USING "CLICK" CHEMISTRY FOR POST-REACTING WELL-DEFINED POLYMER CHAINS PREPARED BY ATOM TRANSFER RADICAL POLYMERIZATION.

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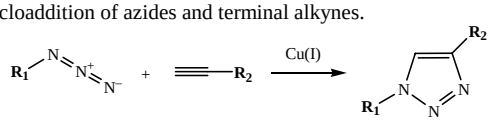
Introduction

The copper catalyzed 1,3 dipolar cycloaddition of azides and terminal alkynes (Scheme 1), was proven to be an almost ideal method for molecular ligation in organic synthetic chemistry.[1, 2] This regioselective reaction can be performed in high yields, in multiple solvents (including water) and in the presence of numerous other functional groups. Thus, the 1,3 dipolar cycloaddition of azides and alkynes is the archetype of a "click" reaction as defined by Sharpless.[3]

Scheme 1. Regioselective formation of a triazole ring via copper catalyzed 1,3 dipolar cycloaddition of azides and terminal alkynes.



Undoubtedly, such "click" chemistry also possesses a huge potential in material science. The latter was first illustrated by the very influential works of Hawker and Fréchet concerning dendrimers and dendronized polymers.[4-6] Hence, "click" chemistry could be used as a general tool for macromolecular engineering (e.g. for modifying side-groups or end-groups of polymers or for coupling preformed polymeric segments). In particular, innovative materials could be prepared by involving tailor-made polymers prepared by atom transfer radical polymerization (ATRP) in "click" reactions.[7-10] This combination of ATRP and "click" chemistry is very tempting since both synthetic methods are versatile and easy.[1, 11] In the present work, we investigated the 1,3 dipolar cycloaddition of azides and terminal alkynes as a general tool for synthesizing either end-functional polymers or block copolymers from polymers prepared by ATRP.

Experimental

Materials. Propargyl alcohol (99%), propiolic acid (96%), 2-methyl-1-buten-3-yne (99%), N,N,N',N',N" pentamethyldiethylenetriamine (PMDETA) (99%), methyl 2-bromopropionate (MBP) (98%) and sodium azide (99%) were purchased from Aldrich and used as received. Styrene (Aldrich, 99%) was passed through a basic activated aluminum oxide (50-200 microns) column prior to use. Copper(I) bromide (Acros, 95%) was washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol and dried. 4,4'-Di-(5-nonyl)-2,2'-bipyridine (dNbipy) was synthesized according to previously reported procedure.[12]

Preparation Of Bromine End-Functional Polystyrene (1) Via Atom Transfer Radical Polymerization. Copper bromide (156 mg, 1.09 mmol) and styrene (10 ml, 87.2 mmol) were added to a flask sealed with a septum. The styrene suspension was purged with dry argon for 20 minutes. Then, PMDETA (187 mg, 1.09 mmol) was added through the septum with a syringe and the mixture turned homogeneous. Last MBP (364 mg, 2.18 mmol) was added via a syringe. The mixture was heated at 90°C in an oil bath. After 4 hours, the experiment was stopped by opening the flask and exposing the catalyst to air. The final mixture was diluted in tetrahydrofuran (THF) and passed through a short silica column in order to remove copper catalyst. The mixture was concentrated by rotary evaporation and subsequently precipitated in methanol. The precipitated polystyrene was filtrated and dried under vacuum.

Preparation Of Azide End-Functional Polystyrene. The procedure for transforming bromide end-functional polystyrene into azide end-functional polystyrene was adapted from the literature.[13] Typically, the bromine end-functional polystyrene (2700 g·mol^{-1}) (500 mg, 0.18 mmol), sodium azide (13 mg, 0.2 mmol) and dimethyl formamide (3 mL) were added in a flask. The clear homogeneous solution was stirred at room temperature for 3 hours. Then polystyrene was precipitated in methanol, filtered and dried under vacuum.

General Procedure For The "Click" Coupling Of An Azide End-Functional Polystyrene And A Low Molecular Weight Alkyne. In a flask, azide end-functional polystyrene (300 mg, 0.11 mmol), copper bromide (47 mg, 0.33 mmol) and dNbipy (272 mg, 0.66 mmol) were added. The flask was capped with a septum and purged with argon for 2 minutes. Then 3 mL of degassed THF was added via a degassed syringe and the mixture turned to a homogeneous brown/dark red solution. Last, the functional alkyne (0.33 mmol for all alkynes except for the very volatile 2-methyl-1-buten-3-yne (boiling point = 32°C), where a bigger excess was used: 1.1 mmol) was added via a microliter syringe. The mixture was stirred overnight at room temperature. Functionalized polystyrene was precipitated in methanol, filtered and dried under vacuum.

