-MALLS. From these observations we hypothesized that the presence of a strong nucleophile (DBU) results in zwitterionic polymerization of maleimide occurring faster than the desired thiol−maleimide Michael addition. Accordingly, it is also possible that other nucleophiles (e.g., amines and trialkylphosphines) commonly used during "one-pot" aminolysis/thiol−maleimide modification of RAFT polymers can promote similar side reactions. Recently, Azechi et al. showed that 1°, 2°, and 3° amines initiate the anionic polymerization of N-substituted maleimides in highly polar aprotic solvents (DMSO and DMF), whereas no polymerization is observed in less polar solvents (THF).[40] Additionally, trialkylphosphines have been used as initiators for the zwitterionic polymerizations of methylene malonic esters,[41] cyanoacrylates,[42] and N-substituted maleimides.[43] We therefore chose to first investigate the effect of solvent on the reactions of N-substituted maleimides with nucleophiles commonly employed during "one-pot" aminolysis/thiol−maleimide RAFT polymer reactions.

Reaction of N- and P-Based Nucleophiles with *N*-Methylmaleimide. Initially, we examined the effect of solvent on the reactions of representative N- and P-based nucleophiles with *N*-methylmaleimide (NMM) in the absence of thiol as outlined in Figure 1. A stoichiometric excess of maleimide relative to nucleophile ($[Nu]_0$:$[Mal]_0$ = 1.0:10) was chosen to reflect the relative concentrations of these reagents used during RAFT polymer end-group thiol−maleimide reactions and to elucidate whether or not maleimide polymerization was occurring. The time-dependent fractional change in maleimide concentration ($[Mal]/[Mal]_0$) was monitored using *in situ* ^{1}H NMR by following the peak area of the maleimide olefin protons relative to the peak area of an internal standard (CH_2Cl_2).

Figure 1a shows the time-dependent $[Mal]/[Mal]_0$ plots for the reactions of hexylamine (HexAM) and NMM in different solvents with the dashed line ($[Mal]/[Mal]_0$ = 0.9) representing the theoretical decrease in $[Mal]/[Mal]_0$ predicted for the reaction of HexAM and NMM via single aza-Michael addition. Reactions conducted in MeCN, EtOH, and CH_2Cl_2 show decreases in $[Mal]/[Mal]_0$ to a value of 0.9, beyond which no change is observed up to 12 h, indicating exclusive aza-Michael addition takes place in these solvents. Conversely, reaction of HexAM with NMM in DMSO results in rapid maleimide consumption, corresponding to an average of 7.5 maleimides per amine within the first 3 min. Subsequently, no change in $[Mal]/[Mal]_0$ is observed up to 12 h. These results are consistent with those reported by Azechi et al.[40] and confirm that HexAM can initiate the anionic polymerization of NMM in polar solvents such as DMSO whereas exclusive aza-Michael addition takes place in less polar solvents. The effect of solvent polarity on the reaction of HexAM with NMM is also readily observed in Figure 1a by noting the increase in reaction rate with increasing solvent polarity in the order of CH_2Cl_2 (ε = 8.93) < EtOH (ε = 24.5) < MeCN (ε = 37.5) < DMSO (ε = 46.7).

The time-dependent $[Mal]/[Mal]_0$ plots for the reactions of DBU and NMM are shown in Figure 1b. Again, reaction rate increases with increasing solvent polarity with 100% NMM conversion reached in <10 min in DMSO. Quantitative consumption of NMM was also observed for reactions performed in EtOH and MeCN while 71% maleimide conversion was measured in CH_2Cl_2 after 12 h. Noteworthy is that precipitation was observed during the reaction of DBU and NMM in CH_2Cl_2 after ∼30 min. Limited solubility of the propagating macro zwitterionic enolate in CH_2Cl_2 likely prevents the propagating chain-end from reacting with NMM in solution, accounting for the nonquantitative maleimide conversion observed. Nonetheless, these results indicate that DBU-initiated polymerization of NMM occurs rapidly and extensively in both polar and nonpolar solvents.

Interestingly, the kinetic plots in Figure 1b do not rapidly reach a constant value of $[Mal]/[Mal]_0$ as was observed during the reaction of HexAM and NMM in Figure 1a. Unlike protic nucleophiles (e.g., 1°, 2° amines, and thiols), aprotic nucleophiles such as DBU cannot undergo complete Michael addition due to the lack of a transferable hydrogen from the nucleophile. Therefore, if an active hydrogen-containing compound (e.g., thiol) is in low concentration or completely absent, the nucleophile-derived zwitterionic enolate **1** can initiate maleimide propagation without enolate termination by proton transfer, thus allowing for a living-like polymerization process to occur. Furthermore, termination by nucleophilic displacement in macrozwitterionic polymerizations can regenerate the nucleophilic initiator and increase polymer molecular weight even after complete monomer consumption (Scheme 3).[44] Evidence of this occurring during the reaction of DBU with NMM in DMSO (Figure 1b) was garnered by noting the increased viscosity of the reaction after 12 h and a high molecular weight polymer peak eluting at the exclusion limit of our DMF SEC-MALLS system (Figure S1).

