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

Quentin Laurent,[a] Naomi Sakai,[a] and Stefan Matile*[a]

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 3_{10} helical peptides as unprecedented templates and semicarbazones 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 dynamers 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 dynamers[7–10] 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 cyclic 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).

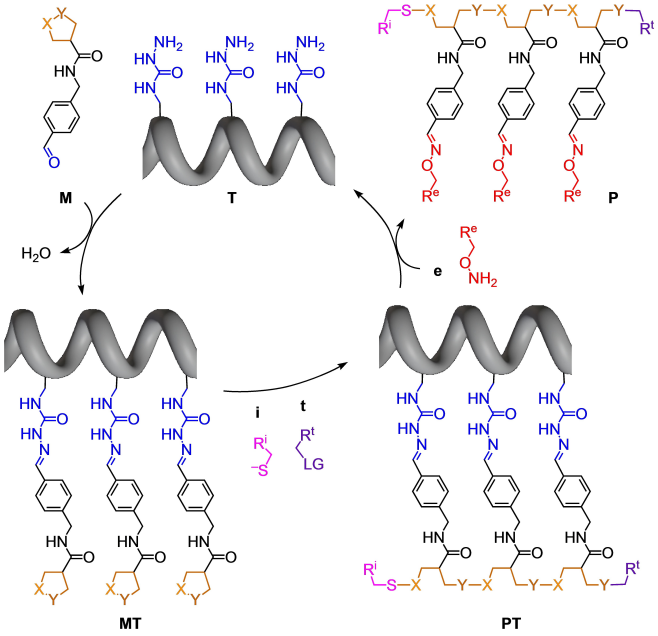

Figure 1. The concept of templated ring-opening polymerization with orthogonal dynamic covalent chemistry: X, Y = S, Se, **M** = monomers, **P** = polymer, **T** = template, **i** = initiator, **t** = terminator, **e** = exchanger, LG = leaving group.

Templated polymerization has been explored to produce oligomers of precise length[26–35] and, at best, also defined sequence.[1,26,27] DC chemistry has been used to attach ROP monomers like norbonene[36] or cyclobutenes[37] 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,[2] but more challenging to synthesize because the polymerization

[a] *Dr. Q. Laurent, Dr. N. Sakai, Prof. S. Matile*
Department of Organic Chemistry
University of Geneva
1211 Geneva (Switzerland)
E-mail: stefan.matile@unige.ch
Homepage: www.unige.ch/sciences/chiorg/matile/

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process is reversible. Several reports exist on templated DC polymerization,[35,38–40] and DC-TROP of 1,2-dithiolanes using vesicles,[12] DNA,[9] and π stacks as templates.[2,41] 3_{10} 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.[42–51] Whereas macrocyclization across one turn, also referred to as stapling, is very common with helical peptides for different applications,[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.[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.[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^e 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 aminoisobutyrates (Aib) to promote folding into a 3_{10} helix[42,44–46] and one newly devised artificial amino acid with a semicarbazide side chain. Substitution of one out of three amino acids in Aib homopolymers has been shown not to disturb the 3_{10} helicity of the peptides.[47,60,61]

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,[62] and occurs under acidic conditions without interfering with the orthogonal 1,2-dithiolanes exchange under basic conditions.[17,18,20] In the **MT** conjugate **3**, these 1,2-dithiolanes align along one face of the 3_{10} 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**), methoxyamine **6** as the exchanger (**e**) to detach the products as irreversible oximes,[63] and Kool's amine-buffer, *N*,*N*-dimethylethylenediamine (DMEN), as a catalyst (Schemes S10 and S11, Figures S4 and S5).[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 generate 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

