One-pot approach for multi-step, iterative synthesis of sequence-defined oligocarbamates

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Abstract

A high-yielding and scalable method for iterative synthesis of sequence-defined macromolecules is a great challenge in modern polymer chemistry. Sequence-defined macromolecules are fabricated by multi-step iterative processes that involve high reagents and solvents consumption. Moreover, every step causes yield losses that result in low overall yield. Despite the envisioned valuable functions and applications of sequence-defined polymers, the synthetic limitations constitute a barrier for the exploitation of their practical potential. Here, we investigated the one-pot synthesis of oligocarbamates without the purification of intermediates. To control the monomer sequence without isolation, we introduced a monitoring feedback loop to fuel the exact amount of reagents to the reaction mixture, assuring full conversion of each reaction. Based on a one-pot strategy, we have developed a facile approach for the preparation of uniform, oligocarbamates with full control of monomer order and defined stereochemistry. The great advantage of the presented methodology is the scalability of the process (demonstrated for synthesis of 50 g) and high yield (up to 90%). Oligomers obtained on a large scale can be further used as precursors for the synthesis of polymers with high molar mass. One-pot methods combined with chemoselective reactions bear the potential to overcome existing synthesis limitations and unlock the practical use of sequence-defined macromolecules. The presented concept might be further extended to different multi-step processes.

Introduction

Sequence-defined polymers (SDPs) are discrete macromolecules with controlled monomer order.[1-3] The control of primary structure in synthetic polymers gives rise to unexplored regulation of macromolecule properties.[4-8] During the last decade SDPs have been successfully used in, catalysis[9-11], drug delivery[12], sensing[6], and selective binding[13]. Furthermore, the emergence of a new type of macromolecules has opened up completely novel application directions, such as data storage materials,[14-17] information encrypting[18, 19] and anti-counterfeiting.[20-22]

Oligocarbamates with defined monomer order, commonly known as oligourethanes, are an interesting material due to their characteristic, e.g., chemical stability, capability to permeate cell membranes, and depolymerization on demand. Carbamate bond is related to amide-ester hybrid features, therefore oligocarbamates can be regarded as peptidomimetic derivatives. They have found applications as, e.g., data storage materials[17, 23-25], taggants in security technologies[21, 26, 27], molecular transporters[28, 29] or peptidomimetic foldamers.[30, 31] For each of these applications, the monomer sequence was critical to achieving the desired properties of the oligomers.

The synthesis of sequence-defined oligocarbamates involves multi-step processes accompanied by various limitations, resulting from the need for the purification of intermediates after each synthesis step. They are obtained by either solid-phase synthesis[17, 23, 28] or solution synthesis.[17, 32-34] The synthesis conducted on a solid support, e.g., polymer resin simplifies purification between steps. However, solid support hinders couplings, thus, demands high reagent excesses, up to 10-fold.[23] Moreover, it consumes a huge amount of organic solvents due to the necessity of thorough resin washings between reactions. Costs of solid support, reagent wastage, and solvent consumption impede this approach from practical use. Alternatively, they can be fabricated by solution synthesis accompanied by purification between steps.[17, 32, 33] Recently, it was demonstrated that purification can be restricted to simple extraction that enables high-scale synthesis.[27, 32] However, isolation of intermediates

causes yield losses that result in low overall yield and impede the achievement of high molar mass products. Solution phase synthesis appears to be a more attractive approach, as long as the cumbersome purification between steps is eliminated.[35]

One-pot synthesis,[36, 37] where a cascade of reactions occurs in just one reactor without the need for intermediates purification, seems to be a highly attractive approach for the synthesis of sequence-defined macromolecules. The approach is greener due to the reduction of required work-up procedures and purification steps. The one-pot methodology was applied, e.g., in oligonucleotide synthesis[38], oligosaccharides fabrication[39, 40], native chemical ligation[41] multicomponent reactions[42], and multicatalytic processes.[43-45] However, it has not been extended to multiple monomer iterations providing full control over monomer order, without isolation between steps, so far.

Here, we have considered that the concept of one-pot synthesis of sequence-defined macromolecules would be the key to overcoming the limitations of multi-step synthesis and performing the process efficiently. We assumed that supplying exactly the requisite portions of reagents is critical for a high yield and full sequence control of final macromolecules. The amounts of reagents and time, needed to push each reaction to full conversion, can be determined based on chromatography monitoring of synthesis progress. In our approach, the whole multi-step synthesis process is performed in one reaction vessel without the isolation of intermediate products.

