Macromolecules

Article

pubs.acs.org/Macromolecules

One-Step Synthesis of Azlactone-Functionalized SG1-Based Alkoxyamine for Nitroxide-Mediated Polymerization and Bioconjugation

Vianney Delplace,[†] Simon Harrisson,[‡] Hien The Ho,[§] Antoine Tardy,[∥] Yohann Guillaneuf,[∥] Sagrario Pascual,[§] Laurent Fontaine,[§] and Julien Nicolas*[,†]

[†]Institut Galien Paris-Sud, CNRS UMR 8612, Université Paris-Sud, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, F-92296 Châtenay-Malabry Cedex, France

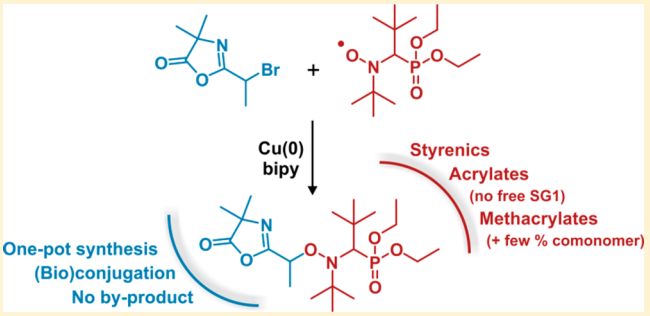
[‡]Université de Toulouse, CNRS, Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique UMR 5623, 118 route de Narbonne, 31062 Toulouse, France

[§]L'UNAM, Institut des Molécules et Matériaux du Mans, Equipe Méthodologie et Synthèse des Polymères, UMR CNRS 6283, Université du Maine, Avenue Olivier Messiaen, 72085 Le Mans Cedex 9, France

[∥]Aix-Marseille Université, CNRS, Institut de Chimie Radicalaire UMR 7273, Avenue Escadrille Normandie-Niemen, 13397 Marseille Cedex 20, France

Supporting Information

ABSTRACT: The one-step synthesis of azlactone-functionalized SG1-based alkoxyamine (AzSG1) for the design of functional polymers by nitroxide-mediated polymerization (NMP) is reported. At 347.7 K, its dissociation rate constant, k_d, was determined to be 2.72 × 10^{-4} s^{-1}, leading to an activation energy, E_a, of 119.5 kJ mol^{-1}, which represents the lowest value ever reported for a secondary SG1-based alkoxyamine without any activation by an external stimulus. This was ascribed to enhanced stabilization of the released radical compared to other secondary alkyl radicals. The AzSG1 alkoxyamine was successfully used for the NMP for styrene, *n*-butyl acrylate, and methyl methacrylate with the addition of a small amount of acrylonitrile as a comonomer, without the need for free SG1. In all cases, first-order kinetics, good control with low dispersities ($Đ$ = 1.2−1.4), and high living chain fractions (LF ∼90%) were obtained. As a proof of concept, the conjugation of azlactone-functionalized polymers to benzylamine and lysozyme was successfully demonstrated. This work may be of high interest for conjugation as the azlactone functionality is also known to react with other nucleophiles such as alcohols or thiols.



INTRODUCTION

Nitroxide-mediated polymerization (NMP)[1,2] is a very simple yet efficient reversible deactivation radical polymerization (RDRP) technique. With atom-transfer radical polymerization (ATRP)[3,4] and reversible addition−fragmentation chain transfer (RAFT)[5,6] polymerization—to mention only the most widely used—these techniques have revolutionized the field of macromolecular synthesis by enabling the design of well-defined, complex, and functional architectures. The most efficient procedure to perform NMP is to use a preformed alkoxyamine,[1] which undergoes reversible thermal homolysis to produce an initiating radical and a persistent nitroxide. The NMP mechanism is then governed by a reversible activation−deactivation equilibrium where the role of the nitroxide is to reversibly deactivate the growing radicals into an alkoxyamine dormant functionality.[1,7]

Early drawbacks of NMP (e.g., requirement for high temperatures, applicability to a limited number of monomers) have been substantially overcome by the development of more active second-generation alkoxyamines derived from 2,2,5-trimethyl-4-phenyl-3-azahexane-3-oxyl (TIPNO),[8] 2,2,5,5-tetramethyl-4-(diethylphosphono)-3-azahexane-3-oxyl[9] (SG1),[10−12] or other structures.[13−15] Yet, among the aspects of NMP that would benefit from improvement is the ease of access to functionalized alkoxyamines for direct coupling. Compared to the broad range of readily available functionalized ATRP initiators and RAFT agents, a very limited number of second-generation, functionalized alkoxyamines have been reported. For instance, the BlocBuilder alkoxyamine based on the SG1 nitroxide (Figure 1a), which is one of the most potent alkoxyamines developed so far,[12,16−19] possesses a carboxylic acid moiety that enables only low degrees of coupling with

Received: January 27, 2015
Revised: March 6, 2015
Published: April 1, 2015

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

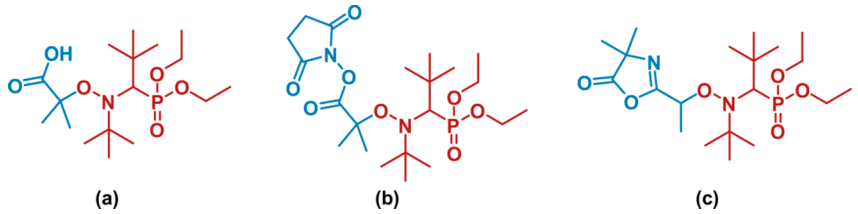


Figure 1. Structure of BlocBuilder (a), NHS-BlocBuilder (b), and azlactone-functionalized SG1-based (AzSG1) alkoxyamines (c).

various alcohols using carbodiimide-based catalysis.[20] The low yields observed are likely due to both the crowded structure of the carboxylic acid moiety and the higher lability of the alkoxyamine once the carboxylic acid group is activated.[20,21] Low to moderate coupling efficiencies were also reported using preactivated alcohols, highlighting the difficulty to prepare ester derivatives of the BlocBuilder alkoxyamine.[21] To confer higher coupling abilities, the BlocBuilder alkoxyamine can be converted into its *N*-succinimidyl (NHS) ester derivative (Figure 1b),[22] which is susceptible to reaction with bulkier amine-containing substrates such as peptides/proteins[23] or silica particles.[24,25] Further derivatizations of the NHS alkoxyamine lead to a variety of different amine-containing linkers bearing functional groups, such as alkene for thiol−ene coupling,[26] hydroxyl for initiating the ring-opening polymerization of lactide,[22] or (3-aminopropyl)triethoxysilane[20] for the surface grafting of silica particles. Alternatively, the use of very efficient coupling agents such as (benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) allows the direct coupling of BlocBuilder to various amines, in particular the *N*-terminus moiety of peptides.[27] Despite these achievements, the previously mentioned synthetic strategies lack simplicity as the functionalized alkoxyamines require either multistep syntheses or the assistance of expensive coupling agents (which also generate byproducts). In consequence, the direct synthesis of functionalized alkoxyamines is still an important challenge that needs to be overcome.

Azlactones are lactone-based functional groups that undergo ring-opening reactions in the presence of nucleophiles such as primary amines, alcohols, or thiols.[28−30] Polymers bearing azlactone moieties, either as side groups along the polymer backbone or positioned on the chain end, can react rapidly, at room temperature, and in the absence of a catalyst (or the generation of byproducts) with primary amine-functionalized (macro)molecules.[31−35] Coupling of azlactone-functionalized polymers proceeds in a broad range of organic solvents as well as in aqueous solution. Additionally, the azlactone group is relatively stable to hydrolysis, a substantial advantage compared to activated ester polymers containing NHS groups.

In this context, we report the one-step synthesis of azlactone-functionalized SG1-based alkoxyamine for the design of functional polymers by NMP (Figure 1c). While the synthesis of an azlactone-functionalized alkoxyamine based on the 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) nitroxide has previously been reported,[36] no control ($M_w/M_n \sim 2$) was obtained over the NMP of styrene. Therefore, the present work represents not only an important step toward easy access to functionalized, SG1-based alkoxyamines, but it is also the first time that the azlactone moiety has successfully been used in combination with NMP, which advantageously diversifies the coupling methods of the NMP toolbox.

