E-Selective Ring-closing Metathesis in α-Helical Stapled Peptides Using Carbocyclic α,α-Disubstituted α-Amino Acids

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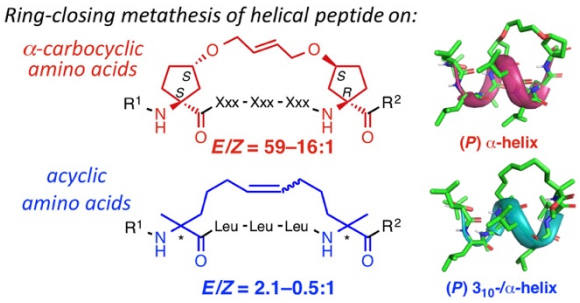
Supporting Information Placeholder



ABSTRACT: We present an *E*-selective ring-closing metathesis reaction in α-helical stapled peptides at *i* and *i* + 4 positions. The use of two chiral carbocyclic α,α-disubstituted α-amino acids, (1*S*,3*S*)-Ac$_5$c^{3OAll} and (1*R*,3*S*)-Ac$_5$c^{3OAll} provide a high *E*-selectivity of up to a 59:1 *E*/*Z* ratio, while mixtures with a *E*/*Z* of 2.1–0.5:1 were produced with standard acyclic (*S*)-(4-pentenyl)alanine amino acids. A stapled octapeptide composed of (1*S*,3*S*)- and (1*R*,3*S*)-Ac$_5$c^{3OAll} amino acids showed a right-handed α-helical crystal structure.

The function of a protein strictly depends on its higher-order structure formed by protein folding. To achieve a similar functionality using short oligopeptides, it is important to constrain their secondary structures to a specific state. This issue can be resolved by the concept of foldamers.[1] Helical foldamers, including peptides,[2] oligoureas,[3] and polymers,[4] are representative foldamers that can be used as organocatalysts, chiral ligands, drug delivery tools, etc. The hydrocarbon stapling of a peptide is a powerful and convenient approach to stabilize the secondary structure of helical peptide foldamers and to enhance their functionality.[5] The first example of helical hydrocarbon-stapled peptide was reported by Blackwell and Grubbs using *O*-allyl L-homoserine-containing peptide as a cyclization precursor (Figure 1A).[6] The ring-closing metathesis (RCM) reaction of this peptide using the first-generation Grubbs catalyst gave the desired 3$_{10}$-helical stapled peptides in high yields as an inseparable *E*/*Z* mixture with a ca. 5:1 ratio. In 2000, Verdine and co-workers reported all-hydrocarbon stapling with (*S*)-(4-pentenyl)alanines [Ala(4-Pte)] at *i* and *i* + 4, as well as *i* + 7 positions in α-helical peptides (Figure 1B).[7] The all-hydrocarbon stapling became one of the most commonly used strategies to induce helicity, although their *E*/*Z*-selectivities as well as crystallographic structures have not been elucidated. A joint group of Toniolo, Grubbs, and O'Leary reported the first example of *E*-selective hydrocarbon-stapling formation with a *E*/*Z* of over 20:1 at *i* and *i* + 3 positions in 3$_{10}$-helical peptide foldamers (Figure 1C).[8] They used *O*-allyl L-serine in a peptide sequence of α-aminoisobutyric acid (Aib), which preferred a 3$_{10}$-helical secondary structure.[9] Grubbs, O'Leary et al. reported *Z*-selective all-hydrocarbon stapling in α-helical peptides using a *Z*-selective ruthenium catalyst (*E*:*Z* = <1:9).[10] However, there are no reports on *E*-selective hydrocarbon-stapling at *i* and *i* + 4 positions of peptides, while development of *E*-selective macrocyclization by RCM has attracted increased attention in the last

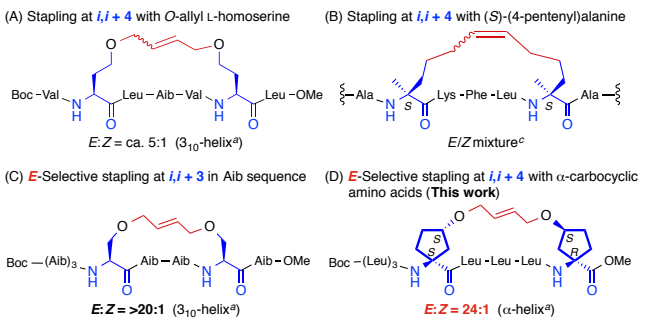


Figure 1. Helical stapled peptides obtained by the RCM reaction ([a]crystallographic structure of peptides, [b]conversion, [c]the *E*/*Z*-ratio was not determined)