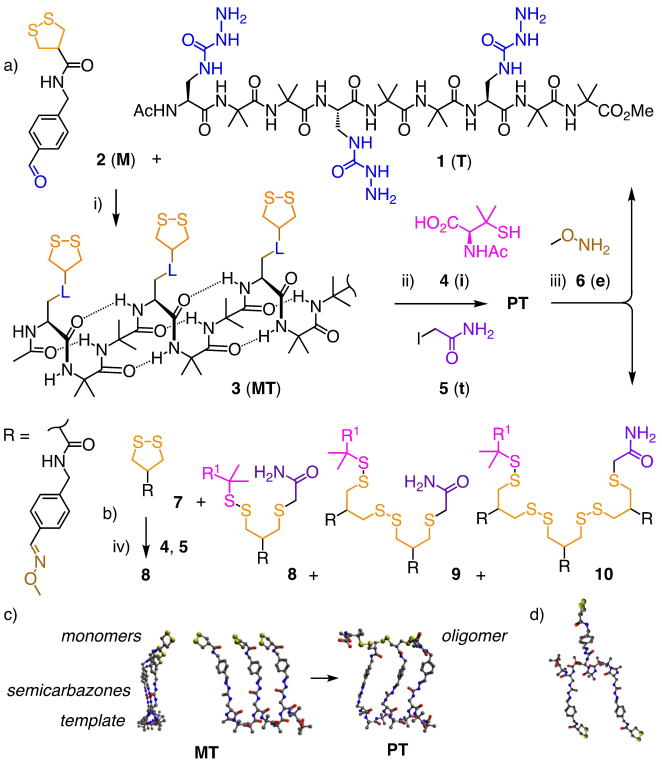

Figure 2. a) ODC-TROP of 1,2-dithiolane **2** on template **1** with initiator **4**, terminator **5** and exchanger **6** to products **7** (no ring opening), **8** (no templation), dimer **9** and trimer **10** (operational templation). b) Control exchange without template, affording only ring-opened 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, H_2O, pH 5.0, rt, 16 h, 62%; ii) 100 μM **3**, 1–5 mM **4**, 10 mM Tris, 1 mM EDTA in H_2O/TFE, pH, T variable, 30 min, then addition of **5**; iii) 20 μM **PT**, 100 mM **6**, 50 mM DMEN, 150 mM NaCl, H_2O, pH 5.0, rt, 16 h; iv) 100 μM–30 mM **7**, 2 mM 4, 10 mM Tris, TFE/H_2O 1:1, pH 9.0, rt, 30 min, then addition of **5**.

products **7**–**10** after treatment with **6** (Figure S8). This includes isomers like PT **11**, originating from the exchange of initiator **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 methoxyamine **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 RO = 100%-η_7, where η_7 is the yield η of ring-closed product **7**. Templation was quantified from the ratio of the yields of templated oligomers and the ring-opened monomer, that is, $T_f = \log\ (\Sigma\eta_T/\eta_{ROM})$, here $T_f = \log[(\eta_{10}+\eta_9)/\eta_8]$. Being a product of failed ring-opening, product **7** was not considered to calculate templation. Minimum acceptable templation was fixed arbitrarily to $\Sigma\eta_T = \eta_{10}+\eta_9 = 20\%$ yield of templated products, that is, $T_f^{min} = -0.60$. The effective templation was then defined as $T_{eff} = T_f - T_f^{min}$ (Table S1 and S2). This definition ensured $T_{eff} > 0$ for efficient templation over 20% and $T_{eff} < 0$ for anti-templation effects.