EXPERIMENTAL PART

Materials. Sodium hydroxide (≥98%), 2-methylalanine (98%), hydrochloric acid (37%), triethylamine (TEA, ≥99%), ethyl chloroformate (97%), 2-bromopropionyl bromide (97%), oligo(ethylene glycol) methyl ether acrylate (OEGA, M_n = 454 g mol^{-1}), methyl methacrylate (MMA, 99%), *n*-butyl acrylate (*n*BA, ≥99%), styrene (S, 99%), acrylonitrile (AN, 99+%), diethyl phosphite (DEP, 98%), benzylamine (99%), copper wire (1.0 mm diameter, 99.9+%), magnesium sulfate ($MgSO_4$, ≥99.5%), sodium chloride (NaCl, ≥99.5%), and anhydrous toluene (99.8%) were purchased from Sigma-Aldrich and used as received. Copper(II) sulfate ($CuSO_4$) and 2,2′-bipyridine (bipy) were purchased from VWR and used as received. Deuterated chloroform ($CDCl_3$) was obtained from Eurisotop. All other solvents were purchased from Carlo-Erba and used as received. The *N*-*tert*-butyl-*N*-(1-diethylphosphono-2,2-dimethylpropyl) nitroxide (SG1, 86%) was kindly supplied by Arkema. Bromoazlactone (AzBr) was synthesized as reported elsewhere.[32]

Analytical Techniques. ^{1}H NMR spectroscopy was performed in 5 mm diameter tubes in $CDCl_3$ on a Bruker Avance-300 (300 MHz) spectrometer. The chemical shift scale was calibrated on the basis of the solvent peak (δ = 7.26 ppm). ^{31}P NMR spectroscopy was performed in 5 mm diameter tubes in $CDCl_3$ on a Bruker Avance-400 (400 MHz) spectrometer. Diethyl phosphite (DEP, δ = 7.1 ppm) was used as internal reference to calibrate the chemical shift scale. Size exclusion chromatography (SEC) was performed at 30 °C with two columns from Polymer Laboratories (PL-gel MIXED-D; 300 × 7.5 mm; bead diameter, 5 μm; linear part, 400−4 × 10^5 g mol^{-1}) and a differential refractive index detector (Spectrasystem RI-150 from Thermo Electron Corp.), using chloroform ($CHCl_3$) as eluent, a Waters 515 pump at a flow rate of 1 mL min^{-1}, and toluene as a flow-rate marker. The conventional calibration curve was based on poly(methyl methacrylate) (PMMA) standards (peak molar masses, M_p = 625−625 500 g mol^{-1}) or polystyrene (PS) standards[37] (peak molar masses, M_p = 162−523 000 g mol^{-1}) from Polymer Laboratories. This technique allowed M_n (number-average molar mass), M_w (weight-average molar mass), and M_w/M_n (dispersity, $Đ$) to be determined.

Computational Details. All calculations were performed on a HP cluster composed of 16 HP Proliant BL460c computing nodes. For AzSG1, the lowest energy geometry was first determined by simulated annealing with the semiempirical AM1 method of the Ampac 9.2 software, and density functional theory (DFT) calculations were then performed with the Gaussian 09 package[38] to obtain energy values. The details of the calculations are provided in the Supporting Information.

Methods. *Synthesis of Azlactone-Functional SG1-Based Alkoxyamine (AzSG1).* In a 10 mL round-bottom flask, 503 mg of *N*-*tert*-butyl-*N*-(1-diethylphosphono-2,2-dimethylpropyl) nitroxide (SG1, 1.71 × 10^{-3} mol), 401 mg of AzBr (1.71 × 10^{-3} mol), and 526 mg of 2,2′-bipyridine (3.37 × 10^{-3} mol) were introduced and dissolved in 4 mL of acetonitrile. The solution was fitted with a rubber septum and a magnetic bar and deoxygenated under stirring by nitrogen bubbling for 5 min at room temperature. A 107 mg sample of copper wire (1.69 × 10^{-3} mol) was then added, and the solution was deoxygenated again under stirring by nitrogen bubbling for 5 min at room temperature. The solution was finally stirred overnight under nitrogen. To purify the product, the mixture was first diluted in 100 mL of ethyl acetate and filtered. The solution was then washed 3 times with 50 mL of water, 3 times with 50 mL of a 5% $CuSO_4$ solution, and 3 times with

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

50 mL of a saturated NaCl solution. The colorless organic solution was dried over $MgSO_4$ and filtered before evaporation of the solvent to obtain a mixture of both diastereoisomers (66:34, measured by integrating the protons between 3.27 and 3.43 ppm) as a crystallized white powder and a sticky brown compound. An additional recrystallization of a week at −20 °C in pentane was performed to eliminate the free SG1, giving 403 mg of pure products (yield: 39%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.0−1.2 (m, $C(CH_3)_3$, 18 H), 1.2−1.35 (dt, $PCH_2(CH_3)_2$, 6H), 1.35−1.45 (s, $NC(CH_3)_2$, 6H), 1.57−1.63 (d, $OCCH_3$, 3H), 3.27−3.36 (d, N(CH)P, 1H), 3.32−3.43 (d, N(CH)P, 1H), 3.9−4.3 (dq, $PCH_2(CH_3)_2$, 4H), 4.85−5.05 (dq, $NOCH(CH_3)$, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, δ, ppm). Major diastereomer: 181.4 (O−C═O), 163.3 (O−C═N), 74.0 ($OCH(CH_3)CNO$), 69.5 (CP, d, J = 105 Hz), 65.1 ($NC(CH_3)_2$), 61.8 ($NC(CH_3)_3$), 61.8 (OCH_2CH_3), 59.3 (OCH_2CH_3) 35.5 ($CHC(CH_3)_3$, d, J = 2.8 Hz), 30.6 ($PCHC(CH_3)_3$), 28.3 ($NC(CH_3)_3$), 24.5 ($NC(CH_3)(CH_3)$), 24.1 ($NC(CH_3)(CH_3)$) 17.8 ($OCHCH_3$), 16.7 ($POCH_2CH_3$, d, J = 3.8 Hz), 16.4 ($POCH_2CH_3$, d, J = 4.5 Hz). Minor diastereomer: 181.4 (O−C═O), 164.0 (O−C═N), 74.0 ($OCH(CH_3)CNO$), 69.9 (CP, J = 104 Hz), 65.1 ($NC(CH_3)_2$), 61.8 ($NC(CH_3)_3$), 61.8 (OCH_2CH_3), 59.4 (OCH_2CH_3), 35.7 ($CHC(CH_3)_3$, d, J = 3.9 Hz), 30.4 ($PCHC(CH_3)_3$), 28.3 ($NC(CH_3)_3$), 24.5 ($NC(CH_3)(CH_3)$), 23.8 ($NC(CH_3)(CH_3)$), 18.3 ($OCHCH_3$), 16.7 ($POCH_2CH_3$, d, J = 3.8 Hz), 16.4 ($POCH_2CH_3$, d, J = 4.5 Hz). ^{31}P NMR (400 MHz, $CDCl_3$, δ, ppm): 23.49 and 24.29. MS (ESI): 435 $(M + H)^+$. Calcd for $C_{20}H_{40}N_2O_6P^+$: 435.4.

Determination of the Dissociation Rate Constant (k_d). Electron spin resonance (ESR) experiments were performed on a Bruker EMX 300 spectrometer. The appearance of the nitroxide was followed during the thermolysis of AzSG1 alkoxyamine ($[AzSG1]_0 = 1.0 \times 10^{-4}$ M) in *tert*-butylbenzene as solvent (0.5 mL). O_2 was used as radical scavenger. The time evolution of the doubly integrated ESR signal of the SG1 nitroxide radical was followed by ESR spectroscopy at 347.7 K for 10 h. A first-order fit of the signal allows the determination of the k_d value. The activation energy E_a was calculated from k_d via the Arrhenius equation with $A = 2.4 \times 10^{14}$ s^{-1}.[39]

Polymerization of Styrene (S). A typical bulk polymerization procedure (experiment 2) is as follows. In a 5 mL vial, fitted with a rubber septum and a magnetic bar, a mixture of S (2.0 g, 1.92×10^{-2} mol) and AzSG1 (25.3 mg, 5.82×10^{-5} mol) was deoxygenated under stirring by nitrogen bubbling for 10 min at room temperature. The vial was placed in a preheated oil bath at 120 °C, corresponding to the time zero of the reaction (according to the small volume of solution and its quasi-instantaneous heating). Samples were periodically taken and dried to follow S conversion by ^{1}H NMR spectroscopy and the molar mass and the dispersity evolutions by SEC (PS calibration). The polymer was then precipitated once in cold methanol and dried under high vacuum until constant weight. Conversion (3 h) = 57%, $M_{n,SEC}$ = 23 070 g mol^{-1}, M_w/M_n = 1.23 (raw polymer). The same procedure was followed for experiments 1 and 3 with 1.31×10^{-4} mol and 4.60×10^{-5} mol of AzSG1, respectively.

Polymerization of n-Butyl Acrylate (nBA). A typical solution polymerization procedure (experiment 4) is as follows. In a 5 mL vial, fitted with a rubber septum and a magnetic bar, a mixture of *n*BA (802 mg, 6.25×10^{-3} mol), AzSG1 (20.1 mg, 4.62×10^{-5} mol), and anhydrous toluene (806 mg, 0.93 mL) was deoxygenated under stirring by nitrogen bubbling for 10 min at room temperature. The mixture was then immersed in a preheated oil bath at 110 °C, corresponding to the time zero of the reaction (according to the small volume of solution and its quasi-instantaneous heating). Samples were periodically taken and dried to follow *n*BA conversion by ^{1}H NMR spectroscopy and the molar mass and the dispersity evolutions by SEC (PS calibration). The polymer was then precipitated once in cold diethyl ether and dried under high vacuum until constant weight. Conversion (3 h) = 64%, $M_{n,SEC}$ = 9690 g mol^{-1}, M_w/M_n = 1.31 (raw polymer). The same procedure was followed for experiments 5 and 6 with 4.55×10^{-5} and 2.32×10^{-5} mol of AzSG1, respectively.