Results and discussion

To evaluate the devised concept in the synthesis of sequence-defined oligocarbamates, we have chosen two reactions: (i) activation of the hydroxyl group by N,N-disuccinimidyl carbonate (DSC), and (ii) chemoselective coupling of an amino alcohol, where amine group reacts preferably with active carbonate. The synthesis scheme is presented in Figure 1.

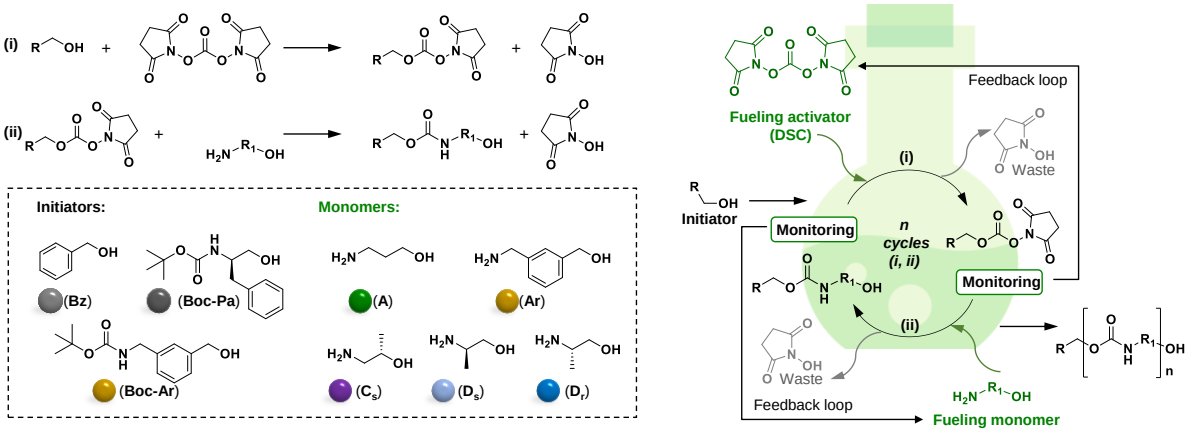

Figure 1. Scheme of sequence-defined oligocarbamates synthesis using a one-pot, reagent-fueled approach. The synthesis involves two reactions (i) activation of the hydroxyl group by DSC and (ii) chemoselective coupling of amino alcohol monomer. Steps i and ii are subsequently repeated by the sequential addition of fresh reagent portions: DSC or monomer. Synthesis is supported by HPLC reaction monitoring that delivers feedback information about reaction conversions. The final product is isolated from the reaction mixture by extraction.

Upon evaluation of reactions in the one-pot approach, we found that the active carbonate (i) reacts quantitatively with the supplied portion of amino alcohol (ii), despite the presence of formed N-hydroxysuccinimide by-product, thus, there is no need for purification after step (i).[46] Therefore, the two-step monomer attachment can be easily performed in a one-pot manner, and we could continue the synthesis by adding a fresh portion of DSC to the system. Despite the 2-fold excess of N-hydroxysuccinimide by-product in the reaction mixture, we were able to achieve full conversion in the following step (i). Although the supplied activator can react with N-hydroxysuccinimide, the reaction will lead to a regeneration of the activator molecule, hence the process can be carried on. Formed N-hydroxysuccinimide does not inhibit the steps (i), however, can influence the reaction time and yield. We speculate that part of DSC undergoes a reaction with N-hydroxysuccinimide by-product, thus, locally reducing the number of DSC molecules available for reaction with the oligomer hydroxyl group. While adding a new portion of the next monomer, the subsequent quantitative coupling has been observed. We could successfully carry on the synthesis by constantly supplying appropriate portions of reagents:

DSC and monomers. We were able to perform up to eight consecutive steps in one pot, without any purification, which yielded uniform pentamer products (Tab. 1, Entries O1-O7).

