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Thiol−Ene Click Functionalization and Subsequent Polymerization of 2-Oxazoline Monomers

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ABSTRACT: Polymers of 2-oxazolines are a class of polymers that can exhibit water solubility and biocompatibility depending upon the *N*-acyl side-chain functionality and can be readily modified at both the end-groups and backbone, making them appealing alternatives to poly(ethylene glycol) (PEG). Methods to introduce a range of functionality onto oxazoline monomers are of interest due to the limited availability of functionalized oxazoline monomers. Prepolymerization thiol−ene coupling between a side-chain thiol and 2-isopropenyl-2-oxazoline was used to synthesize three different monomers followed by polymerization to yield 2-substituted oxazoline polymers with aryl, ester, protected amine, and protected carboxylic acid side chains. The resulting polymers exhibited molecular weights ranging form 3000 to 8000 and polydispersities typically below 1.3. The deprotection of these polymers was also achieved to yield polymers with reactive functionalities, including a cysteine with either the amine or carboxylic acid exposed on each repeat unit.

Introduction

The general cationic ring-opening polymerization reaction of 2-oxazolines follows a living mechanism that leads to well-defined poly(*N*-acylethylenimine) polymers (PNAIs) with low polydispersities.[1] The ease with which the backbone and end-groups of poly(oxazolines) can be modified makes this family of polymers attractive for broad applications.[2−4] In addition, the high water solubility[5−7] and biocompatibility of the short side-chain derivatives of 2-oxazolines (2-methyl- and 2-ethyl-) make these *N*-acyl derivatives attractive alternatives to PEG.[2−4] For example, Zalipsky and co-workers[8,9] have demonstrated that the replacement of PEG with poly(2-methyl-2-oxazoline) or poly-(2-ethyl-2-oxazoline) provided similar advantages for polymer-modified liposomes, including increased plasma lifetimes, decreased blood clearance, and increased solubility in organic solvents. PNAIs have also been successfully used as a component in amphiphilic polymer micelles for the encapsulation of Doxorubicin,[10] as polymer-drug conjugates for high and low molecular weight biomolecules such as trypsin and Ara-C,[11] as ^{111}In-enriched scaffolds for radiotherapy and tumor imaging,[12] as non-ionic polymer surfactants, and as the hydrophilic component of cross-linked hydrogels.[1]

In contrast to PEG, which only has the capacity for end-group functionalization, the *N*-acyl side chain of oxazoline polymers enables diverse functionalization along the polymer backbone. As a result, the chemical properties and solubility of these polymers are readily tunable based on the functionality of the side chain at the substituted 2-position.[1,3,13] Though a number of 2-substituted oxazolines are commercially available, the modular syntheses of oxazolines containing a wider range of functional side chains would be advantageous for both biomedical and materials science applications.

The polymerization of cyclic imino ethers is thermodynamically favored due to the favorable isomerization of the imino ether group to the amide functionality and elimination of monomer ring strain. The cationic ring-opening polymerization of 2-oxazolines can follow either ionic or covalent mechanisms depending on the relative nucleophilicity of the counteranion derived from the initiator versus the monomer. Initiators with triflate or tosylate counterions tend to follow the ionic route while halide initiators propagate using a covalent intermediate.[14] Termination occurs following the addition of a strong nucleophile or adventitious reactions with water. This versatility in initiation and termination allows for the introduction of different functionalities at either end of the polymer chain.

Side-chain functionality can be introduced to a polymer either by attachment of the desired functionality to the monomer before polymerization or by postpolymerization modification of a reactive functionality on the polymer backbone.[13,15−18] Post-polymerization functionalization of the repeat units can enable access to functional PNAIs with high degrees of polymerization; however, this route can suffer from incomplete side-chain modification. In particular, thiol−ene chemistry has been used in the postpolymerization modifications of poly(oxazolines) as reported by Schlaad and co-workers to tune the LCSD properties of polyoxazoline.[13] On the other hand, the direct polymerization of modified oxazoline monomers should exhibit quantitative functionalization, however, and requires protection of side-chain functionalities that are incompatible with the cationic ring-opening polymerization. In addition, this route is expected to be prone to steric retardation of polymerization rates, resulting in lower degrees of polymerization. Because only a limited diversity of functionalized oxazoline monomers are commercially available, thiol−ene coupling between the readily available 2-isopropenyl-2-oxazoline and a library of thiols was explored as a route toward a wider range of oxazoline monomers, and their resultant polymerizations reactions were investigated.

The utilization of highly efficient "click reactions", as termed by Kolb et al.,[19] has been widespread due to their high specificity, near-quantitative yields, and near-perfect fidelity in the presence of most functional groups.[20] The Cu(I)-catalyzed [3 + 2] cycloaddition reaction between an azide and an alkyne has been the most utilized of the click reactions because it is fast, high-yielding, functional group tolerant, and compatible with a range of

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solvents. However, this type of click reaction is complicated by the Cu catalyst, which complexes strongly to electron-donating functionalities (such as amines, thiols, alcohols, etc.) and can result in unacceptable levels of contamination (single ppms) for both biological and materials applications.[21] Different methods have been investigated to overcome this complication, such as catalyst-free triazole couplings;[22,23] however, the strained or fluorinated alkyne substrates require substantial synthetic effort and the absence of a catalyst offers limited control over coupling. Recently, the addition of thiols to alkenes (thiol−ene) has emerged as an attractive complementary and metal-free click process.[24,25] This reaction can be catalyzed photochemically or by nucleophiles such as amines depending on the electrophilicity of double bond with near-quantitative yields in a period of seconds at ambient temperatures while showing compatibility with a wide range of functional groups in a variety of different solvents.[24−28] The high efficiency of the thiol−ene reaction has enabled the preparation of well-defined films of uniform molecular networks exhibiting high glass transition temperatures as well as highly cross-linked networks.[24,26,27] Recently, many examples of the thiol−ene reaction in other areas of material science have been reported,[29] including the syntheses of dendrimers[30] and star polymers[26] and functionalization of polymer end-groups and surfaces.[13,15,26,31] This type of chemistry has also been studied for use in biomedical and pharmaceutical applications[32] including the synthesis of hydrogels[33,34] and controlled drug release systems.[35] In addition, postpolymerization modifications of poly(oxazolines) using thiol−ene reactions have been performed by Schlaad and co-workers.[13] The absence of the Cu catalyst makes the thiol−ene coupling attractive for biological applications and therefore was applied toward the preparation of functionalized 2-substituted oxazoline monomers.

Herein, we report the functionalization and polymerization of three different 2-substituted oxazoline monomers utilizing the amine-catalyzed thiol−ene click reaction between the commercially available 2-isopropenyl-2-oxazoline and three thiols: thiophenol, methyl thioglycolate, and *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester.

Experimental Section

Materials. Merrifield's peptide resin (1% cross-linked) was purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (reagent grade, Fisher Scientific) was purified by distillation at 85 °C and placed over molecular sieves until used. Methyl tosylate (98%) was purified by distillation at 140 °C and placed over molecular sieves until used. 2-Isopropenyl-2-oxazoline (≥99%), thiophenol (97%), methyl thioglycolate (95%), *N*-(*tert*-butoxyarbonyl)-L-cysteine methyl ester (97%), butylamine (99.5%), trifluoroacetic acid (99%), and sodium trifluoroacetate (98%) were purchased from Aldrich and used without further purification. 1,8,9-Anthracenetriol (puriss. for MALDI-MS) was purchased from Fluka and used without further purification. Ammonium hydroxide (reagent grade) was purchased from Fisher Scientific and used without further purification. All other solvents were reagent grade, purchased from Fisher Scientific, and used without further distillation or purification.

Instrumentation. Mass spectral data were acquired using a Bruker Autoflex III (Billerica, MA) matrix-assisted laser desorption ionization time of flight mass spectrometer (MALDI) with delayed extraction using the reflector and positive ion mode. Mass spectral data were also obtained with a Bruker MicrOTOF electrospray ionization (ESI) mass spectrometer with an Agilent 1200 series binary pump (Santa Clara, CA).