Polymerization of Oligo(ethylene glycol) Methyl Ether Acrylate (OEGA). A typical solution polymerization procedure (experiment 10) is as follows. In a 5 mL vial, fitted with a rubber septum and a magnetic bar, a mixture of OEGA (800 mg, 1.76×10^{-3} mol), AzSG1 (20.1 mg, 4.62×10^{-5} mol), and anhydrous toluene (800 mg, 0.93 mL) was deoxygenated under stirring by nitrogen bubbling for 10 min at room temperature. The mixture was then immersed in a preheated oil bath at 110 °C, corresponding to the time zero of the reaction (according to the small volume of solution and its quasi-instantaneous heating). Samples were periodically taken and dried to follow OEGA conversion by ^{1}H NMR spectroscopy and the molar mass and the dispersity evolutions by SEC (PS calibration). The polymer was then precipitated once in cold hexane and dried under high vacuum until constant weight. Conversion (1 h) = 40%, $M_{n,SEC}$ = 7840 g mol^{-1}, M_w/M_n = 1.19 (purified polymer).

Copolymerization of Methyl Methacrylate (MMA) with a Small Amount of Acrylonitrile (AN). A typical solution copolymerization procedure (experiment **9**) is as follows. In a 5 mL vial, fitted with a rubber septum and a magnetic bar, a mixture of MMA (1.52 g, 1.52×10^{-2} mol), AN (80 mg, 1.50×10^{-3} mol, f_{AN0} = 0.09), AzSG1 (10.1 mg, 2.32×10^{-5} mol), and anhydrous toluene (1.60 g, 1.85 mL) was deoxygenated under stirring by nitrogen bubbling for 10 min at room temperature. The mixture was then immersed in a preheated oil bath at 90 °C, corresponding to the time zero of the reaction (according to the small volume of solution and its quasi-instantaneous heating). Samples were periodically taken and dried to follow MMA conversion by ^{1}H NMR spectroscopy and the molar mass and the dispersity evolutions by SEC (PMMA calibration). The copolymer was then precipitated once in cold diethyl ether and dried under high vacuum until constant weight. Conversion (8 h) = 50%, $M_{n,SEC}$ = 26 620 g mol^{-1}, M_w/M_n = 1.30 (raw copolymer). The same procedure was followed for experiments 7 and 8 with 4.55×10^{-5} and 4.64×10^{-5} mol of AzSG1, respectively.

Conjugation of Benzylamine to Azlactone−Poly(methyl methacrylate) (AzPMMA). In a 5 mL vial, fitted with a rubber septum and a magnetic bar, 49.8 mg of an AzPMMA (experiment 7, M_n = 12 090 g mol^{-1}, 4.12×10^{-6} mol) and 5 mg of benzylamine (4.7×10^{-5} mol, 11 equiv) were dissolved in 2.5 mL of THF and reacted together for 3 days at 30 °C, under stirring. The product was then purified by precipitation in cold diethyl ether and dried under high vacuum until constant weight, prior to analysis by SEC and ^{1}H NMR in acetone-d_6.

Conjugation of Azlactone−Poly[oligo(ethylene glycol) Methyl Ether Acrylate) (AzPOEGA) to Lysozyme. Lysozyme (4.3 mg, 3.0×10^{-7} mol) and AzPOEGA from experiment 10 (47.7 mg, 6.1×10^{-6} mol, 20.2 equiv with respect to lysozyme) were dissolved in dimethyl sulfoxide (DMSO, 2.0 mL). Triethylamine (TEA, 0.10 mL) was then added. The reaction was stirred for 3 days at 25 °C. A solution of methanol/water (50:50 v/v, 20.0 mL) was then added to the crude reaction. This solution was subsequently dialyzed against methanol/water (50:50 v/v) for 1 day (MWCO = 3500 Da). The final product was recovered by lyophilization. The bioconjugate was then dissolved in 1.0 mL of water and was further analyzed by SDS-PAGE.

RESULTS AND DISCUSSION

Synthesis of Azlactone-Functionalized SG1-Based Alkoxyamine (AzSG1). Among the strategies that have been developed for the synthesis of alkoxyamines,[1,40] the combination of an alkyl radical (generated *in situ*) with a stable nitroxide radical is the most popular.[41−43] The alkyl radical is typically generated by homolytic scission of alkyl halides by reaction with CuBr (either generated *in situ* by comproportionation of Cu(0) and $CuBr_2$, or directly added in combination with Cu(0)). In line with this concept, we recently proposed an optimized procedure in which a stoichiometric amount of Cu(0) is reacted very rapidly at room temperature with the alkyl bromide, with no need for additional copper(I) or copper(II) species.[44]

We applied this method to the reaction of SG1 nitroxide and the functional alkyl bromide 2-(1-bromoethyl)-4,4-dimethyl-4*H*-oxazolin-5-one (AzBr) (Figure 2). AzBr has recently been

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

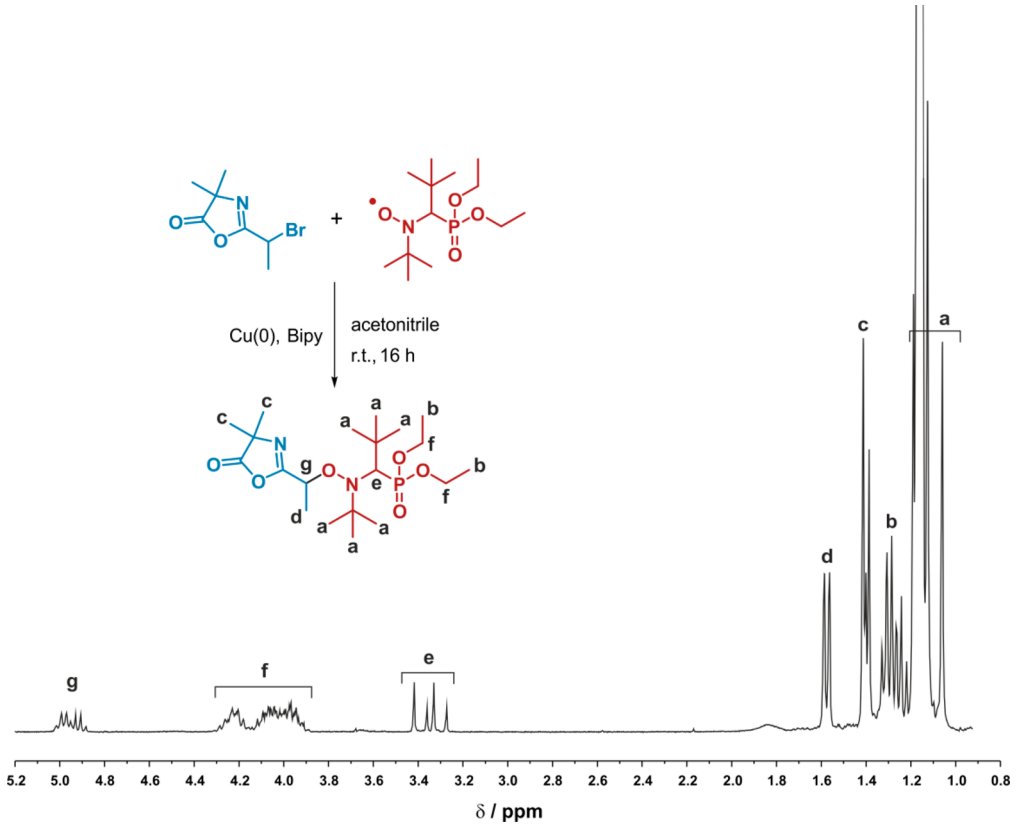


Figure 2. Synthesis of the azlactone-functionalized SG1-based alkoxyamine (AzSG1) and ^{1}H NMR spectrum in $CDCl_3$ in the 0.9−5.2 ppm region.

used as precursor to multifunctional coupling agents that could find interesting applications in different areas of bioconjugation (e.g., labeling, diagnostic).[45] Therefore, we thought it could be advantageous to prepare a SG1-alkoxyamine functionalized with the Az moiety. AzBr was reacted overnight at room temperature with the SG1 nitroxide in the presence of Cu(0) in stoichiometric conditions with 2 equiv of 2,2′-bipyridine (bipy) as the ligand in acetonitrile. The formation of the desired alkoxyamine was observed by NMR spectroscopy and mass spectrometry (see Experimental Part). The ^{1}H NMR spectrum showed all of the characteristic peaks of the AzSG1 alkoxyamine (Figure 2). Purified AzSG1 was obtained as a mixture of two diastereoisomers (66:34, see peaks in the 3.25−3.45 ppm region) with an overall yield of about 40%. This demonstrates the versatility of the synthetic pathway employed here as functional halide moieties can be directly linked to nitroxides to form functional alkoxyamines in one step.

It has been shown that, for a given monomer and nitroxide, the rate of dissociation of the initiating alkoxyamine determines the degree of control over the polymerization.[12] DFT calculations were performed in order to evaluate the bond dissociation energy (BDE) of AzSG1 (see the Supporting Information). The BDE and the activation energy of the homolytic dissociation are expected to be similar since the reverse reaction is estimated to be barrierless.[46,47] The calculations were carried out with two different levels of theory (i.e., B3LYP and BMK) since the selected method may lead to discrepancies compared to experimental data.[48,49] These levels of theory are less time-consuming than the more accurate G3MP2RAD level recommended by Coote and co-workers[48] but are still very useful to determine the relative BDE of the C−ON bond. The calculations predict a very low BDE for AzSG1 (119.1 kJ mol^{-1} for B3LYP and 123.8 kJ mol^{-1} for BMK) and thus a very low activation energy for the dissociation reaction.

