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Flow-IEG Enables Programmable Thermodynamic Properties in Sequence-Defined Unimolecular Macromolecules

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Monodisperse oligomers are important intermediates for studying structure–property relationships in soft materials but are traditionally laborious to synthesize. A semi-automated synthetic system that combines the benefits of telescoped reactions in continuous flow with iterative exponential growth (IEG) greatly expedites this process and makes the rapid synthesis of structurally diverse oligomer libraries practical. Herein, the coupling chemistry in the Flow-IEG system has been upgraded and expanded to include both 1,4- and 1,5-triazole linkages between monomers through an improved copper-catalyzed azide–alkyne cycloaddition (CuAAC) and a newly-optimized ruthenium-catalyzed azide–alkyne cycloaddition (RuAAC), respectively. Improvements to the Flow-IEG framework enabled the library synthesis of monodisperse oligomers with variations in triazole connectivity. These discrete oligomers allowed the systematic evaluation of the consequences of triazole sequence on material properties. The crystallization properties of these macromolecules were highly dependent on both their monomer sequence and triazole substitution pattern.

Introduction

The synthesis of monodisperse macromolecules of intermediate size is inherently challenging. Oligomers are too large for practical stepwise synthetic approaches, which can require days to produce a single oligomer, and traditional polymerization techniques lack the fine control of molecular structure necessary to produce unimolecular, sequence-defined materials. However, the ability to control the molecular structure and assembly of sequence-defined synthetic polymers holds significant potential in a number of applied areas and this demand has spurred many creative approaches to this contemporary challenge.1−7

A valuable method for the synthesis of sequence-defined unimolecular polymers is iterative exponential growth (IEG). First developed by Whiting,8,9 and expanded by Moore,10,11 Tour,12 Hawker,13,14 and many others,15 IEG methods combine a series of orthogonal deprotection and coupling steps to produce unimolecular, sequence-defined polymers. Fundamental to the success of this iterative approach are orthogonal and high-yielding reactions. As a result, the azide–alkyne 1,3-dipolar cycloaddition, first developed by Huisgen16 and made catalytic by both Sharpless et al.17 and Meldal et al.,18 has found great utility in IEG.15,19−22 Despite its utility and long history, IEG has not become a routine method in polymer synthesis due to its laborious and repetitive nature, which requires three reaction and purification steps for each doubling of molecular weight.

Continuous-flow chemistry was recognized as a promising technology to simplify the redundancy of IEG due to its throughput, reproducibility, and ability to telescope reactions.23−25 Our group previously reported the application of flow chemistry to IEG to create a continuous-flow system that performs one growth cycle (two deprotection steps, an in-line purification, and one coupling step) in an uninterrupted fashion26 (Figure 1). Referred to as Flow-IEG, the first generation continuous system demonstrated the utility of this
hybrid approach through the synthesis of an alternating polyester/oligo(ethylene glycol) hexadecamer with a molecular weight of 4,023 g/mol in a 28% overall yield from the monomer in four cycles.

With the concept of Flow-IEG established, there exist significant opportunities to exploit the molecular-level control over sequence and connectivity to synthesize materials with systematic variations in structure and enable a semi-automated method to tune the thermal and mechanical properties of soft materials. Herein we report an improved Flow-IEG platform that incorporates new coupling chemistry and facilitates the rapid synthesis of a library of structurally isomeric oligomers with systematic variations in both sequence and connectivity. Enabled by this synthetic technology, the structure–property relationships of these soft materials were investigated with the ultimate goal of understanding the consequence of monomer sequence and connectivity on material function. The second-generation Flow-IEG system described herein offers a number of inherent advantages in pursuit of this goal, namely the plug-and-play nature of the platform and its expanded reactivity, which enables facile variation in sequence (through the use of different starting monomers) and connectivity (through the introduction of new chemical reactions).

