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Versatile Precursors of Functional RAFT Agents. Application to the Synthesis of Bio-Related End-Functionalized Polymers

Maël Bathfield,[†,‡] Franck D'Agosto,*[,‡] Roger Spitz,[‡] Marie-Thérèse Charreyre,*[,†] and Thierry Delair[†]

Unité Mixte CNRS-bioMérieux, École Normale Supérieure de Lyon, 46 allée d'Italie, 69364 Lyon Cedex 07, France, and The Laboratoire de Chimie et Procédés de Polymérisation, UMR 140 CNRS/ESCPE, Bât 308 F, 43 Boulevard du 11 Novembre 1918, BP 2077, 69616 Villeurbanne Cedex, France

Received November 15, 2005; E-mail: mtcharre@ens-lyon.fr; franck.dagosto@lcpp.cpe.fr

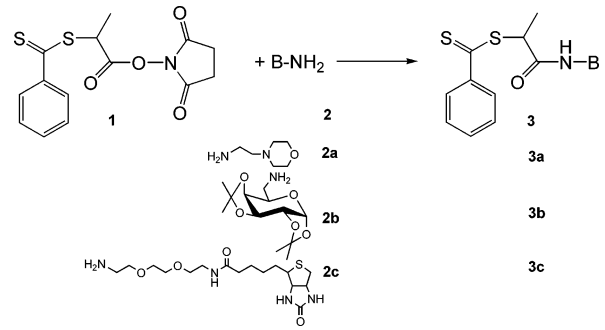
Biomolecule−polymer conjugates are widely used in medicine and biotechnology,[1−3] with the biomolecule being either a macromolecule, such as a nucleic acid sequence, a peptide, or a protein, or a molecular species, such as a sugar, a lipid, or a biotin. Usually, the biomolecule−polymer conjugate is designed such that multiple copies of the biomolecule are randomly bound all along the polymer chain. Another interesting conjugate architecture consists of the presence of a single biomolecule located, for instance, at one chain end. Such a structure may (i) favor an oriented binding of the polymer chain onto a specific support exhibiting complementary moieties to the chosen biomolecule; (ii) promote the occurrence of various types of recognition events on a single chain, if another kind of biomolecule is bound along the chain; (iii) lead to a great variety of conjugates, bearing, for instance, a biomolecule at one end and non-bio-related compounds along the polymer chain, such as colorimetric or fluorescent labels.

The usual strategy to introduce a molecule of interest at a polymer chain end consists in binding an appropriate derivative of that molecule onto a reactive group located at the polymer chain end.[4−6] However, the binding yield depends on the reactivity of the two counterparts and decreases with the increase in the polymer chain length. Another strategy consists in introducing the molecule of interest in a compound which will be used to initiate the polymer chains. Hence, each polymer chain will bear one biomolecule at the α-end.[7−9] In addition, if a homogeneous chain size distribution is required, it will be necessary to use a so-called living/controlled polymerization process.

In recent years, the field of free-radical polymerization has been revolutionized by the development of techniques for controlling the molecular weight and the architecture of polymer chains. Among the most versatile approaches, the reversible addition−fragmentation chain transfer (RAFT) polymerization is carried out with thiocarbonylthio compounds (of the general formula Z−C(═S)−SR, known as RAFT agents), which reversibly react with growing radicals via chain transfer reactions.[10,11] Consequently, chains undergo successive active/dormant cycles that minimize radical−radical termination processes and lead to a simultaneous growth of all chains. Besides exhibiting a narrow molar mass distribution and a controlled chain length, the obtained polymer chains are characterized by the presence of the R and Z groups−from the RAFT agent−at their α- and ω-ends, respectively.

Then, the modification of the structure of the RAFT agent, that is, the R and Z groups, appears as a highly powerful means for introducing a molecule of interest at polymer chain ends. As the Z group may easily be removed from the chain via hydrolysis or aminolysis, focusing on R group modifications seems to be a promising strategy. Several RAFT agents bearing a molecule of interest in the R group were recently synthesized with either a fluorophore,[12] carbohydrates,[13] or a natural polymer, such as cotton.[14] However, in these various examples, the link between that molecule and the thiocarbonylthio moiety was an ester, a labile group imparting a limited stability to the material. In any case, the modification chemistry should preserve the thiocarbonylthio moiety responsible for the control of the polymerization. For instance, chemistry involving amine groups should be proscribed since they rapidly degrade the thiocarbonylthio function by thioamidation.[15]

Scheme 1. Functional RAFT Agent Synthesis from Precursor **1**



The approach we propose relies on the design of RAFT agent precursor, **1**, bearing an activated ester in the R group (Scheme 1).[16] Succinimidyl ester moieties readily react with nucleophiles in a one-step reaction;[17] hence the amidation reaction of **1** by amino derivatives, **2**, should be favored rather than the thioamidation. Moreover, the resulting RAFT agent, **3**, would present a more stable link compared to esters.

This synthetic approach was first assessed with a model compound, *N*-aminoethylmorpholine, **2a**. A careful optimization of the conditions led to functional RAFT agent **3a** (68% yield after purification, full characterization in Supporting Information), without any degradation of the thiocarbonylthio moiety as long as the **2**/**1** molar ratio remained lower than 1.

The successful synthesis of the model compound prompted us to explore the use of amino derivatives of biomolecules, a carbohydrate derivative, 6-amino-6-desoxy-1,2:3,4-di-*O*-isopropylidene-6-α-D-galactopyranose, **2b**, and a biotin derivative, (+)-biotinyl-3,6-dioxaoctanediamine, **2c**. They reacted with **1** under the same conditions as for **2a** (Scheme 1). After purification, pure RAFT agents **3b** and **3c** were isolated (66 and 72% yield, respectively) and characterized by ^{1}H and ^{13}C NMR, elemental analyses, and mass spectrometry (Figure 1 and Supporting Information).