Gas chromatography−mass spectrometry (GCMS) was carried out on a Varian 450-GC gas chromatograph with a Varian 300-MS SQ mass spectrometer (Palo Alto, CA). For polymer **4**, solutions of 1,8,9-anthracenetriol matrix (20 mg mL^{-1}) in chloroform, NaTFA (2 mg mL^{-1}) as the counterion in THF, and 2 mg mL^{-1} of sample in THF were used. MALDI samples were then prepared by using a matrix solution/counterion solution/sample solution ratio of 1:1:1. The pulsed ion extraction delay was set to 0 ns, with ion source 1 voltage of 19.00 kV and ion source 2 voltage of 16.47 kV, a lens voltage of 8.30 kV, a low mass gate at 800 amu, and a laser power set to 55%. For polymer **5**, solutions of 1,8,9-anthracenetriol matrix (20 mg mL^{-1}) in chloroform, NaTFA (2 mg mL^{-1}) as the counterion in THF, and 2 mg mL^{-1} of sample in THF were used. MALDI samples were then prepared by using a matrix solution/counterion solution/sample solution with a ratio of 1:2:1. The pulsed ion extraction delay was set to 50 ns, with ion source 1 voltage of 19.00 kV and ion source 2 voltage of 16.70 kV, a lens voltage of 6.40 kV, a low mass gate at 600 amu, and a laser power set to 31%. For polymer **6**, solutions of galvinoxyl, free radical matrix (10 mg mL^{-1}) in THF, NaTFA (2 mg mL^{-1}) as the counterion in THF, and 2 mg mL^{-1} of sample in THF were used. MALDI samples were then prepared using a matrix solution/counterion solution/sample solution with a ratio of 1:0.7:1. The pulsed ion extraction delay was set to 0 ns, with ion source 1 voltage of 19.00 kV and ion source 2 voltage of 8.30 kV, a lens voltage of 8.30 kV, a low mass gate at 800 amu, and a laser power set to 55%. Number-average molecular weight (M_n) and polydispersity (PDI) were calculated using PolyTools software.

Gel permeation chromatography (GPC) was carried out on a Waters model 1515 series pump (Milford, MA) with three-column series from Polymer Laboratories, Inc., consisting of PLgel 5 μm Mixed D (300 mm × 7.5 mm, molecular weight range 200−400 000), PLgel 5 μm 500 Å (300 mm × 7.5 mm, molecular weight range 500−30 000), and PLgel 5 μm 50 Å (300 mm × 7.5 mm, molecular weight range up to 2000) columns. The system was fitted with a model 2487 differential refractometer detector, and anhydrous tetrahydrofuran was used as the mobile phase (1 mL min^{-1} flow rate) at 25 °C. The resulting molecular weight was based on calibration using linear polystyrene standards.

Infrared (IR) spectroscopy was implemented using a NEXUS 670 FT-IR ESP (Madison, WI). Samples were made using ∼4 mg of polymer and 5 mg of KBr which was ground into a fine powder by mortar and pestle and compacted into a pellet.

Proton (and carbon) nuclear magnetic resonance (NMR) spectra were obtained from a 400 MHz (100 MHz) Varian Mercury spectrometer (Palo Alto, CA), using TMS = 0.00 ppm calibration and a 500 MHz (125 MHz) Bruker spectrometer (Billerica, MA), using TMS = 0.00 ppm calibration with deuterated solvents at room temperature.

Synthesis of Butylamine Resin.[36] Butylamine (29.26 g, 400 mmol) was added to 75 mL of DMF in a round-bottom flask containing a magnetic stir bar. Merrifield's peptide resin (20 g, 80 mmol) was then added to the reaction flask and refluxed overnight. The solid resin was then filtered and washed with 250 mL of dichloromethane (DCM) until no unreacted butylamine remained. The butylamine resin was then dried under vacuum overnight, and an IR was taken.

General Procedure for the Functionalization of 2-Isopropenyl-2-oxazoline Monomer. 2-Isopropenyl-2-oxazoline (1.06 g, 9.078 mmol) was dissolved in 5 mL of dry tetrahydrofuran (THF) in a round-bottom flask with a magnetic stir bar. The desired thiol (e.g., thiophenol 1.002 g, 9.078 mmol) was added, and the reaction mixture was stirred under N_2 gas for 5 min. The butylamine resin (1.54 g, 4.7896 mmol) was then added, and the reaction was allowed to stir overnight at room temperature. The butylamine resin was filtered off and washed with DCM. The filtrate was concentrated, characterized, and used without further purification (yield: 95%). Representative characterization (racemic) data for thiophenol (**1**) follow.

Characterization of 1. The monomer **1** was isolated as its racemate (yield: 95%). ^{1}H NMR ($CDCl_3$, δ, ppm): 1.30 (d, 3H,

J = 6.8 Hz, (−CH_3)), 2.70 (sx, 1H, J = 6.96 Hz (−$C_{oxazoline}$-CH(CH_3)CH_2−)), 2.934 (dd, 1H, J = 7.6 Hz, 13.4 (−CHCH_aH$_b$S−)), 3.29 (dd, 1H, J_1 = 6.4 Hz, J_2 = 13.2 Hz (−CHCH$_a$$H_b$S−), 3.77 (t, 2H, J = 9.6 Hz (−NCH_2CH$_2$O−)), 4.13 (t, 2H, J = 9.6 Hz (−NCH$_2$CH_2O−)), 7.16 (m, 1H, J = 7.2 Hz (Ar(H)), 7.26 (m, 2H, J = 7.0 Hz (Ar(H)), 7.35 (m, 2H, J = 6.0 Hz (Ar(H)). ^{13}C NMR (CDCl$_3$, δ, ppm): 18.5 (−CH$_3$), 33.0 (−$C_{oxazoline}$$CHCH_2$S−), 38.0 (−CH$CH_2$S−), 54.0 (−N$CH_2$-CH$_2$O−), 68.0 (−NCH$_2$$CH_2$O−), 126.0 (Ar($C$)), 129.0 (Ar($C$)), 131.0 (Ar($C$)), 136.0 (Ar($C$)), 170.0. (−N=$C$−). IR: 1600 (Ar(C=C)), 1670 (N=C), 3080 (Ar(C−H)), 3050 (Ar(C−H).

Characterization of ***2***. The monomer **2** was isolated as its racemate (yield 90%). ^{1}H NMR (CDCl$_3$, δ, ppm): 1.25 (d, 3H, J = 6.4 Hz (−CH_3)), 2.725 (dd, 2H, J_1 = 6.4 Hz, J_2 13.6 Hz (−CHCH_2S−)), 2.94 (m, 1H, J = 6.56 ($C_{oxazoline}$$CHCH_3CH_2$−)), 3.23 (s, 2H (−SC$H_2$COOC$_3$)), 3.71, (s, 3H (−COOC$H_3$)), 3.82 (t, 2H, J = 9.6 Hz (−NCH_2CH$_2$O−)), 4.22 (t, 2H, J = 9.2 Hz (−NCH$_2$CH_2O−). ^{13}C NMR (CDCl$_3$, δ, ppm): 17.0 (−CH$_3$), 34.0 (−$C_{oxazoline}$$CHCH_2$S−), 34.0 (−CH$CH_2$S−), 37.0 (−S$CH_2$-COOCH$_3$), 52.0(−COO$CH_3$), 54.0 (−N$CH_2CH_2$O−), 67.5 (−NCH$_2$$CH_2$O−), 170.0 (−N$C$−), 171.0 (−CH$_2$$C$OOCH$_3$). IR: 1250 (ester (O−C)), 1670 (N=C), 1740 (C=O).