The dissociation rate constant (k_d) of AzSG1 was then determined experimentally. The release of the SG1 nitroxide from the alkoxyamine upon heating was monitored by ESR using oxygen as radical scavenger to prevent the recombination reaction. The experiment was carried out twice, and following standard practice,[39] the activation energy E_a was estimated using the averaged frequency factor $A = 2.4 \times 10^{14}$ s^{-1}. At 347.7 K, the k_d value was determined to be 2.72×10^{-4} s^{-1}, suggesting an E_a of 119.5 kJ mol^{-1}. This value is in good agreement with the DFT calculations and is also the lowest value ever reported for a secondary alkoxyamine since the E_a of the ester analogue (the so-called MONAMS) is 128.4/130.8 kJ mol^{-1} for the two diastereoisomers.[46] Such a low E_a is usually associated with sterically hindered SG1-based alkoxyamines such as those bearing tertiary alkyl moieties (e.g., the BlocBuilder alkoxyamine for which E_a = 112.3 kJ mol^{-1})[12] or secondary alkyl moieties with bulky substituents.[50] Low E_a has also been observed with (photo)chemically activated secondary alkoxyamines.[51,52] The 10 kJ mol^{-1} difference would induce a 20 times higher k_d value at 120 °C for AzSG1 compared to MONAMS.

The k_d value is governed by the structure of the alkyl moiety, with a combination of polar, steric, and stabilization effects.[42] A strong electrophilic group such as Az is thus likely to have a significant influence on the k_d value. Coote and co-workers developed linear free energy relationships to describe the dissociation of alkoxyamines using parameters calculated by high-level *ab initio* calculations that quantify the electronic, steric, and radical stabilization characteristics of the alkyl part.[53] By using this approach, the dissociation of MONAMS and

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

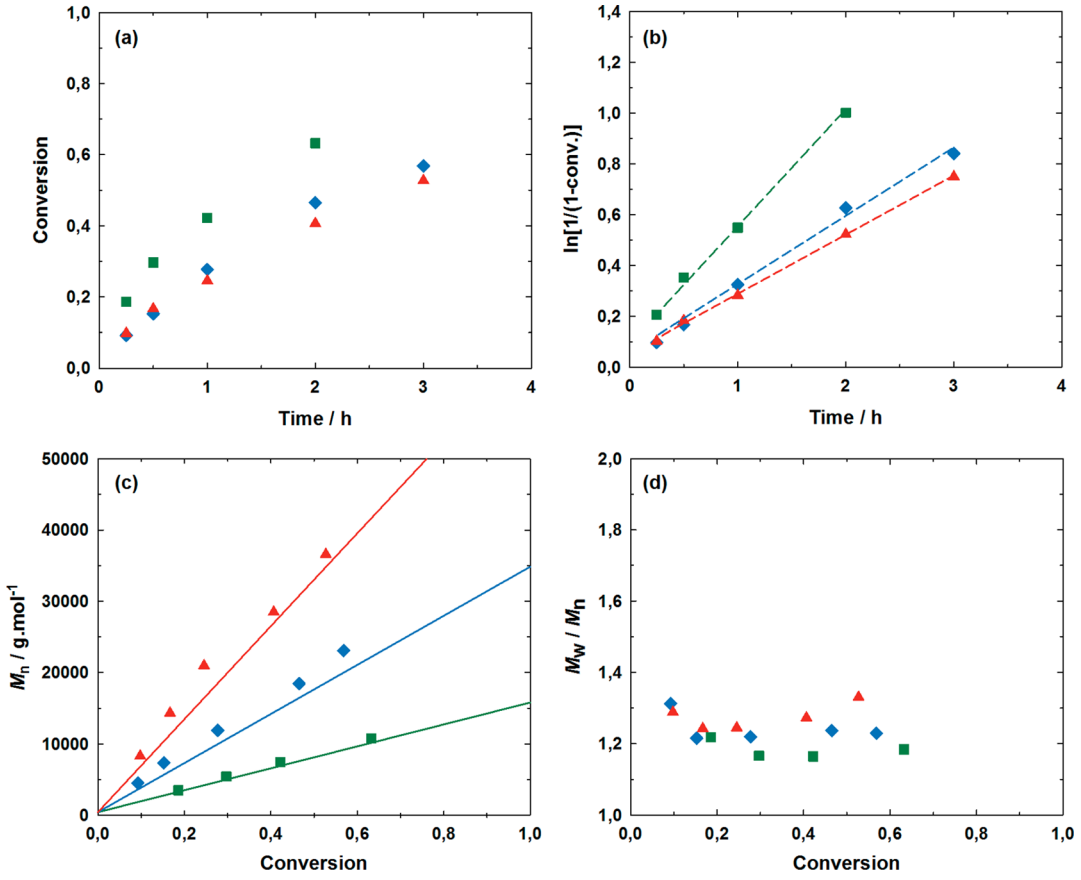


Figure 3. Bulk NMP of styrene initiated by the azlactone-functionalized alkoxyamine (AzSG1) at 120 °C as a function of the targeted number-average degree of polymerization ($DP_{n,th}$): ■, experiment 1 ($DP_{n,th}$ = 148); ◆, experiment 2 ($DP_{n,th}$ = 331); ▲, experiment 3 ($DP_{n,th}$ = 627). (a) Evolution of S conversion (conv) with time (t); (b) evolution of $\ln[1/(1 - \text{conv})]$ with time (t); (c) evolution of the number-average molar mass (M_n) with conv and (d) evolution of the dispersity ($Đ$) with conv. The full lines represent the theoretical M_n, and lines connecting data points are guides for the eye only.

AzSG1 can be compared to obtain a better insight into the influence of the Az moiety over the k_d (see the Supporting Information). First, the two molecules have similar Tolman's cone angles, which measure the steric hindrance of the alkyl radical (the values are 2.34 rad for the 1-ethoxycarbonylethyl radical and 2.43 rad for the Az moiety). Their electronic properties, as measured by the vertical ionization potential of the alkyl radical, are also very close (8.92 eV for the 1-ethoxycarbonylethyl radical and 8.58 eV for the Az moiety). Conversely, the molecules differ significantly with regard to radical stabilization: the standard radical stabilization energy of the Az radical is 65.4 kJ mol^{-1} compared to 41.2 kJ mol^{-1} for the 1-ethoxycarbonylethyl radical. Thus, the difference in dissociation rates is primarily due to improved stabilization of the Az radical.

As a consequence, the AzSG1 alkoxyamine was anticipated to be a good initiator for the NMP of a wide range of monomers.

NMP of Vinyl Monomers Initiated by the AzSG1 Alkoxyamine. We then investigated the ability of the AzSG1 alkoxyamine to control the polymerization of different vinyl monomers. To study the flexibility of the synthesis, the initial monomer/AzSG1 molar ratio was varied in order to target different molecular weights.

NMP of Styrene (S). We first performed the bulk polymerization of styrene (S), which is one of the most widely used monomers in radical polymerization. Polymerizations were performed at 120 °C without any addition of free SG1 (experiments 1−3). S conversions of 50−65% were achieved in 3 h, and first-order kinetics were obtained for the all three targeted DP_n (Figure 3a,b), indicating constant concentrations of propagating radicals throughout the polymerizations, as already observed for fast dissociating alkoxyamines.[12] As measured by the slope of the different $\ln[1/(1 - \text{conv})]$ vs time curves, higher polymerization rates were obtained when lower DP_ns were targeted, in good agreement with the equation describing the kinetics of NMP.[1] The evolution of the M_n with the monomer conversion was linear and in excellent agreement with the predicted values, giving high initiating efficiencies of 86−95% (Figure 3c,d). Dispersities decreased with the monomer conversion and were as low as 1.18−1.33 at the end of the polymerizations. These results establish that S polymerization initiated by AzSG1 is well controlled.

NMP of *n*-Butyl Acrylate (*n*BA). SG1 is an efficient controlling agent for the polymerization of a broad variety of different acrylic esters.[1] A representative monomer of the acrylic ester family is *n*-butyl acrylate (*n*BA). Many studies have reported the SG1-mediated polymerization of *n*BA in many different conditions.[16,54−56] The AzSG1-initiated NMP of *n*BA was thus performed in toluene at 110 °C. Similarly to S, NMP of *n*BA yielded up to ∼65% conversions in 3 h with first-order kinetics (Figure 4a,b). The polymerizations proceeded in a controlled fashion, leading to linear evolutions of M_n vs *n*BA conversion, in good agreement with the theoretical values, and decreasing dispersities of 1.3−1.4 (Figure 4c,d). Lower