Table 1. Sequence-defined oligocarbamates obtained by the one-pot approach

No.	Oligomer[a]	M_{mi}[b]	m/z[c]	Purity[d] [%]	Y[e] [%]	Scale[f] [g]
O1	Bz-AAAAr	574.26	575.27	92.3	90	0.40
O2	Boc-ArAAAA	641.33	642.34	99.6	75	3.80
O3	Boc-PaD_rAAA	655.34	656.35	97.1	90	4.40
O4	Boc-PaD_sAAA	655.34	656.35	96.5	92	1.30
O5	Boc-Pa$C_sC_sC_sC_s$	655.34	656.35	96.0	95	2.27
O6	Boc-ArD_sAAA	641.33	642.34	96.2	86	1.36
O7	Boc-PaAAAA	655.34	656.35	94.1	94	50.40

[a]Building blocks: 3-Amino-1-propanol (A), 3-(aminomethyl)benzyl alcohol (Ar), benzyl alcohol (Bz), (S)-1-Amino-2-propanol (Cs), (S)-2-Amino-1-propanol (Ds), (R)-2-Amino-1-propanol (Dr), (S)-2-Amino-3-phenyl-1-propanol (Pa); [b]monoistopic molar mass; [c]LC-MS analysis; [d]purity - calculated based on HPLC UV (220 nm); [e]yields calculated for crude products isolated by extraction; [f]scale - the weight of the obtained product, [g]HPLC analysis performed using MeOH instead of ACN as phase B.

The key to controlling the monomer sequence is the full conversion of initiator-based substrates. Therefore, the conversion on each step of activation (i) and coupling (ii) was monitored by chromatography (Fig. 2 a). Thanks to the significant polarity difference between activated and free alcohol products, we can follow the synthesis progress by reverse-phase HPLC. It is crucial to carry out the reactions using a slight excess of the supplied reagents to drive the conversions to completions. For example, in the initiation step (i), full activation of the benzyl alcohol hydroxyl group was only reached using 1.2 equivalent of DSC (Fig. S27). During carbamate synthesis in solution, a small excess of DSC is commonly used to ensure full alcohol transformation.[47]

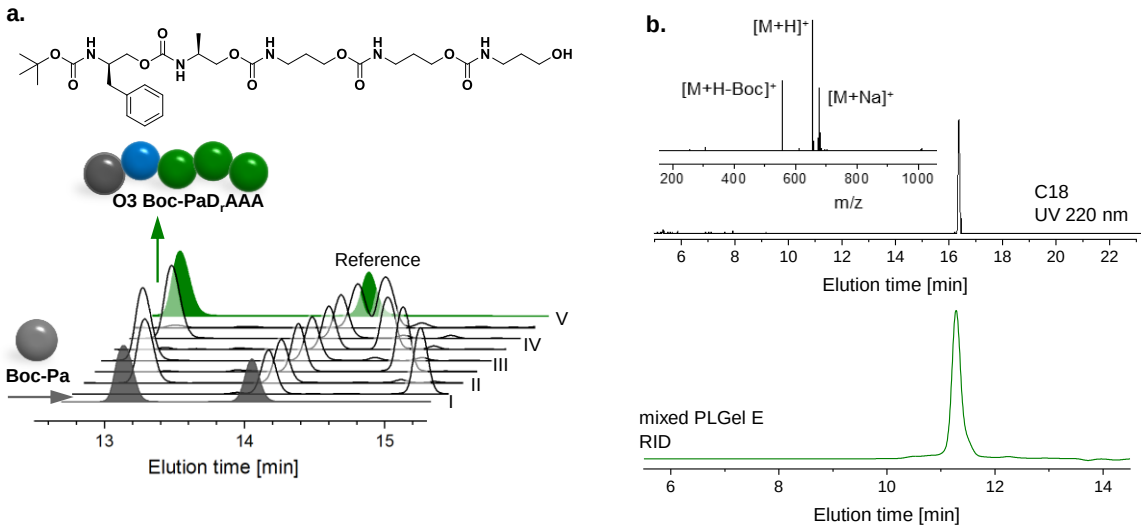

Figure 2. Representative characterization of O3 Boc-PaDrAAA. (a) HPLC monitoring of the synthesis; I – synthesis start, Boc-Pa is used as the initiator, II – coupling of D_r; III, IV, V – coupling of A; chromatograms between monomer couplings are corresponding to activation steps; signal at 14 min of elution time corresponds to reference anisole signal. (b) LC-MS and GPC data. The final product was isolated by extraction, no chromatography was performed for purification before analyses.

