Summer Scholar Report

Synthesis of Heterotelechelic Polymers via RAFT Polymerization for Tagging Red Blood Cells as Drug Carriers

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Abstract

Heterotelechelic polymers with functionalized chain ends were synthesized using the reversible addition-fragmentation chain transfer (RAFT) chemistry. A trithiocarbonyl chain transfer agent was designed to introduce reactive polymer chain ends for future bioconjugation to red blood cells. NMR and gel permeation chromatography (GPC) were used to study the molecular weight, dispersity, and chain end group fidelity.

Introduction

Tagging red blood cells (RBC) using nanoparticles can offer great advantages for potential applications in drug delivery. The abundance, biocompatibility, and longevity in circulation made the RBC an excellent candidate as a drug shuttle[1]. Also, the drug-carrying agents attached to RBC can be cleared through the pathways that normally eliminate old and damaged red blood cells, which provides a novel approach for targeting these pathways.[2] Therefore, anchoring the drug-loading nanoparticle to the surface of RBCs via a synthetic polymer linker is a promising strategy to improve the solubility of the loaded drugs, extend the time of their circulation, reduce the unintended side effects of the drug, and enhance their efficacy.

It has been widely studied and developed that controlled radical polymerization (CRP) gives predictable molecular weights, low polydispersity (*Đ*) and precise control of the reaction rate.[3] For reversible addition-fragmentation chain transfer (RAFT) reaction, the reaction kinetics is controlled by the chain transfer agent (CTA). The result of RAFT polymerization is a well-defined polymer with low *Đ*.[4]

In this work, we propose to synthesize heterotelechelic polymers with thiol and alkyne functionalities on the chain ends. The thiol can form conjugate with a drug-loading agent via thiol-maleimide reaction and the alkyne can undergo click chemistry with an azide-functionalized affinity reagent.

The Experimental Section

Materials. All organic solvents used were from Sigma and used as received. All commercially available chemicals were from Sigma and TCI. Azobisisobutyronitrile (AIBN) was recrystallized in methanol. 2-hydroxyl acrylate (HEA) was purified according to literature. Poly(ethylene glycol) acrylate(PEGA, Mw=480) and *N,N*-dimethylacrylamide (DMA) were purified by alumina column.

Characterization. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, on either a Varian Gemini-600 (600 MHz) or Varian Inova-500 (500 MHz) and calibrated residual solvent peaks. Size-exclusion chromatography (SEC) measurements were performed using Tosoh's high-performance SEC system HLC-8320GPC with TSKgel Alpha-M columns at 50^0C and a flow rate of 1 mL /min. HPLC grade dimethylformamide (DMF) with 0.01 M LiBr (anhydrous, purchased from Sigma-Aldrich) as used as the eluent. Polystyrene standards (Ready-Cal Kit, Sigma-Aldrich#81434) were used to determine the molecular weight and molecular weight distribution of polymers. The polymers were dissolved in the above DMF solution and filtered through a 0.20 μm PTFE filter before being injected into the SEC system.

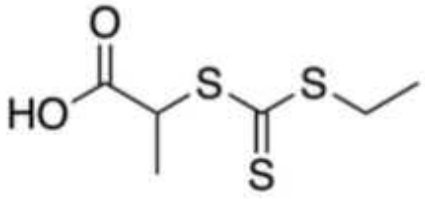


Figure 1. *Structure of PAETC*

Synthesis of 2-(propionic acid)ylethyl trithiocarbonate (PAETC). Sodium hydroxide (6.25 M, 3.20 mL) and ethanethiol (1.28 g, 20 mmol, 1.53 mL) were combined in H_2O (20 mL) and acetone (50mL). methanedithione (1.83 g, 24.00 mmol, 1.45 mL) was added dropwise. The reaction mixture was then left to react at room temperature for 40 min, affording a dark orange solution. 2-bromopropanoic acid (3.37 g, 22.00 mmol, 1.98 mL) was added dropwise on ice bath over 10min. The resulting yellow reaction mixture was stirred overnight. 5.0mL of 10M HCl was added, and the reaction mixture is extracted into ethyl acetate. The organic layer was washed with 100mL brine three times and dried with a layer of sodium sulfate. The residue was concentrated in vacuo and recrystallized in hexane at 50^0C to yield 2-ethylsulfanylcarbothioylsulfanyl-propanoic acid (2.89 g, 13.74 mmol, 68.70% yield). ^{1}H-NMR (500Hz, $CDCl_3$) δ4.86 (1H, q, $CH_3CH(S)COOH$), 3.38 (2H, q, CH_3CH_2S), 1.63 (3H, d, $CH_3CH(S)COOH$), 1.36 (3H, t, CH_3CH_2S).

