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Sequence-Controlled Oligomers

Multifunctionalized Sequence-Defined Oligomers from a Single Building Block**

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Two decades of progress in the field of living and controlled polymerizations, combined with the elaboration of efficient conjugation reactions, have greatly contributed to the elegant preparation of functionalized macromolecular architectures.[1] However, these state-of-the-art methodologies, while providing a high degree of structural and topological control, are inadequate tools for controlling the polymer microstructure. Crucial parameters like primary structure (i.e. monomer sequence) and tacticity largely remain unmastered by current man-made approaches. Expectations for the next generation of synthetic polymers include performance as single chains, ability to fold and self-regulate, and ability to sense specific molecules and/or catalyze reactions.[2] These precisely functionalized linear polymers should exhibit sharply defined and tailored structure–activity relationships analogous to nature's delicately engineered macromolecules. Therefore, progress towards reliable sequence-controlled polymerization, enabling the preprogrammed distribution of multiple functional groups along the backbone, is drawing attention in a growing number of research groups worldwide.[3,4]

Pioneering efforts to control the primary structure of functionalized polymers have been based on several approaches, using different reactivity ratios of vinyl monomers,[5] spatial prearrangement of monomers on a (macromolecular) template[6] or, as recently demonstrated, the action of a small-molecule machine.[7] Other attempts use (automated) sequential addition of building blocks on a solid[8] or liquid[9] support, leading to sequence control as a result of iterative coupling steps, thereby omitting the need for preorganization. These methods, established for peptide[10] and oligonucleotide[11] synthesis, present considerable drawbacks for sequence-controlled polymerization: they generally require the use of protecting groups, and the restricted number of readily available building blocks ("monomer alphabet") equipped with the appropriate functional handle can further hamper the preparation of tailor-made functionalized sequences. The development of new chemical methods for chain elongation, often on a solid support, that result in sequence-defined (macro)molecular structures with unique backbones and side chain functionalities, or fragments thereof that could be combined to obtain sequence-controlled polymers,[4] is therefore highly desirable.

Herein, we report on a new coupling strategy for the controlled generation of sequence-defined multifunctionalized oligomers on solid support by using thiolactone-based chemistry. This protecting group free approach was inspired by the 'submonomer' synthetic method for the preparation of functionalized peptoids.[12] Although the generated oligomers are small in size, reconstitution approaches could further allow the synthesis of larger chains featuring the designed and repetitive display of carefully selected and well-positioned functional entities.

The synthetic scope of thiolactones as reactive precursors for thiols in various polymeric systems was recently reported by us[13,14] and other groups.[15] These cyclic thioesters are susceptible to selective aminolysis, releasing a thiol and thus providing a functional handle for subsequent efficient conjugation reactions.[16] From this, we reasoned that immobilization of a thiolactone unit on a solid support should enable chain extension after on-resin aminolysis. Indeed, using a judiciously selected thiolactone building block to reinstate the thiolactone functionality would allow the start of a next iterative reaction sequence (Scheme 1). This two-step aminolysis/chain extension method does not make use of any protecting groups and relies on a single thiolactone-containing building block for chain extension. Most importantly, a myriad of functionalities can be introduced by using the corresponding readily available amines.

The main requirement for this approach is the preparation of a suitable thiolactone-based building block for chain extension. Since such a reactant will typically be used in

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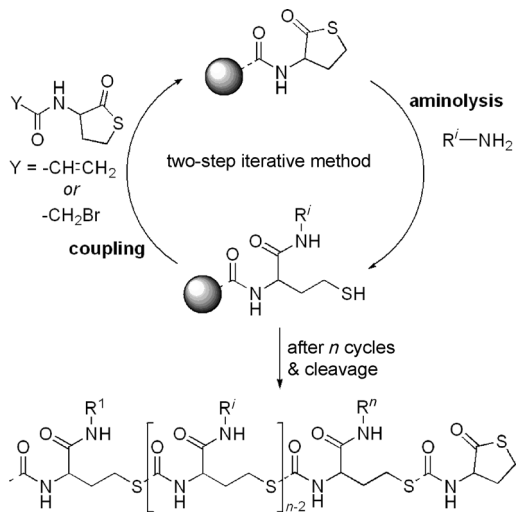
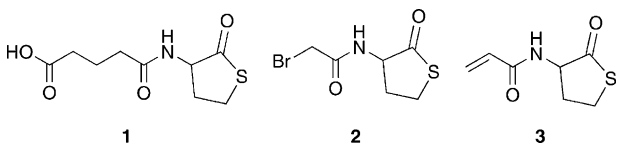


Scheme 1. Two-step iterative method for the synthesis of functionalized oligomers on solid support: aminolysis of the resin-bound thiolactone followed by coupling of a thiolactone-containing building block that selectively reacts with the generated thiol.

high excess when applying solid-support methods, its synthesis has to be high yielding on a multigram scale. Different building blocks (Figure 1) were designed and could be synthesized in a one-step procedure, with a straightforward purification, from the readily available racemic DL-homocysteine thiolactone (Schemes S1, S2, and S3 in the Supporting Information). Together with compound **1**, which is used for the initial loading of a thiolactone unit onto a suitable solid support, building blocks **2** and **3** were evaluated as chain extension moieties.

