Rapid Metal-Free Macromolecular Coupling via *In Situ* Nitrile Oxide-Activated Alkene Cycloaddition

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ABSTRACT: Nitrile oxide 1,3 dipolar cycloaddition is a simple and powerful coupling methodology. However, the self-dimerization of nitrile oxides has prevented the widespread use of this strategy for macromolecular coupling. By combining an *in situ* nitrile oxide generation with a highly reactive activated dipolarophile, we have overcome these obstacles and present a metal-free macromolecular coupling strategy for the modular synthesis of several ABA triblock copolymers. Nitrile oxides were generated *in situ* from chloroxime terminated poly(dimethylsiloxane) B-blocks and coupled with several distinct hydrophilic (poly(2-methyloxazoline) and poly(ethylene glycol)), and poly(*N*-isopropylacrylamide) or hydrophobic (poly(L-lactide) A-blocks terminated in activated dipolarophiles in a rapid fashion with high yield. This methodology overcomes many drawbacks of previously reported metal-free methods due to its rapid kinetics, versatility, scalability, and ease of introduction of necessary functionality. Nitrile oxide cycloaddition should find use as an attractive macromolecular coupling strategy for the synthesis of biocompatible polymeric nanostructures. © 2014 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2014**, *52*, 3134–3141

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INTRODUCTION Polymeric amphiphiles capable of self-assembling into higher-ordered structures have emerged as an important class of soft materials in the biomedical field. Their amphiphilic nature leads to self-assembly upon hydration, allowing for the compartmentalization of therapeutics or other biomediscally useful agents.[1–4] These are often block copolymers that can self-assemble into a wide variety of morphologies, including micelles, vesicles, and tubular structures, under appropriate conditions.[5–7] Vesicular structures are particularly attractive due to their shell-like membrane surrounding an aqueous core, which can encapsulate therapeutics.[8,9] It is critical to generate nanostructures with well-defined physical parameters such as size, morphology, and shape, since these features influence their interactions with the complement system determining their immune response.[10] The molecular structure of block copolymers dictates the physical properties of resulting nanostructures, with molecular weight and hydrophobic/hydrophilic ratios being of utmost importance.[5,6,11,12] Therefore a precise and well-controlled synthesis is essential, which is often difficult to achieve with common methods such as macroinitiation.

Click chemistry has emerged as a powerful tool for the synthesis of polymeric architectures via polymer-polymer coupling.[13–16] Polymeric blocks may be clicked in a modular fashion, often in near-quantitative yields using molar equivalents of reactants resulting in minimal purification. By allowing for the synthesis and characterization of individual blocks prior to coupling, well-defined polymeric architectures can be designed. Furthermore, the click approach allows for the systematic investigation into the effects of single parameter variations, such as block length or identity, on the resulting nanostructure morphology or biocompatibility. Although the click approach is an exceptional method for block copolymer synthesis, not all click reactions meet the stringent requirements for use in biomedical applications.

The optimal polymer-polymer coupling strategy should be clickable, scalable, and versatile in reaction scope, and provide biocompatible nanostructures. We have previously demonstrated the synthesis of amphiphilic ABA triblock copolymers containing a hydrophobic poly(dimethylsiloxane) (PDMS) B-block flanked by hydrophilic A-blocks using two distinct click methods. Our initial efforts used the prevalent

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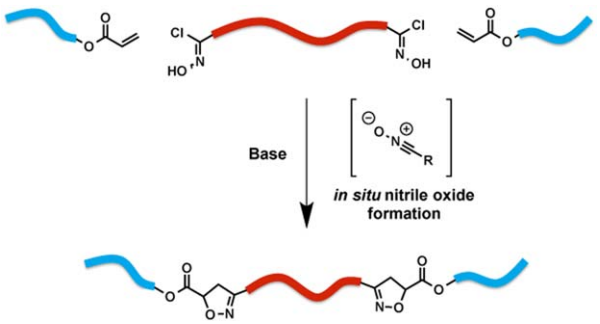


SCHEME 1 1,3-dipolar cycloaddition between polymeric blocks terminated in nitrile oxides and activated-alkene end-groups to provide ABA triblock copolymers. Due to their inherent instability, nitrile oxides are formed *in situ* from chloroximes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

copper-catalyzed azide-alkyne cycloaddition (CuAAC) to synthesize poly(oxazoline)-poly(siloxane)-poly(oxazoline) (*Ox*-ABA) and poly(ethylene glycol)-poly(siloxane)-poly(ethylene glycol) (*P*-ABA) triblock copolymers.[17] Although CuAAC demonstrated scalability and is known to be wide in scope, trace amounts of residual copper diminished pivotal *in vitro* stealth and biocompatibility attributes of polymer vesicles (polymersomes) assembled from this method. This led to the development of a metal-free coupling strategy using the strain-promoted azide-alkyne cycloaddition (SPAAC) for the synthesis of *P*-ABA and *Ox*-ABA triblocks.[18] Polymersomes assembled via SPAAC demonstrated superior *in vitro* biocompatibility and stealth properties compared with their copper-catalyzed counterparts, confirming the requirement for a metal-free click methodology for these structures. However, the cyclooctyne component is cumbersome to synthesize and commercially expensive to purchase, therefore SPAAC does not lend itself to scale-up.