Tributylphosphine (TBP) also initiates polymerization of NMM in all solvents tested (Figure 1c). Reaction rates increase with solvent polarity with 95% maleimide conversion reached in 2.5 min in DMSO. Precipitation is also observed during reactions performed in EtOH and CH_2Cl_2 after ∼5 min following the addition of TBP. Poor solubility of the propagating phosphonium zwitterionic enolate is again a likely reason for the nonquantitative maleimide conversions achieved in EtOH and CH_2Cl_2 during the time frame of these kinetic experiments.

From these results it is apparent that DBU- and TBP-initiated maleimide polymerization is unavoidable, even in low

DOI: 10.1021/acs.macromol.6b01512
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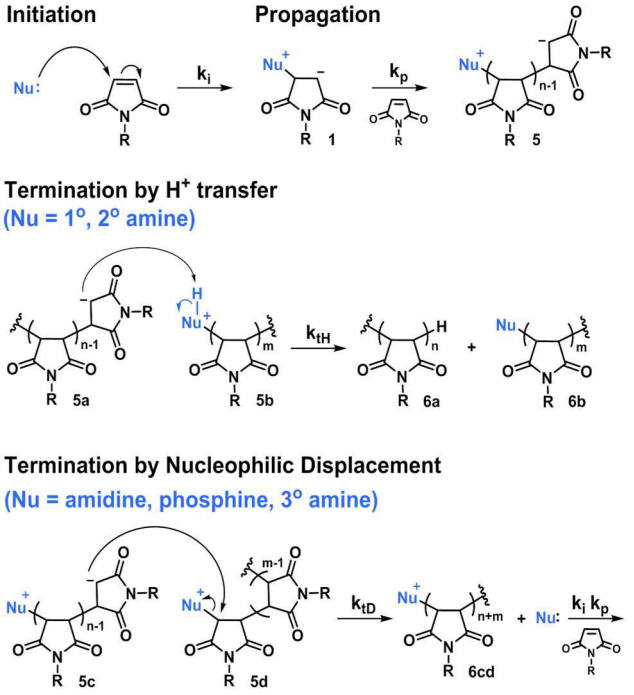
Scheme 3. Proposed Mechanisms of Initiation, Propagation, and Termination for the Nucleophile-Initiated Anionic Polymerization of N-Substituted Maleimides



polarity solvents. While DBU is optional during "one-pot" aminolysis/thiol−maleimide modification of RAFT polymers, mild reducing agents such as TBP are generally required to prevent disulfide formation from occurring between polymeric thiols during the RAFT agent aminolysis step.[20,26] Recently, Ho et al. reported the use of trialkyl phosphites as cheaper and less toxic alternatives to trialkylphosphines as reducing agents during "one-pot" RAFT polymer aminolysis/thiol−ene reactions.[45] While trialkyl phosphites can undergo conjugate addition to electron-deficient olefins, they are less nucleophilic than phosphines and typically require elevated temperatures (100 °C) for such reactions to occur.[46,47] As shown in Figure 1d, the reaction of trimethyl phosphite (TMP) with NMM results in no measurable change in $[Mal]/[Mal]_0$ in MeCN, EtOH, and CH_2Cl_2. Only after prolonged reaction times (12 h) in DMSO is a 65% decrease in $[Mal]/[Mal]_0$ observed. Consequently, TMP is a suitable alternative to phosphines as a reducing agent during thiol−maleimide reactions when used in less polar solvents. It should also be noted that trace amounts of water must be present for trialkyl phosphite (and phosphine) reduction of disulfides to occur.[48] Therefore, rigorous anhydrous conditions should be avoided during RAFT polymer aminolysis when using trialkyl phosphites as reducing agents.

Reaction of Thiols with *N*-Methylmaleimide. It is evident from the kinetic plots shown in Figure 1 that strong N- and P-based nucleophiles can react with maleimides to form either the Michael addition product or polymaleimide depending upon the nucleophile type (protic or aprotic) and solvent polarity. Therefore, it is plausible that other strong nucleophiles such as thiolates can initiate polymerization of maleimides in polar solvents. To this end, we chose to investigate the reaction of ethyl 2-mercaptopropionate (E2MP) with NMM under conditions identical to those outlined in Figure 1, except with the addition of TEA to generate the nucleophilic thiolate species. E2MP was chosen as a model thiol due to its structural similarity to the polymeric thiol that would be produced upon aminolysis of a polyacrylate RAFT polymer.