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

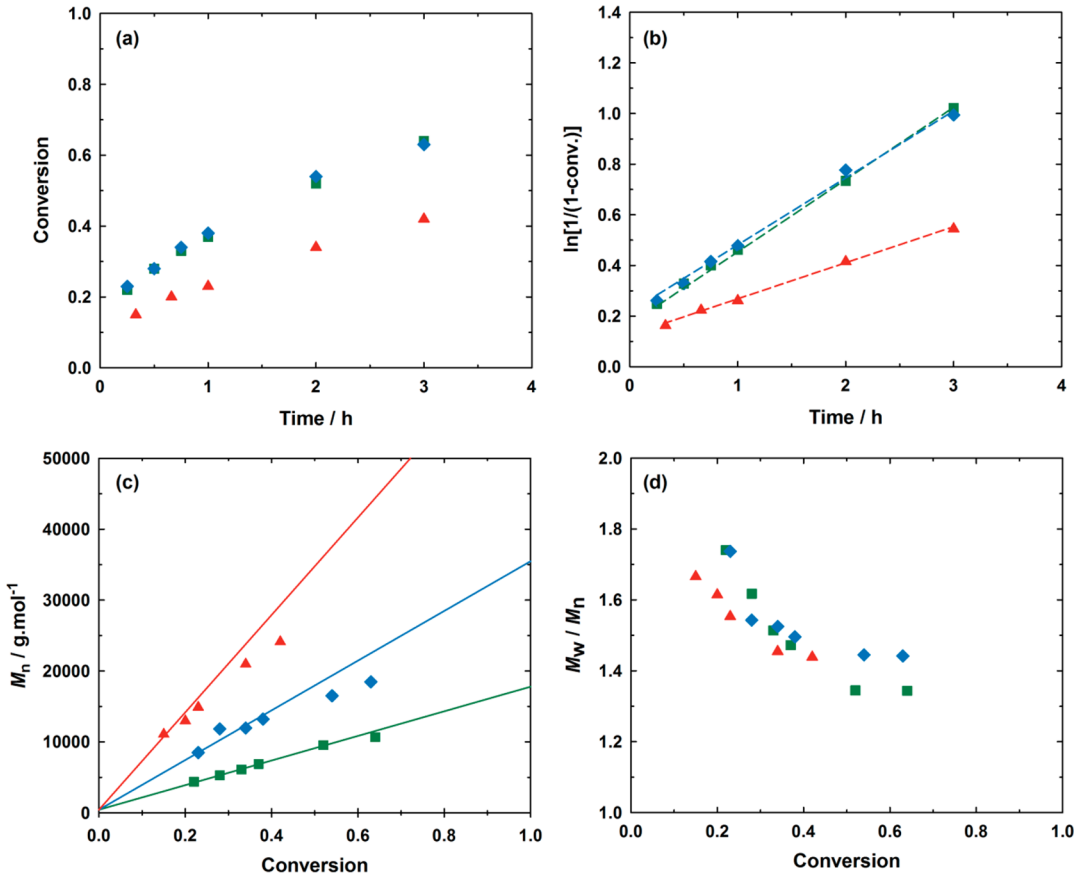


Figure 4. Solvent NMP of *n*BA initiated by the azlactone-functionalized alkoxyamine (AzSG1) at 110 °C, as a function of targeted number-average degree of polymerization ($DP_{n,th}$): ■, experiment 4 ($DP_{n,th}$ = 135); ◆, experiment 5 ($DP_{n,th}$ = 274); ▲, experiment 6 ($DP_{n,th}$ = 536). (a) Evolution of *n*BA conversion (conv) with time (t); (b) evolution of $\ln[1/(1 - \text{conv})]$ with time (t); (c) evolution of the number-average molar mass (M_n) with conv and (d) evolution of the dispersity ($Đ$) with conv. The full lines represent the theoretical M_n, and lines connecting data points are guides for the eye only.

dispersities can be obtained by addition of free SG1 to the polymerization medium.[12,55] A similar experiment (experiment 4′) performed under a slight excess of free SG1 (10 mol %) led to a drastic decrease in the polymerization rate (21% after 4 h) but gave substantially better control (M_n = 4300 g mol^{-1}, $Đ$ = 1.23, initiation efficiency = 96%) when compared to experiments 4−6 at a similar monomer conversion. These results are in good agreement with previous work describing the importance of the initiation on the control of the polymerization in the presence of alkoxyamines.[12] Polymerizations of *n*BA initiated with the MONAMS alkoxyamine without excess free nitroxide usually fail due to the low rate of decomposition of MONAMS. Conversely, the same polymerizations initiated by the BlocBuilder alkoxyamine give good control due to a 100-fold increase of the k_d value. Although AzSG1 has a secondary initiating group like MONAMS, its k_d is 20 times higher. AzSG1 dissociates rapidly enough to afford good control of the polymerization of *n*BA, though the dispersities obtained are higher than with the BlocBuilder alkoxyamine. As for MONAMS, the addition of extra SG1 is beneficial for the control but decreases the polymerization rate.

NMP of Methyl Methacrylate (MMA). The NMP of methacrylic esters has always been challenging.[57] With SG1, the activation−deactivation equilibrium strongly favors the production of propagating radicals,[58,59] resulting in a high level of irreversible termination reactions.[18,59−61] However, the addition of a small amount of S during the SG1-mediated polymerization of methyl methacrylate (MMA) initiated with a high dissociation rate constant alkoxyamine, such as BlocBuilder, allowed well-defined and living PMMA-rich copolymers to be synthesized.[18,62,63] This copolymerization approach was shown to be versatile as it has been successfully extended to other methacrylic esters[64−69] and "controlling" comonomers.[70,71]

We were therefore interested to investigate whether AzSG1 was also able to control the polymerization of methacrylic esters via the copolymerization approach. We selected acrylonitrile (AN) as a "controlling" comonomer[70] and performed the copolymerization of MMA and AN (f_0 = 9 mol %) in toluene at 90 °C without adding free SG1. Whatever the targeted DP_n, the different polymerizations exhibited all the features of a controlled system. First-order kinetics were obtained for the three polymerizations (Figure 5a,b). Linear evolutions of the experimental M_n, in good agreement with predicted values, were observed as well as dispersities which decreased as the polymerization proceeded, reaching 1.3 after 50% conversion (Figure 5c,d). This is an important result as the AzSG1 is shown to dissociate at sufficiently low temperature to quantitatively initiate the copolymerization of MMA and AN, giving well-defined copolymers, despite its secondary leaving group. Good control in NMP depends on the ability of the initiating alkoxyamine to fully decompose at the beginning of the polymerization. Therefore, its k_d should be higher or at least similar to the one of the corresponding macroalkoxyamine. For

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

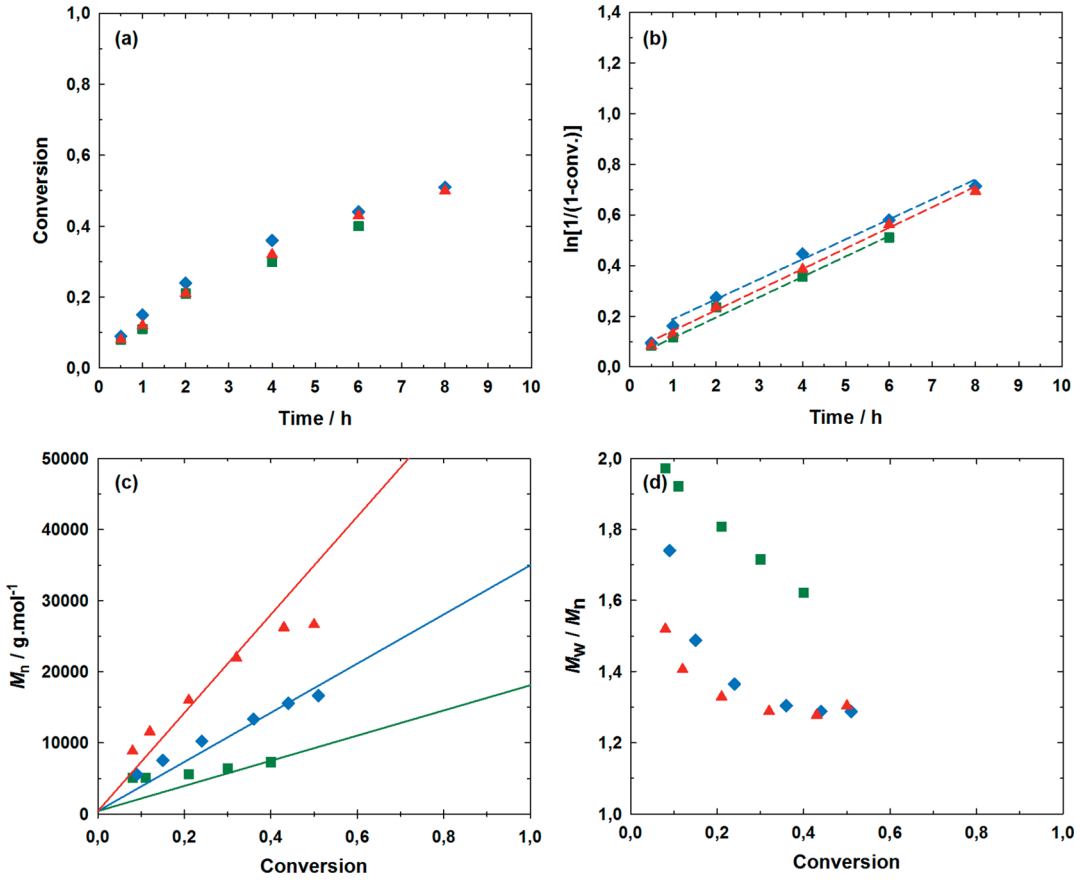


Figure 5. Solvent NMP of methyl methacrylate (MMA) with a small amount of acrylonitrile (AN, f_0 = 9 mol %) initiated by the azlactone-functionalized alkoxyamine (AzSG1) at 90 °C, as a function of the targeted number-average degree of polymerization ($DP_{n,th}$): ■, experiment 7 ($DP_{n,th}$ = 185); ◆, experiment 8 ($DP_{n,th}$ = 360); ▲, experiment 9 ($DP_{n,th}$ = 720). (a) Evolution of MMA conversion (conv) with time (t); (b) evolution of ln[1/(1 − conv)]) with time (t); (c) evolution of the number-average molar mass (M_n) with conv and (d) evolution of the dispersity ($Đ$) with conv. The full lines represent the theoretical M_n, and lines connecting data points are guides for the eye only.