Characterization of ***3***. The monomer **3** was isolated as its racemate (yield 97%). ^{1}H NMR (CDCl$_3$, δ, ppm): 1.23 (d, 3H, J = 6.28 Hz (−CH_3)), 1.425 (s, 9H (−OC(CH_3)$_3$)), 2.64 (m, 2H ($C_{oxazoline}$CHCH_2S−)), 2.84 (m, 1H, ($C_{oxazoline}$$CHCH_3CH_2$−)), 2.95 (d, 2H, J = 4.8 Hz (−SCH_2CH−)), 3.70 (s, 3H (−COOCH_3)), 3.82 (t, 2H, J = 9.6 Hz (−NCH_2CH$_2$O−)), 4.22 (t, 2H, J = 9.6 Hz (−NCH$_2$CH_2O−)), 4.51 (m, 1H (−CH$_2$−NHCHCOOCH$_3$). ^{13}C NMR (CDCl$_3$, δ, ppm): 17.0 (−CH$_3$), 28.0 (−OC(CH$_3$)$_3$), 34.0 (−$C_{oxazoline}$$CHCH_3CH_2$S−), 35.0 (−$C_{oxazoline}$CHCH$_3$$CH_2$S−), 37.0 (−S$CH_2$CH), 52.5 (−COO$CH_3$), 54.0 (−SCH$_2$$C$HCOOCH$_3$), 54.3 (−N$CH_2$-CH$_2$O−), 67.5 (−NCH$_2$$CH_2$O−), 80.0 (−NHCOO$C$(CH$_3$)$_3$), 155.5 (−NH$C$OOC−), 170.0 (−N$C$−), 172.0 (−C$C$OOCH$_3$). IR: 1250 (ester (O−C)), 1670 (N=C), 1720 (C=O), 1760 (carbamate (O = C)), 3200 (N−H), 3350 (N−H).

General Procedure for the Polymerization of Functionalized 2-Isopropenyl-2-oxazoline. The desired 2-isopropenyl-2-oxazoline functionalized monomer was dried under vacuum overnight to remove any water. All glassware was flame-dried. Distilled acetonitrile (4−6 mL) was added to the reaction flask under N_2 gas with a syringe. Varying initiator to monomer ratios were studied including 1:50, 1:100, 1:150, and 1:200. In this example, an initiator to monomer ratio of 1:100 was employed. Distilled methyl tosylate (0.0126 g, 0.0678 mmol) was added to the reaction via syringe. Functionalized 2-isopropenyl-2-oxazoline monomer (e.g., monomer **1**, 1.5 g, 6.785 mmol) was added via syringe, and the reaction was stirred for 24 h at 70 °C. After 24 h the reaction was cooled in an ice bath, and 0.5 mL of H_2O was added and stirred overnight at room temperature. The polymer was precipitated into diethyl ether and filtered. The polymer was washed with $NaHCO_3$ to remove any tosylate anion (conversion: 19%).

For the determination of monomer conversion versus molecular weight, the polymerization procedure described above was employed with a 1:130 initiator (0.0129 g, 0.0696 mmol) to monomer (monomer **1**, 2 g, 9.0462 mmol) ratio with acetonitrile as solvent (4 mL). The reaction was heated to 65 °C. Aliquots (0.25 mL) of the bulk reaction were removed every 2 h with a disposable syringe. The samples were cooled in an ice bath, and 0.1 mL of H_2O was added. The sample was then concentrated to remove acetonitrile with a rotary evaporator. No further purification was preformed on the samples before acquiring GPC data (Figure 7).

Characterization of ***4***. MALDI-TOF MS was taken at a matrix/counterion/sample ratio of 1:1:1, with 1,8,9-anthracenetriol matrix (20 mg/mL), NaTFA (2 mg/mL) as the counterion, and 2 mg/mL of sample. Representative characterization data follow. ^{1}H NMR (CDCl$_3$, δ, ppm): 1.0−1.4 (br (−CH_3)), 2.6−2.8 (br (−CHCH_2S−)), 2.8−3.1 (br (−CCHCH$_3$CH$_2$−)), 3.1−3.8 (br (−NCH_2CH_2−)), 6.9−7.4 (br (CH from (Ph))). ^{13}C NMR (CDCl$_3$, δ, ppm): 19 (−CH$_3$), 37 (−CCHCH$_3$CH$_2$−), 38 (−CHCH$_2$S−), 48 (−NCH$_2$CH$_2$−), 126 (Ar(C)), 137 (Ar(C)), 139 (Ar(C)), 146 (Ar(C)), 186 (−NCOCH−). IR: 1600 (Ar(C=C)), 1640 (tertiary amide(C−N)), 3050 (Ar(C−H), 3080 (Ar(C−H)), 3300 (O−H), 3500 (O−H).

Characterization of ***5***. MALDI-TOF MS was taken at a matrix/counterion/sample ratio of 1:2:1, with 1,8,9-anthracenetriol matrix (20 mg/mL), NaTFA (2 mg/mL) as the counterion, and 2 mg/mL of sample. Representative characterization data follow. ^{1}H NMR (CDCl$_3$, δ, ppm): 1.10−1.30 (br (−CH_3)), 2.55−2.70 (br (−CHCH_2S−)), 2.85−3.10 (br (−CCHCH$_3$CH$_2$−)), 3.10−3.30 (br (−SCH_2C−)), 3.40−3.60 (br (−NCH_2CH_2−)) 3.60−3.75 (br (−COOCH_3)). ^{13}C NMR (CDCl$_3$, δ, ppm): 19 (−CH$_3$), 34 (−CCHCH$_3$CH$_2$−), 36 (−CCH$_2$S−), 37 (−SCH$_2$CO-OCH$_3$), 46 (−NCH$_2$$CH_2$−), 52 (−COO$CH_3$), 172 (−CH$_2$$C$OO-CH$_3$), 176 (−N$C$OC−). IR: 1640 (tertiary amide (C−N)), 1735 (ester (C=O)), 3500 (O−H), 3200 (O−H).

For the purification of polymer **5f** (Table 1), a different workup was employed to isolate the highest molecular weight fraction of the polymer. The above-described polymerization procedure was employed with a 1:130 initiator (0.0132 g, 0.0709 mmol) to monomer (monomer **2**, 2 g, 9.2133 mmol) ratio with acetonitrile as solvent (4 mL) followed by the addition of H_2O (0.5 mL), precipitation in diethyl ether, filtration, and wash (conversion: 14%); M_n (GPC, before collection of high molecular weight fraction): 4000. The polymer (0.1500 g, 0.0375 mmol) was then dissolved in toluene (150 mL) with a small amount of dichloromethane (25 mL). Diethyl ether was then added dropwise until cloudy (~50 mL). The solution was then heated using a hot air gun until clear and allowed to precipitate overnight overnight. The precipitate was then collected. This was repeated twice (overall yield: 4.8%).

Characterization of ***6***. MALDI-TOF MS was taken at a matrix/counterion/sample ratio of 1:0.7:1, with galvinoxyl, free radical (10 mg/mL) matrix, NaTFA (2 mg/mL) as the counterion, and 2 mg/mL of sample. Representative characterization data follow. ^{1}H NMR (CDCl$_3$, δ, ppm): 1.00−1.20 (br (−CH_3)), 1.25−1.50 (br (−C(CH_3)$_3$)), 2.40−2.65 (br (−CHCH_2S−)), 2.65−3.10 (br (−CCHCH$_3$CH$_2$−)); (−SCH_2CH−), 3.40−3.60 (br (−NCH_2CH_2−)), 3.61−3.78 (br (−COOCH_3)), 4.35−4.55 (br (−SCH$_2$CH−)). ^{13}C NMR (CDCl$_3$, δ, ppm): 19.0 (−CH$_3$), 29.0 (C(CH$_3$)$_3$), 35.0 (−CHCH$_2$S−), 37.0 (−CHCH$_2$S−), 39.0 (−SCH$_2$CH−), 44.0 (−NCH$_2$CH$_2$−), 52.0 (−COOCH$_3$), 54.0 (−SCH$_2$$C$H−), 81.0 (−COO$C$(CH$_3$)$_3$), 156.0 (−NH$C$OOC-(CH$_3$)$_3$), 171.5 (−CH$C$OOCH$_3$−), 177.0 (−N$C$OOCH−). IR: 1640 (tertiary amide (−N−)), 1710 (C−O), 1760 (carbamate (O=C)), 3350 (O−H, N−H), 3200 (O−H, N−H).