As shown in Figure 2, only the reaction of E2MP and NMM in CH_2Cl_2 gives the change in $[Mal]/[Mal]_0$ expected for

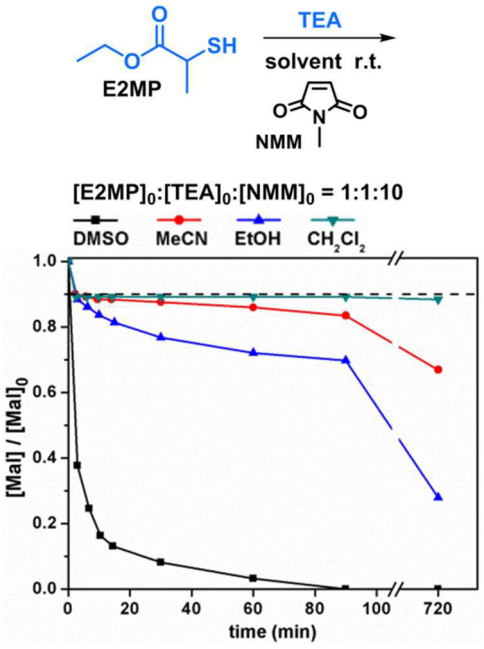


Figure 2. Effect of solvent on the time-dependent fractional change in $[Mal]/[Mal]_0$ during the TEA-catalyzed reaction of E2MP with NMM as measured by *in situ* ^{1}H NMR analysis.

exclusive thiol−maleimide Michael addition ($[Mal]/[Mal]_0$ = 0.9, dashed line). Meanwhile, the reactions conducted in more polar solvents (DMSO, EtOH, and MeCN) show a continued decrease in $[Mal]/[Mal]_0$ up to 12 h, with 100% maleimide conversion reached in 90 min in DMSO. Initially, we anticipated the kinetic profiles for the reactions of E2MP and NMM (Figure 2) to rapidly reach a constant $[Mal]/[Mal]_0$ value due to rapid enolate termination by means of proton transfer, as previously observed for the reactions of HexAM and NMM (Figure 1a). However, a continued decrease in $[Mal]/[Mal]_0$ up to 12 h is evidence of either slow reaction of thiol or an additional reaction pathway.

Closer inspection of the kinetic profiles for reactions conducted in EtOH and MeCN reveals an inflection point at the first time point (2.5 min) when $[Mal]/[Mal]_0 \approx 0.9$, indicating that thiol−maleimide Michael addition likely occurs faster than subsequent maleimide propagation in these solvents. Furthermore, *in situ* ^{1}H NMR analysis was used to show 100% thiol conversion was reached by the first time point (2.5 min) for reactions conducted in DMSO, MeCN, and CH_2Cl_2 (Figures S2−S4, respectively). Thiol conversion could not be measured for the reaction performed in EtOH-d_6 due to deuterium exchange between the solvent and sulfhydryl proton. Quantitative thiol consumption early in each reaction requires that proton transfer from thiol to enolate also occur rapidly. Therefore, the continued decrease in $[Mal]/[Mal]_0$ observed at longer reaction times in more polar solvents is not due to slow reaction of thiol and is indicative of an alternate maleimide reaction pathway.

One possible explanation for the continued decrease in $[Mal]/[Mal]_0$ at longer reaction times is TEA-initiated

DOI: 10.1021/acs.macromol.6b01512
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maleimide polymerization. The kinetic plots for the reactions of TEA with NMM in DMSO and EtOH (Figure S5) show a decrease in $[Mal]/[Mal]_0$ of 0.07 and 0.04, respectively, after 12 h. Meanwhile, no change in $[Mal]/[Mal]_0$ was observed in MeCN and CH_2Cl_2 after 12 h. While this confirms TEA can initiate polymerization of NMM in polar solvents, the minimal change in $[Mal]/[Mal]_0$ observed after 12 h in DMSO and EtOH, and the lack of reaction in MeCN, is insufficient to account for the decrease in $[Mal]/[Mal]_0$ that occurs after the same time period during the reactions of E2MP with NMM in these solvents.