The devised method is compatible with a variety of building blocks (Fig. 1, Tab. 1). In the synthesis of oligomers we have successfully used different initiators: alcohol (BzOH), amino alcohol with Boc-protected amine group (Boc-Ar, Boc-Pa) and various amino alcohol monomers: aromatic (Ar, Pa), aliphatic with primary (A, D_s, D_r) and secondary alcohol (C_s) group. By selecting the appropriate chiral building blocks (Pa, D_s, D_r, C_s), we can control the stereochemistry of resulting oligomers.

All obtained products are characterized by a uniform structure of high purity as demonstrated by LC-MS (Fig. 2 b, S1-S7), ^{1}H NMR (Fig. S11-S17), and GPC (Fig. 2 b, S19-S25). The crude oligomer's purity was usually about ~95% as determined by HPLC with UV detection (Tab. 1). The overall synthesis yields are usually above 95%, as calculated based on HPLC chromatograms. The average isolated yields are close to 90% thus much higher in comparison

to the conventional multi-step solution synthesis (~30%) and depend on the sequence that regulates the polarity and influences the efficiency of final isolation by extraction (Tab. 1).

The method is feasible for large-scale, e.g., tens-gram syntheses. Here, we demonstrate the synthesis of 50 g of oligomer Boc-PaAAAA (entry O7, Tab. 1). The synthesis scale can be further increased since the process does not require heating and is technically simple to carry out.

The conversion of steps (i) and (ii) depends on monomer sequence and iteration number (Fig. 3). As expected, for most examples with the elongation of the oligomer, larger DSC excess is needed to achieve quantitative transformations (Tab. S1). Accumulated waste and growing macromolecule chains hinder the reagent access to the active end groups. In the case of step (ii) chemoselective coupling of monomer, the reaction usually proceeds quantitatively using an equimolar amount of reagents. Only some sequences needed excess amino alcohol. Usually, if the preceding step (i) the excess of DSC (more than 1.2 eq) was needed, the extra portion of amino alcohol was necessary. Since surplus DSC was present in the system, it could react with a free monomer precluding full conversion of active carbonate.

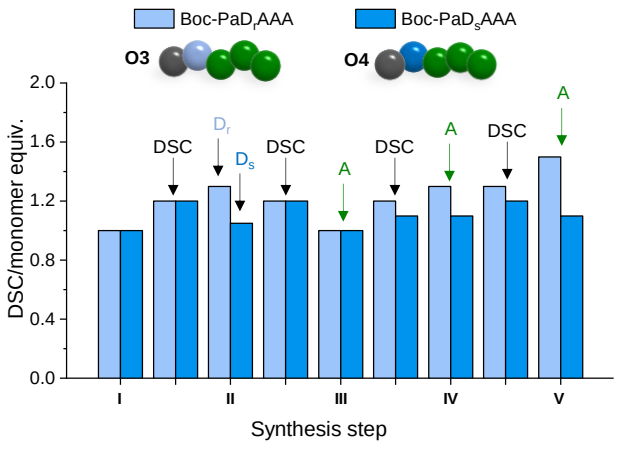

Figure 3. Amount of reagents used for the synthesis of O3 and O4 of different configurations of stereocenters.

In general, reactions of rigid structures enriched with chiral monomers (C_s, D_s, D_r) required higher reagent excess to yield uniform macromolecules. It is plausible that the course of

particular reaction steps is related to the shape and exposition of the reactive terminus of the molecules. However, there is no clear indication of the secondary structure displayed by discrete oligocarbamates, hence the correlation between the sequence and the course of the reactions remains unclear at present.

Compared with other methods of oligocarbamate solution synthesis, the one-pot approach leads to higher synthesis yield (Fig. 4). On average, about 15% of the product is lost with each extraction that leads to only 30% of overall yield after four monomer couplings when the extraction is performed after each step (i) and (ii). Elimination of the isolation step between reactions (i) and (ii) gives 50% of the overall yield.[46] Using a one-pot procedure we can reach a significantly higher yield, up to 90% on average. Moreover, the synthesis consumes significantly less organic solvents, making the process greener (Fig. 4).