Although several clickable metal-free polymer-polymer coupling methods have been reported, each has drawbacks that hinder their scope as an ideal and universally applicable methodology. A conventional Diels-Alder reaction has been used for macromolecular coupling, however this reversible cycloaddition required harsh conditions and nonequimolar reactant ratios.[19] The hetero-Diels-Alder reaction has found use for coupling macromolecules synthesized via reversible-addition-fragmentation transfer polymerization,[20] but introduction of the activated dithioester dienophile moiety does not lend itself to other polymerization techniques such as cationic ring-opening polymerization (CROP). Tetrazine-norbornene polymer-polymer coupling has demonstrated versatility in synthesizing several diblock copolymers. However, tetrazine synthesis is low yielding and cumbersome, thus undesirable for scale-up.[21] Despite its popularity, thiol-ene metal-free click coupling was found to be an unsuitable method for block copolymer coupling due to poor conversion.[22] Aldehyde-hydrazine coupling was achieved via a Schiff base linker to form diblock copolymers under harsh reaction conditions.[23] The resulting acylhydrazone linker is readily cleaved at low pH, and though useful for stimuli responsiveness, the cleavable linker is undesirable for other applications. Inverse electron demand Diels-Alder reactions between tetrazine and transcyclooctene display rapid kinetics, but the reactants require cumbersome synthetic techniques, rapidly decompose in water, and have low nucleophilic group tolerance.[24] Furthermore, there are no reports of macromolecular coupling using this methodology.

The 1,3-dipolar cycloaddition between nitrile oxide dipoles and dipolarophiles offers an appealing strategy for the synthesis of block copolymers via metal-free click coupling. The nitrile oxide cycloaddition (NOC) couples nitrile oxides with activated-alkenes to provide isoxazoline heterocycles. Due to the inherent instability of nitrile oxides, they are derived *in situ* from chloroxime precursors using an organic base. These highly reactive dipoles cyclize with a variety of activated-alkene dipolarophiles with attractive reaction kinetics, are easily installed moieties, and lead to highly regioselective products.[25–27] They have found use as metal-free click reactions for polymer end group modification,[28,29] biomolecular conjugation, and as monomers for polymerizations via polycycloaddition,[30–32] but their utility as macromolecular coupling agents has not yet been established. Herein, we demonstrate the first report of using NOC as a metal-free macromolecular coupling strategy for the modular synthesis of several ABA triblock copolymers (Scheme 1). However, the difficulty of obtaining the precise molecular weight of the polysiloxane B-block used in this work prevents the equimolar addition of the compounds. Thus, we refrain from referring to this as a click coupling strategy. However, it should be stressed this is due to the choice of B-block and not a drawback of the coupling methodology.

EXPERIMENTAL

Materials

p-Toluenesulfonyl chloride (TsCl; Alfa Aesar, 98%), 4-(dimethylamino)pyridine (DMAP; Fluka, 99%), sodium hydroxide (NaOH; Fisher Chemical), sodium sulfate anhydrous (Na_2SO_4; Fisher Chemical, 99%), potassium carbonate anhydrous (K_2CO_3; Fisher Chemical), 1,3-*bis*(4-hydroxybutyl)tetramethyldisiloxane (Gelest, 95%), sulfuric acid (H_2SO_4; Fisher Chemical, 98%), triethylamine (TEA; Fisher Chemical), di-*tert*-butyl dicarbonate [$(Boc)_2O$] (Aldrich, 99%), acryloyl chloride (Aldrich, 97%), 4-formylbenzoic acid (Aldrich, 97%), hydroxylamine hydrochloride (Sigma-Aldrich, 99%), pyridine (Fisher Chemical, 99%), *N*-chlorosuccinimide (NCS; Aldrich, 98%), *N,N*′-dicyclohexylcarbodiimide (DCC; Aldrich, 99%), poly(*N*-isopropylacrylamide), maleimide terminated (NIPAM-maleimide; Aldrich, M_n 2.00 k), and poly(L-lactide), 2-hydroxyethyl methacrylate terminated (PLA-methacrylate; Aldrich, $M_n = 2.00$ k, PDI ≤ 1.1) were used as received. Poly(ethylene glycol) methyl ether was a gift from Prof. Dr. Craig Hawker. PEG-acrylate ($M_n = 2.00$ k) was prepared from poly(ethylene glycol) methyl ether and acryloyl chloride following a previously reported protocol.[33] Acrylic acid was distilled over the radical quencher *p*-methoxyphenol and distilled under reduced pressure. Silica gel (Silicycle, 230–400

mesh) was deactivated by spinning in an appropriate amount of TEA:Hexanes v/v 5:95 solution for 10 min followed by filtration and several washes with hexanes. 2-Methyl-2-oxazoline (Aldrich, 98%) was purified by stirring in calcium hydride (CaH_2) overnight then fractionally distilling through a 10 cm vigreux column under argon. Octamethylcyclotetrasiloxane (D_4; Gelest, 95%) was distilled from CaH_2 under reduced pressure. Tetrahydrofuran (THF), dichloromethane (DCM), toluene (PhMe), acetonitrile (MeCN), and *N,N′*-dimethylformamide (DMF) were obtained from a PureSolv solvent purification system.

Instrumentation

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DMX500MHz SB NMR Spectrometer or Varian VNMRS 600MHz SB NMR Spectrometer using solutions of samples in deuterated chloroform. Gel permeation chromatography (GPC) was performed in chloroform (0.25% TEA) on a Waters equipped with a refractive index detector. Molecular weights of polymers were calculated relative to linear polystyrene standards.