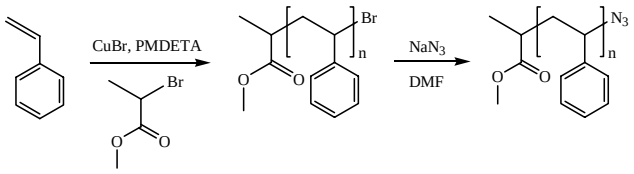
General Procedure For The "Click" Coupling Of An Azide End-Functional Polystyrene With An Alkyne Functional Polypeptide. In a flask, azide end-functional polystyrene, alkyne functional TAT peptide, copper bromide and dNbipy were added. The flask was capped with a septum and purged with argon for 10 minutes. Then, degassed NMP was added via a syringe and the mixture turned to a homogeneous brown/dark red solution. The mixture was stirred overnight at room temperature.

Measurements And Analysis. ***Size Exclusion Chromatography, SEC.*** Molecular weights and molecular weight distributions were determined by SEC performed in either THF or NMP as eluent. For calibration, linear polystyrene standards were used. ***^{1}H NMR.*** ^{1}H NMR Spectra were recorded in $CDCl_3$ on a Bruker DPX-400 operating at 400.1 MHz.

Results and Discussion

Synthesis Of End-Functional Polymers.[8] Controlling the chain-end functionality of synthetic polymers is very important since end-groups can be used for performing further modifications such as reinitiating polymerizations, conjugating macromolecules or adsorbing polymers on surfaces. Combining ATRP and "click" chemistry is an interesting pathway for synthesizing end-functional polymers, since the chain-ends of polymers prepared using ATRP can be easily transformed to azides (Scheme 2) and several functional alkynes are commercially available. In order to illustrate the versatility of such method, a polystyrene sample prepared via ATRP was involved in "click" reactions with various low molecular weight functional alkynes.

Scheme 2. Transformation of the chain-end of well-defined polymers prepared by ATRP into an azide functionality.



First, a polystyrene sample was prepared by ATRP in the presence of the catalyst system Cu(I)Br/PMDETA. The formed polymer possessed a well-defined molecular structure (M_n = 2700 g·mol^{-1}, M_w/M_n = 1.11). After purification, the polymer was analyzed by ^{1}H NMR. Two signals due to each chain-end of the polystyrene chains were detected: a signal from 3.35 to 3.60 ppm due to the methyl ester protons from the MBP moiety and a signal from 4.30 to 4.65 ppm due to the methine proton neighboring the bromine chain-end.[14, 15] Integration of both signals allowed to calculate that 85 ± 5% of the initiated chains remain effectively capped by a bromine atom after polymerization.[14, 15]

In order to perform a 1,3 dipolar cycloaddition azide/alkyne at the ω-chain-end, the bromo functional polystyrene was transformed in an azide ω-functional polymer by nucleophilic substitution (Scheme 2).[13] This reaction was found to be quantitative, as evidenced by ^{1}H NMR. After three hours of reaction, the signal of the methine neighboring the bromine (4.30-4.65 ppm) completely shifted upfield (3.80-4.10 ppm) due to the quantitative substitution of bromine against azide. Integration of the new signal confirmed that the chain-end transformation was quantitative. The obtained azide ω-functional

polystyrene was subsequently involved in "click" reactions with various functional alkynes (propargyl alcohol, propiolic acid or 2-methyl-1-buten-3-yne) in order to prepare ω–hydroxy, ω–carboxyl or ω–methyl-vinyl functional polystyrene. A combination of copper(I) bromide and the ligand 4,4'-di-(5-nonyl)-2,2'-bipyridine (dNbipy) was tested as a new catalytic system for the cycloaddition azide/alkyne in non-polar media. The latter was found to be an excellent catalyst for the 1,3 dipolar cycloaddition of azide functional polystyrene and functional alkynes in THF. Figure 1 shows the ^{1}H NMR spectra recorded for the polystyrene sample prepared from the reaction with propargyl alcohol. After reaction, the signal due to the methine proton neighboring the azido group (H_c: 3.80-4.10 ppm) completely disappeared and was replaced by two new broad signals at 4.50-4.80 ppm and 4.95-5.25 ppm. The first region was assigned to the two methylene protons neighboring the alcohol function (H_d), whereas the second one was assigned to the methine proton neighboring the triazole ring (H_c). Integration of both signals confirmed quantitative transformation of azide end-groups into hydroxymethyl-triazole end groups. Hence, the whole two-step transformation of ATRP chain-ends into hydroxy moieties was found to be quantitative.