As the repetitive aminolysis and chain extension steps occur in basic medium, an acid-labile linkage was foreseen for final cleavage from the solid support. Consequently, the carboxylic acid functionalized thiolactone linker **1** was coupled to a 2-chlorotrityl resin using standard conditions (Figure S4 in the Supporting Information).[17] The thiolactone building block for chain extension should be equipped with a functional handle to allow reaction with the on-resin generated thiol preferentially in a nucleophilic fashion. Therefore, we have chosen *N*-(2-bromoacetyl) homocysteine-γ-thiolactone (**2**) and *N*-(acryloyl) homocysteine-γ-thiolactone (**3**), which are susceptible to thio-bromo substitution and thiol-Michael addition, respectively .



1 **2** **3**

Figure 1. Thiolactone-containing building blocks for loading (**1**) and chain extension (**2** and **3**).

Building blocks **2** and **3** were subsequently tested in the aminolysis/chain extension method. Aminolysis of the resin-bound thiolactone was performed by overnight treatment of the swollen resin with an excess of benzylamine in CH_2Cl_2, thus guaranteeing full thiolactone conversion. LC–MS analysis of the sample after acidic cleavage revealed quantitative consumption of the thiolactone, but only the corresponding disulfide of the expected thiol could be identified (Figure 2 a). This inevitable disulfide formation during aminolysis is independent of the identity of the amine because the corresponding disulfide was detected for a variety of (functionalized) amines (see below). As a free thiol is required for the subsequent coupling step, a first attempt to avoid disulfide formation involved lowering the amine concentration. However, the thiolactone conversion was negatively influenced while the disulfide remained the dominant reaction product. The disulfides formed could be partially cleaved in a post-aminolysis treatment by using dimethylphenylphosphine (Me_2PhP) as a reducing agent (Figure 2 b). However, their complete reduction and the subsequent conservation of the

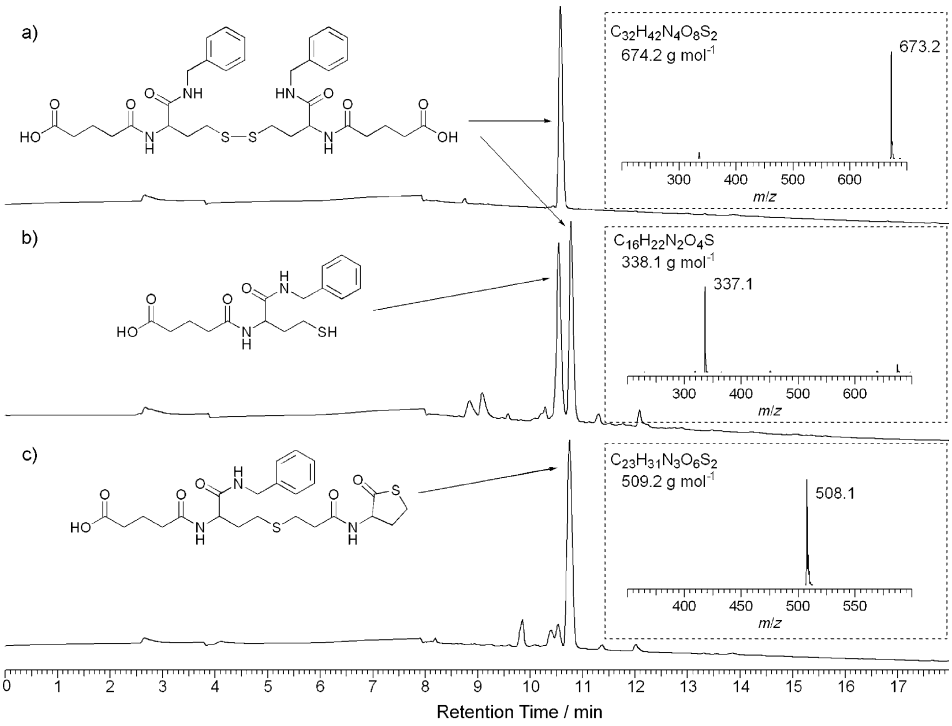


Figure 2. LC–MS analysis of cleaved samples after a) aminolysis of the resin-bound thiolactone with benzylamine, generating the corresponding disulfide, b) on-resin reduction of the formed disulfide by phosphine treatment, and c) on-resin reduction of the formed disulfide and sequential one-pot chain extension through thiol–acrylamide conjugation. Inserts: ESI-MS spectra of the dominant species (negative mode).

obtained resin-bound thiols appeared challenging. The synthetic strategy was thus adapted to consider the resin-bound disulfides generated upon aminolysis as stable intermediates. With respect to the two-step iterative method (Scheme 1), reduction of the disulfide by phosphine treatment followed by immediate in situ reaction of the generated thiol[18] with the next monomer building block is indeed an alternative for the proposed chain extension. However, these conditions were found not to be applicable when using building block **2** owing to the incompatibility of a bromide leaving group with a trialkylphosphine. On the other hand, considering chain extension by using building block **3**, the phosphine reagent can fulfill a dual role: both as a reducing agent for the disulfide generated upon the aminolysis and as an efficient catalyst for the thiol-Michael addition.[19] Cleavage of the resin-bound disulfide and Michael addition of the thiol to acrylamide **3** could indeed be successfully performed in the presence of an excess of Me_2PhP and building block **3**, as demonstrated by the HPLC trace of the obtained conjugated product (Figure 2c).