Synthesis of *Bis*-Benzaldehyde Disiloxane End-Blocker (3)

A solution of 1,3-*bis*(4-hydroxybutyl)tetramethyldisiloxane (5.40 mL, 18.0 mmol, 1.0 equiv) and 4-formylbenzoic acid (6.74 g, 450 mmol, 2.5 equiv) in DCM (90 mL) was cooled to 0 °C. DCC (10.2 g, 50.0 mmol, 2.8 equiv) was then added portion wise followed by DMAP (44 mg, 0.36 mmol, 0.02 equiv). The mixture was allowed to warm to room temperature and stirred overnight after which time TLC showed consumption of the starting material. The slurry was diluted with Et_2O, filtered through celite, and concentrated to give pale yellow oil. This crude oil was purified by column chromatography on silica gel (EtOAc:Hexanes, 15/85) to give 3 as a colorless oily solid. Yield: 6.54 g (67%). ^{1}H NMR (500 MHz, $CDCl_3$, δ):10.1 (s, 2H, CHO), 8.18 (d, $J = 8.0$ Hz, 4H, ArH), 7.94 (d, $J = 8.5$ Hz, 4H, ArH), 4.36 (t, $J = 6.75$ Hz, 4H, CH_2), 1.81 (m, 4H, CH_2), 1.49 (m, 4H. CH_2), 0.59 (t, 4H, CH_2), 0.057 (s, 12H, CH_3).^{13}C NMR (600 MHz, $CDCl_3$, δ):191.8, 165.8, 139.2, 135.6, 130.3, 129.6, 65.5, 32.2, 20.0, 18.1, 0.486.

Synthesis of *Bis*-Benzaldehyde PDMS (4)

Bis-benzaldehyde end-blocker (**3**) (0.700 g, 1.29 mmol, 1.0 equiv) was added to an oven-dried round bottom flask with a stir bar and stirred under reduced pressure for 30 min at room temperature to remove trace water. The end-blocker was first dissolved in PhMe (1 mL), then freshly distilled D4 (8.00 mL, 25.8 mmol, 20 equiv) was added, and the mixture was heated to 80 °C to fully solubilize the reagents. Concentrated H_2SO_4 (0.030 mL) was added dropwise, at which time the solution became viscous. After 21 h an aliquot was taken for ^{1}H NMR, which indicated complete monomer consumption. The reaction was cooled to room temperature, diluted with hexanes, quenched with Et_3N (1 mL), and stirred for 30 min. The solvent was concentrated *in vacuo*. The polymer was dissolved in hexanes, washed with MeOH (3 times), and dried over anhydrous Na_2SO_4 and concentrated to give pale yellow viscous oil. Yield: 7.00 g (80%), $M_{n,1HNMR}$ 7.96 k. $M_{n,\ GPC}$ 7.18 k, PDI 1.50. ^{1}H NMR (500 MHz, $CDCl_3$, δ):10.1 (s, 2H, CHO), 8.19 (d, $J = 8.5$ Hz, 4H, ArH), 7.94 (d, $J = 8.5$ Hz, 4H, ArH), 4.36 (t, $J = 6.5$ Hz, 4H, CH_2), 1.82–1.87 (m, 4H, CH_2), 1.51 (m, 4H, CH_2), 0.62 (t, 4H, CH_2), 0.070 (s, 612H, CH_3).

Synthesis of *Bis*-Aldoxime PDMS (5)

Bis-benzaldehyde PDMS (**4**) (3.45 g, 0.434 mmol, 1.0 equiv) was dissolved in THF (20 mL) then pyridine (0.20 mL, 2.60 mmol, 6.0 equiv) was added. A solution of hydroxylamine hydrochloride (0.12 g, 1.74 mmol, 4.0 equiv) in MeOH (10 mL) was added dropwise at room temperature. The solution was stirred overnight, after which ^{1}H NMR demonstrated complete conversion to the *bis*-aldoxime. The reaction was diluted with hexanes, washed with MeOH (3 times), dried over anhydrous Na_2SO_4 and concentrated to give clear oil. Yield: 3.14 g (91%). $M_{n,1HNMR}$ 8.58 k. $M_{n,\ GPC}$ 7.44 k, PDI 1.52. ^{1}H NMR (500 MHz, $CDCl_3$, δ):8.16 (s, 2H, CNOH), 8.04 (d, $J = 8.5$ Hz, 4H, ArH), 7.64 (d, $J = 8.5$ Hz, 4H, ArH), 4.33 (t, $J = 6.5$ Hz, 4H, CH_2), 1.80–1.85 (m, 4H, CH_2), 1.51 (m, 4H, CH_2), 0.61 (t, 4H, CH_2), 0.070 (s, 660H, CH_3).

Synthesis of *Bis*-Chloroxime PDMS (6)

To a solution of *bis*-aldoxime PDMS (**5**) (2.14 g, 0.250 mmol, 1 equiv) in THF (12 mL) was added DMF (4 mL). The solution was cooled to 0 °C then NCS (0.080 g, 0.60 mmol, 2.4 equiv) was added. The solution was allowed to warm to room temperature and stirred overnight after which ^{1}H NMR demonstrated complete conversion to the *bis*-chloroxime. The reaction was diluted with hexanes, transferred to a separatory funnel and a small amount of MeOH (about 1 mL) was added to promote phase separation. The DMF/MeOH layer was removed and the hexanes layer was washed with MeOH (6 times), dried over anhydrous Na_2SO_4 and concentrated to give clear oil. Yield: 2.00 g (93.4%). $M_{n,\ 1HNMR}$ 9.54 k.^{1}H NMR (500 MHz, $CDCl_3$, δ):8.06 (d, $J = 8.5$ Hz, 4H, ArH), 7.92 (d, $J = 8.5$ Hz, 4H, ArH), 4.34 (t, $J = 6.5$ Hz, 4H, CH_2), 1.78–1.83 (m, 4H, CH_2), 1.51 (m, 4H, CH_2), 0.61 (t, 4H, CH_2), 0.070 (s, 732H, CH_3).