Deprotection of Carboxylic Acid Functionality of ***5***. Ammonium hydroxide (3 mL) was added to methyl thioglycolate functionalized 2-isopropenyl-2-oxazoline polymer (25 mg, 5.6818 mmol) in a 5 mL vial. The vial was then capped and heated in a sand bath at 90 °C overnight. The ammonium hydroxide and any water present were then evaporated, and the sample was further dried under vacuum. Representative characterization data follow. ^{1}H NMR (D_2O, δ, ppm): 1.20−1.26 (br (−CH_3)), 2.60−2.80 (br (−CHCH_2S−)), 2.80−2.90 (br (−CCHCH$_3$CH$_2$−)), 3.00−3.20 (br (−SCH_2C−)), 3.20−3.60 (br (−NCH_2CH_2−)). ^{13}C NMR (CD_3OD, δ, ppm): 19.0 (−CH$_3$), 36.0 (−CCHCH$_3$CH$_2$−), 37.0 (−CCH$_2$S−), 37.5 (−SCH$_2$COOCH$_3$), 48.0 (−NCH$_2$$CH_2$−), 175.0 (−CH$_2$$C$OOH), 178.5 (−N$C$OC−). IR: 1640 (tertiary amide (C−N)), 1660 (ester (C=O)), 3500 (O−H), 3200 (O−H), 3310 (O−H).

Deprotection of Carboxylic Acid Functionality of ***6***. Ammonium hydroxide (3 mL) was added to *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester functionalized 2-isopropenyl-2-oxazoline polymer (25 mg, 3.5714 mmol) in a 5 mL vial. The vial was then capped and heated in a sand bath at 90 °C overnight.

Table 1. Initiator to Monomer Ratio (I:M_{ono}), Monomer Concentration ([M_{ono}]), Temperature, Time, Number-Average Molecular Weight (M_n), Polydispersity Index (PDI = M_n/M_w), and Degrees of Polymerization (DP = M_n/[Mass Repeat Unit]) Data for Different Trials of the Three Functionalized Oxazoline Polymer Samples[a]

					M_n		PDI		DP	
polymer/trial	I:M_{ono}	[M_{ono}], mmol	temp (°C)	time (h)	GPC	MALDI	GPC	MALDI	GPC	MALDI
				thiophenol-functionalized 2-isopropenyl-2-oxazoline polymer (**4**)						
4a	1:100	6.7850	70	24	4300	4400	1.17	1.11	19	20
4b	1:150	13.5693	80	48	3800	3600	1.15	1.10	17	16
4c	1:150	11.3070	90	48	3800	4300	1.16	1.07	17	19
4d	1:150	11.3078	70	48	3900	4300	1.17	1.07	18	19
4e	1:150	11.3078	70	36	4000	4200	1.17	1.23	18	19
				methyl thioglycolate -functionalized 2-isopropenyl-2-oxazoline polymer (**5**)						
5a	1:50	9.2133	65	24	3700	4000	1.31	1.11	16	18
5b	1:100	6.9099	70	24	3800	4200	1.15	1.11	17	19
5c	1:100	7.3706	70	24	3900	4600	1.16	1.13	18	21
5d	1:150	11.5176	70	48	4700	4500	1.24	1.16	22	21
5e	1:150	13.8198	80	48	5000	4400	1.29	1.13	23	20
5f	1:130	9.2133	70	48	9100	n/a	1.27	n/a	42	n/a
				N-(*tert*-butoxycarbonyl)-L-cysteine methyl ester-functionalized 2-isopropenyl-2-oxazoline polymer (**6**)						
6a	1:50	3.7556	70	24	4000	n/a	1.18	n/a	11	n/a
6b	1:100	13.7510	80	48	4700	n/a	1.32	n/a	13	n/a
6c	1:100	7.2159	70	24	5000	n/a	1.30	n/a	14	n/a
6d	1:200	7.2159	90	24	2800	n/a	1.01	n/a	8	n/a

[a] Polymer trial **5f** was synthesized using the same procedure as trails **5a**−**5e**, followed by a separation of the highest molecular weight fraction as described in Experimental Section. For numbering of polymers, see Scheme 1.

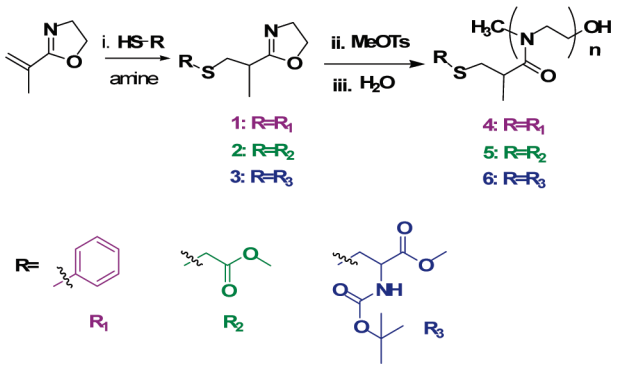
The ammonium hydroxide and any water were then evaporated, and the sample was then further dried on a vacuum pump. Representative characterization data follow. ^{1}H NMR (D_2O): δ 0.9−1.00 (b (−CH_3)), 2.4−2.56 (b (−C(CH_3)$_3$)), 2.58−2.72 (b (−CHCH_2S−)), 3.02−3.06 (b (−CCHCH$_3$CH$_2$−)); (−SCH_2-CH−), 3.2−3.6 (b (−NCH_2CH_2−)), 4.05 (br (−SCH$_2$CH−)). ^{13}C NMR (CD_3OD): δ 19 (−CH$_3$), 29 (C(CH$_3$)$_3$), 35 (−CCHCH$_3$CH$_2$S−), 37 (−CHCH$_2$S−), 38 (−SCH$_2$CH−), 46 (−NCH$_2$CH_2−), 52 (−SCH$_2$$C$H−), 80 (−COO$C$(CH$_3$)$_3$), 157 (−NH$C$OOC(CH$_3$)$_3$), 176 (−CH$C$OOH), 178 (−N$C$OOCH−). IR: 1640 (tertiary amide (C−N)), 1710 (C═O), 1760 (carbamate (C═O)), 3350 (O−H, N−H), 3200 (O−H, N−H).

Deprotection of Amine Functionality of ***6****.* *N*-(*tert*-Butoxycarbonyl)-L-cysteine methyl ester functionalized 2-isopropenyl-2-oxazoline (25 mg, 3.5724 mmol) was either stirred with trifloroacetic acid (TFA) (3 mL) with DCM as solvent or HCl (1 mL) in methanol at room temperature overnight. The reaction was then concentrated with a rotary evaporator. The solid was dissolved in methanol, filtered, and precipitated in diethyl ether. The solid was collected. Representative characterization data follow. ^{1}H NMR (CD_3OD, δ, ppm): 1.0−1.2 (b (−CH_3)), 2.4−2.65 (b (−CHCH_2S−)), 2.65−3.1 (b (−CCHCH$_3$CH$_2$−)); (−SCH_2CH−), 3.4−3.6 (b (−NCH_2CH_2−)), 3.61−3.78 (b (−COOCH_3)), 4.35−4.55 (b (−SCH$_2$CH−)). ^{13}C NMR (CD_3-OD, δ, ppm): 19 (−CH$_3$), 35 (−CHCH$_2$S−), 37 (−CHCH$_2$S−), 38 (−SCH$_2$CH−), 46 (−NCH$_2$CH_2−), 54 (−COOCH$_3$), 55 (−SCH$_2$$C$H−), 170 (−CH$C$OOCH$_3$−), 178 (−N$C$OOCH−). IR: 1640 (tertiary amide (C−N)), 1680 (ester (C═O)), 3350 (O−H, N−H), 3200 (O−H, N−H).

Results and Discussion

To demonstrate the versatility of utilizing the thiol−ene to prepare a range of functional oxazoline monomers, thiols of varying reactivity were screened. Three representative thiols were selected: thiophenol to first verify the coupling procedure, methyl thioglycolate to investigate the compatibility of the polymerization with hydrogen bond accepting functionalities, and *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester as a route toward incorporating peptides or proteins.

Scheme 1. Synthetic Scheme of the Functionalization and Polymerization of 2-Isopropenyl-2-oxazoline[a]



[a] Reagents and conditions: (i) HS-R, butylamine resin, tetrahydrofuran (THF), room temperature; (ii) MeOTs, acetonitrile, 70 °C, 24 h.