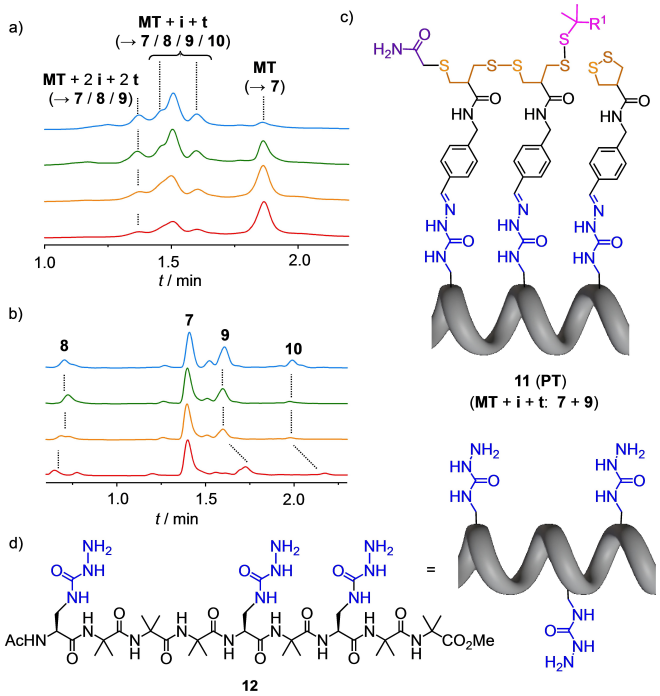

Figure 3. a) HPLC signatures of reaction mixtures obtained from **3** with 20, 20, 15, 10 equivalents of **4** (top to bottom; $c_i/c_M = 6.7, 6.7, 5.0, 3.3$) in buffer with 80% (top) and without TFE (rest) after termination with **5**; with assignments according to MS, and products expected after detachment. b) HPLC signatures of reaction mixtures in (a) after detachment from the template with **6**. c) Notional structure of incomplete PM **11**. d) Structure of anti-template **12**.

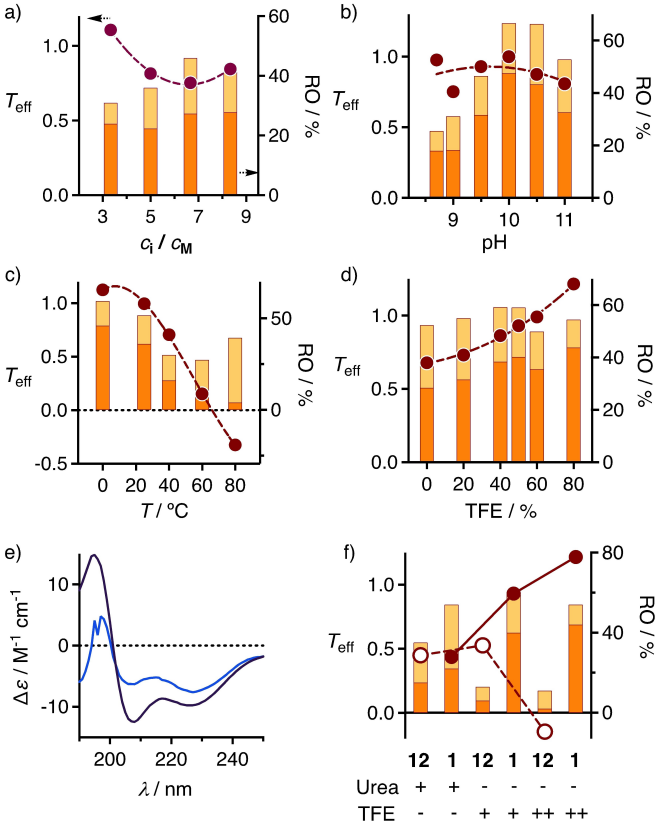

Figure 4. a–d, f) Dependence of ring-opening percentage RO (bars, consisting of η_8: yellow, and η_{9+10}: orange) and templation efficiency T_{eff} (circles) on a) c_i/c_M ratio, b) pH, c) temperature, and d, f) the presence of denaturant (urea 1.0 M) and helix promoter (TFE, +: 50%, ++: 80%) with template **1** (filled circles) and anti-template **12** (open circles). e) CD spectra of **3** in buffer without (cyan, $R = \Delta\varepsilon_{220}/\Delta\varepsilon_{208} = 0.97$) and with 80% TFE (dark blue, $R = 0.73$).