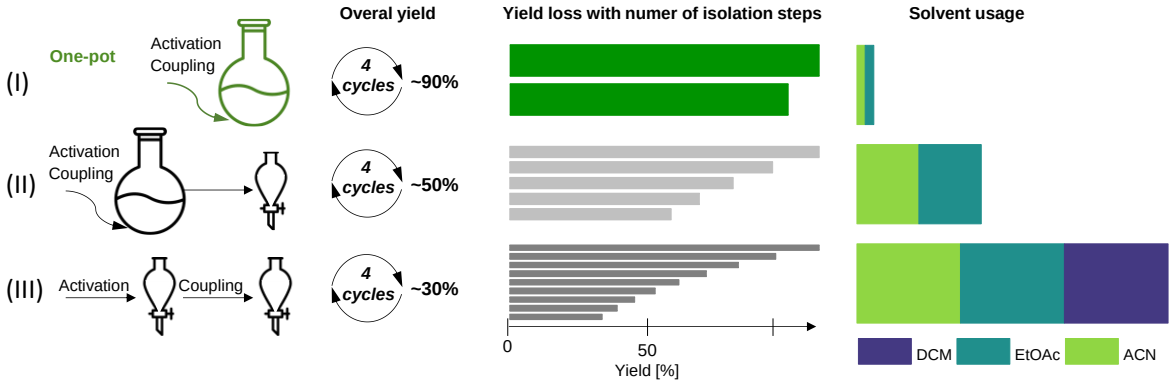

Figure 4. Comparison of solution synthesis methods yielding sequence-defined oligocarbamates: (I) one-pot approach, (II) activation and monomer coupling reactions (i, ii) are performed in one pot followed by extraction, and (III) intermediate products are isolated after each step by extraction. For comparison, the solvent usage was calculated for four monomer couplings assuming a constant concentration of solvents used for reaction and purification to exclude the scale effect, values were divided by the average overall yield. Average overall yields were calculated based on reported data.[27, 46]

The devised, one-pot approach enables the performance of up to eight consecutive reactions that yield uniform pentamers (Table 1, O1-O7), which can be further used as precursors for

the synthesis of sequence-defined polymers of higher molar mass. The pentamers can be coupled together using reactions (i) and (ii) since Boc protecting group can be easily removed without decomposing the product (Fig. S18, S26). Appling oligomers in the exponential growth approach and taking advantage of the one-pot strategy, we can increase the oligomer molar mass by having full control over the monomer sequence and overcoming limitations connected with dramatic yield losses of multi-step synthesis (Figure 5).

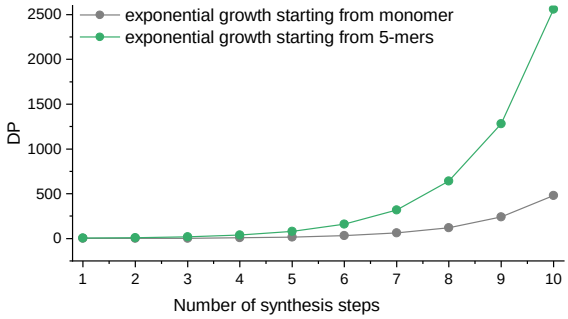

Figure 5. Comparison of polymer molar mass increase in iterative exponential growth depending on used starting material (monomer or 5-mers).

Conclusions

The multi-step synthesis of sequence-defined macromolecules can be carried out in one pot, providing a controlled supply of reagents and time to assure full conversion of each reaction. The approach offers a facile protocol, does not demand any purification between steps, is easy to scale up, and provides sequence-defined macromolecules with a high yield. Synthesis consumes less solvents, which makes the process greener in comparison to existing methods. Thanks to high yield the strategy opens the possibility to fabricate high molar mass polycarbamates using oligomers as precursors. In a broader context, the methodology can be matched with other multi-step processes that involve orthogonal reactions, leading to different classes of sequence-defined macromolecules to perform syntheses efficiently.

Author Contributions

R. Szweda conceived and designed the study, wrote a paper, evaluated data, and supervised experimental work. P. Cwynar performed experimental work on the synthesis of oligocarbamates. P. Pasikowski performed LC-MS analyses. All authors helped to prepare the manuscript, analysed and discussed the data. All authors have approved the final version of the manuscript.

Conflicts of interest

R. Szweda and P. Cwynar are inventors on patent application no. PCT/IB2021/057872 related to this work filed by Łukasiewicz Research Network – PORT (filed 27 August 2021).

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Data availability

Experimental details, NMR spectra, LC-MS data, GPC chromatograms are contained in Electronic Supplementary Information.

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