Another potential explanation for the continued decrease in $[Mal]/[Mal]_0$ observed in the kinetic plots of Figure 2 involves deprotonation of the thiol−maleimide Michael adduct by TEA to regenerate the nucleophilic enolate species. Initially, this seems unlikely since the pK_a of TEA in DMSO (10.75) is much lower than that of most succinimide-derived enolates (~25). However, enolate formation of 2-aminosuccinimide residues in peptides has been shown to occur under mildly basic conditions in aqueous media (pH = 7.4), indicating that heteroatom substitution of the succinimide α-carbon can reduce enolate pK_a compared to unsubstituted succinimides.[49]

Thiol−Maleimide Adducts as Nucleophiles. To test whether thiol−maleimide Michael addition products are capable of reinitiating maleimide polymerization in the presence of TEA, the Michael adduct of benzyl mercaptan and NMM (7) was synthesized and purified by column chromatography. It should be noted that the Michael adduct of E2MP and NMM was not synthesized due to potential complications arising from the presence of chemically distinct diastereomers and the complexity in identifying the ^{1}H NMR chemical shifts of the resulting four stereoisomers. Meanwhile, the reaction of benzyl mercaptan and NMM affords a racemic mixture of chemically indistinguishable enantiomers.

As shown in Figure 3, $[Mal]/[Mal]_0$ decreases with time during the TEA-catalyzed reactions of 7 and NMM in DMSO, EtOH, and MeCN whereas no change in $[Mal]/[Mal]_0$ is observed in CH_2Cl_2 up to 12 h. The kinetic profiles obtained in each solvent for the reactions of 7 and NMM in Figure 3 accurately reflect the kinetic profiles observed below $[Mal]/[Mal]_0 = 0.9$ (dashed line) for the analogous reactions of E2MP and NMM shown in Figure 2. This is not a surprising result since the dashed line in Figure 2 represents the change in $[Mal]/[Mal]_0$ predicted for formation of the thiol−maleimide Michael adduct. Any decrease in $[Mal]/[Ma]_0$ below a value of 0.9 in Figure 2 would presumably occur as a result of TEA-promoted regeneration of the nucleophilic enolate species and therefore resemble the kinetic profiles in Figure 3.

Also worth noting is that TEA has little influence on the time-dependent $[Mal]/[Mal]_0$ plots for the reaction of HexAM and NMM in DMSO as seen in Figure S6 of the Supporting Information. Only after 12 h was a minimal decrease in $[Mal]/[Mal]_0$ observed for the TEA-catalyzed reaction of HexAM and NMM relative to the $[Mal]/[Mal]_0$ values measured for the same reaction performed in the absence of TEA. This indicates that TEA-catalyzed reactions of thiol−maleimide adducts with additional maleimide may be unique among heteroatom−maleimide Michael addition products.

We next sought to provide direct evidence that TEA is a strong enough base to generate the enolate of 7 in polar solvents. To this end, we used *in situ* ^{1}H NMR analysis to measure the relative rates of hydrogen−deuterium (H−D) exchange of 7 in a DMSO-d_6/D_2O mixture in the presence of

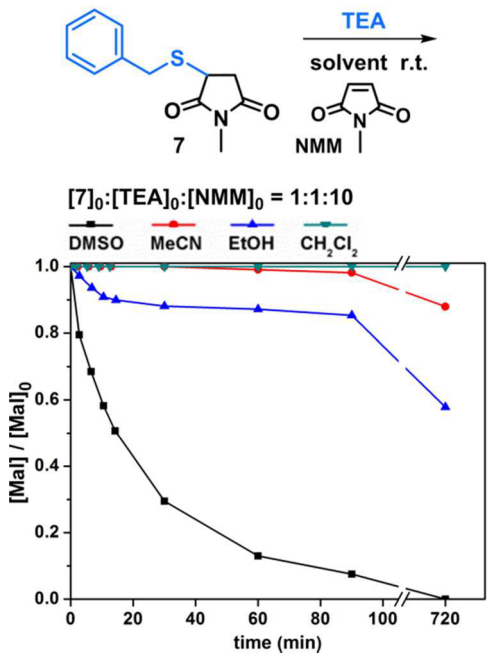


Figure 3. Effect of solvent on the time-dependent fractional change in $[Mal]/[Mal]_0$ during the TEA-catalyzed reaction of 7 with NMM as measured by *in situ* ^{1}H NMR analysis.

TEA. Figure 4a shows the time-dependent fractional change in peak area (A_t/A_0) for the three chemically distinct protons of 7 which could be abstracted by TEA to form an enolate on either the α-carbon (H_a) or the β-carbon (H_b and H_c) relative to the benzylsulfanyl group. For simplicity, only the structure of the S enantiomer is shown in Figure 4a. Interestingly, H−D exchange was only observed for proton H_a with a 97% decrease in A_t/A_0 occurring by 34 min. Sigma withdrawing effects by the adjacent thioether are most likely responsible for the increased acidity of H_a, leading to exclusive enolate formation at the α-carbon. Figure 4b shows the ^{1}H NMR spectral overlay of select time points during the H−D exchange experiments with 7. The peak corresponding to H_a decreases in area with time while maintaining the doublet of doublets (dd) splitting pattern that arises from spin−spin coupling with H_b and H_c. Meanwhile, the peaks corresponding to the geminal protons H_b and H_c do not change in area, but rather show a change in splitting pattern from dd to d as spin−spin coupling with H_a is diminished through deuterium exchange. Also apparent in Figure 4b is the significant downfield chemical shift of H_a (3.78 ppm) relative to H_b (3.07 ppm) and H_c (2.46 ppm) that arises from the deshielding (sigma withdrawing) effects of the benzylsulfanyl group.