With increasing initiator/monomer ratio in buffer, ring opening yields increased to maximal RO = 47% at $c_i/c_M = 6.7$ (Figures 3a, b and 4a). Maximal $T_{eff} = 1.11$ was obtained at lowest $c_i/c_M = 3.3$, coinciding with RO = 31% (Figure 4a, filled circles). Increasing initiator c_i naturally increased ring opening but decreased templation down to $T_{eff} = 0.75$ at $c_i/c_M = 6.7$ due to increasing double initiation (Figure 4a).

At constant $c_i/c_M = 6.7$, ring-opening yield increased with pH to maximal RO = 67% at pH = 10.0, while templation remained stable around $T_{eff} \sim 0.8$ (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_i/c_M 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.[26,27] 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.[65] 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 3.6_{13} α 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[47,60] 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_{13} α 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_{13} to 3_{10} helical uptwisting 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 periph-

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_{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_{eff} = 0.93 at RO = 59%, while the T_{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_{eff} = 1.22, RO = 54%), anti-template **12** afforded negative T_{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_{10} helix **12** that is too far apart for ODC-TROP. Interestingly, peptide **12** in 3.6_{13} α helical conformation should favor rather than disfavor the formation of the dimerization product **9**. Consistent with the 3_{10} 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).[7] 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.[15] 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.

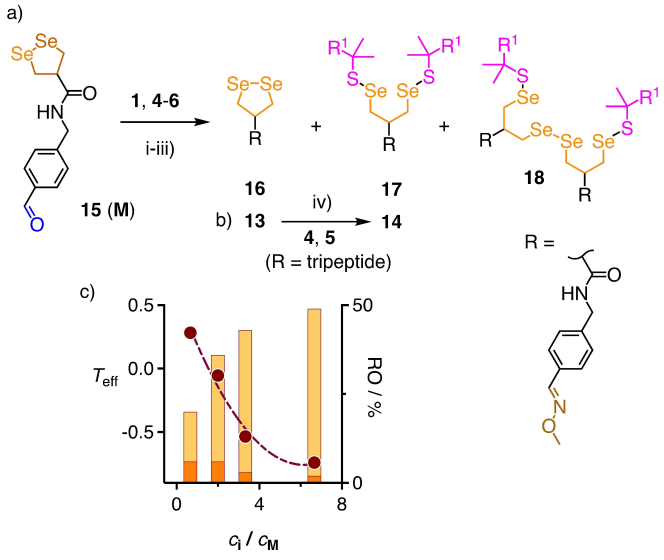

Figure 5. a) ODC-TROP of 1,2-diselenolane **15** on template **1** with initiator **4**, terminator **5** and exchanger **6** to products **16** (no ring-opening), **17** (no templation) and dimer **18** (operational templation). b) Control exchange without the template, affording only ring-opened monomer **14** (R: see Figure S6). c) T_{eff} templation factor (filled circles) and ring-opening percentage (bars, consisting of η_{17}: yellow, and η_{18}: orange) as a function of c_i/c_M ratio. Conditions i–iv) analogous to Figure 2.

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[15,66,67] that oxidation is faster than termination (Figure 5a). Dimer **18** with initiator selenosulfide 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_i/c_M ratio with minimal RO = 20% (Figure 5c, Table S3). A respectable T_{eff} = 0.28 was found under these conditions. With increasing c_i/c_M ratio, T_{eff} rapidly decreased, while RO increased correspondingly to result in no more templation above c_i/c_M = 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.

Keywords: diselenides · disulfides · dynamers · orthogonal dynamic covalent chemistry · ring-opening dynamic covalent polymerization · templated polymerization