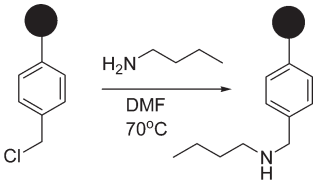
The functionalized monomers were prepared by reaction with the desired thiol and 2-isopropenyl-2-oxazoline to afford three different functionalized 2-oxazoline monomers (Scheme 1: monomers **1**−**3**). While this reaction can be catalyzed by a solution phase amine, an amino solid support was used to simplify purification. This resin was prepared by reacting Merrifield's resin, an insoluble polymer support consisting of chloromethylated polystyrene (1% cross-linked), with butylamine (Scheme 2).[36] The presence of the secondary amine on the solid support was verified using IR spectroscopy. Though initially the thiol−ene reactions were stirred overnight, later kinetic studies show that the reactions were complete in less than 1 min. The three functionalized monomers were then polymerized by cationic ring-opening mechanism with initiation from methyl tosylate followed by termination with water (Scheme 3) to yield three functionalized polymers (Scheme 1: polymers **4**−**6**).

The thiol−ene coupling reactions were carried out in THF at room temperature under nitrogen gas with a slight excess of 2-isopropenyl-2-oxazoline. The crude reaction mixtures were purified solely by filtration to remove the solid support and

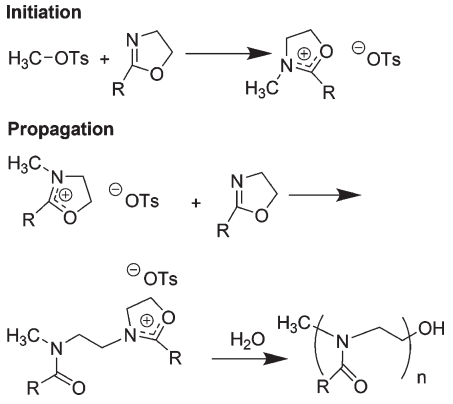
concentration *in vacuo* to remove both the solvent and unreacted 2-isopropenyl-2-oxazoline.

The functionalized monomers were characterized using ^{1}H (Figure 1) and ^{13}C (Supporting Information Figure S1) nuclear magnetic resonance (NMR) spectroscopy, and infrared (IR) spectroscopy (Figure 2), and gas chromatography−mass spectrometry (GCMS) (Supporting Information Figures S2 and S3) to confirm purity of the monomer and electrospray ionization (ESI) mass spectroscopy (Supporting Information Figure S4) to provide higher resolution mass spectral data. ^{1}H NMR (Figure 1) indicated quantitative coupling in the synthesis of the three monomers by the shift of the methylene protons of 2-isopropenyl-2-oxazoline from singlets (Figure 1, b) at 5.4 and 5.7 ppm to multiplets between the 2.7 and 2.9 ppm range for all three monomers. Also, the singlet attributed to the methyl protons adjacent to the double bonds (Figure 1, a) in 2-isopropenyl-2-oxazoline at 1.9 ppm is shifted to 1.2 ppm and is split to form a doublet. ^{13}C NMR (Figure S1) verified the shift of the terminal carbon of the alkene from 122 ppm between 31 and 35 ppm for the corresponding methylene adjacent to the thio ether. The disubstituted vinylic carbon with a resonance of 133 ppm shifts between 34 and 37 ppm corresponding to a methyne carbon in the product. Finally, the methyl at 19 ppm shifts to 17 ppm due to loss of the adjacent double bond.

Scheme 2. Synthesis of Secondary Amine Solid Support Catalyst in Dimethylformamide (DMF), 70 °C



Scheme 3. Polymerization Scheme of Functionalized 2-Isopropenyl-2-oxazolines[a]



[a] All polymers were purified by precipitation in diethyl ether.

GC-MS spectroscopy (Figures S2 and S3) was used to validate the purity of the 2-isopropenyl-2-oxazoline monomers. The gas chromatograph showed a shift in retention time for the three functionalized monomers with no contaminations by the starting materials. The mass spectra exhibited the expected molecular ions for all the monomers: 221.0 m/z (theoretical m/z: 221.3) for **1**, 217.3 m/z (theoretical m/z: 217.3) for **2**, and 346.5 m/z (theoretical m/z: 346.4) for **3**.

The use of ESI mass spectroscopy (Figure S4) allowed for increased resolution of the mass spectra relative to the GC-MS to provide further evidence for the quantitative thiol−ene coupling. The ESI spectrum of **1** shows the monoisotopic molecular ion peak at 244.078 m/z (theoretical m/z: 244.078) corresponding to $C_{12}H_{15}NOS$ + Na and a monoisotopic peak at 222.0925 m/z (theoretical m/z: 222.0945) corresponding to $C_{12}H_{15}NOS$ + H. The spectra of **2** show the monoisotopic molecular ion peak at 240.070 m/z (theoretical m/z: 240.067) corresponding to $C_9H_{15}NO_3S$ + Na and a monoisotopic peak at 218.088 m/z (theoretical m/z: 218.085) corresponding to $C_9H_{15}NO_3S$ + H. The spectra of **3** show the monoisotopic molecular ion peak at 369.152 m/z (theoretical m/z: 369.156) corresponding to $C_{15}H_{26}N_2O_5S$ + Na and a monoisotopic peak at 347.170 m/z (theoretical m/z: 347.164) corresponding to $C_{15}H_{26}N_2O_5S$ + H, all in agreement with the predicted values.

The IR of **1** (Figure 2) shows the loss of the alkene absorbance of the 2-isopropenyl-2-oxazoline starting material and new absorbances at 3080 and 3050 cm^{-1} corresponding to the aromatic

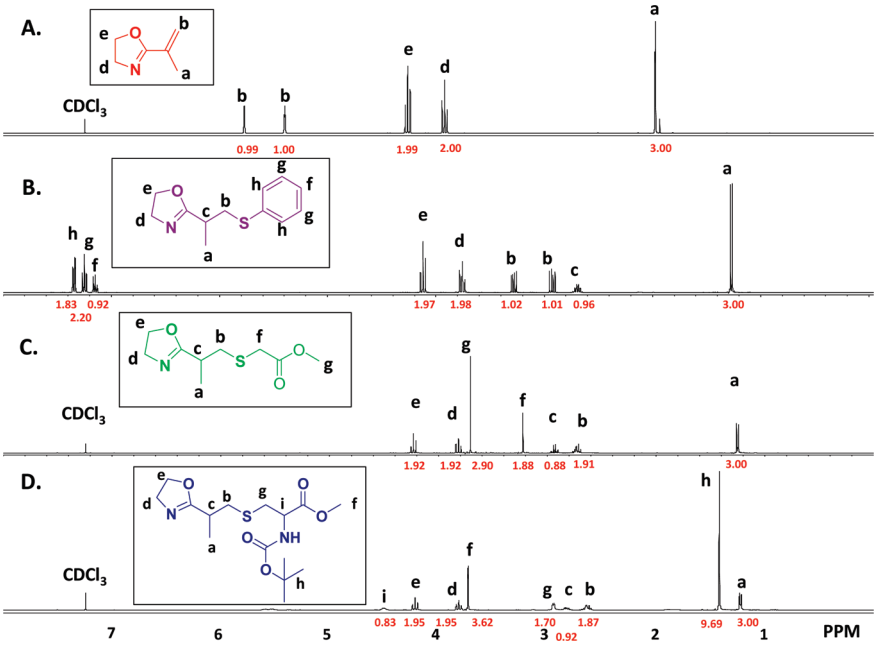


Figure 1. ^{1}H NMR spectra of functionalized 2-oxazoline monomers ($CDCl_3$): (A) 2-isopropenyl-2-oxazoline; (B) monomer **1**; (C) monomer **2**; (D) monomer **3**.