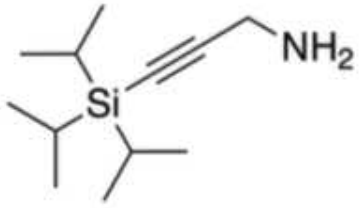


Figure 2. *Structure of TIPS-PA*

Synthesis of 3-triisopropylsilylprop-2-yn-1-amine (TIPSPA).
prop-2-yn-1-amine (851.73 mg, 15 mmol, 990.38 uL) in anhydrous THF (40 mL) was cooled to -78 ÆC. butyllithium (2.5 M, 6.00 mL) were added dropwise. The solution was stirred for 30 minutes and warmed to 0^0C. chloro(triisopropyl) silane (3.58 g, 18.00 mmol, 3.97 mL) was added dropwise. The reaction was left to react overnight and concentrated in vacuo. 50mL of H_2O were added and the aqueous layer was extracted with ethyl acetate 3 times. The combined organic phase was dried over sodium sulfate. The residue was purified by chromatography on silica gel (1:20 to 1:1 gradient EtOAc: Hexane) to give 3-triisopropylsilylprop-2-yn-1-amine (2.18 g, 10.31 mmol, 69% yield) as a yellow oil. ^{1}H NMR (600Hz, $CDCl_3$) δ1.05- 1.09 (21 H, m, 3x Si-CH(CH_3)2), 1.43 (2H, br s, NH_2), 3.45 (2H, s, CH_2). ^{13}C NMR (600Hz, $CDCl_3$) δ13.20 (CH_3CH_2S), 16.68 (CH_3CHS), 31.95 (CH3CH2S), 47.71 (CH3CHS), 177.66 (CHCOOH), 221.77 (SC(S)S)

Synthesis of PAETC-alkyne-TIPS protected (PAETC-yn).
3-triisopropylsilylprop-2-yn-1-amine (1.16 g, 5.5 mmol), 1- hydroxypyrrolidine-2,5-dione (575.45 mg, 5.00 mmol), *N,N'*- dicyclohexylmethanediimine (1.13 g, 5.50 mmol) were dissolved in DCM (30 mL). The solution was cooled to 0^0C and stirred for 15 minutes. Then the reaction was allowed to stir at room temperature for one hour, during which it turned cloudy. The reaction was then filtered, cooled to 0^0C, then 3-triisopropylsilylprop-2-yn-1-amine (1.16 g, 5.5 mmol) was added. The reaction was then stirred overnight and washed with brine three times. The organic layer was dried over anhydrous sodium sulfate. The residue was purified by silica gel column chromatography (1: 9 to 1:1 gradient EtOAc:Hexane) to yield 2-ethylsulfanyl-carbothioylsulfanyl-N-(3-triisopropylsilylprop-2-ynyl)propanamide (2.00 g, 4.95 mmol, 99%yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.05- 1.09 (21 H, m, 3x Si-CH(CH_3)$_2$), 1.36 (3H, t, CH_3CH_2S), 1.63 (3H, d, CH_3CH(S)COOH), 3.37 (2H, dd, CH_2NH), 4.05 (2H, q, CH_3CH_2S), 4.76 (1H, q, CH_3CH(S)COOH). ^{13}C NMR (600 MHz, $CDCl_3$) δ 219.35 (SC(S)S), 172.49 (NHCOCH), 102.45 (Si-CC), 84.84 (CC-CH_2NH), 47.37 ($COCH_2S$), 31.79 (CCH_2NH), 30.14 (S-CH_2CH_3), 18.52 (Si-CH(CH_3)$_2$), 16.00(SCH(O)CH_3), 11.26 (S-CH_2CH_3), 11.08 (Si-CH(CH_3)$_2$). DART+(m/z) [M+H]+ calculated for $C_{18}H_{34}SiNOS_3$ 404.7492 found 404.1572.