Experimental

Materials

All commercially obtained solvents and reagents were used without further purification, with the exception of the tetrabutylammonium azide (TBAA), which was dissolved in tetrahydrofuran (THF) and filtered through a VWR 13 mm Syringe Filter with 0.2 μm PTFE membrane prior to use. (3-Bromoprop-1-yn-1-yl)trisopropylsilane, the oligoethylene glycol monomer, and 3-(trisopropylsilyl)prop-2-yn-1-yl 11-bromoundecanoate were prepared according to previously published procedures. Anhydrous THF, toluene, and acetonitrile (ACN) were purified in a 5G Water USA solvent column system. Harvard Apparatus syringe pumps, model PHD2000, with 8 mL stainless steel syringes (four syringes in two pumps) were used to flow the substrate solutions and the tetrabutylammonium fluoride (TBAF) and TBAA solutions. VICI Model M6 Liquid Handling Pumps were used to flow the CuAAC or RuAAC solution and the water utilized for separation. FloZET™ software was used to control the flow rates of the M6 pumps. A 6.6 mm ID Omnifit™ Glass Column purchased from Fisher Scientific was used to hold the molecular sieves. All PFA tubing and connectors, mixers, etc. were purchased from IDEX Health & Science. PFA tubing with a 0.03 in inner diameter was used unless otherwise noted. Copper-metal tubing of 0.0345 in inner diameter and 1/16 in outer diameter was purchased from Small Parts, and activated by injection of 10 mL 1 M HCl through the reactor before use. T-mixers with an internal diameter of 0.02 in were used for mixing all of the reagents. Heating the reactors was accomplished by immersion in an oil bath with temperature regulation provided by an IKA hot plate equipped with a thermocouple probe. Reactions were conducted safely above the boiling points of the solvents through the use of Zaiput® back-pressure regulators. In-line liquid–liquid separations were conducted using Zaiput® separators equipped with a 0.5 μm PTFE Membrane.

Sample preparation and setup

Reactions generating alkyl ester tetramers were conducted at monomer concentrations of 0.15 M in chlorobenzene to maintain solubility in the flow system. For reactions involving the alkyl ester/oligo(ethylene glycol) series, a substrate concentration of 0.32 M in toluene was employed. The TBAF solution was prepared by dissolving 1.1 eq TBAF•3H2O in a 2:1 THF/toluene solvent mixture. The TBAA solution was prepared with 1.5 eq TBAA dissolved in THF, then filtered.

CuAAC system: A solution of 5 mol% CuBr2 and 15 mol% tris[2-(dimethylamino)ethyl]amine (Me2TREN) (compared to substrate) in ACN was prepared. Reagents were loaded into syringes and pumped at constant flow rates. Temperatures of the various reactors and flow rates were as noted in Scheme 1. The system was allowed to equilibrate for three residence times to reach steady state before the product was collected for analysis.

RuAAC system: General setup was as above, with the following alterations: catalyst solution of 5 mol% Cp*RuCl(COD) (compared to substrate), and all substrates and reagents degassed by sparging with argon for 20 min prior to use. (See supporting information for detailed explanation.)

Results and Discussion

Design and Optimization of Flow-IEG Click Reactors

In pursuit of a robust and user-friendly Flow-IEG system, we first sought to modify the copper-catalyzed azide–alkyne cycloaddition (CuAAC) utilized in the first-generation Flow-IEG platform. Despite its efficiency and utility, the choice of copper(I) iodide as a catalyst for the CuAAC presented a number of challenges: the iodide counterion participated in a Finkelstein reaction with the bromide functionality of the growing polymer chain, the Cu(l)/ligand complex became increasingly insoluble over the course of hours, and the system required oxygen-free conditions. We sought to develop a continuous CuAAC that was homogeneous, chemoselective, tolerant to air, and could run uninterrupted.

There are a number of reports in the literature of using copper(0) metal to generate an active copper(I) catalytic species for the CuAAC reaction. In a typical batch procedure, the coupling partners are introduced to a polar, aprotic solvent along with an elemental copper catalyst and stirred for 12–48 h. This procedure can be accelerated by a variety of techniques, including: microwave heating, continuous flow, and even mechanochemical conditions. We initiated our studies under continuous-flow conditions employing copper tubing as both a reactor and catalyst source. All reactions were conducted in a 1:1 mixture of toluene/polar...
solvent with the temperature, time, and ligand identity noted in Table 1. The flow reactors were pressurized to 100 psi using a Zaiput® backpressure regulator to allow the superheating of solvents. Conducting the reaction in the absence of a multidentate amine ligand did not generate the 1,4-disubstituted 1,2,3-triazole product (Table 1, entry 1) and the addition of tris[2-(dimethylamino)ethyl]amine (Me₆TREN)⁸ as a ligand did not appreciably improve conversion (entry 2). Changing the identity of the ligand to tris(2-benzimidazolylmethyl)amine [(Bim)₃], a ligand reported to be

Table 1. Optimization table of the CuAAC reaction in flow with copper metal tubing.