Synthesis of 3-Boc-Aminobenzyl Alcohol (8)

To a solution of 3-aminobenzyl alcohol (12.9 g, 105 mmol, 1.0 equiv) in THF (112 mL) was added 2M NaOH aq. (66 mL, 132 mmol, 1.2 equiv) at room temperature. The suspension was cooled to 0 °C and solution of Boc_2O (25.7 g, 117 mmol, 1.1 equiv) in THF (112 mL) was added dropwise. The reaction was allowed to warm to room temperature and stirred overnight, after which TLC showed consumption of the starting material. The slurry was filtered through celite, diluted with ethyl acetate then placed in a separatory funnel and the aqueous layer was removed. The organic layer was washed with H_2O, brine, dried over anhydrous Na_2SO_4, and concentrated. The resulting crude oil was purified by column chromatography on deactivated silica gel (EtOAc:Hexanes, 30/70) to give **8** as white solid. Yield: 13.9 g (59%). 1H NMR (500 MHz, $CDCl_3$, δ):7.45 (s, 1H, ArH), 7.28 (t, $J = 7.8$ Hz, 1H, ArH), 7.21 (t, $J = 7.2$ Hz, 1H, ArH), 7.04 (d, $J = 6.6$ Hz, 1H, ArH), 6.47 (s, 1H, NH), 4.67 (m, 2H, CH_2), 1.63 (t,

1H, OH), 1.52 (s, 9H, CH_3). ^{13}C NMR (600 Hz, $CDCl_3$, δ):152.9, 142.1, 138.7, 129.3, 121.6, 117.9, 117.1, 65.3, 28.5.

Synthesis of 3-Boc-Aminobenzyl Tosylate (9)

To a solution of 3-Boc-aminobenzyl alcohol (**8**) (4.05 g, 18.1 mmol, 1.0 equiv) and DCM (90 mL) cooled to 0 °C was added TsCl (3.62 g, 19.0 mmol, 1.05 equiv) and DMAP (0.22 g, 1.81 mmol, 0.1 equiv) followed by Et_3N (3.80 mL, 27.2 mmol, 1.5 equiv) dropwise. The solution was stirred at 0 °C, and after 1 hr TLC showed consumption of the staring material. The reaction was quenched with saturated $NaHCO_3$ aq. and the organic layer was washed with H_2O, brine, dried over anhydrous Na_2SO_4, and concentrated. The resulting gummy solid was purified by column chromatography on deactivated silica gel (EtOAc:Hexanes, 30/70) to give **9** as white solid. Yield: 3.24 g (47%). ^1H NMR (500 MHz, $CDCl_3$, δ):7.79 (d, $J = 10.2$, 2H, ArH), 7.33–7.27 (m, 4H, ArH), 7.22 (t, $J = 9.3$ Hz, 1H, ArH), 6.93 (d, $J = 9.0$ Hz, 1H, ArH), 6.44 (s, 1H, ArH), 5.02 (s, 2H, CH_2), 2.44 (s, 3H, CH_3), 1.52 (s, 9H, CH_3). ^{13}C NMR (600 MHz, $CDCl_3$): $\delta = 152.7$, 144.9, 138.8, 134.4, 133.4, 130.0, 129.5, 128.1, 123.0, 119.0, 118.4, 28.5, 21.8.

Synthesis of PMOXA-Acrylate (10)

3-Boc-aminobenzyl tosylate (**9**) (1.62 g, 4.29 mmol, 1.0 equiv) was added to an oven-dried round bottom flask with a stir bar and stirred at room temperature under reduced pressure overnight to remove trace water. MeCN (5.5 mL) was added and the reaction was heated to 45 °C to solubilize the initiator, at which time freshly distilled 2-methyl-2-oxazoline (5.5 mL, 64.4 mmol, 15 equiv) was added. The temperature was increased to 80 °C and the reaction was stirred for 21 h at which time ^1H NMR demonstrated full initiation and complete monomer consumption. Additional MeCN (6 mL) was added to decrease the reactions viscosity. The reaction was cooled to 0 °C and freshly distilled acrylic acid was added (0.42 mL, 6.14 mmol, 1.4 equiv) followed by Et_3N (1.2 mL, 8.37 mmol, 1.95 equiv). The reaction was heated to 60 °C and stirred overnight, after which ^1H NMR demonstrated complete termination. The reaction was cooled to room temperature, precipitated into cold Et_2O, filtered, and the solid was washed with Et_2O then collected with DCM and concentrated. The solid was dissolved in acetone, K_2CO_3 (10 g) was added, and the mixture was stirred at room temperature overnight. The solution was then concentrated, dissolved in DCM, filtered through celite, and the filtrate was concentrated. The solid was redissolved in a minimal amount of DCM and precipitated into cold Et_2O, filtered, and the solid was washed with Et_2O then collected with DCM and concentrated to yield PMOXA-acrylate (**10**) as a white solid in quantitative yield. $M_{n,\ 1H\ NMR}$ 2.32 k, $M_{n,\ GPC}$ 1.73 k, PDI 1.42.^1H NMR (500 MHz, $CDCl_3$, δ):6.95–7.73 (m, 3H, ArH), 6.85 (m, 1H, ArH), 6.38–6.46 (m, 1H, Acrylate), 6.10–6.16 (m, 1H, Acrylate), 5.88–5.95 (m, 1H), 4.49 (t, 2H, CH_2), 4.29 (s, 2H, CH_2), 3.46-3.50 (br. m, 96H, CH_2), 2.15 (br. m, 72H, CH_3), 1.52 (s, 9H, CH_3).