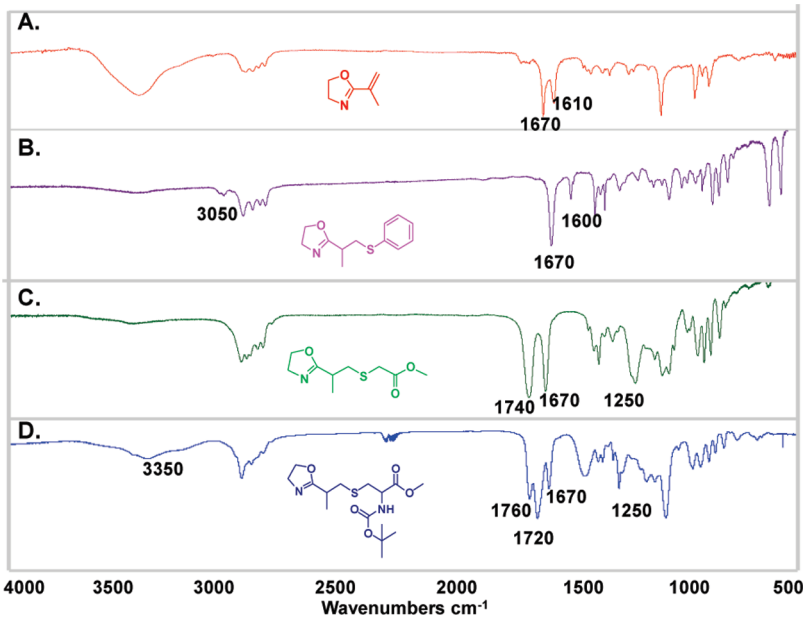


Figure 2. IR spectra of functionalized 2-isopropenyl-2-oxazoline monomers: (A) 2-isopropenyl-2-oxazoline; (B) monomer **1**; (C) monomer **2**; (D) monomer **3**.

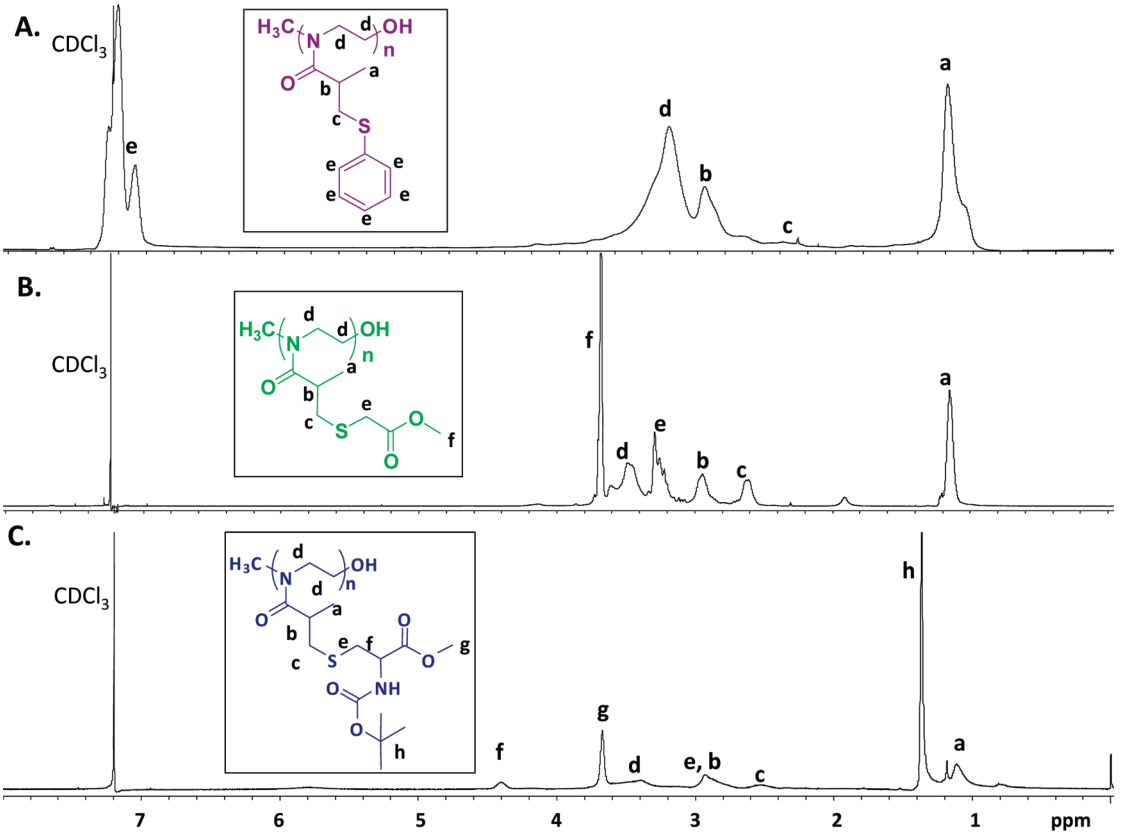


Figure 3. ^{1}H NMR spectra of functionalized 2-isopropenyl-2-oxazoline polymers ($CDCl_3$): (A) polymer **4**; (B) polymer **5**; (C) polymer **6**.

C−H bond stretch, 1600 cm^{-1} corresponding to the aromatic double bond stretches, and 1670 cm^{-1} corresponding to the imine bond stretch. The IR of **2** shows new absorbances at 1740 cm^{-1} corresponding to the carbonyl bond stretch, 1250 cm^{-1} corresponding to the ester O−C bond stretch, and 1670 cm^{-1} corresponding to the imine bond stretch. The IR of **3** shows new absorbances at 1720 cm^{-1} corresponding to the carbonyl bond stretch, 1250 cm^{-1} corresponding to the ester O−C bond stretch, 1760 cm^{-1} corresponding to the acylic amine O−C bond stretch, 3200 and 3350 cm^{-1} corresponding to the N−H bond stretch, and 1670 cm^{-1} corresponding to the imine bond stretch.

Once the functionalized 2-isopropenyl-2-oxazoline monomers were synthesized, they were then polymerized through a cationic ring-opening mechanism by initiation from methyl tosylate followed by termination with water (Scheme 3). The successful polymerization of all three monomers and preservation of

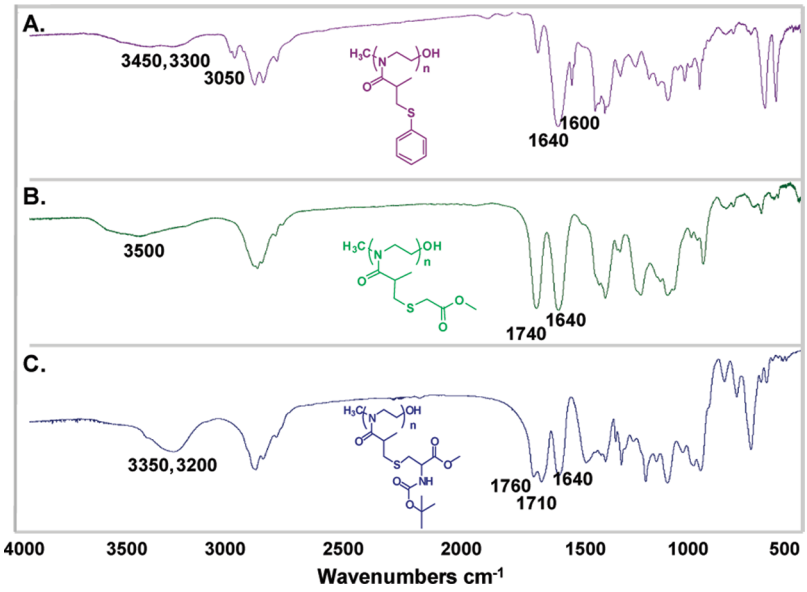


Figure 4. IR spectra of functionalized 2-isopropenyl-2-oxazoline polymers: (A) polymer **4**; (B) polymer **5**; (C) polymer **6**.

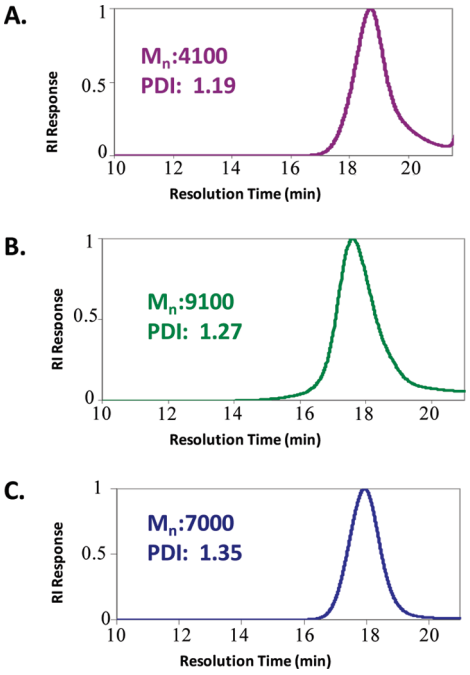


Figure 5. Representative gel permeation chromatograms of functionalized 2-isopropenyl-2-oxazoline polymers (THF as eluent, 2 mg/mL sample in THF): (A) polymer **4**; (B) polymer **5**; (C) polymer **6**.