To assess the control efficiency of the new functional RAFT agents, the polymerization of a biocompatible acrylamide derivative, *N*-acryloylmorpholine (NAM), was performed in the presence of **3a** using previously optimized conditions for NAM.[18] A conversion

[†] École Normale Supérieure de Lyon.
[‡] Laboratoire de Chimie et Procédés de Polymérisation.

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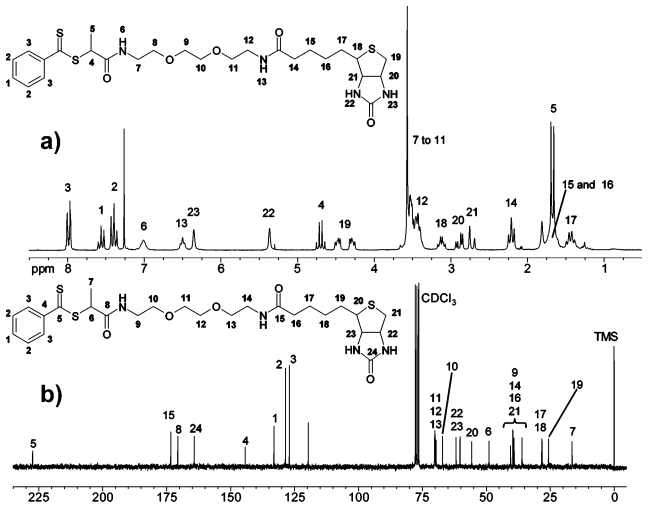

Figure 1. (a) ^{1}H NMR (200 MHz) and (b) ^{13}C NMR (50 MHz) spectra of **3c** in $CDCl_3$ with the corresponding assignments.

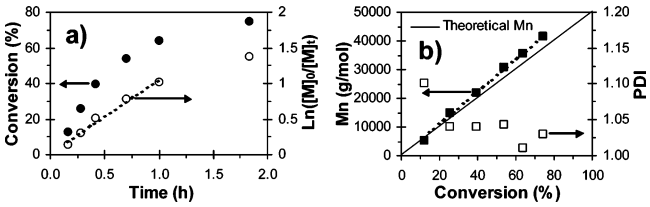

Figure 2. *N*-Acryloylmorpholine (NAM) polymerization mediated by **3a** in dioxane at 90 °C; $[NAM]_0 = 1.6$ mol·L^{-1}; $[NAM]_0/[\mathbf{3a}]_0 = 355$; $[\mathbf{3a}]_0/[AIBN]_0 = 10$. (a) Kinetics plots. (b) Evolution of molar masses and polydispersity indexes (PDI) versus conversion.

Table 1. Theoretical and Experimental Molar Masses Obtained by Size Exclusion Chromatography (using light scattering detection) for *N*-Acryloylmorpholine RAFT Polymerization Mediated by **1** or **3b** or **3c** in Dioxane at 90 °C; $[NAM]_0 = 1.6$ mol·L^{-1}; $[NAM]_0/[\text{RAFT Agent}]_0 = 355$; $[\text{RAFT Agent}]_0/[AIBN]_0 = 10$

RAFT agents	conversion (%)	theoretical M_n (g.mol^{-1})	SEC M_n (g.mol^{-1})	PDI
1	42	21200	28200	1.07
	82	41000	42300	1.11
3b	43	22200	22600	1.02
	74	37300	38200	1.03
3c	28	14800	15500	1.02
	63	32100	31800	1.09

higher than 70% was reached in less than 2 h, and the linearity of the first-order kinetic plot confirmed that steady-state conditions were fulfilled (Figure 2a). The linear increase of the molar masses versus conversion (Figure 2b, obtained by aqueous size exclusion chromatography (SEC) using a three angle light scattering detector; see Supporting Information) as well as the low PDI values evidenced the controlled behavior of the polymerization.

Then, the RAFT homopolymerization of NAM was carried out in the presence of **3b** and **3c**. Again, an excellent agreement was observed between experimental and theoretical molar masses together with low PDI values (Table 1). Finally, MALDI-ToF mass spectrometry analyses confirmed the presence of the biomolecule at the chain end: in the case of a poly(NAM)-carbohydrate of polymerization degree 18, a molar mass of 3029.3 mass units was obtained for a theoretical value of 3029.6 mass units (3144.5 mass units for 3144.6 mass units in the case of a poly(NAM)-biotin).

As shown in Table 1, it is worth mentioning that the precursor **1** itself does control the RAFT polymerization of NAM, leading to polymer chains with a succinimidyl ester α-end group.

In conclusion, we have developed a new straightforward approach to prepare functional RAFT agents for applications in various fields. The use of a precursor RAFT agent bearing a succinimidyl activated ester group favors the reaction of amino derivatives without any competitive degradation of the thiocarbonylthio function. The generated amido link makes these RAFT agents very stable. In addition to precursor **1** (leading to a secondary fragment radical during the RAFT process), another precursor leading to a tertiary fragment radical has been synthesized (**4**; see Supporting Information).[16] This molecule was prepared at the same time by Pan et al. to elaborate dithioester-terminated dendrimers.[19] A wide range of monomers may be polymerized with the two RAFT agent precursors or with their amido-linked derivatives, giving rise to polymer chains carrying a succinimidyl ester or a biomolecule as the α-end group. Other amino derivatives, either bio-related (nucleotides, lipids, peptides) or non-bio-related (fluorophores, silanes, polymer chains) may be introduced into RAFT agents according to this very general strategy.

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Supporting Information Available: Materials and methods and characterizations of the different RAFT agents. This material is available free of charge via the Internet at http://pubs.acs.org.

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