Scheme 4 shows the proposed reaction pathways for the TEA-catalyzed thiol−maleimide reaction when a stoichiometric excess of maleimide relative to thiol is employed in polar solvents. Thiolate addition to maleimide forms the β-enolate **3** which can either abstract a proton from thiol or $^+NH(Et)_3$ to give the thiol−maleimide adduct **4** or react directly with maleimide to form the propagating species **8** when conditions favor propagation over termination (i.e., $[Mal] \gg [thiol]$). Deprotonation of **4** by TEA forms the α-enolate **9** which can reversibly terminate by proton transfer or react with maleimide to form the propagating species **10**. The propagating species **8** and **10** derived from β- and α-enolates, respectively, can continue to react with maleimide until termination occurs by

DOI: 10.1021/acs.macromol.6b01512
Macromolecules 2016, 49, 6193−6202

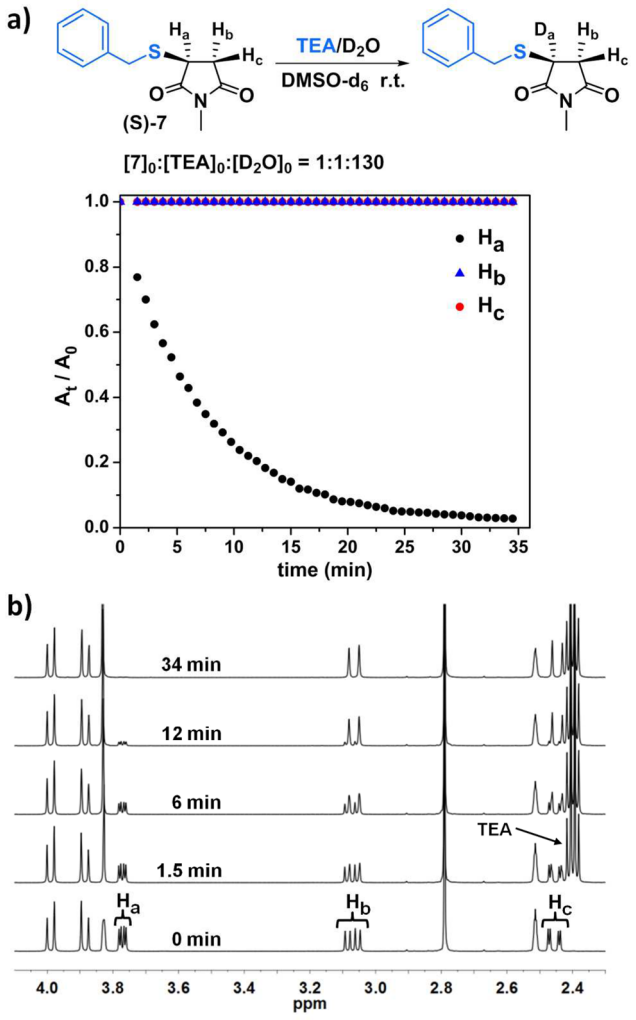
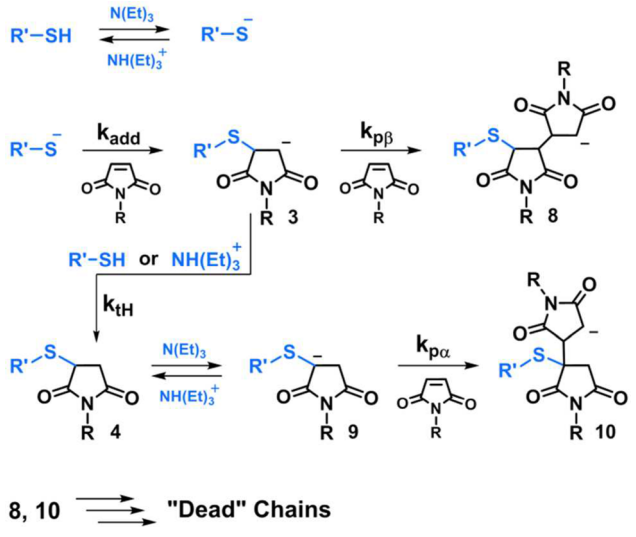


Figure 4. (a) Time-dependent fractional change in peak area (A_t/A_0) for protons H_a, H_b, and H_c during TEA-catalyzed H−D exchange of **7** in DMSO. (b) ^{1}H NMR spectral overlay of select time points during H−D exchange experiments with **7**.

