An Orthogonal Dynamic Covalent Chemistry Tool for Ring-Opening Polymerization of Cyclic Oligochalcogenides on Detachable Helical Peptide Templates

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Abstract: A model system is introduced as a general tool to elaborate on orthogonal templation of dynamic covalent ring-opening polymerization (ODC-TROP). The tool consists of \( \text{310} \) helical peptides as unprecedented templates and semi-carbazones as orthogonal dynamic covalent linkers. With difficult-to-control 1,2-dithiolanes, ODC-TROP on the level of short model oligomers occurs with high templation efficiency, increasing and diminishing upon helix stabilization and denaturation, respectively. Further, an anti-templated conjugate with mispositioned monomers gave reduced templation upon helix twisting. Even with the “unpolymerizable” 1,2-diselenolanes, initial studies already afford mild templation efficiency. These proof-of-principle results promise that the here introduced tool, recyclable and enabling late-stage side chain modification, will be useful to realize ODC-TROP of intractable or unknown cyclic dynamic covalent monomers for dynamer materials as well as cellular uptake and signaling applications.

Introduction

Dynamic covalent ring-opening polymerization (DC-ROP) is attracting attention because the dissipative properties of the resulting dynomers enable broad applications in chemistry, biology and the materials sciences.[1–10] Examples include recyclable, self-sorting,[1,2] and self-healing materials,[3–6] for artificial photosystems,[2] cell-penetrating dynomers[2–10] and dynamic-covalent DNA mimics.[1] Thiolactones[1,6] and particularly lipoic acid derivatives[3–5,7–12] are increasingly explored as dynamic covalent cyclic monomers. However, controlled, thiol-initiated DC-ROP of the more reactive asparagusic acid (AspA) derivatives in solution has been already challenging,[7] and achieved mainly on solid surfaces.[2] Many more dynamic covalent monomers exist,[8,13] including 1,2-diselenolanes,[8,14–16] which have resisted DC-ROP or not been explored, and intriguing new monomer motifs are conceivable. Here, we harness the power of orthogonal dynamic covalent chemistry[17–25] to enable templated ring-opening polymerization (ODC-TROP) and use the new tool to elaborate on the oligomerization of otherwise intractable dynamic covalent rings under controlled conditions (Figure 1).

Templated polymerization has been explored to produce oligomers of precise length[26–25] and, at best, also defined sequence.[1,26,27] DC chemistry has been used to attach ROP monomers like norbornene[28] or cyclobutanes[29] through reversible ester linkages, the ROP however is taking place through irreversible transformations. However, DC polymers are better suited toward such goals because of their self-repairability,[30] but more challenging to synthesize because the polymerization...
process is reversible. Several reports exist on templated DC polymerization and DC-TROP of 1,2-dithiolanes using vesicles, \cite{53,32–40} and DC-TROP of 1,2-dithiolanes using DNA, \cite{30} and π stacks as templates. \cite{34,47} 3₁₀ Helices were our templates of choice for ODC-TROP because of the simplicity of their triad repeat, which aligns the side chains of every third amino acid separated by ~6 Å. \cite{52–57} Whereas macrocyclization across one turn, also referred to as stapling, is very common with helical peptides for different applications, \cite{52–57} continuing oligomerization along several turns is less explored, particularly not with dynamic covalent exchange cascades. Orthogonal dynamic covalent (ODC) chemistry was envisioned to connect the monomers to the template. \cite{17,18,22,58} Generally mild exchange conditions of ODC chemistry promised efficient template recycling with coinciding late-stage side-chain modification of the oligomer, and broad substrate tolerance compared to covalent chemistry. \cite{26,27,59}

**Results and Discussion**

Our ODC-TROP tool was designed as follows: Monomers M are loaded on a template T, then initiator i and terminator t are added to form a DC polymer attached to the template PT, which then releases polymer P upon treatment with orthogonal exchangers e, while introducing side chains R⁻ of free choice (Figure 1). As a proof-of-principle template, peptide 1 was selected and synthesized (Figure 2, Schemes S1 and S2). It consists of three repeats of a triad comprising two amino-isobutyrates (Aib) to promote folding into a 3₁₀ helix and occurs under acidic conditions without interfering with the orthogonal 1,2-dithiolanes exchange under basic conditions. \cite{32,44–46} The semicarbazides in template 1 condense with the aldehyde in monomer 2, which contains AspA unit, to afford the semicarbazones in the MT conjugate 3. DC exchange of semicarbazones is similar to hydrazones but more robust, \cite{62} and occurs under acidic conditions without interfering with the orthogonal 1,2-dithiolanes exchange under basic conditions. \cite{17,18,26} In the MT conjugate 3, these 1,2-dithiolanes align along one face of the 3₁₀ helix, separated by ~6 Å (Figures 1, 2 and S1).