<table>
<thead>
<tr>
<th>#</th>
<th>CuBr₂, mol%</th>
<th>Polar Solvent*</th>
<th>Ligand</th>
<th>Ligand, mol%</th>
<th>Temperature, °C</th>
<th>Residence Time, min</th>
<th>Conversion, %^c</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>DMSO</td>
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<td>7.5</td>
<td>0</td>
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<tr>
<td>2</td>
<td>0</td>
<td>DMSO</td>
<td>Me₆TREN</td>
<td>25</td>
<td>150</td>
<td>7.5</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>DMSO</td>
<td>(Bim)₃</td>
<td>25</td>
<td>150</td>
<td>7.5</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>DMSO</td>
<td>Me₆TREN</td>
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</tr>
<tr>
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<td>Me₆TREN</td>
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</tr>
<tr>
<td>6</td>
<td>10</td>
<td>ACN</td>
<td>–</td>
<td>0</td>
<td>150</td>
<td>3.8</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>ACN</td>
<td>Me₆TREN</td>
<td>15</td>
<td>150</td>
<td>2.5</td>
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<tr>
<td>8</td>
<td>10</td>
<td>ACN</td>
<td>Me₆TREN</td>
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<td>9</td>
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<td>Me₆TREN</td>
<td>15</td>
<td>140</td>
<td>3.3</td>
<td>94</td>
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</tbody>
</table>

* All reactions conducted in 1:1 toluene/polar solvent. ^ Conversion of starting material to product 3 was determined through ¹H NMR. ^ Although quantitative conversion of starting material was achieved, significant byproduct formation occurred. Additionally, CuBr₂ is less soluble in absence of the ligand. ^ PFA tubing of equal internal volume was used in lieu of copper tubing.
highly effective in CuAAC reactions,\textsuperscript{39} was likewise ineffective (entry 3). To increase the concentration of active catalyst in solution, we supplied a soluble, external copper(II) source. Previous work has demonstrated that the comproportionation of copper(II) and copper(0) can generate a soluble and active copper(II) catalyst in solution.\textsuperscript{32,36,40}

Initial trials using 10 mol% of copper(II) bromide (CuBr\textsubscript{2}) passed through the copper tubing at 150 °C produced 85% conversion of starting material terminal alkyne (1) and aliphatic azide (2) into the 1,4-disubstituted triazole (3), with no side products detected (entry 4). We discovered that the replacing dimethyl sulfoxide (DMSO) with acetonitrile (ACN) resulted in a further increase in conversion (entries 5–9). Moreover, we found that this method was tolerant of oxygen in solution, negating the need to rigorously degas the reagents prior to use and thus making the system operationally simpler and more amenable to translation. Subsequent optimization of temperature, solvent, residence time, ligand amount, and catalyst loading allowed us to lower the amount of CuBr\textsubscript{2} while still achieving high conversion (Table 1, entries 5–7). Reagent concentration was varied from 0.1 to 0.5 M with no decrease in reactivity. Minimal conversion was obtained in the absence of copper tubing (Table 1, entry 8). For practical utility in the telescoped sequence of reactions, we selected the conditions in entry 9 for the CuAAC reaction. Our efforts in catalyst development have significantly improved the flow-IEG system. The air-stable nature of the catalyst system makes setup more facile and substantially less time-consuming. The improved reaction conditions have yielded consistent results during continuous operation for 11 hours, retaining high conversion and yields throughout the length of the run.

In order to synthesize designer materials with tunable thermal and mechanical properties, we sought to develop a complementary coupling chemistry that alters the physical properties of the synthesized oligomers while remaining isostructural in composition. The ruthenium-catalyzed azide–alkyne cycloaddition (RuAAC), developed by Fokin et al.,\textsuperscript{41} held strong appeal for this objective as the reaction utilizes the same starting materials but produces a 1,5-disubstituted 1,2,3-triazole, which is a structural isomer of the CuAAC product. The consequences of the structural differences between these linkages has been demonstrated in their incorporation into proteins; the 1,5-triazole was utilized as a surrogate for cis-prolyl peptide bonds, whereas installation of the 1,4-isomer was found to alter the native structure of the protein.\textsuperscript{42} Therefore, we envisioned that incorporating one or more of the 1,5-triazole linkages in an oligomer would lead to significant differences in physical properties.

We elected to use Cp*RuCl(COD) (COD = 1,5-cyclooctadiene) as a catalyst due to the reported regioselective generation of the 1,5-triazole from alkyl azides and terminal alkenes.\textsuperscript{43} Optimization of catalyst loading, temperature, solvent, and reaction time under continuous-flow conditions (Table 2, entries 1–4) led to a method that produced high conversion from 1 and 2 to 4 at 70 °C in only 1 min (Table 2, entry 5).