[1] S. Mavila, B. T. Worrell, H. R. Culver, T. M. Goldman, C. Wang, C.-H. Lim, D. W. Domaille, S. Pattanayak, M. K. McBride, C. B. Musgrave, C. N. Bowman, *J. Am. Chem. Soc.* **2018**, *140*, 13594–13598.
[2] M. Lista, J. Areephong, N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2011**, *133*, 15228–15231.
[3] X. Zhang, R. M. Waymouth, *J. Am. Chem. Soc.* **2017**, *139*, 3822–3833.
[4] Q. Zhang, D.-H. Qu, B. L. Feringa, H. Tian, *J. Am. Chem. Soc.* **2022**, *144*, 2022–2033.
[5] Y. Liu, Y. Jia, Q. Wu, J. S. Moore, *J. Am. Chem. Soc.* **2019**, *141*, 17075–17080.
[6] W. Xiong, W. Chang, D. Shi, L. Yang, Z. Tian, H. Wang, Z. Zhang, X. Zhou, E.-Q. Chen, H. Lu, *Chem* **2020**, *6*, 1831–1843.
[7] E.-K. Bang, G. Gasparini, G. Molinard, A. Roux, N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2013**, *135*, 2088–2091.
[8] Q. Laurent, R. Martinent, B. Lim, A.-T. Pham, T. Kato, J. López-Andarias, N. Sakai, S. Matile, *JACS Au* **2021**, *1*, 710–728.
[9] J. Zhou, L. Sun, L. Wang, Y. Liu, J. Li, J. Li, J. Li, H. Yang, *Angew. Chem. Int. Ed.* **2019**, *58*, 5236–5240; *Angew. Chem.* **2019**, *131*, 5290–5294.
[10] J. Guo, T. Wan, B. Li, Q. Pan, H. Xin, Y. Qiu, Y. Ping, *ACS Cent. Sci.* **2021**, *7*, 990–1000.
[11] J. Lu, H. Wang, Z. Tian, Y. Hou, H. Lu, *J. Am. Chem. Soc.* **2020**, *142*, 1217–1221.
[12] A. Sadownik, J. Stefely, S. L. Regen, *J. Am. Chem. Soc.* **1986**, *108*, 7789–7791.
[13] J. G. Felber, L. Zeisel, L. Poczka, K. Scholzen, S. Busker, M. S. Maier, U. Theisen, C. Brandstädter, K. Becker, E. S. J. Arnér, J. Thorn-Seshold, O. Thorn-Seshold, *J. Am. Chem. Soc.* **2021**, *143*, 8791–8803.
[14] Q. Laurent, N. Sakai, S. Matile, *Helv. Chim. Acta* **2019**, *102*, e1800209.
[15] N. Chuard, A. I. Poblador-Bahamonde, L. Zong, E. Bartolami, J. Hildebrandt, W. Weigand, N. Sakai, S. Matile, *Chem. Sci.* **2018**, *9*, 1860–1866.
[16] E. Bartolami, D. Basagiannis, L. Zong, R. Martinent, Y. Okamoto, Q. Laurent, T. R. Ward, M. Gonzalez-Gaitan, N. Sakai, S. Matile, *Chem. Eur. J.* **2019**, *25*, 4047–4051.
[17] A. Wilson, G. Gasparini, S. Matile, *Chem. Soc. Rev.* **2014**, *43*, 1948–1962.
[18] J. F. Reuther, S. D. Dahlhauser, E. V. Anslyn, *Angew. Chem. Int. Ed.* **2019**, *58*, 74–85; *Angew. Chem.* **2019**, *131*, 76–88.
[19] B. M. Matysiak, P. Nowak, I. Cvrtila, C. G. Pappas, B. Liu, D. Komáromy, S. Otto, *J. Am. Chem. Soc.* **2017**, *139*, 6744–6751.
[20] M. J. Barrell, A. G. Campaña, M. von Delius, E. M. Geertsema, D. A. Leigh, *Angew. Chem. Int. Ed.* **2011**, *50*, 285–290; *Angew. Chem.* **2011**, *123*, 299–304.