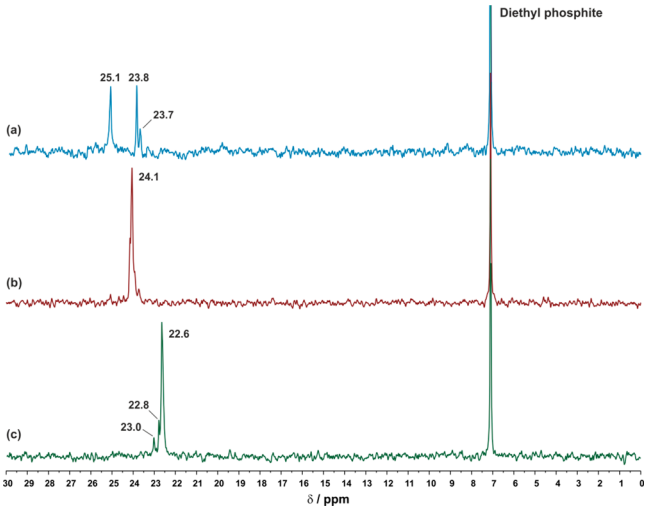


Figure 6. ^{31}P NMR spectra in $CDCl_3$ of (a) Az-PS-SG1 (experiment 2); (b) Az-P*n*BA-SG1 (experiment 4), and (c) Az-P(MMA-*co*-AN)-SG1 (experiment 7) synthesized by SG1-mediated polymerization from AzSG1 alkoxyamine.

the NMP of MMA in the presence of a small amount of AN, the dissociation rate of the macroalkoxyamine is governed by the MMA-AN-SG1 end group.[63,70] By comparison of model alkoxyamines, its E_a should be higher by a few kJ mol^{-1} (~2−5 kJ mol^{-1}) than that of MMA-S-SG1.[1,72] This is also supported by a lower polymerization rate of MMA in the presence of AN than that in the presence of S under identical experimental conditions.[70] The model compound HOOC-C$(CH_3)_2$-S-SG1, similar to the MMA-S-SG1 macroalkoxyamine, has an E_a of 115.5 kJ mol^{-1}.[50] We can thus expect the E_a of MMA-AN-SG1 to be around 117−120 kJ mol^{-1}, which is similar to that of AzSG1. This would explain the ability of AzSG1 to control the polymerization of the MMA in the presence of a small amount of AN (or S). A strong influence of the targeted DP_n on the evolution of the dispersity was also noticed (i.e., the higher the targeted DP_n, the lower $Đ$), which could be explained by a decrease in radical concentration when $[AzSG1]_0$ was decreased, leading to a higher concentration of released SG1 due to the persistent radical effect. This behavior has been observed experimentally[18,70] and confirmed by kinetic modeling.[73]

These results showed that under comparable experimental conditions the AzSG1 alkoxyamine gave a level of control similar to that which could be obtained using the BlocBuilder alkoxyamine.

Determination of the Living Chain Fraction. To investigate the end-group fidelity of the resulting polymer, ^{31}P NMR spectroscopy was performed. This has proven to be a convenient and accurate method for determination of the living chain fraction (LF) by quantifying the presence of the phosphorus-containing SG1 nitroxide end-group using diethyl

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

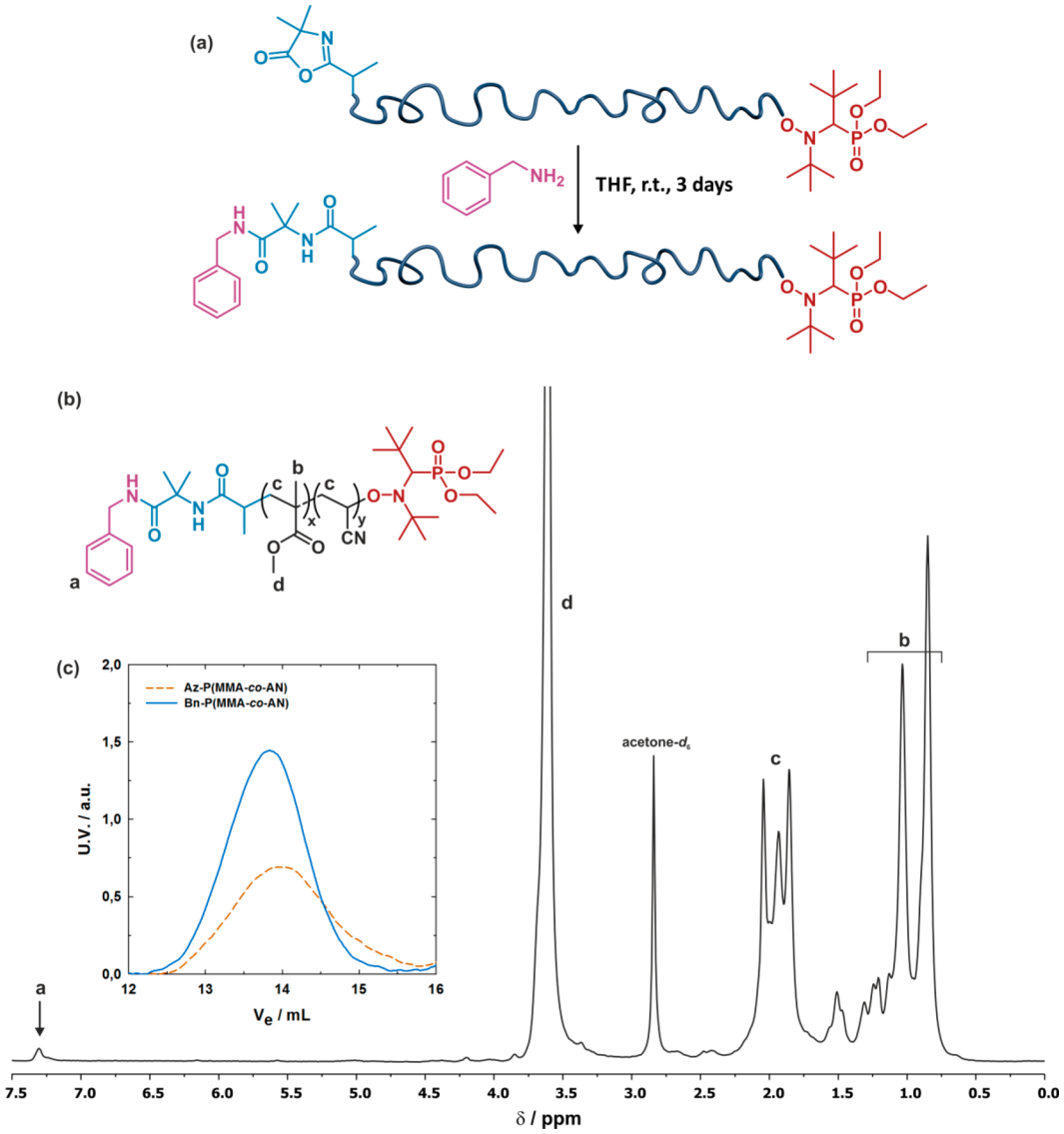


Figure 7. (a) Coupling reaction between benzylamine and a Az-P(MMA-*co*-AN)-SG1 copolymer. (b) ^{1}H NMR spectrum in acetone-d_6 in the 0−7.5 ppm region of the benzylamine-P(MMA-*co*-AN)-SG1 copolymer. (c) Size exclusion chromatography traces (UV signal normalized on the RI signal) of the Az-P(MMA-*co*-AN)-SG1 copolymer (experiment 7) and the benzylamine-P(MMA-*co*-AN)-SG1 copolymer.

phosphite (DEP) as internal reference.[62,74] A purified Az-PS-SG1 (experiment 2) gave a spectrum typical of PS-SG1,[75] and its LF was calculated to be 90%, which demonstrates the living nature of the polymer (Figure 6a). Similarly, the LF of an Az-P*n*BA-SG1 polymer (experiment 4) was 93% (Figure 6b). This value is typical of SG1-based alkoxyamines[12] and confirms that the livingness is determined by the structure of the nitroxide and does not depend on the initiating group. For P(MMA-*co*-AN)-SG1 copolymers, LF values of ∼90% and ∼70% were calculated for the polymers of experiments 7 (targeted DP_n =185) and 8 (targeted DP_n = 360), respectively (Figure 6c). This not only confirms their living character but also is an excellent agreement with both theoretical predictions and experimental LF values for a P(MMA-*co*-S)-SG1 copolymer (which is similar to a P(MMA-*co*-AN)-SG1 copolymer in terms of kinetics) initiated by similar concentrations of the BlocBuilder alkoxyamine.[62,63] Increasing the initial concentration of alkoxyamine (i.e., targeting lower DP_n) results in a significant increase of the reversible termination reactions and therefore gives higher LF.