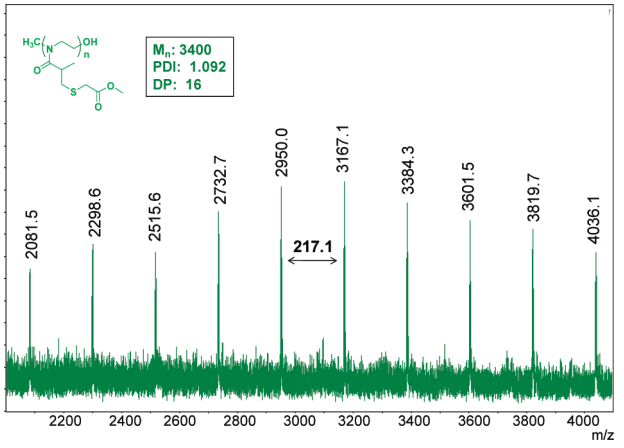


Figure 6. Representative matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectroscopy for polymer **5**. MALDI distribution exhibits a repeat unit mass of 217.1 (theoretical 217.1) and an average residual mass (when corrected for ionization by complexation with Na^+) of 105.0 *m*/*z*, which corresponds closely to the expected chain transfer end-groups of −H and $-SCH_2COOCH_3$ with a combined theoretical mass of 106.0 *m*/*z*.

side-chain functionalities were confirmed by ^{1}H (Figure 3) and ^{13}C NMR (Supporting Information Figure S5), IR (Figure 4), gel permeation chromatography (GPC) (Figure 5, Table 1), and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectroscopy (Figure 6, Supporting Information Figure S6, Table 1).

^{1}H NMR (Figure 3) indicated polymerization by the broadening of all the proton peaks of the three monomers combined with the shift of the proton resonances between the 3.75−3.85 ppm range (Figure 1, d) and the 4.15−4.2 ppm range (Figure 1, e) corresponding to methylene units in the 5-membered ring to one broad peak spanning 3.1−3.6 ppm (Figure 3A, d) for **4**, 3.4−3.6 ppm (Figure 3B, d) for **5**, and 3.1−3.4 ppm (Figure 3C, d) for **6**. ^{13}C NMR (Figure S5) also showed a shift of the carbon resonances from 54 and 68 ppm corresponding to the two adjacent methylene carbons in the 5-membered ring to one broad signal at 45−48 ppm assigned to the methylene carbons of the polymer backbone. There is also a slight shift in signal of the tertiary carbon of the 5-membered ring from 170 to 176 ppm with the formation of an amide carbonyl during the ring-opening.

IR was also used to verify the rather substantial functional group changes during the polymerizations (Figure 4). The loss of the imine absorbance at 1670 cm^{-1} and the appearance of the tertiary amide stretching at 1640 cm^{-1} for all three polymers verify the ring-opening polymerization. Also, the appearance of peaks in all three polymers between 3300 and 3500 cm^{-1} corresponds to the hydroxyl terminus of the polymer and water bound to the hydrophilic backbone. The IR also shows expected absorbance of the side-chain functionalities for the phenyl thioether **4** at 3080 and 3050 cm^{-1} corresponding to aromatic C−H stretch and 1600 cm^{-1} corresponding to the aromatic double bonds. For the methyl thioglycolate side chains of **5**, an additional ester carbonyl absorbance was observed at 1735 cm^{-1}.

For the protected cysteine **6**, a series of new absorbances were observed at 1710 cm^{-1} corresponding to the carbonyl stretch, 1760 cm^{-1} corresponding to the acylic amine O−C stretch, and 3200 and 3350 cm^{-1} corresponding to the N−H stretch.

Attempts to polymerize these functional oxazoline monomers yielded consistent molecular weights with low degrees of polymerization (DP) and narrow polydispersities (PDI). Table 1 documents the number-average molecular weights (M_n), PDIs, and DPs with varying reaction conditions for a series of polymerizations as reported by GPC (representative, Figure 5) for the three polymers and MALDI-TOF MS (Figure S6 (**4**), Figure 6 (**5**)) for the polymers from the benzyl **4** and methyl thioglycolate **5** functionalized. Though other groups have shown that polymers of 2-oxazolines can be polymerized with molecular weights of up to 15 000 with the use of a microwave reactor[37] and without microwave catalysis for the case 2-ethyl-2-oxazoline,[38] attempts to prepare thioether-functionalized polymers without microwave catalysis yielded polymers with more modest molecular weights. The polymerization of the phenylsulfide-functionalized monomer, **1**, resulted in polymers **4a**−**e**, which exhibited low molecular weights with a range between 3800 and 4300, degrees of polymerization from 16 to 19, and polydispersities below 1.3. The acid-catalyzed polymerization of the methyl thioglycolate-functionalized monomer, **2**, resulted in polymers **5a**−**e** with a similar molecular weight range of 4000−4600, degrees of polymerization from 16 to 23, and polydispersities below 1.4. However, a higher molecular weight polymer sample (M_n = 9100) could be isolated by fractionation of polymer **5f** (detailed work-up in Experimental Section). Finally, the sterically bulky *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester-functionalized monomer, **3**, yielded polymers **6a**−**d** which again exhibited a modest molecular weight range from 2800 to 5000, degrees of polymerization between 8 and 14, and polydispersities below 1.4. For all three monomers, all attempts failed to prepare higher molecular weight polymers despite variations in the reaction conditions, suggesting the possibility of chain transfer or other side reactions. Recently, Kempe et al.[39] proposed that the cationic ring-opening polymerization of a 2-oxazoline monomer containing a thioether bond was not living due to the nucleophilic character of that functionality. The thioether was proposed to carry out nucleophilic attack on the propagating oxazolium species, generating a sulfonium ion, followed by a chain transfer event. The possibility of chain transfer was supported by MALDI-TOF MS end-group analysis which in the case of polymer **5e** afforded a combined residual mass of 105 Da, close to the expected 106 Da for initiation from H^+ and termination with thioglycolate. In addition, a much weaker signal was observed which corresponded to the original initiation from the methyl cation and termination with thiophenol for polymer 4 (Figure S6). Kinetic studies which compared the conversion of monomer **1** to the molecular weight of polymer **4** exhibited a molecular weight that sharply increased at low monomer conversion and then plateaued despite continued conversion of monomer, providing further evidence of the nonliving character of the polymerization (Figure 7).

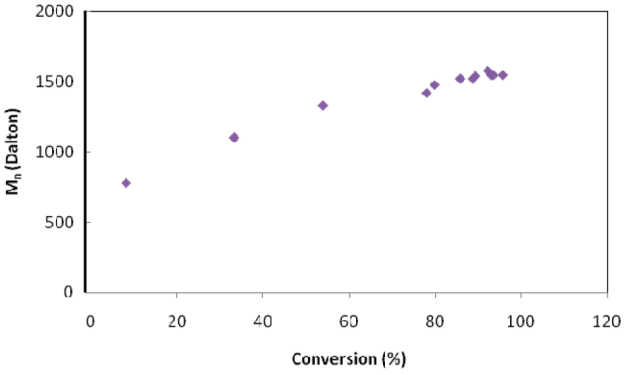


Figure 7. Number-average molecular weight (M_n) and conversion plot for the polymerization of **4** in acetonitrile at 65 °C.