Synthesis of PMOXA-PDMS-PMOXA (11)

To a solution of PMOXA-acrylate (**10**) (0.106 g, 0.0460 mmol, 2.2 equiv) and *bis*-chloroxime PDMS (6) (0.200 g, 0.0209 mmol, 1 equiv) in $CHCl_3$ (2 mL) was added Et_3N (0.030 mL, 0.209 mmol, 10 equiv). The solution was stirred for 1 h at room temperature. On removal of the solvent, diethyl ether (20 mL) was added. The solution was filtered and filtrate was concentrated. The residue was dissolved in THF, filtered to remove the TEA salt, and concentrated to yield **11** as a colorless solid, yield 91.2%. ^1H NMR (600 MHz, $CDCl_3$, δ):8.30–6.95 (m, 6H, ArH), 8.08 (d, 4H, ArH), 7.72 (d, 4H, ArH), 6.83 (m 2H, ArH), 5.24 (m, 2H, CH), 4.48 (t, 4H, CH_2), 4.33 (m, 8H, CH_2), 3.45-3.49 (br. m, 192H, CH_2), 2.15 (br. m, 144H, CH_3), 1.80 (m, 4H, CH_2), 1.50 (s, 18H, CH_3) 0.61 (t, 4H, CH_2), 0.066 (s, 732H, CH_3). GPC M_n 21.2 k; PDI 1.2.

Synthesis of PLA-PDMS-PLA (12)

Same procedures were followed as preparation of **11** except the reaction was stirred for 2 h at room temperature. PLA-PDMS-PLA (**12**) was obtained as a white solid, yield 90.9%. 1H NMR (500 MHz, $CDCl_3$, δ): 8.06 (d, $J = 8.5$ Hz, 4H, ArH), 7.71 (d, $J = 8.0$ Hz, 4H, ArH), 5.16–6.12 (m, 68 H, CH), 4.32–4.46 (m, 14H), 1.96 (m, 4H, CH_2), 1.72 (s, 6H, CH_3), 1.48–1.60 (m, 208H, CH_3), 0.61 (t, 4H, CH_2), 0.070 (s, 732H, CH_3). GPC $M_n = 15.4$ k; PDI 1.26.

Synthesis of PEG-PDMS-PEG (13)

Same procedures were followed as preparation of **11**. PEG-PDMS-PEG (**13**) was obtained as a colorless solid, yield 91.5%. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.07 (d, $J = 8.5$ Hz, 4H, ArH), 7.74 (d, $J = 8.5$ Hz, 4H, ArH), 5.23–5.26 (m, 2H, CH), 4.32–4.38 (m, 8H, CH_2), 3.63–3.79 (m, 324H, CH_2), 3.38 (s, 6H, CH_3), 1.81–1,85 (m, 4H, CH_2), 1.48–1.52 (m, 4H, CH_2), 0.61 (m, 4H, CH_2), 0.070 (s, 732H, CH_3). GPC, M_n 21.5 k; PDI 1.13.

Synthesis of PNIPAM-PDMS-PNIPAM (14)

Same procedures were followed as preparation of 11 except TEA was added at 0 °C and the reaction was stirred for 1 h at 0 °C and another hour at room temperature. PNIPAM-PDMS-PNIPAM (**14**) was obtained as a light yellow solid, yield 93%. ^1H NMR (600 MHz, $CDCl_3$, δ): 8.02–8.08 (m, 8H,ArH), 7.56 (m, 2H, CH), 6.34 (br, 36H, CH), 4.31–4.33 (m, 4H, CH_2), 4.00 (m, 36H, CH), 3.58–3.69 (m, 4H, CH_2), 1.32–2.18 (m, 348H, CH_3), 0.069 (s, 732H, CH_3). GPC M_n 14.2 k; PDI 1.30.

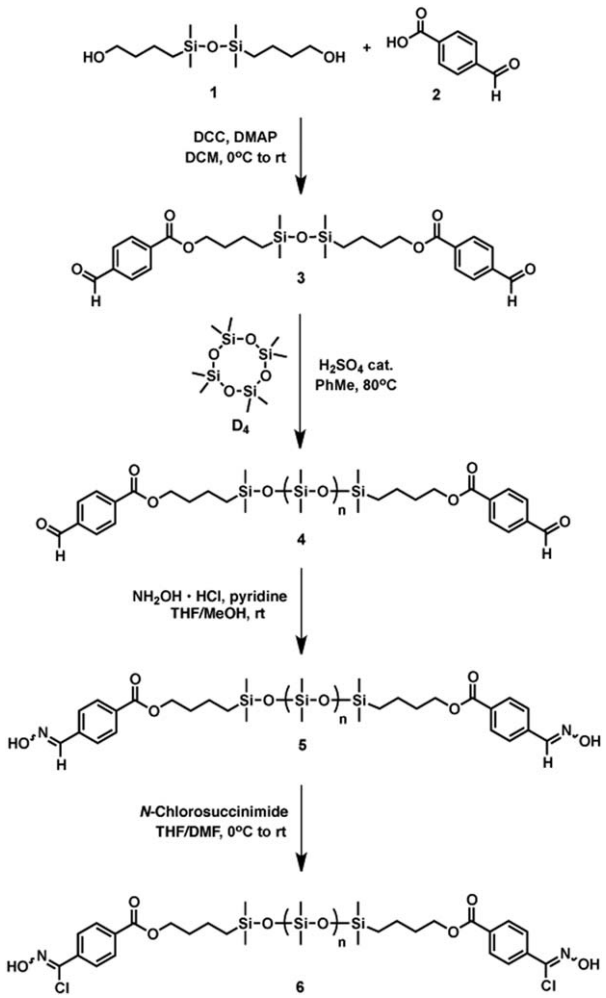
Synthesis of High M_n PMOXA-PDMS-PMOXA (19)