Availability of the Azlactone Functionality for Coupling. The use of an alkoxyamine bearing a functional group on the initiating moiety enables the design of *α*-functional polymer chains. According to the generally high LF values we previously obtained from the series of polymerizations performed with different monomers, we can therefore anticipate the synthesis of well-defined *α*,*ω*-functional polymers exhibiting an Az starting moiety and a high proportion of SG1 end-group. To probe the availability of the Az functionality toward reactive groups, we reacted an Az-P(MMA-*co*-AN)-SG1 copolymer with benzylamine, a convenient model amine-containing molecule due to its aromatic protons that facilitate the characterization of the resulting conjugates (Figure 7a). The Az-P(MMA-*co*-AN)-SG1 copolymer (experiment 7, M_n = 12 kg mol^{-1}, $Đ$ = 1.3) was reacted with 10 equiv of benzylamine for 3 days at 30 °C in THF. After a precipitation step in cold diethyl ether to remove the excess of benzylamine, the conjugate was analyzed by SEC and ^{1}H NMR in acetone-d_6. SEC qualitatively confirmed the successful coupling, as shown by the increased UV trace of the conjugated polymer compared

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

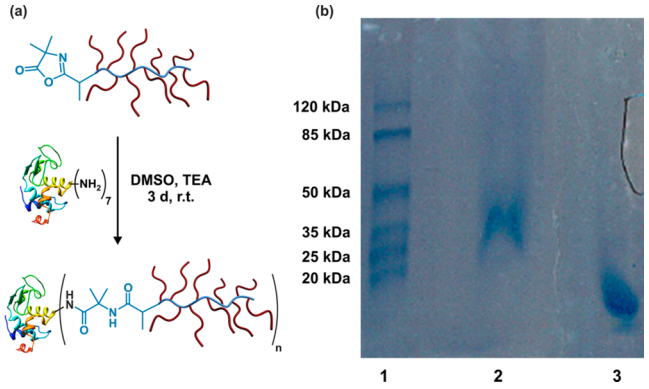


Figure 8. (a) Conjugation pathway of azlactone-poly[oligo(ethylene glycol) methyl ether acrylate] (Az-POEGA) to lysozyme in DMSO/TEA. (b) SDS-PAGE of (1) protein standards, (2) the conjugation of lysozyme with Az-POEGA (experiment 10), and (3) native lysozyme.

to that of the unreacted product (Figure 7c). Aromatic protons are also visible in the 7.0−7.5 ppm region of the ^{1}H NMR spectrum. From the purified copolymer composition determined by SEC and NMR and from the aromatic protons of the benzylamine moiety, a nearly quantitative coupling yield of 98% was calculated (Figure 7b). This result confirmed the availability of the Az moiety and raises the possibility of easy conjugations to azlactone-initiated polymers prepared by NMP.

Application to Protein PEGylation. Conjugation of synthetic polymers to proteins and peptides is an area of high interest. The best-known example is PEGylation, consisting of the covalent linkage of poly(ethylene glycol) (PEG) to protein/peptide-based therapeutics.[76,77] The resulting bioconjugates exhibit improved biodistribution and pharmacokinetics, better stability and solubility, reduced immunogenicity, and longer plasma half-life due to both reduced renal filtration and proteolysis.[78] In the past decade, as introduced by Haddleton and co-workers,[79] RDRP methods have been extensively employed in the field of protein/peptide PEGylation, especially by ATRP and RAFT.[80,81] Application of NMP has been limited to the use of NHS-terminated PEG-based polymers.[23] Additionally, there has been relatively little attention, so far, to the use of the azlactone functionality for protein conjugation from RDRP-derived polymers.[31]

In this context, a well-defined Az-functional PEG-based polymer was prepared from the NMP of oligo(ethylene glycol) methyl ether acrylate (OEGA) initiated by the AzSG1 alkoxyamine at 110 °C in toluene. Lysozyme was chosen as a model protein for polymer−protein conjugation with the resulting Az-POEGA (experiment 10, $M_{n,SEC}$ = 7840 g mol^{-1}, M_w/M_n = 1.19) in anhydrous DMSO/TEA mixture (95/5; v/v) (Figure 8a). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to monitor the conjugation process. SDS-PAGE analysis revealed the presence of high molar mass conjugates of about ∼40 kDa and the absence of residual lysozyme (Figure 8b). Considering the molar mass of the lysozyme (∼14.3 kDa) and the M_n of Az-POEGA, this suggests a ratio of 3−4 polymer chains per protein (in fact, lysozyme has six lysine residues in addition to the terminal amine).

CONCLUSION

A new SG1-based alkoxyamine bearing an azlactone moiety was synthesized in one step and employed as an initiator for the NMP of styrene, *n*-butyl acrylate, and methyl methacrylate with the addition of a small amount of acrylonitrile. Despite its secondary structure, there was no need to perform the polymerization in the presence of excess free SG1. First-order kinetics together with good control, low dispersities, and high livingness were observed in all cases. The conjugation to a model molecule was shown to be quantitative, opening the door to easy functionalization and bioconjugation. The latter was illustrated by the successful coupling between an azlactone PEG-based polymer and a model protein, lysozyme. The present work represents not only an important step toward the easy access to functionalized SG1-based alkoxyamines but also the first time the azlactone moiety has successfully been used in combination with NMP, which advantageously diversifies the possible coupling methods of the NMP toolbox.

ASSOCIATED CONTENT

Supporting Information

UB3LYP/6-31g(d)//UB3LYP/6-31g(d), UBMK/6-31g(d)//UBMK/6-31g(d), and G3MP2RAD calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail julien.nicolas@u-psud.fr; Twitter: @julnicolas; Tel +33 1 46 83 58 53; Fax +33 1 46 83 55 11 (J.N.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Alexandre Bénard is gratefully acknowledged for technical assistance in the preparation of AzBr. We also thank the French National Research Agency (ANR-11-JS08-0005) for the financial support of the PhD thesis of V.D. Arkema is warmly acknowledged for kindly providing the BlocBuilder MA alkoxyamine and the SG1 nitroxide. All computations were performed at "Centre Régional de Compétences en Modélisation Moléculaire (CRCMM)" in Marseille. The CNRS and the French Ministry of Research are also acknowledged for financial support.

REFERENCES

(1) Nicolas, J.; Guillaneuf, Y.; Lefay, C.; Bertin, D.; Gigmes, D.; Charleux, B. *Prog. Polym. Sci.* **2013**, *38*, 63−235.
(2) Grubbs, R. B. *Polym. Rev.* **2011**, *51*, 104−137.
(3) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921−2990.
(4) Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* **2001**, *101*, 3689−3745.
(5) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2009**, *62*, 1402−1472.
(6) Perrier, S.; Takolpuckdee, P. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 5347−5393.
(7) Goto, A.; Fukuda, T. *Prog. Polym. Sci.* **2004**, *29*, 329−385.
(8) Benoit, D.; Chaplinski, V.; Braslau, R.; Hawker, C. J. *J. Am. Chem. Soc.* **1999**, *121*, 3904−3920.
(9) Note that the following nomenclature is often used in the literature: *N-tert*-butyl-*N*-[1-diethylphosphono-(2,2-dimethylpropyl)] nitroxide.
(10) Benoit, D.; Grimaldi, S.; Robin, S.; Finet, J.-P.; Tordo, P.; Gnanou, Y. *J. Am. Chem. Soc.* **2000**, *122*, 5929−5939.
(11) Nicolas, J.; Charleux, B.; Guerret, O.; Magnet, S. *Macromolecules* **2004**, *37*, 4453−4463.
(12) Chauvin, F.; Dufils, P.-E.; Gigmes, D.; Guillaneuf, Y.; Marque, S. R. A.; Tordo, P.; Bertin, D. *Macromolecules* **2006**, *39*, 5238−5250.