The successful prepolymerization introduction of functionality via thiol−ene coupling was confirmed for a diversity of functional monomers. However, because of the incompatibility of many basic or nucleophilic functionalities with the cationic polymerization conditions, protecting groups had to be used with side chains including both amines and carboxylic acids. The deprotection reactions of **5** to remove the methyl ester as well as the removal of the amine and carboxylic acid functionalities of **6** were next explored and characterized. The deprotection of the methyl ester of **5** was verified by a loss of the methyl ester resonance at 3.4−3.6 ppm in the ^1H NMR (Figure 8, f) and at

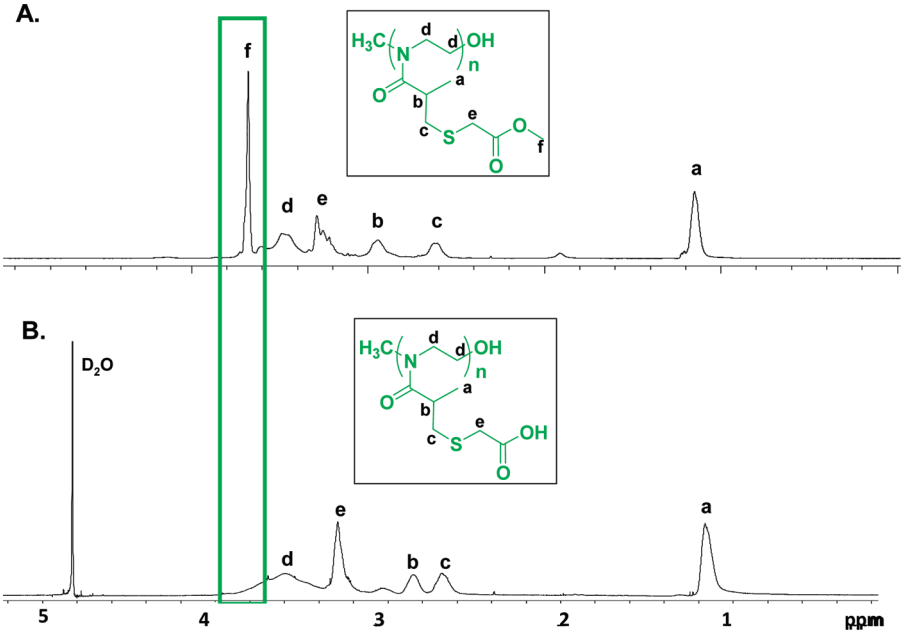


Figure 8. ^1H NMR spectra of (A) polymer **5** ($CDCl_3$) and (B) deprotected thioglycolate-functionalized 2-isopropenyl-2-oxazoline polymer (D_2O). The absence of the methyl ester protons (f) suggests the deprotection occurred.

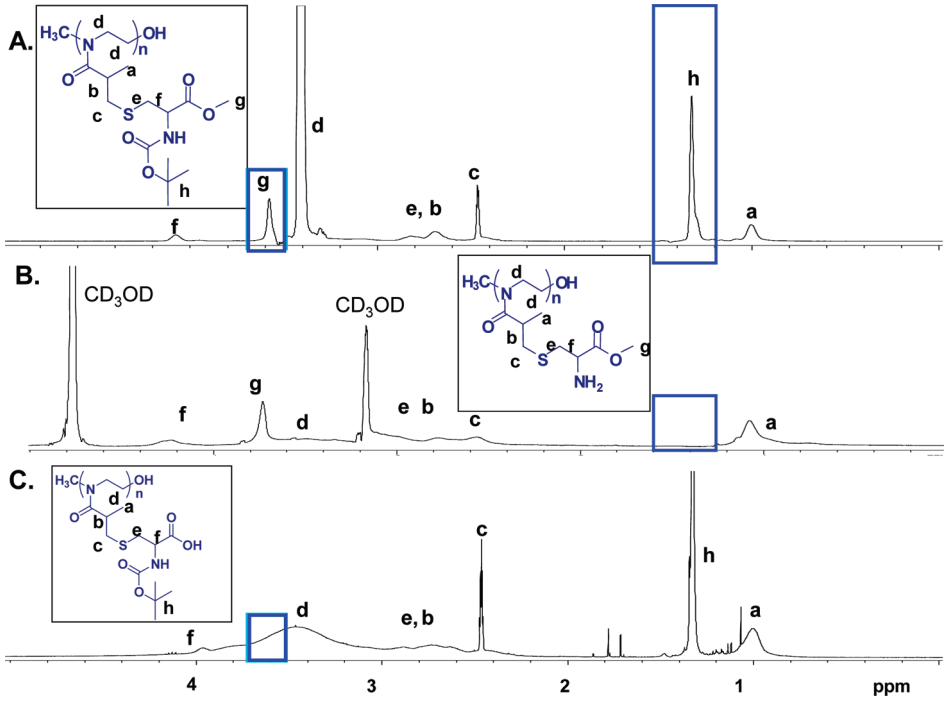


Figure 9. ^{1}H NMR spectra of (A) polymer **6** (DMSO) and (B) deprotected L-cysteine methyl ester-functionalized 2-isopropenyl-2-oxazoline polymer (CD_3OD). The absence of the methyl *tert*-butyl protons (h) indicates the deprotection occurred. (C) Deprotected *N*-(*tert*-butoxycarbonyl)-L-cysteine-functionalized 2-isopropenyl-2-oxazoline polymer (DMSO). The absence of the methyl ester protons (g) indicates the deprotection occurred.

51 ppm in the ^{13}C NMR (Supporting Information, Figure S7, f) as well as a shift in the IR (Supporting Information, Figure S8) from 1735 cm^{-1} for the carbonyl stretching to 1660 cm^{-1}.

After the deprotection of the *tert*-butoxycarbonyl group on **6**, a loss of the *tert*-butyl protons at 1.25−1.5 ppm in the ^{1}H NMR (Figure 9A,B, h) and the carbon resonances at 29 ppm in the ^{13}C NMR (Supporting Information, Figure S9A,B, i) was observed. The loss of the tertiary carbon resonance at 81 ppm (Figure S9A, B, j) and the carbonyl carbon resonance at 156 ppm is also observed (Figure S9A,B, k). The loss of the acylic amine O−C bond stretch at 1760 cm^{-1} observed in the IR (Supporting Information, Figure S10) further suggests the deprotection of the *tert*-butyl group. Characterization of the methyl ester deprotection of **6** shows an absence of the methyl ester protons at 3.61−3.78 ppm in the ^{1}H NMR (Figure 9A,C, g) and at 52 ppm in the ^{13}C NMR (Figure S9A,C, f). The IR shows a shift from 1710 cm^{-1} corresponding to the carbonyl bond stretch to 1680 cm^{-1} (Figure S10). After quantitative deprotection of the methyl ester functionality of **5** and the amine and the carboxylic acid functionalities of **6**, these diverse polymers now have the potential for use in biological applications, including the further reaction of the cysteine functionalized polymer **6** to yield peptide or protein conjugates.

Conclusions

The prepolymerization functionalization of oxazoline monomers has been investigated for the installation of diverse functionality along the poly(*N*-acylethylenamine) backbone. Through the use of thiolene coupling between a thiol and the pendant alkene of 2-isopropenyl-2-oxazoline, the monomer functionalization route has enabled the preparation of PNAI with aryl, ester, amine, and carboxylic acid side chains. The resulting polymers could be prepared with molecular weights ranging from 3000 to 8000 and polydispersities typically below 1.3. Of particular interest, a protected cysteine amino acid was successful coupled to the 2-isopropenyl-2-oxazoline monomer and polymerized. The selective deprotection of either the *t*-BOC protection group to expose an N-terminus on each repeat unit or the methyl ester protection group to expose a carboxylic acid on each repeat unit was demonstrated, highlighting the compatibility of this coupling and polymerization chemistry with conjugation to peptides and proteins. The diversity of side-chain modifications combined with the water solubility of biocompatibility of the poly(oxazoline) backbone make this synthetic route an attractive method for preparing polymer bioconjugates. Because the attachment occurs through the side chains rather than the end groups of the polymer, a much higher degree of modification can be obtained than with traditional poly(ethylene glycol) end-group conjugation.

Acknowledgment. The authors thank the Tulane University for support of this research and the Louisiana Board of Regents for a graduate fellowship (MAC). The authors also acknowledge the National Science Foundation (NSF-MRI 0619770) for enabling mass spectral characterization.

Supporting Information Available: ESI spectra of monomers, GCMS of starting materials and monomers, ^{13}C NMR of all monomers and polymers, representative MALDI-TOF spectrum of polymer **4**, and IR of the deprotection reactions of polymers **5** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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