The water sensitivity of the Cp*RuCl(COD) catalyst presented challenges not observed in the copper system. Although the RuAAC reaction functions with high conversion in isolation, the telescoped Flow-IEG system necessitates that the coupling reaction be conducted after an in-line liquid–liquid extraction. Initial experiments running the Flow-IEG system with the RuAAC gave only low conversions (~10–15%) of starting material to the 1,5-triazole product. We discovered that ~2500 ppm of water (quantified by Karl Fischer titration) remained in the reaction stream after in-line liquid–liquid separation, which hindered reactivity in our initial experiments. This challenge was overcome by the addition of a column of 3 Å molecular sieves immediately after the in-line separation, which reduced the water content to <120 ppm and restored reactivity commensurate with Table 2, entry 5. Reactions were run under these conditions for up to 2 h with no decrease in reactivity or yield.

<table>
<thead>
<tr>
<th>#</th>
<th>Cp*RuCl(COD), mol%</th>
<th>Temperature, °C</th>
<th>Solvent</th>
<th>Residence Time, min</th>
<th>Conversion, %</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Toluene</td>
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<td>59</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>60</td>
<td>Toluene</td>
<td>2.5</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>70</td>
<td>Toluene</td>
<td>1.5</td>
<td>97</td>
</tr>
<tr>
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<td>2.5</td>
<td>80</td>
<td>Toluene</td>
<td>1.5</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>70</td>
<td>4:1 Toluene/THF</td>
<td>1</td>
<td>96</td>
</tr>
</tbody>
</table>

Conversion of starting material to product 4 was determined through \textsuperscript{1}H NMR. The RuAAC reaction has been reported to proceed with internal alkenes.\textsuperscript{43,44} However, control experiments with substrates that contain a bulky, TIPS-protected alkyne show no reactivity for cycloaddition under the conditions described.
Library Synthesis of Oligomers and Investigation of Structure–Property Relationships

With two distinct modes of reactivity now amenable to Flow-IEG, we sought to systematically vary connectivity in monodisperse oligomers in order to investigate its influence on material properties. For the di-functional aliphatic ester depicted in Figure 2, its tetrameric species have eight possible structural isomers derived solely from differences in triazole connectivity. Incorporating the newly optimized coupling reactions into the Flow-IEG framework, we were able to rapidly synthesize a library of oligoesters with a variety of 1,4- and 1,5-triazole linkages (Figure 2).

The initial deprotection steps of in-flow oligomer synthesis are the same for both isomers. The reagents are loaded into syringes, then pumped into two separate reactors. In the first, a trisopropylsilyl ether (TIPS) protecting group is removed by a soluble fluoride source to reveal a terminal alkyne, while concurrently in the other reactor the bromide end group is substituted by an azide (Scheme 1). Water is introduced directly after the deprotection reactions to quench excess deprotection reagents and remove tetrabutylammonium salts. The alkyne and azide streams are combined and passed through an in-line, membrane-based, liquid–liquid separator. The aqueous stream containing excess TBAF and TBAA is collected as waste. For the formation of the 1,4-triazole, the organic stream containing the deprotected monomers then mix with a stream of CuBr and Me$_2$TREN catalyst and proceed directly into the copper tubing. Alternatively, to form the 1,5-triazole, the organic stream passes through a column of 3 Å molecular sieves immediately after the in-line separator to reduce water content in the organic phase, and mixes with a flow stream containing the ruthenium catalyst. In the final system, comprised of three reactions and one in-line purification, 1,4- and 1,5-triazoles can be synthesized in just 10 and 15 minutes, respectively. By combining three reactions into one cycle of this semi-automated system, tetramers can be produced quickly and efficiently in two cycles. Using this divergent Flow-IEG system, all eight aliphatic ester tetramers were synthesized, with isolated yields averaging 53–92% for the second run through the Flow-IEG system (Figure 2). For the experiments described, the system was run to yield up to 0.52 g/h of tetramer product and the reported yields were reproducible over numerous experiments. As reported previously, the Flow-IEG system can easily be scaled up to 2.75 g/h, with little change in the system engineering controls. 26

We characterized all oligomers by $^1$H and $^{13}$C NMR (Figure 3), gel-permeation chromatography (GPC), differential scanning calorimetry (DSC), high-resolution mass spectrometry (HR-MS), and thermogravimetric analysis (TGA). The GPC traces of the 1,4-tetramers displayed sharp, monomodal peaks that matched with previously determined values 26 and confirmed the unimolecular nature of the oligomers. The dispersity (D) of each oligomer was ≤1.01 according to polystyrene standards. However, due to the nature of the coupling, the small quantities of impurities (<2.5%) are always predictably either residual dimer or small amounts of octamer. The $^1$H NMR spectra revealed distinct triazole peaks (7.69 ppm for 1,5 triazoles, 7.57 ppm for 1,4 triazoles, Figure 3 inset) and propargylic peaks that aided in confirming structures of the new oligomers and synthetic intermediates. Concurrently with the method development and optimization of these platforms, we wanted to explore the thermal properties of the