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

(13) Greene, A. C.; Grubbs, R. B. *Macromolecules* **2010**, *43*, 10320−10325.
(14) Guillaneuf, Y.; Gigmes, D.; Marque, S. R. A.; Astolfi, P.; Greci, L.; Tordo, P.; Bertin, D. *Macromolecules* **2007**, *40*, 3108−3114.
(15) Greene, A. C.; Grubbs, R. B. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 6342−6352.
(16) Nicolas, J.; Charleux, B.; Guerret, O.; Magnet, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 6186−6189.
(17) Dufils, P.-E.; Chagneux, N.; Gigmes, D.; Trimaille, T.; Marque, S. R. A.; Bertin, D.; Tordo, P. *Polymer* **2007**, *48*, 5219−5225.
(18) Charleux, B.; Nicolas, J.; Guerret, O. *Macromolecules* **2005**, *38*, 5485−5492.
(19) Charleux, B.; Nicolas, J. *Polymer* **2007**, *48*, 5813−5833.
(20) Gigmes, D.; Vinas, J.; Chagneux, N.; Lefay, C.; Phan, T. N. T.; Trimaille, T.; Dufils, P.-E.; Guillaneuf, Y.; Carrot, G.; Boue, F.; Bertin, D. *ACS Symp. Ser.* **2009**, *1024*, 245−262.
(21) Brémond, P.; Kabytaev, K.; Marque, S. R. A. *Tetrahedron Lett.* **2012**, *53*, 4543−4547.
(22) Vinas, J.; Chagneux, N.; Gigmes, D.; Trimaille, T.; Favier, A.; Bertin, D. *Polymer* **2008**, *49*, 3639−3647.
(23) Chenal, M.; Boursier, C.; Guillaneuf, Y.; Taverna, M.; Couvreur, P.; Nicolas, J. *Polym. Chem.* **2011**, *2*, 1523−1530.
(24) Parvole, J.; Ahrens, L.; Blas, H.; Vinas, J.; Boissiere, C.; Sanchez, C.; Save, M.; Charleux, B. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 173−185.
(25) Chevigny, C.; Gigmes, D.; Bertin, D.; Jestin, J.; Boue, F. *Soft Matter* **2009**, *5*, 3741−3753.
(26) Bernhardt, C.; Stoffelbach, F.; Charleux, B. *Polym. Chem.* **2011**, *2*, 229−235.
(27) Trimaille, T.; Mabrouk, K.; Monnier, V.; Charles, L.; Bertin, D.; Gigmes, D. *Macromolecules* **2010**, *43*, 4864−4870.
(28) Buck, M. E.; Lynn, D. M. *Polym. Chem.* **2012**, *3*, 66−80.
(29) Ho, H. T.; Levere, M. E.; Fournier, D.; Montembault, V.; Pascual, S.; Fontaine, L. *Aust. J. Chem.* **2012**, *65*, 970−977.
(30) Heilmann, S. M.; Rasmussen, J. K.; Krepski, L. R. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 3655−3677.
(31) Ho, H. T.; Levere, M. E.; Pascual, S.; Montembault, V.; Casse, N.; Caruso, A.; Fontaine, L. *Polym. Chem.* **2013**, *4*, 675−685.
(32) Ho, H. T.; Leroux, F.; Pascual, S.; Montembault, V.; Fontaine, L. *Macromol. Rapid Commun.* **2012**, *33*, 1753−1758.
(33) Levere, M. E.; Ho, H. T.; Pascual, S.; Fontaine, L. *Polym. Chem.* **2011**, *2*, 2878−2887.
(34) Sun, B.; Liu, X.; Buck, M. E.; Lynn, D. M. *Chem. Commun.* **2010**, *46*, 2016−2018.
(35) Fontaine, L.; Lemele, T.; Brosse, J.-C.; Sennyey, G.; Senet, J.-P.; Wattiez, D. *Macromol. Chem. Phys.* **2002**, *203*, 1377−1384.
(36) Fansler, D. D.; Gaddam, B. N.; Lewandowski, K. M.; Wendland, M. S. Ring-opened azlactone initiators for nitroxide-mediated polymerization. WO 2004072139 A1, 2004.
(37) The PS calibration is appropriate for P*n*BA samples as shown by the Mark−Houwink−Sakurada parameters: actually, it leads to an error of about 3−5%, which is within the accepted range for SEC analysis. Indeed, the MHS parameters in THF at 30 °C are the following: K_{PS} = 11.4 × 10^{-5} dL g^{-1} and α_{PS} = 0.716 for PS [see: Hutchinson, R. A.; Paquet, D. A., Jr.; McMinn, J. H.; Beuermann, S.; Fuller, R. E.; Jackson, C. *Dechema Monogr.* **1995**, *131*, 467.] K_{PnBA} = 12.2 × 10^{-5} dL g^{-1} and α_{PnBA} = 0.700 for P*n*BA [see: Beuermann, S.; Paquet, D. A., Jr.; McMinn, J. H.; Hutchinson, R. A. *Macromolecules* **1997**, *29*, 1918].
(38) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*; Gaussian, Inc.: Wallingford, CT, 2009.
(39) Bertin, D.; Gigmes, D.; Marque, S. R. A.; Tordo, P. *Chem. Soc. Rev.* **2011**, *40*, 2189−2198.
(40) Greene, A. C.; Grubbs, R. B. *ACS Symp. Ser.* **2009**, *1024*, 81−93.
(41) Matyjaszewski, K.; Woodworth, B. E.; Zhang, X.; Gaynor, S. G.; Metzner, Z. *Macromolecules* **1998**, *31*, 5955.
(42) Bertin, D.; Gigmes, D.; Marque, S. R. A.; Tordo, P. *Macromolecules* **2005**, *38*, 2638−2650.
(43) Bertin, D.; Gigmes, D.; Le Mercier, C.; Marque, S. R. A.; Tordo, P. *J. Org. Chem.* **2004**, *69*, 4925−4930.
(44) Harrisson, S.; Couvreur, P.; Nicolas, J. *Polym. Chem.* **2011**, *2*, 1859−1865.
(45) Fontaine, L.; Ho, T. H.; Pascual, S.; Montembault, V. Multifunctional coupling reagents having an azlactone function. WO2014060357, 2014.
(46) Marque, S.; Le Mercier, C.; Tordo, P.; Fischer, H. *Macromolecules* **2000**, *33*, 4403−4410.
(47) Bagryanskaya, E. G.; Marque, S. R. A. *Chem. Rev.* **2014**, *114*, 5011−5056.
(48) Izgorodina, E. I.; Coote, M. L.; Radom, L. *J. Phys. Chem. A* **2005**, *109*, 7558−7566.
(49) Zhang, I. Y.; Wu, J.; Luo, Y.; Xu, X. *J. Chem. Theory Comput.* **2010**, *6*, 1462−1469.
(50) Bertin, D.; Dufils, P.-E.; Durand, I.; Gigmes, D.; Giovanetti, B.; Guillaneuf, Y.; Marque, S. R. A.; Phan, T.; Tordo, P. *Macromol. Chem. Phys.* **2008**, *209*, 220−224.
(51) Versace, D. L.; Lalevee, J.; Fouassier, J. P.; Guillaneuf, Y.; Bertin, D.; Gigmes, D. *Macromol. Rapid Commun.* **2010**, *31*, 1383−1388.
(52) Brémond, P.; Koïta, A.; Marque, S. R. A.; Pesce, V.; Roubaud, V.; Siri, D. *Org. Lett.* **2012**, *14*, 358−361.
(53) Hodgson, J. L.; Lin, C. Y.; Coote, M. L.; Marque, S. R. A.; Matyjaszewski, K. *Macromolecules* **2010**, *43*, 3728−3743.
(54) Farcet, C.; Nicolas, J.; Charleux, B. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 4410−4420.
(55) Lacroix-Desmazes, P.; Lutz, J.-F.; Chauvin, F.; Severac, R.; Boutevin, B. *Macromolecules* **2001**, *34*, 8866−8871.
(56) Nicolas, J.; Charleux, B.; Magnet, S. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 4142−4153.
(57) Guégain, E.; Guillaneuf, Y.; Nicolas, J. *Macromol. Rapid Commun.* **2015**, submitted.
(58) Ananchenko, G. S.; Souaille, M.; Fischer, H.; Le Mercier, C.; Tordo, P. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 3264−3283.
(59) Guillaneuf, Y.; Gigmes, D.; Marque, S. R. A.; Tordo, P.; Bertin, D. *Macromol. Chem. Phys.* **2006**, *207*, 1278−1288.
(60) Dire, C.; Belleney, J.; Nicolas, J.; Bertin, D.; Magnet, S.; Charleux, B. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 6333−6345.
(61) McHale, R.; Aldabbagh, F.; Zetterlund, P. B. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 2194−2203.
(62) Nicolas, J.; Dire, C.; Mueller, L.; Belleney, J.; Charleux, B.; Marque, S. R. A.; Bertin, D.; Magnet, S.; Couvreur, L. *Macromolecules* **2006**, *39*, 8274−8282.
(63) Nicolas, J.; Mueller, L.; Dire, C.; Matyjaszewski, K.; Charleux, B. *Macromolecules* **2009**, *42*, 4470−4478.
(64) Nicolas, J.; Couvreur, P.; Charleux, B. *Macromolecules* **2008**, *41*, 3758−3761.
(65) Lessard, B.; Maric, M. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 2574−2588.
(66) Lessard, B.; Marić, M. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 5270−5283.
(67) Lessard, B.; Tervo, C.; De Wahl, S.; Clerveaux, F. J.; Tang, K. K.; Yasmine, S.; Andjelic, S.; D'Alessandro, A.; Maric, M. *Macromolecules* **2010**, *43*, 868−878.
(68) Lessard, B. H.; Ling, E. J. Y.; Marić, M. *Macromolecules* **2012**, *45*, 1879−1891.

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097

(69) Ting, S. R. S.; Min, E.-H.; Escale, P.; Save, M.; Billon, L.; Stenzel, M. H. *Macromolecules* **2009**, *42*, 9422−9434.
(70) Nicolas, J.; Brusseau, S.; Charleux, B. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 34−47.
(71) Lessard, B.; Ling, E. J. Y.; Morin, M. S. T.; Marić, M. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 1033−1045.
(72) Denis, B.; Didier, G.; Sylvain, M.; Paul, T. *e-Polym.* **2013**, *3*, 16−24.
(73) Gigmes, D.; Bertin, D.; Lefay, C.; Guillaneuf, Y. *Macromol. Theory Simul.* **2009**, *18*, 402−419.
(74) Lefay, C.; Belleney, J.; Charleux, B.; Guerret, O.; Magnet, S. *Macromol. Rapid Commun.* **2004**, *25*, 1215−1220.
(75) Delplace, V.; Harrisson, S.; Tardy, A.; Gigmes, D.; Guillaneuf, Y.; Nicolas, J. *Macromol. Rapid Commun.* **2014**, *35*, 484−491.
(76) Roberts, M. J.; Bentley, M. D.; Harris, J. M. *Adv. Drug Delivery Rev.* **2002**, *54*, 459−476.
(77) Veronese, F. M.; Harris, J. M. *Adv. Drug Delivery Rev.* **2002**, *54*, 453−456.
(78) Harris, J. M.; Chess, R. B. *Nat. Rev. Drug Discovery* **2003**, *2*, 214−221.
(79) Lecolley, F.; Tao, L.; Mantovani, G.; Durkin, I.; Lautru, S.; Haddleton, D. M. *Chem. Commun.* **2004**, 2026−2027.
(80) Nicolas, J.; Mantovani, G.; Haddleton, D. M. *Macromol. Rapid Commun.* **2007**, *28*, 1083−1111.
(81) Le Droumaguet, B.; Nicolas, J. *Polym. Chem.* **2010**, *1*, 563−598.

DOI: 10.1021/acs.macromol.5b00178
Macromolecules 2015, 48, 2087−2097