Same procedures were followed as preparation of **11**. PMOXA-PDMS-PMOXA (**19**) was obtained as a colorless solid. ^1H NMR (600 MHz, $CDCl_3$, δ): 8.30–6.95 (m, 6H, ArH), 8.08 (d, 4H, ArH), 7.72 (d, 4H, ArH), 6.83 (m, 2H, ArH), 5.24 (m, 2H, CH), 4.48 (t, 4H, CH_2), 4.33 (m, 8H,CH_2), 3.45 (br. m, 140H, CH_2), 2.15 (br. m, 102H, CH_3), 1.50–1.66 (s, 18H, CH_3), 0.59 (t, 4H, CH_2), 0.060 (s, 2160H, CH_3). GPC, M_n 34.6 k; PDI 1.96.

RESULTS AND DISCUSSION

Synthesis of *Bis*-Chloroxime PDMS

Due to their inherent instability, nitrile oxides were prepared from chloroximes *in situ*, which were readily installed as

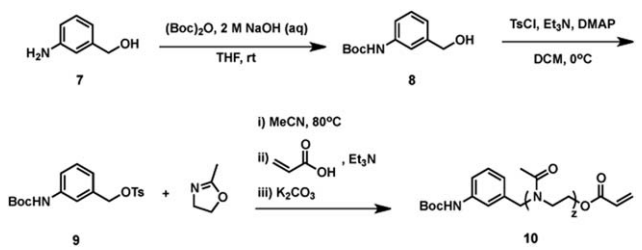


SCHEME 2 Synthesis of the nitrile oxide precursor *bis*-chloroxime PDMS (**6**) via CROP of cyclic siloxane monomer using the end-blocker method.

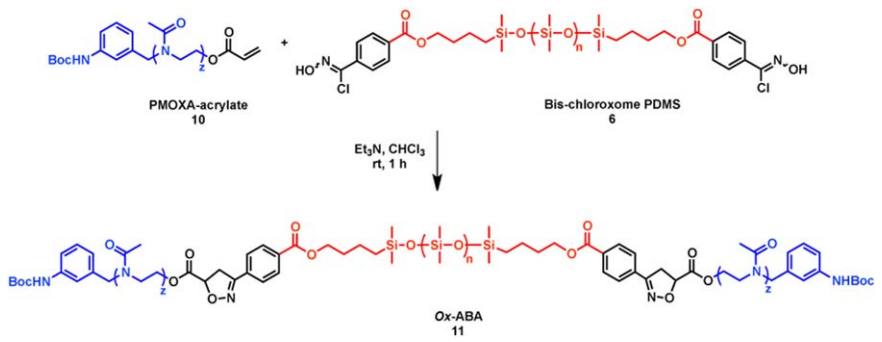
end-groups on a PDMS B-block (Scheme 2). By initially synthesizing a *bis*-benzaldehyde PDMS B-block, we envisioned the desired *bis*-chloroximes could be constructed via postpolymerization functional group modification. The synthesis of *bis*-aldehyde PDMS was accomplished via CROP of cyclic siloxane monomers using the end-blocker method.[34] This short-chain disiloxane, referred to as the end-blocker, serves as the end-groups of the poly(siloxane) polymer while terminating the polymerization. *Bis*-aldehyde end-blocker (**3**) was synthesized by coupling 1,3-*bis*(4-hydroxybutyl)tetramethyldisiloxane (**1**) with 4-formylbenzoic acid (**2**) in the presence of N,N-dichclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). Using benzaldehyde end-blocker (**3**) and octamethylcyclotetrasiloxane (D_4) as the cyclic siloxane monomer, bifunctional *bis*-aldehyde PDMS (**4**) was synthesized via CROP using H_2SO_4 as the catalyst. After workup, the PDMS B-block was provided in good yield with M_n 7.96 k as determined by ^1H NMR using end-group analysis and M_n 7.19 k, PDI 1.50 from GPC. By means of end-group modification, the nitrile oxide precursor *bis*-chloroxime PDMS (**6**) was readily synthesized in two steps using standard procedures. Condensation of *bis*-aldehyde PDMS (**4**) was performed with hydroxylamine in the presence of pyridine to generate *bis*-aldoxime PDMS (**5**) in good yield ($M_{n,\ 1H\ NMR}$ 8.58 k; $M_{n,GPC}$, 7.44 k, PDI 1.52). Chlorination of the *bis*-aldoxime with NCS led to quantitative conversion to *bis*-chloroxime PDMS (**6**), as demonstrated by ^1H NMR with $M_{n,\ 1H\ NMR}$ 9.54 k. The inability to obtain the precise molecular weight of the PDMS B-block, using GPC, prevents the equimolar addition of polymers during the coupling step and a 2.2 equivalents (1.1 for each side) of the A block was used. For all subsequent coupling reactions, the molecular weight of the B-block was carefully estimated by increasing the relaxation times in the ^1H NMR.