Scheme 4. Proposed Reaction Pathways for the TEA-Catalyzed Thiol−Maleimide Reaction in Which Reversible Enolate Formation Is Operational

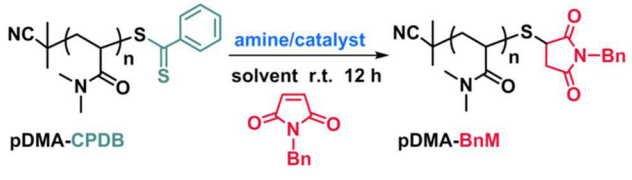


proton transfer. Also, the termination products of **8** can in theory reinitiate maleimide polymerization by α-enolate formation. These additional reaction pathways can account for the continued decrease in $[Mal]/[Mal]_0$ following initial formation of the thiol−maleimide Michael adduct for the TEA-catalyzed reactions of E2MP with NMM shown in Figure 2. It should also be noted that under these conditions exclusive enolate formation at the α-carbon rules out the possibility of TEA-catalyzed β-elimination (retro Michael addition) as a means of regenerating the nucleophilic thiolate species after formation of the thiol−maleimide Michael adduct.

"One-Pot" Aminolysis/Thiol−Maleimide End-Group Functionalization of RAFT Polymers. It is apparent that many of the reagents (e.g., amines and phosphines) commonly used during "one-pot" RAFT polymer aminolysis/thiol−maleimide reactions can react with maleimides to form polymaleimide or Michael addition byproducts and potentially outcompete the desired polymeric thiol−maleimide Michael reaction. We have also shown that intermediate strength bases such as TEA can deprotonate thiol−maleimide Michael adducts in polar solvents to form a nucleophilic α-enolate capable of subsequent reaction with maleimide. However, the effects of these side reactions on RAFT polymer end-group functionalization efficiency are not yet well understood. Also worth investigating is the influence of aminolysis method on end-group functionalization efficiency. The simplest method involves simultaneous aminolysis of the RAFT polymer in the presence of maleimide and does not require the use of a reducing agent to prevent disulfide formation. However, the competing amine−maleimide aza-Michael addition could prevent quantitative RAFT agent aminolysis and subsequently reduce the degree of end-group functionalization. Alternatively, a sequential method allows for complete aminolysis of the RAFT agent to occur prior to the addition of maleimide. This route minimizes side reactions between the amine and maleimide but necessitates the use of a reducing agent to prevent polymeric disulfide formation from occurring during the aminolysis stage.

To investigate the effects of solvent, catalyst, reducing agent, and aminolysis method on RAFT polymer end-group functionalization efficiency, we first synthesized the water-soluble polymer poly(*N*,*N*-dimethylacrylamide) (pDMA-CPDB) using the RAFT agent 2-cyano-2-propyl benzodithioate. Low dispersities ($Đ$ = 1.06) and excellent agreement between the number-average molecular weights determined by size exclusion chromatography (M_n(SEC) = 3360 g/mol) and by ^{1}H NMR (M_n(NMR) = 3220 g/mol) are evidence of a controlled polymerization and high dithiobenzoate chain-end fidelity. "One-pot" reactions of pDMA-CPDB with *N*-benzylmaleimide (BnM) were conducted at room temperature for 12 h using the initial molar ratios of $[pDMA\text{-}CPDB]_0$:$[HexAM]_0$:$[BnM]_0$ = 1.0:2.5:5.0 (Scheme 5). End-group analysis by ^{1}H

Scheme 5. "One-Pot" Aminolysis/Thiol−Maleimide Modification of PDMA-CPDB with BnM



DOI: 10.1021/acs.macromol.6b01512
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NMR was performed in D_2O by comparing the integrated peak area of the benzyl aromatic protons (7.50−7.15 ppm) to the known integrated peak area of the pDMA *N*,*N*-dimethyl side chain and methyne backbone protons (3.30−2.20 ppm) (Figures S7−S27). The poor solubility of BnM and its nucleophile-initiated byproducts (i.e., poly(BnM)) in D_2O allows for accurate quantification of only BnM that is covalently attached to pDMA.

Table 1 summarizes the results of the "one-pot" aminolysis/thiol−maleimide reactions of pDMA-CPDB with BnMA with the last column indicating the color exhibited by each reaction after 12 h. It should be noted that all reactions performed in this work that resulted in maleimide polymerization also became red in color. Addition of trifluoroacetic acid to these reactions gave near colorless solutions, thus indicating that the red coloration is likely the result of a persistent enolate concentration and allows for reaction color to be used as a qualitative indicator of zwitterionic/anionic maleimide polymerization.