The reaction conditions were optimized using simple monomers before ODC-TROP to identify penicillamine 4 as the initiator (i), methoxamine 6 as the exchanger (e) to detach the products as irreversible oximes, \cite{63} and Kool’s amine-buffer, N,N-dimethyllethylendiamine (DMEN), as a catalyst (Schemes S10 and S11, Figures S4 and S5). \cite{64} Thus, DC disulfide exchange oligomerization of AspA monomers in 3 was initiated by 4 and terminated by iodoacetamide (5), followed by the semicarbazone-oxime exchange using 6 to produce products including monomer 7, ring-opened monomer 8, and oligomers 9 and 10, indicative of failed opening, failed, and operational templated oligomerization, respectively.

The reaction was monitored using LC–MS (Figure 3a). Several products arising from the addition of one initiator 4 and one terminator 5 on 3 were observed, which should give products 7–10 after treatment with 6 (Figure S8). This includes isomers like PT 11, originating from the exchange of inhibitor 4 with the central monomer, and preventing complete polymerization (Figure 3c). A peak was also observed for products containing two equivalents each of 4 and 5 added to MT 3, which could lead only to dimer 9, not trimer 10.

Upon detachment of the ODC-TROP products from the template with methoxamine 6, the expected products 7–10 appeared in the LC–MS profiles (Figure 3b) and were assigned by MS. HPLC profiles were analyzed to give ring-opening yield, defined as \( R_O = 100\% \cdot \eta_T \) where \( \eta_T \) is the yield of ring-closed product 7. Template was quantified from the ratio of the yields of templated oligomers and the ring-opened monomer, that is, \( \eta_T = \log \left( \frac{\Sigma T}{\Sigma MT} \right) \). Here \( T_I = \log \left( \eta_T + \eta_R / \eta_T \right) \). Being a product of failed ring-opening, product 7 was not considered to calculate template. Minimum acceptable template was fixed arbitrarily to \( \Sigma T = \eta_T + \eta_R = 20\% \) yield of templated products, that is, \( T_I = -0.60 \). The effective template was then defined as \( T_{eff} = T_I - T_{min} \) (Table S1 and S2). This definition ensured \( T_{eff} > 0 \) for efficient templation over 20% and \( T_{eff} < 0 \) for anti-templation effects.

![Figure 2](image-url)

Figure 2. a) ODC-TROP of 1,2-dithiolane 2 on template 1 with initiator 4, terminator 5 and exchanger 6 to products 7 (no ring opening), 8 (no templation), dimer 9 and trimer 10 (operational templation). b) Control exchange without template, affording only ring-opened monomer 8. c) Molecular model of MT (top and side view) and PT. d) Model of monomer-loaded anti-template. i) 50 mM DMEN, 150 mM NaCl, H₂O, pH 5.0, rt, 16 h, 62%; ii) 100 μM 3, 1–5 mM 4, 10 mM Tris, 1 mM EDTA in H₂O/TFE, pH 7, T variable, 30 min, then addition of 5; iii) 20 μM PT, 100 mM 6, 50 mM DMEN, 150 mM NaCl, H₂O, pH 5.0, rt, 16 h; iv) 100 μM–30 mM 7, 2 mM 4, 10 mM Tris, TFE/H₂O 1:1, pH 9.0, rt, 30 min, then addition of 5.
With increasing initiator/monomer ratio in buffer, ring opening yields increased to maximal RO = 47% at c/cm = 6.7 (Figures 3a, b and 4a). Maximal T_{eff} = 1.11 was obtained at lowest c/cm = 3.3, coinciding with RO = 31% (Figure 4a, filled circles). Increasing initiator c naturally increased ring opening but decreased templation down to T_{eff} = 0.75 at c/cm = 6.7 due to increasing double initiation (Figure 4a).

At constant c/cm = 6.7, ring-opening yield increased with pH to maximal RO = 67% at pH = 10.0, while templation remained stable around T_{eff} ~ 0.8 (Figure 4b). This increasing RO was consistent with the activation of initiator 4 by thiolate formation. At pH < 8.0, RO was too low to properly analyze the HPLC traces. At constant c/cm and pH, templation and RO decreased significantly from T_{eff} = 1.13 and RO = 59% at 0°C to T_{eff} = −0.32 and RO = 39% at 80°C (Figure 4c). This temperature dependence corroborated the importance of 3_10 helical structure in the template, which denatures by heat. Using urea, chemical denaturation was also explored, resulting in an expected drop to T_{eff} = 0.44 (Figure 4f), corresponding to the templation on a linear two-dimensional oligomer.[47,60] Low 2D T_{eff} as compared to those achieved on the helical template highlight the efficiency of the 3D templation.