To demonstrate the general potential of this methodology in terms of versatility and tolerance towards functional groups, a small library of functionalized sequences was prepared by two iterations of the elaborated two-step method. A random selection of nonfunctionalized primary or secondary amines (*n*-propylamine, benzylamine, and pyrrolidine) for the first ring-opening step and functionalized primary amines (allylamine, propargylamine, furfurylamine, and *N*-(3-aminopropyl) morpholine) for the consecutive aminolysis allowed for the synthesis of six functionalized dimers (Figures S5–10, upper part). By reversing the order in which the amines were employed, the isomeric sequences for each combination were obtained in good purity, thus illustrating the possibility to control the sequence without significant interference from the introduced functional groups. (Figures S5–10, lower part). Different functional handles could consequently be incorporated into a single, longer oligomeric motif through the application of an extra aminolysis/chain extension cycle. This was demonstrated by extension of the benzylamine–propargylamine sequence (Figure S10) through a third iteration using furfurylamine during the aminolysis, thus yielding a multifunctionalized sequence-defined oligomer suitable for orthogonal modification.[14,20]

To further define the scope and limitations of the described method, the maximum number of repetitions of the synthetic cycle to generate longer sequences was determined. While the consecutive overnight reactions in the current method render the overall process time-consuming, it was demonstrated that heating through microwave irradiation significantly reduces the reaction times of both steps (Figure S11). This accelerated microwave-assisted method was applied to the preparation of two pairs of structurally related trimer and tetramer sequences in good purity (Table S1 in the Supporting Information). Attempts to extend the tetramer to a multifunctionalized pentamer were only partially successful because side reactions tend to become predominant (Figure S12).

The trimers and tetramer (Table S1) were purified and subjected to HRMS (Figures S25–28) and 2D NMR (500 and 700 MHz) analysis, thus enabling full characterization of the obtained oligomeric species (see the Supporting Information). The appearance of amide N–H resonances in the ^{1}H NMR spectra ($\delta = 7.8$–8.5 ppm) is highly specific (like a fingerprint) for each oligomer as a result of the significant influence of the amine residue on the chemical shifts of the amide protons in both the side chain and the backbone (Figure 3).

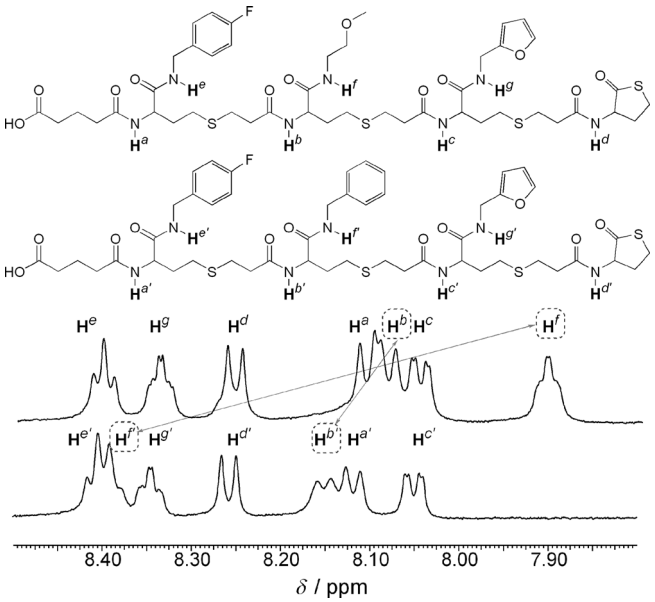


Figure 3. The amide signals in the ^{1}H NMR spectra (500 MHz, [D$_6$]DMSO) of two structurally related trimeric species.

In summary, a thiolactone-based approach for the solid-supported preparation of multifunctionalized sequence-defined oligomers was successfully developed. The elaborated two-step iterative method, consisting of an on-resin aminolysis and subsequent chain extension through Michael addition, does not require protecting groups and relies on the use of a single, readily available thiolactone acrylamide building block. Several functionalized short (up to tetrameric) sequences were obtained by consecutive use of a variety of commercially available amines. The obtained sequence-defined motifs featuring a unique backbone and a preprogrammed organization of functionalized side chains were fully characterized by NMR spectroscopy and HRMS. These species could potentially exhibit tunable folding and self-association properties, predominantly determined by the nature of the functionalized amines. In this regard, high-throughput automation and control over the stereochemistry, induced by the use of enantiomerically pure building blocks, will be further explored. Moreover, through further application of thiolactones as latent thiol functionalities and installation of appropriate functional end groups, these short heterotelechelic fragments are susceptible to periodic polymerization, a process expected to afford larger sequence-controlled polymers. This promising reconstitution approach[4] will be the focus of our future research efforts.

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