Table 1. Summary of "One-Pot" Aminolysis/Thiol−Maleimide Reactions of PDMA-CPDB with BnM[a]

entry	method[b]	solvent	catalyst/red agent	funct[e] (%)	CTA[f] (%)	color[g] (12 h)
1a	1	CH_2Cl_2	DBU[c]	93	0	R
1b	1	MeCN	DBU	37	0	R
1c	1	EtOH	DBU	25	15	R
1d	1	DMSO	DBU	0	0	R
2a	1	CH_2Cl_2	–	89	0	Y
2b	1	MeCN	–	81	3	O
2c	1	EtOH	–	8	90	O
2d	1	DMSO	–	31	46	R
3a	2	CH_2Cl_2	–	95	0	Y
3b	2	MeCN	–	95	0	R
3c	2	EtOH	–	84	0	Y
3d	2	DMSO	–	72	0	R
4a	2	CH_2Cl_2	TBP[d]	89	0	R
4b	2	MeCN	TBP	86	0	R
4c	2	EtOH	TBP	84	0	R
4d	2	DMSO	TBP	72	0	R
5a	2	CH_2Cl_2	TMP[d]	98	0	Y
5b	2	MeCN	TMP	99	0	Y
5c	2	EtOH	TMP	86	0	Y
5d	2	DMSO	TMP	89	0	R

[a] $[pDMA\text{-}CPDB]_0:[HexAM]_0:[BnM]_0$ = 1.0:2.5:5.0. [b] Method 1: simultaneous aminolysis/thiol−maleimide; method 2: sequential aminolysis/thiol−maleimide. [c] $[pDMA\text{-}CPDB]_0:[DBU]_0$ = 1.0:1.0. [d] $[pDMA\text{-}CPDB]_0:[TBP \text{ or } TMP]_0$ = 1.0:5.0. [e] Percent end-group functionalization of pDMA-CPDB with BnM as measured by ^1H NMR. [f] Percent CTA remaining after reaction of pDMA-CPDB with BnMA as measured by ^1H NMR. [g] Reaction color after 12 h: red (R), orange (O), or yellow (Y).

Entries 1a−1d reflect our initial attempts to catalyze thiol−maleimide end-group functionalization reactions with DBU as a catalyst and were performed using the simultaneous aminolysis method (method 1). A general trend of decreasing end-group functionalization efficiency with increasing solvent polarity was observed with 0% functionalization achieved in DMSO. These results are relatable to the trend observed for the reactions of DBU and NMM shown in Figure 1b, where the rate of DBU-initiated maleimide polymerization increases with solvent polarity. These results confirm that the cause of failure for our preliminary DBU-catalyzed RAFT polymer functionalization reactions was DBU-initiated maleimide polymerization outcompeting the desired polymeric thiol−maleimide reaction. Also worth noting is the incomplete aminolysis observed for the reaction performed in EtOH (entry 1c) with 15% of dithibenzoate end-groups remaining after 12 h and can be attributed to the amine−maleimide aza-Michael addition reaction competing with CTA aminolysis.

Entries 2a−2d in Table 1 show the effect of simultaneous aminolysis/thiol−maleimide Michael addition in the absence of DBU on end-group functionalization efficiency. Incomplete aminolysis was observed in all solvents except for CH_2Cl_2 (2a) with reactions performed in DMSO (2d) and EtOH (2c) retaining 46% and 90%, respectively, of the original dithiobenzoate functionality. Accordingly, only 31% and 8% end-group functionalization was observed in DMSO and EtOH, respectively. These results show that the amine−maleimide aza-Michael addition can occur faster than CTA aminolysis in more polar solvents and are consistent with the effect of solvent polarity on the reaction rates of HexAM with NMM shown in Figure 1a.

The reactions performed in entries 3a−3d of Table 1 were identical to those performed in entries 2a−2d except the HexAM was allowed to react with pDMA-CPDB for 30 min prior to the addition of BnM (method 2). CTA aminolysis was qualitatively confirmed to occur within 30 min by noting the change in color that takes place as the dithiobenzoate end-groups (orange) are aminolyzed to the corresponding *N*-hexylthiobenzamide (yellow). As seen in entries 3a−3d, 100% CTA aminolysis was accomplished in all solvents while end-group functionalization efficiencies were significantly improved compared to reactions conducted using the simultaneous aminolysis method (entries 2a−2d). High end-group functionalization (95%) was achieved in the less polar aprotic solvents CH_2Cl_2 (3a) and MeCN (3b) while only moderate degrees of functionalization were obtained in EtOH (84%, 3c) and DMSO (72%, 3d). While these results are promising, it is well-known that disulfide coupling of polymeric thiols can occur during CTA aminolysis, resulting in both reduced end-group functionalization efficiencies and high molecular weight impurities, thus necessitating the use of a reducing agent.[20]