Synthesis of Poly(PEGA) and Poly(HEA): RAFT polymerization was conducted using standard Schlenk techniques. Monomer, CTA and AIBN were loaded in a Schlenk tube with 1mL DMF as solvent. The sealed tube was subjected to three freeze-pump-thaw cycles. The polymerization was initiated at 70 C. The reaction was stopped by exposure to air at certain time points. The polymers were purified against H_2O (MWCO 3500 Da). Polymer conversion was calculated from the signal from vinyl protons in the polymer

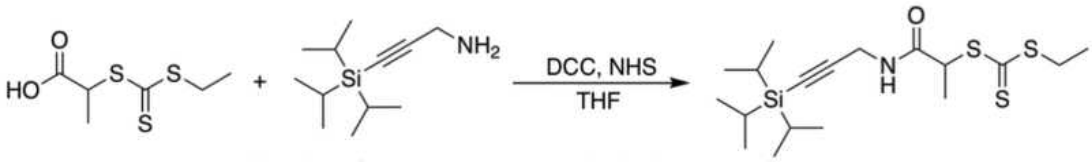
versus the monomer in 1H NMR ($CDCl_3$). Polymer molecular weight and the polydispersity (*Đ*) was measured by SEC.

Synthesis of Poly(DMA): Reactions were carried in a 3 mL clear glass vial with PP Hole Cap & PTFE/Silicone Septa with all reaction components. The via was covered in aluminum foil and degassed by N_2 for 30 minutes, then irradiated by a blue LED strip (4.8 Watts, λmax = 435 nm) at room temperature. The polymer was analyzed by ^{1}H NMR ($CDCl_3$) and GPC to measure the conversions, number average molecular weights (Mn) and *Đ*.

Result and Discussions:

CTA Design and Synthesis

The CTA was designed to contain a trisopropylsiyl (TIPS) protected-alkyne group and a trithiocarbonate group. The TIPS protecting group can be conveniently deprotected by tetrabutylammonium. The alkyne will couple with the antibody that with an azide modification via copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC). Trithiocarbonate chain transfer agent was chosen for its high reactivity with acrylates and acrylamide monomers.[5] The thiocarbonate chain end could be removed to yield a free thiol group, which then can be coupled to a maleimide-containing poly(lactic-co-glycolic acid) (PLGA) drug loading molecule. The synthesis of 2-(ethyl trithiocarbonate) propionic acid was conducted following literature procedures. The resulting trithiocarbonate was subsequently coupled using *N,N'*-dicyclohexylcarbodiimide and *N*-hydroxysuccinimide to form the final CTA in high yield (Scheme 1).



Scheme 1. *Synthesis of TIPS protected chain-transfer agent.*

Synthesis of Poly(PEGA) and Poly(HEA) via RAFT Polymerization:

The CTA was used to polymerize monomers poly(ethylene glycol) acrylates (PEGA), and 2-hydroxyl acrylate (HEA) using azobisisobutyronitrile (AIBN) as initiator to form poly(PEGA) and poly(HEA) (Table 1, entry 1-4). With [PEGA]:[CTA]:[AIBN] = 200:1:0.1, from ^{1}H-NMR the monomer conversion is calculated to be 77% after 2 hours . *Đ* was found to be 1.43 for the poly(PEGA) from SEC. The broad *Đ* occurred in the polymerization of PEGA is a sign that the reaction started to lose control at higher molecular weight (Table 1, entry 1). A lower degree of polymerization at the ratio of [PEGA]:[CTA]:[AIBN] = 50:1:0.1 was subsequently studied.

Entry	M	Initiator	[M]:[CTA]:[I]	Conv (%) ^{1}H NMR	$M_{n\,(NMR)}$	Conv(%) SEC	$M_{n\,(SEC)}$	$Đ$
1	PEGA	AIBN	200/1/0.1	77	74,100	68	34,500	1.43
2	PEGA	AIBN	50/1/0.1	63	15,600	50	12,800	1.29
3	HEA	AIBN	200/1/0.1	74	17,700	64	12,200	1.38
4	HEA	AIBN	50/1/0.1	69	4,400	68	2,600	1.27
5	DMA	$Ru(bpy)_3Cl_2$	$400/1/1x10^{-4}$	68	27.600	55	22,300	1.23
6	DMA	$Ru(bpy)_3Cl_2$	$200/1/1x10^{-4}$	76	15,600	56	11,500	1.14
7	DMA	$Ru(bpy)_3Cl_2$	$50/1/1x10^{-4}$	63	3,550	57	3,300	1.08