[21] S. Lascano, K.-D. Zhang, R. Wehlauch, K. Gademann, N. Sakai, S. Matile, *Chem. Sci.* **2016**, *7*, 4720–4724.
[22] H. M. Seifert, K. Ramirez Trejo, E. V. Anslyn, *J. Am. Chem. Soc.* **2016**, *138*, 10916–10924.
[23] S. Ulrich, *Acc. Chem. Res.* **2019**, *52*, 510–519.
[24] A. Fuertes, M. Juanes, J. R. Granja, J. Montenegro, *Chem. Commun.* **2017**, *53*, 7861–7871.
[25] C. Gehin, J. Montenegro, E.-K. Bang, A. Cajaraville, S. Takayama, H. Hirose, S. Futaki, S. Matile, H. Riezman, *J. Am. Chem. Soc.* **2013**, *135*, 9295–9298.
[26] D. Núñez-Villanueva, M. Ciaccia, G. Iadevaia, E. Sanna, C. A. Hunter, *Chem. Sci.* **2019**, *10*, 5258–5266.
[27] D. Núñez-Villanueva, C. A. Hunter, *Acc. Chem. Res.* **2021**, *54*, 1298–1306.
[28] Z. Zhou, E. F. Palermo, *Macromolecules* **2018**, *51*, 6127–6137.
[29] C. R. South, M. Weck, *Macromolecules* **2007**, *40*, 1386–1394.
[30] P. J. Milnes, M. L. McKee, J. Bath, L. Song, E. Stulz, A. J. Turberfield, R. K. O'Reilly, *Chem. Commun.* **2012**, *48*, 5614–5616.
[31] J. F. Lutz, M. Ouchi, D. R. Liu, M. Sawamoto, *Science* **2013**, *341*, 1238149.
[32] N. Badi, J.-F. Lutz, *Chem. Soc. Rev.* **2009**, *38*, 3383–3390.
[33] P. K. Lo, H. F. Sleiman, *J. Am. Chem. Soc.* **2009**, *131*, 4182–4183.
[34] R. E. Kleiner, Y. Brudno, M. E. Birnbaum, D. R. Liu, *J. Am. Chem. Soc.* **2008**, *130*, 4646–4659.
[35] P. Luo, J. C. Leitzel, Z.-Y. J. Zhan, D. G. Lynn, *J. Am. Chem. Soc.* **1998**, *120*, 3019–3031.
[36] N.-T. Lin, S.-Y. Lin, S.-L. Lee, C.-H. Chen, C.-H. Hsu, L. P. Hwang, Z.-Y. Xie, C.-H. Chen, S.-H. Luang, T.-Y. Luh, *Angew. Chem. Int. Ed.* **2007**, *46*, 4481–4485; *Angew. Chem.* **2007**, *119*, 4565–4569.
[37] Y.-Z. Ke, S.-L. Huang, G. Lai, T.-Y. Luh, *Beilstein J. Org. Chem.* **2019**, *15*, 44–51.
[38] S. Otto, *Acc. Chem. Res.* **2012**, *45*, 2200–2210.
[39] F. B. L. Cougnon, J. K. M. Sanders, *Acc. Chem. Res.* **2012**, *45*, 2211–2221.
[40] O. Ramström, J.-M. Lehn, *Nat. Rev. Drug Discovery* **2002**, *1*, 26–36.
[41] H. Hayashi, A. Sobczuk, A. Bolag, N. Sakai, S. Matile, *Chem. Sci.* **2014**, *5*, 4610–4614.
[42] J. E. Jones, V. Diemer, C. Adam, J. Raftery, R. E. Ruscoe, J. T. Sengel, M. I. Wallace, A. Bader, S. L. Cockroft, J. Clayden, S. J. Webb, *J. Am. Chem. Soc.* **2016**, *138*, 688–695.
[43] M. De Poli, W. Zawodny, O. Quinonero, M. Lorch, S. J. Webb, J. Clayden, *Science* **2016**, *352*, 575–580.
[44] C. Toniolo, A. Polese, F. Formaggio, M. Crisma, J. Kamphuis, *J. Am. Chem. Soc.* **1996**, *118*, 2744–2745.