Entries 4a−4d of Table 1 summarize the effects of TBP as a reducing agent on end-group functionalization efficiency when using the sequential aminolysis method. The use of TBP as a reducing agent results in decreased end-group functionalization efficiencies for reactions performed in CH_2Cl_2 (4a) and MeCN (4b) compared to analogous reactions conducted without TBP (entries 3a and 3b, respectively). Meanwhile, no effect of TBP was observed on the functionalization efficiencies of reactions performed in EtOH and DMSO (entries 4c and 4d, respectively). From these results and the kinetic plots in Figure 1c, we conclude that trialkylphosphines are not suitable for use as reducing agents during thiol−maleimide end-group modification of RAFT polymers due to competing phosphine-initiated maleimide polymerization. Alternatively, using the less nucleophilic TMP as a reducing agent affords substantially increased degrees of end-group functionalization in all solvents as seen in Table 1 entries 5a−5d with 98% and 99% end-group functionalization achieved in CH_2Cl_2 (5a) and MeCN (5b), respectively.

Stoichiometric Considerations. Efficient and quantitative end-group functionalization of RAFT polymers using aminolysis/thiol−maleimide chemistry also requires consideration

DOI: 10.1021/acs.macromol.6b01512
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of the initial reactant stoichiometry. Ideally, minimal excess of maleimide should be used relative to polymeric thiol to limit the waste of potentially costly N-substituted maleimide compounds. However, inevitable side reactions such as amine−maleimide aza-Michael addition must be taken into account when choosing reactant stoichiometry such that $[Mal]_0 > [P_nSH] + [RNH_2]$ where $[P_nSH]$ and $[RNH_2]$ are the polymeric thiol and unreacted amine concentrations, respectively, after complete RAFT agent aminolysis has occurred. In this work, we found that aminolysis of pDMA-CPDB with HexAM using a molar ratio of $[pDMA\text{-}CPDB]_0:[HexAM]_0$ = 1.0:2.5 results in complete loss of dithiobenzoate end-groups within 30 min. However, other work conducted by our group (not reported herein) has shown that dithiobenzoate-functional polystyrene synthesized by RAFT requires several hours for complete aminolysis to occur using the same dithiobenzoate to amine ratio. Therefore, the reactant feed ratios reported herein should be considered a starting point for stoichiometric optimization of different RAFT polymer systems.

The type of RAFT agent being aminolyzed must also be considered when choosing reactant stoichiometry. Dithiobenzoate-terminated polymers react with 1 equiv of amine to yield polymeric thiol and thiobenzamide byproducts in equimolar amounts. Conversely, trithiocarbonate-terminated polymers can react with 2 equiv of amine to give the polymeric thiol, Z-group derived thiol, and thiourea byproduct in equimolar amounts. In this case, the reactant stoichiometry must allow for $[Mal]_0 > [P_nSH] + [RNH_2] + [ZSH]$, where [ZSH] is the concentration of small molecule Z-group derived thiol.

CONCLUSIONS

New insights into nucleophile-promoted thiol−maleimide side reactions have been provided. Nonquantitative end-group functionalization of RAFT polymers using "one-pot" aminolysis/thiol−maleimide reactions was determined to be the result of nucleophile-initiated maleimide polymerization occurring faster than the desired thiol−maleimide Michael reaction. Furthermore, previously unreported base-catalyzed α-enolate formation of thiol−maleimide Michael adducts in polar solvents was shown to promote multiple maleimide additions per thiol. Ultimately, such side reactions can be prevented by conducting RAFT polymer thiol−maleimide end-group modification reactions in less polar solvents while avoiding the use of strong aprotic nucleophiles such as amidine catalysts and phosphine reducing agents.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.6b01512.

SEC RI chromatogram of DBU-initiated poly(*N*-methylmaleimide), ^{1}H NMR spectra of select time points during reactions of E2MP and NMM in different solvents, kinetic plots for reaction of TEA with NMM in different solvents, kinetic plots for reaction of HexAM with NMM in different solvents, and ^{1}H NMR spectra of *N*-benzylmaleimide end-functionalized poly(*N*,*N*-dimethylacrylamide) (PDF)

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Notes

The authors declare no competing financial interest.
Paper 160 in a series entitled "Water-Soluble Polymers."

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support provided in part by the National Science Foundation Graduate Research Fellowship Program under Grant GM004636/GR04355 and the National Science Foundation's EPSCoR under Agreement IIA1430364.0.

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DOI: 10.1021/acs.macromol.6b01512
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