Trifluoroethanol (TFE) is known to promote 3_10 helical conformation.[8] An increase in templation was observed with increasing TFE, up to a record T_{eff} = 1.22 at RO = 54% in buffer with 80% TFE (Figures 3a, b, cyan and 4d). In principle, helical uptwisting from 3_13 helices toward 3_10 helices can be detected in the circular dichroism (CD) spectra by the decreasing ratio R of the first negative CD Cotton effect at 220 nm divided by the second one at 208 nm.[44] For 3, the R value decreased gradually from R = 0.98 in buffer to R = 0.73 in buffer with 80% TFE, consistent with increasing 3_10 helicity (Figures 4e and S2). The peptide conformation cannot be directly estimated from the R values because the red shift of first negative CD Cotton effect by about 10 nm compared to the typical value of α helices implied interference from overlapping contributions of the phenyl semicarbazone chromophores in the new sidechain. Thus, both R values are presumably overestimates, and R = 0.98 does not indicate a 3_6.1 helix. The 3_10 helicity of Aib peptides with one monosubstituted α amino acid per triad is understood,[47,60] and a monomer separation of ~10 Å in α helix models (Figure S3) is incompatible with the TROP observed.

Excluding the possibility of an intriguing 3_6.1 to 3_10 helical upwisting during TROP, peptide 3 in a hypothetical α helical conformation (Figure S3) should thus serve as an anti-template rather than as a template. To characterize the impact of such anti-templation on TROP, the 3_10 helical peptide 12 with the central semicarbazide pointing by 120° away from the periphery.
eral semicarbazides was synthesized (Schemes S3, S4 and S9, Figures 2d and 3d). In the presence of a chemical denaturant, 1.0 M urea, ODC-ROP along template 1 and anti-template 12 gave identical $T_{\text{eff}} = 0.44/0.45$ (Figure 4f), corresponding to that of a random coil peptide. In buffer with 50% TFE, templation along 1 increased to $T_{\text{eff}} = 0.93$ at RO = 59%, while the $T_{\text{eff}}$ of anti-template 12 did not change significantly, and RO dropped to 13%. Under the best conditions for 1 with 80% TFE in buffer ($T_{\text{eff}} = 1.22$, RO = 54%), anti-template 12 afforded negative $T_{\text{eff}} = -0.15$ and RO = 11%. This drop of templation upon helix formation was in agreement with a stable positioning of monomers along the 3$_1$ helix 12 that is too far apart for ODC-TROP. Interestingly, peptide 12 in 3.6, 0 helical conformation should favor rather than disfavor the formation of the dimerization product 9. Consistent with the 3$_1$ helical nature of template 1 and anti-template 12, this was not observed.

Under the best ODC-TROP conditions, template-free polymerization of monomers 7 did not occur and gave only ring-opened monomer 8 (Figures 2b and S7, Scheme S13). After confirming templated oligomerization of 1,2-dithiolanes that otherwise resist oligomerization, we envisioned ODC-TROP of 1,2-diselenolanes. ROP of 1,2-diselenolanes has remained elusive so far because of their preference to stay closed due to low ring tension and the ease of selenol intermediates to oxidize. Control experiments with template-free monomer 13 confirmed the absence of ROP and the fast oxidation of selenol with another initiator 4 into the mixed selenosulfide 14 (Figures 5b and S6, Scheme S12).

For ODC-TROP with 1,2-diselenolanes, monomer 15 was synthesized (Schemes S5 and S7) and attached to template 1 to afford the MT conjugate as described for 1,2-dithiolanes. Addition of initiator 4 followed by terminator 5 and disassembly of the PT conjugate with ODC exchanger 6 afforded a mixture of products 16–18, confirming that oxidation is faster than termination (Figure 5a). Dimer 18 with initiator selenolufide at both termini was considered as the product of ODC-TROP. The alternative mechanism through oxidation of two selenols of opened monomers to give 18 was unlikely considering that its relative yield was best at the lowest $c/c_{\text{ML}}$ ratio with minimal RO = 20% (Figure 5c, Table S3). A respectable $T_{\text{eff}}$ = 0.28 was found under these conditions. With increasing $c/c_{\text{ML}}$ ratio, $T_{\text{eff}}$ rapidly decreased, while RO increased correspondingly to result in no more templation above $c/c_{\text{ML}} = 3.3$ (Figure 5c).

**Conclusion**

Taken together, this study introduces ODC-TROP to elucidate otherwise inaccessible dynamic covalent ring-opening polymerizations under controlled conditions. The generality of the new tool promises compatibility with diverse cyclic dynamic monomers and sequences, promising, inter alia, access to new dynamer chemistry for materials applications and to transmembrane signal transduction or cellular uptake applications. Recyclable, mild, robust and general, the here introduced tool thus plants a seed that promises to blossom in many different ways.

**Experimental Section**

Please see Supporting Information.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability Statement**

The data that support the findings of this study are openly available: https://doi.org/10.5281/zenodo.6415282.