Synthesis of Acrylate-Functionalized Poly(oxazoline)

The synthesis of bi-functional acrylate-terminated poly(methyloxazoline) (PMOXA) A-block (**10**) was achieved via CROP of 2-methyl-2-oxazoline using an amine-protected benzyl tosylate initiator, followed by termination with an clickable acrylate group (Scheme 3). The acrylate end-group may be clicked with nitrile oxides, while a free amine can be used as a functional handle to affix additional chemical moieties. Tosylate initiator (**9**) was synthesized in two steps starting from 3-aminobenzyl alcohol (**7**). The Boc group is stable to most bases and has high nucleophilic tolerance, but is readily cleaved under mild acidic conditions.[35] Selective protection of the amine with Boc was achieved by using $(Boc)_2O$ in a biphasic solution of 2 M NaOH *aq.*/THF to give 3-Boc-aminobenzyl alcohol (**8**) using a modified literature procedure.[36] Alcohol tosylation with Et_3N and DMAP provided 3-Boc-amino benzyl tosylate (**9**). CROP of 2-methyl-2-oxazoline initiated by tosylate (**9**) was performed in MeCN at 80 °C, and after 21 h ^1H NMR demonstrated full initiation and complete monomer consumption. Using a modified termination procedure, the solution was first cooled to 0 °C and diluted with MeCN to decrease the viscosity of the solution.[37,38] Acrylic acid was added dropwise followed by Et_3N, the reaction was heated to 60 °C and stirred overnight to terminate the polymerization. ^1H NMR demonstrated complete termination, and the polymer was isolated by precipitation into cold Et_2O. The resulting acrylate salt was deprotonated by stirring with K_2CO_3 in acetone. PMOXA-acrylate (**10**) was provided with M_n 2.32 k, as demonstrated by ^1H NMR using



SCHEME 3 Synthesis of acrylate-functionalized poly(methyloxazoline) was achieved via CROP of 2-methyl-2-oxazoline using Boc-protected tosylate initiator **9**, followed by termination with acrylic acid.



SCHEME 4 Synthesis of Ox-ABA triblock copolymer (**11**) from PMOXA-acrylate (**10**) and *bis*-chloroxime PDMS (**6**) using Et_3N for the *in situ* generation of the nitrile oxides. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

end-group analysis and M_n 1.73 k (PDI 1.42) as determined by GPC.

ABA Triblock Copolymers via NOC

Nitrile oxide dimerization is a competing reaction with the desired 1,3-dipolar cycloaddition, thus dipolarophiles with high cycloaddition rates must be used.[25] We therefore chose the activated dipolarophiles acrylate, methacrylate or maleimide, whose electron withdrawing properties enhance the reactivity of the dipolarophiles by lowering the energy gap of the interacting frontier molecular orbitals.[39] ^{1}H NMR was utilized to monitor the progress of all polymer-coupling reactions, by observing the appearance of isoxazoline peaks concurrent with the consumption of dipolarophile peaks. Since GPC columns used typically contain TEA, monitoring the progress of the reaction using GPC is infeasible since the chloroxime is converted to the nitrile oxide in the column leading to dimerization.

We initially investigated the synthesis of PMOXA-PDMS-PMOXA (*Ox*-ABA) triblock copolymers from *bis*-chloroxime PDMS (**6**) and PMOXA-acrylate (**10**) with Et_3N as an organic base, which converts the chloroxime into the reactive nitrile oxide *in situ*. The cycloaddition proceeded

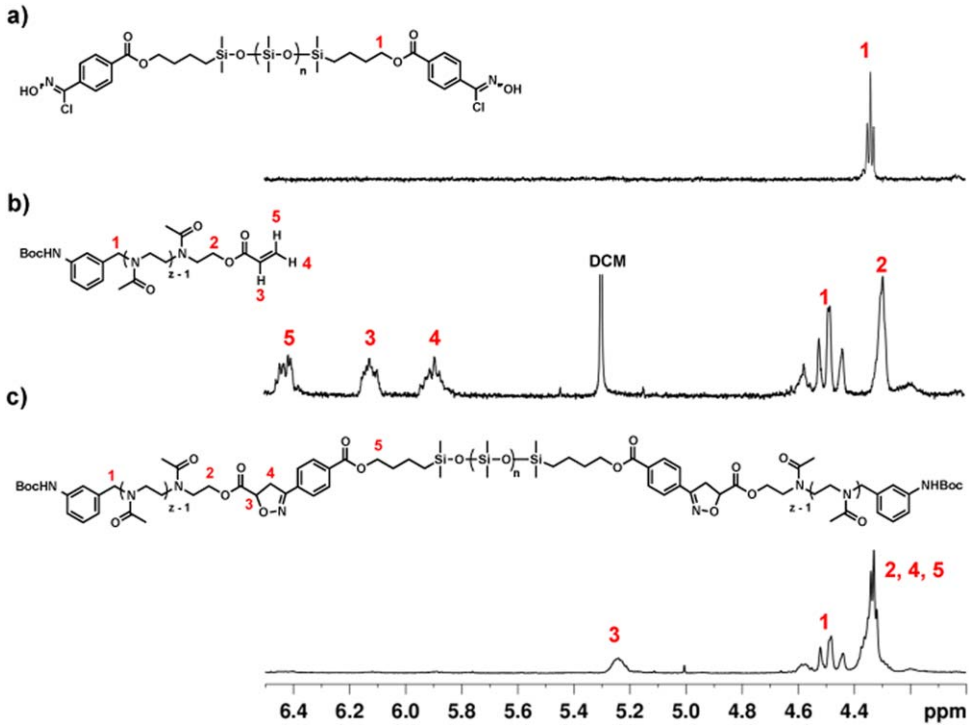
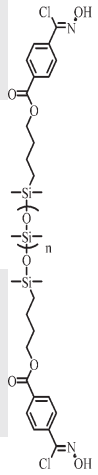
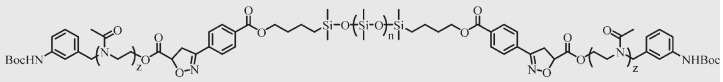
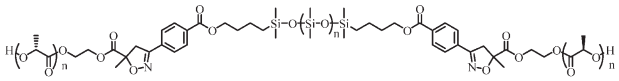
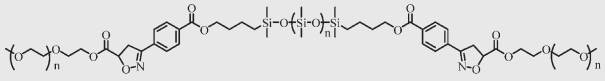
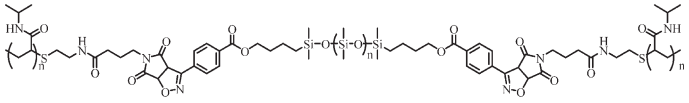