Figure 2. Top: graphical notation key for oligomers. Bottom: yields of oligoesters. Yields are given for the three-step Flow-IEG cycle that directly produced the given oligomer. Oligomers are consistently depicted to imply the existence of the bromide on the left end and the TIPS protecting group on the right end of the molecule. Thus, the 1,4-1,4-1,5 and the 1,5-1,4-1,4 oligomer are not identical.

![Figure 2](image_url)

Figure 3. Overlaid $^1$H NMR and GPC traces of alkyl ester 1,4 and 1,5 oligomers. Inset zoom of the $^1$H NMR spectra of the triazole protons.

![Figure 3](image_url)
oligoesters to examine the variation in thermal properties as a function of both connectivity and structure.

The properties of the all 1,4 oligoester tetramer are distinct from that of the all 1,5 oligoester tetramer. The 1,5 species adopt a smaller hydrodynamic radius than their 1,4 analogues (Figure 4a), most likely due to the change in chain conformation induced by the 1,5- vs. 1,4- triazole linkage. As shown in the HR-MS in Figure 4b, these tetramers are unimolecular species of the expected molecular weight. Furthermore, although the library of eight structurally isomeric tetramers in Figure 4a differ only in the connectivity between monomers, they displayed remarkably different physical properties, namely glass transition temperature ($T_g$), melting temperature ($T_m$), and crystallization behavior (Figure 4c). The $T_g$ of all materials increased proportionally to molecular weight, as expected, and were similar across the series of tetramers. For the oligomers that did crystallize, we observed behavior typical of cold crystallization, with a crystallization event occurring above the $T_g$ but below the $T_m$ during the heating cycle. The traces in Figure 4c display the consequences of cold crystallization with little temperature difference between the $T_g$ and the $T_m$. Further, for many of these materials we observed multiple melting exotherms, likely due to the many secondary intermolecular interactions inherent to these oligomers that contain multiple heterocycles, which could result in multiple metastable crystal forms. Similar crystallization behavior has been seen in polyamides such as the nylon series.46-49

Crystallization behavior was significantly influenced by changes in connectivity between the monomers. The 1,4-1,4-1,4 tetramer displayed a melting point in the DSC, and is a waxy solid at room temperature ($T_m = 69.0 °C$, all DSC data reported from 3rd heating scan at a heating rate of 5 °C/min). All of the species with two 1,4-triazoles and one 1,5-triazole exhibit melting transitions ranging from 48.9 to 58.0 °C, which are lower than those of the parent 1,4-1,4-1,4 tetramer. Only small differences in thermal properties are observed between these three tetramers, indicating that the sequence of triazole connectivity within these oligomers has only a moderate impact on thermal properties. The species with one 1,4-triazole and two 1,5-triazoles, as well as the 1,5-1,5-1,5 tetramer, only display a melting peak during the first heating cycle of the DSC, but do not recrystallize during subsequent heating and cooling cycles. Further, the melting transitions observed during this slow crystallization process range from 54.5–61.1 °C, all surprisingly lower than the slow crystallization

![Figure 4](image-url)

**Figure 4.** Physical properties vary with triazole connectivity. (a) Monomodal GPC traces of alkyl ester tetramers. (b) Representative HR-MS spectrum of 1,4-1,5-1,5 tetramer. (c) DSC traces of alkyl ester tetramers grouped by triazole connectivity.
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This work demonstrates the utility of the Flow-iEG system for making unimolecular, defined macromolecules and the ability to diversify structure through the use of plug-and-play reactors. Oligomers of >1000 g/mol were manufactured quickly, efficiently, and in a semi-automated manner. Production of monodisperse oligomers of this size is inherently challenging: iterative synthetic approaches require days to produce a single oligomer, and polymer chemistry approaches struggle to produce truly monodisperse products. By developing technology for the rapid production of libraries of oligomers, we were able to isolate the variables of sequence and connectivity and study how they independently influence the properties of soft materials. Predictive rules emerged from these studies that can potentially be used to synthesize materials with programmable properties. This work validates Flow-iEG as a uniquely valuable tool in the production of libraries of materials for structure/function analysis, and displays the benefits of merging continuous processes and polymer chemistry to enable fundamental studies that are inaccessible through traditional means.

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References