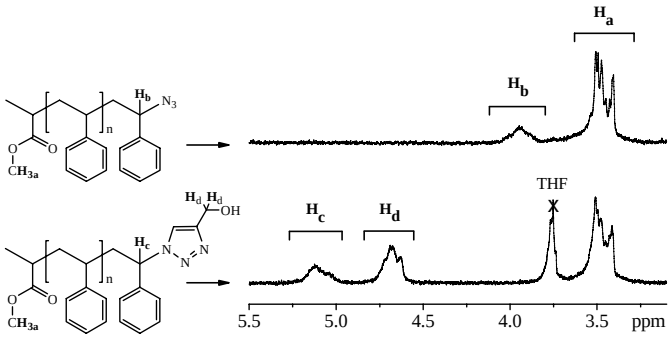


Figure 1. ^{1}H NMR spectrum (zoom of the region 5.5 – 3 ppm) of purified polystyrenes before and after "click reaction". Spectra were recorded at room temperature in $CDCl_3$.

The method was also successfully applied for the preparation of ω–carboxyl and ω–methyl-vinyl functionalized polystyrenes. In both cases, the successful transformation of azide end-groups into functional triazoles was evidenced in ^{1}H NMR by the disappearance of the methine proton neighboring the azido group (3.80-4.10 ppm) and the appearance of the methine proton neighboring the triazole (4.95-5.25 ppm). Moreover, additional regions of the ^{1}H NMR spectra corroborated the efficiency of the "click" reactions. For the ω–carboxyl polystyrene, a signal at 7.45-7.70 ppm was observed. The latter was assigned to the proton of the triazole ring, which appears downfield due to the adjacent carboxylic acid group. For the ω–methyl-vinyl polystyrene, the ethylenic protons of the chain-end were also observable at 5.55-5.75 ppm and 4.95-5.25 ppm. The latter overlaps with the methine proton in α of the triazole. In all cases, the integration of all aforementioned signals confirmed quantitative formation of the functionalized triazole chain-ends

Synthesis of Chimeras Polystyrene-*b*-Polypeptide. Block copolymers composed of a synthetic polymer segment and a defined polypeptide segment (so called molecular chimeras) are very interesting structures for biotechnology.[16, 17] They can be prepared by ATRP, using a polypeptide macroinitiator.[18] However, the direct "click" coupling of a preformed well-defined polymer segment with a preformed sequence-defined polypeptide would be also a very interesting alternative. Van Hest and coworkers already reported that such strategy could be successfully applied for the preparation of diblock copolymers of polystyrene and poly(methyl methacrylate).[9] Thus, in the present work, we investigated the "click" ligation of a well-defined polystyrene prepared by ATRP (M_n = 2200 g·mol^{-1}, M_w/M_n = 1.15) and a model polypeptide (TAT sequence YGRKKRRQRRR including protecting side-groups, M = 3864 g·mol^{-1}) prepared by solid-phase supported synthesis. The polystyrene prepared by ATRP was transformed into an azide functional polymer using the aforementioned procedure. The terminal amino-functionality of the polypeptide was transformed in an alkyne moiety via a coupling reaction with 4-pentynoic acid in the presence of N,N'-dicyclohexylcarbodiimide. The "click" reaction was investigated in solution in *N*-methyl pyrrolidone (the polypeptide was cleaved from the solid support prior to use) in the presence of the catalyst system CuBr/dNbipy. As expected, the coupling between azide functional polystyrene and the alkyne functional polypeptide occurred. Well-defined conjugates polystyrene-*b*-polypeptide could be observed by size exclusion chromatography (apparent M_n = 6900 g·mol^{-1}, M_w/M_n ~ 1.05). The yield of coupling was high (typically 70-80%) but not quantitative.

Conclusions

The copper catalyzed 1,3 dipolar cycloaddition of azides and terminal alkynes was investigated as a new synthetic tool for post reacting well-defined polymers prepared by ATRP. Bromine end-functional polystyrene samples were first transformed into azide functional polymers and subsequently involved in "click" coupling reactions with either low molecular weight or macromolecular compounds bearing an alkyne moiety. With low molecular weight alkynes, in all cases, the chain-end transformation reactions were found to be quantitative. This new technique can therefore be considered as an universal method for preparing end-functional polymers. With alkyne functional polypeptides, the method was also found to be an efficient pathway, although not quantitative, for preparing well-defined conjugates polystyrene-*b*-polypeptides.

Acknowledgements. Fraunhofer society is gratefully acknowledged for financial support. In addition, the authors thank Katharina Otte and Hartmut Rettig for the TAT synthesis, Marlies Gräwert for SEC measurements and Olaf Niemeyer for the NMR measurements.

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