Table 1. *Polymer linkers generated using different hydrophilic monomers.*

However, SEC still showed $Đ$ =1.29 at 63% monomer conversion by NMR (Table 1, entry 2). For poly(PEGA), the Mn calculated by SEC using the polystyrene standards was lower than the predicted value by NMR. Such a disparity was attributed to the structural difference between the branched hydrophilic chain on the poly (PEGA) and polystyrene standards. I reason that the high molecular weight of PEGA monomer limits the monomer concentration and reactivity in polymerization, so I decided to try a smaller monomer HEA. The polymerization at the ratio of [HEA]:[CTA]:[AIBN] = 200:1:0.1 and 50:1:0.1 were studied. However, both polymers still exhibited Đ>1.2 (Table 1, entry 3-4). The short half-life of the AIBN initiator is known to limit the molecular weight of the polymer and cause broad dispersity.[6] To overcome this challenge, I decided to employ the photoelectron transfer-RAFT (PET-RAFT) method to generate the heterotelechelic polymers. As an emerging technology, PET-RAFT allows the continuous single electron transfer from the excited photocatalysts over the course of the irradiation, leading to a constant concentration of the propagating chain ends and excellent control over polymerization for high molecular weight polymers.[7,8] Dimethyl-acrylamide (DMA) was chosen as the monomer due to compatibility for photo-RAFT and water-solubility, which makes a good candidate for making a hydrophilic linker. The photocatalyst tris(2,2'-bipyridine) ruthenium(II) was used to initiate the reaction after irradiated by blue LED light ((4.8W, λ_{max} = 435 nm). Three poly(DMA) polymers were synthesized with [M]:[CTA] ratio between 50 and 400, and the polymers achieved reasonable conversion and low polydispersity even at high degree of polymerization ($Đ$<1.3) (Table 1, entry 5). NMR spectrum and SEC traces are shown in Figure 3. The NMR spectrum of dialyzed polymer shows characteristic signals of functional chain ends (Figure 3A). Triisopropyl groups, propargyl amine, trithiocarbonate signals were confirmed by the characteristic signals at δ 1.09 ppm, δ 3.3 ppm, and δ 4.0 ppm respectively.

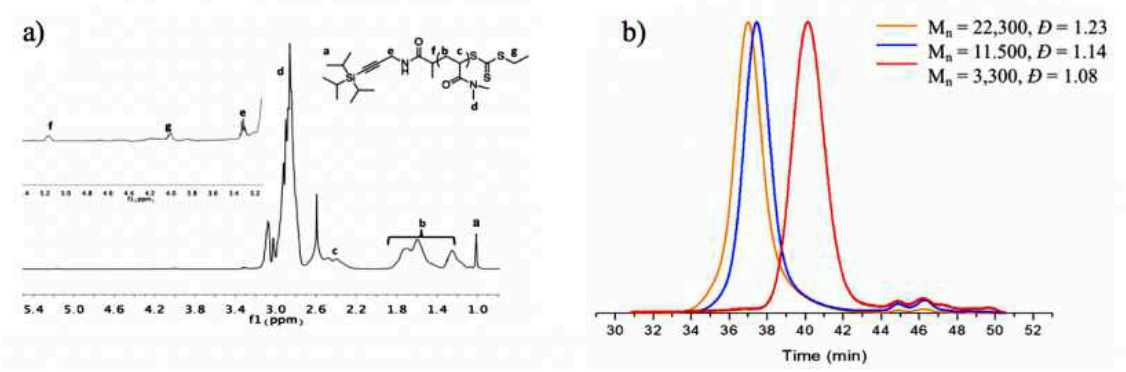


Figure 3. *a)1H NMR Spectrum ($CDC1_3$) of PAECT-poly(DMA). b) GPC result of PAETC-poly(DMA).*

Conclusion and Future directions:

I have developed a simple method to synthesize polymers with an alkyne group for conjugating proteins site-specifically later in the project. The triisopropylsilyl protected poly(PEGA), poly (HEA) and poly(DMA) were successfully synthesized via RAFT chemistry. Since poly(DMA) demonstrated low *Đ* at high molecular weight, poly(DMA) will be deprotected the thiol and alkyne chain ends to conjugate to the drug loading nanoparticle and RBC-binding antibody, respectively. The alkyne would be first deprotected and coupled to the antibody in order to increase the solubility of the polymer complex. Protein SDS gels could be used to detect the presence of the antibody. The polymer-antibody conjugate will then be conjugated to drug-loading PLGA nanoparticles. Transmission electron microscopy will be employed to study the structure of the conjugated nanoparticles. Further investigations for studying for drug delivery agents such as drug loading efficiency, and the stability of the conjugates will be performed in collaboration with Samir Mitragotri's lab at Harvard University School of Engineering.

Bibliographic Citations:

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