FIGURE 1 ^{1}H NMR spectra of *bis*-chloroxime PDMS (**6**) (a), PMOXA-acrylate (**10**) (b), and NOC product *Ox*-ABA (**11**) (c). Consumption of the acrylate peaks in conjunction with the appearance of isoxazoline peaks demonstrates polymer-polymer coupling. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 1 ABA Triblock Copolymers Synthesized via the NOC Coupling Reaction

A-Block	B-Block	Condition	ABA polymers
PMOXA_acrylate		Et_3N, CH_3Cl, rt, 1 h, Yield: 91.2%	 *Ox*-ABA (M_n, 21.2 k) **11**
PLA_methacrylate		Et_3N, CH_3Cl, rt, 2 h, Yield: 90.0%	 *PLA*-ABA (M_n, 15.4 k) **12**
PEG_acrylate		Et_3N, CH_3Cl, rt, 1 h, Yield: 91.5%	 *P*-ABA (M_n, 21.5 k) **13**
PNIPAM_maleimide		Et_3N, CH_3Cl, 0 °C-rt, 2 h, Yield: 93%	 *N*-ABA (M_n, 14.2 k) **14**

rapidly in a solution of chloroform at room temperature (Scheme 4). Samples were taken every hour, ^{1}H NMR demonstrated almost no change of the intensity PMOXA-acrylate (**10**) peaks at 6.36, 6.13, and 5.88 ppm and isoxazoline peak of *Ox*-ABA (**11**) at 5.24 ppm after 1 h and no side products indicative of nitrile oxide dimerization (Fig. 1).

The scope of the NOC was investigated by synthesizing several ABA triblock copolymers with various block identities, molecular weights, and dipolarophiles. We investigated the NOC with hydrophilic A-blocks poly(ethylene glycol) (PEG) and poly(*N*-isopropylacrylamide) (PNIPAM) or hydrophobic poly(L-lactide) (PLA) terminated with acrylate, maleimide or methacrylate dipolarophiles, respectively (Table 1). PEG and PMOXA are known to impart stealth like properties,[18] while PNIPAM block copolymers are known to be thermoresponsive[40] and PLA-PDMS-PLA block copolymers are known to self-assemble into nano dots.[41] In addition, a high molecular weight *bis*-chloroxime PDMS (**18**) B-block (M_n 27.0 k) was used to demonstrate the feasibility of high molecular weight polymer-polymer coupling using the NOC.

In all the cases, the corresponding triblock copolymers were obtained successfully with TEA as base at room temperature using the standard procedure. However, due to the differences in reactivity of end-group dipolarophiles and solvation of polymer blocks, reaction time and temperature required optimization with certain substrates. In the case of PLA-metharylate, conversion was accomplished in 2 h at room temperature to provide PLA-PDMS-PLA (*PLA*-ABA) (**12**), which can be ascribed to the steric hindrance of methyl group of the methacrylate. When the coupling was attempted with PNIPAM-maleimide at room temperature, some amount of nitrile oxide dimerization product was observed via ^{1}H NMR. When the reaction temperature was cooled to 0 °C, PNIPAM-PDMS-PNIPAM (*N*-ABA) (**14**) was synthesized in 2 h with no nitrile oxide dimerization observed. This indicates that the decreased temperature did not hinder the rapid cycloaddition kinetics of the madimide dipolarophile. The NOC reaction of much higher molecular weight (M_n 27.0 k) PDMS B-blocks with PMOXA-acrylate (**10**) was also investigated. *Bis*-chloroxime PDMS (**18**) (M_n 27.0 k) was prepared by means of end-group modification, similar to the procedure used for the low molecular weight PDMS (**6**), starting from commercial available *bis*(3-aminopropyl) terminated PDMS (Supporting Information Scheme S1). The coupling of high molecular weight *bis*-chloroxime PDMS (**18**) with PMOXA-acrylate (**10**) via NOC was efficient under standard reaction conditions demonstrating the reactions utility for coupling high molecular weight polymers.

CONCLUSIONS

We have established the NOC as a robust, rapid, and versatile metal-free polymer-polymer coupling methodology for the modular synthesis of amphiphilic ABA triblock copolymers. Nitrile oxide precursors and various dipolarophiles were readily introduced as end-groups into polymeric blocks, which were then coupled rapidly using mild reaction conditions. We

have successfully clicked nitrile oxide terminated PDMS B-blocks with several distinct hydrophilic (PMOXA, PEG, and PNIPAM) or hydrophobic (PLA) A-blocks terminated in activated dipolarophiles in a straightforward, mild, and rapid fashion. This method overcomes many drawbacks of previous metal-free methods due to its rapid kinetics, versatility, scalability, and ease of introduction of necessary functionality. These results confirm NOC as versatile coupling strategy for the synthesis amphiphilic ABA triblock copolymers. This method should find use as an attractive macromolecular coupling strategy for the synthesis of biocompatible polymeric nanostructures. Efforts to expand this methodology and explore the utilization of polymeric amphiphiles as drug delivery vehicles are currently being undertaken in our laboratory.

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