[45] M. Tomsett, I. Maffucci, B. A. F. Le Bailly, L. Byrne, S. M. Bijvoets, M. G. Lizio, J. Raftery, C. P. Butts, S. J. Webb, A. Contini, J. Clayden, *Chem. Sci.* **2017**, *8*, 3007–3018.
[46] F. Zieleniewski, D. N. Woolfson, J. Clayden, *Chem. Commun.* **2020**, *56*, 12049–12052.
[47] K. Yanagisawa, T. Morita, S. Kimura, *J. Am. Chem. Soc.* **2004**, *126*, 12780–12781.
[48] P. Rossi, F. Felluga, P. Tecilla, F. Formaggio, M. Crisma, C. Toniolo, P. Scrimin, *J. Am. Chem. Soc.* **1999**, *121*, 6948–6949.
[49] K. Sato, T. Umeno, A. Ueda, T. Kato, M. Doi, M. Tanaka, *Chem. Eur. J.* **2021**, *27*, 11216–11220.
[50] R. A. Brown, V. Diemer, S. J. Webb, J. Clayden, *Nat. Chem.* **2013**, *5*, 853–860.
[51] F. G. A. Lister, B. A. F. Le Bailly, S. J. Webb, J. Clayden, *Nat. Chem.* **2017**, *9*, 420–425.
[52] G. J. Hilinski, Y.-W. Kim, J. Hong, P. S. Kutchukian, C. M. Crenshaw, S. S. Berkovitch, A. Chang, S. Ham, G. L. Verdine, *J. Am. Chem. Soc.* **2014**, *136*, 12314–12322.
[53] G. A. Woolley, *Acc. Chem. Res.* **2005**, *38*, 486–493.
[54] J. Ceballos, E. Grinhagena, G. Sangouard, C. Heinis, J. Waser, *Angew. Chem. Int. Ed.* **2021**, *60*, 9022–9031; *Angew. Chem.* **2021**, *133*, 9104–9113.
[55] A.-T. Pham, S. Matile, *Chem. Asian J.* **2020**, *15*, 1562–1566.
[56] A. Lindsey-Crosthwait, D. Rodriguez-Lema, M. Walko, C. M. Pask, A. J. Wilson, *Pept. Sci.* **2021**, *113*, e24157.
[57] Y. H. Lau, P. de Andrade, Y. Wu, D. R. Spring, *Chem. Soc. Rev.* **2014**, *44*, 91–102.
[58] N. Luisier, K. Schenk, K. Severin, *Chem. Commun.* **2014**, *50*, 10233–10236.
[59] M. Ciaccia, D. Núñez-Villanueva, C. A. Hunter, *J. Am. Chem. Soc.* **2019**, *141*, 10862–10875.
[60] M. Pollastrini, G. Marafon, J. Clayden, A. Moretto, *Chem. Commun.* **2021**, *57*, 2269–2272.
[61] S. J. Pike, T. Boddaert, J. Raftery, S. J. Webb, J. Clayden, *New J. Chem.* **2015**, *39*, 3288–3294.
[62] J. Kalia, R. T. Raines, *Angew. Chem. Int. Ed.* **2008**, *47*, 7523–7526; *Angew. Chem.* **2008**, *120*, 7633–7636.
[63] W. Drożdż, C. Bouillon, C. Kotras, S. Richeter, M. Barboiu, S. Clément, A. R. Stefankiewicz, S. Ulrich, *Chem. Eur. J.* **2017**, *23*, 18010–18018.
[64] D. Larsen, A. M. Kietrys, S. A. Clark, H. S. Park, A. Ekebergh, E. T. Kool, *Chem. Sci.* **2018**, *9*, 5252–5259.
[65] P. Luo, R. L. Baldwin, *Biochemistry* **1997**, *36*, 8413–8421.
[66] J. C. Lukesh, B. Vanveller, R. T. Raines, *Angew. Chem. Int. Ed.* **2013**, *52*, 12901–12904; *Angew. Chem.* **2013**, *125*, 13139–13142.
[67] J. Beld, K. J. Woycechowsky, D. Hilvert, *Biochemistry* **2007**, *46*, 5382–5390.

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