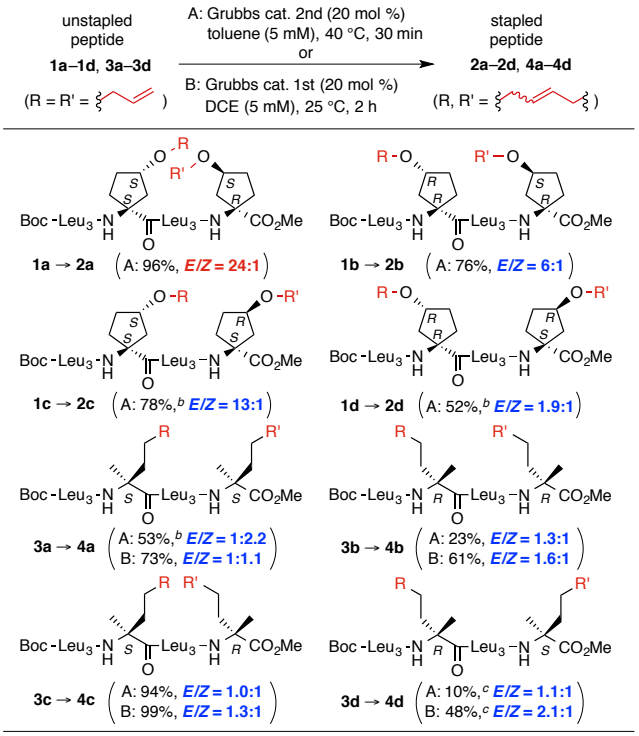
decade.[11] A successive RCM reaction at *i* and *i* + 4 positions of peptides followed by ethenolysis of the undesired *Z*-configured staples reported by Grubbs et al. might be an alternative route to obtain *E*-enriched staples with a *E*/*Z* of up to 24:1.[12] However, the initial *E*/*Z*-selectivities of the RCM products were moderate between 2.4:1 to 4.9:1. Since α-helix is the most abundant secondary structure, α-helix-inducing *i*,*i* + 4-stapled peptides are frequently used for the peptide-based drug discovery.[13] Furthermore, recent studies imply that *E*/*Z*-configuration of stapled peptides is a key to their biological activity.[14] Accordingly, a direct approach to access the *E*-selective hydrocarbon-stapled peptides at *i*,*i* + 4 positions has been demanded.[15]

A possible reason of poor *E*-selectivities of hydrocarbon-stapling at *i* and *i* + 4 positions is a flexible nature of the tethered side chains of L-homoserine and (*S*)-Ala(4-Pte), which easily form both *E*- and *Z*-hydrocarbon stapling. If the conformation of tethered side chains of the stapling precursor is properly constrained, selective hydrocarbon-stapling of thermodynamically more stable *E*-isomer at *i* and *i* + 4 positions in α-helical peptides is possible. Accordingly, carbocyclic α,α-disubstituted α-amino acids (dAAs)[16,17] are suitable for this purpose because of a rigid carbocyclic ring and existence of the second stereogenic center on the ring. Furthermore, carbocyclic dAAs enhance the efficacy of peptide coupling reaction,[9b,18] while side-chain stereogenic centers control a helical screw direction, as well as a secondary structure of their peptides.[19] We have recently reported the synthesis of L-arginine-enriched stapled peptides containing 3-allyloxy-1-aminocyclopentanecarboxylic acids (Ac_5c^{3OAll}) possessing a high cell permeability, as well as a high stability against peptidases.[20] However, a detailed structural analysis of peptides has not been performed, particularly for their *E*/*Z*-selectivity and crystallographic structures. This work is aimed at the synthesis of stapled L-leucine-based Ac_5c^{3OAll}-containing peptides and determination of the impact of Ac_5c^{3OAll} stereochemistry. A peptide containing Verdine's Ala(4-Pte) was also synthesized as a control. Herein, we report *E*-selective RCM in α-helical peptides stapled at *i* and *i* + 4 positions and analysis of their structure by X-ray diffraction (Figure 1D).

We synthesized four Ac_5c^{3OAll} (Figure S1) from (1*S*,3*S*)- and (1*R*,3*S*)-1-amino-3-hydroxycyclopentanecarboxylic acids.[21,22] These Ac_5c^{3OAll} were successfully introduced to L-leucine-based peptides to provide **1a** octapeptides as RCM precursors.[22] Further, RCM between tethered allyloxy groups of compound **1a** was performed by Grubbs catalyst to produce stapled peptides **2a**. After optimization of the reaction conditions (see Table S1),[22] the reaction of **1a** using 20 mol % of the second-generation Grubbs catalyst in toluene completed within 30 min gave **2a** with an excellent *E*/*Z* of 24:1 and 96 % isolated yield. The amount of catalyst could be reduced to 10 mol % preserving a high *E*-selectivity of 21:1.

Under the optimized conditions, however, other peptides **1b**–**1d** possessing different stereochemistry on Ac_5c^{3OAll} provided moderate to low *E*-selectivities (*E*/*Z* = 1.9–13:1, Scheme 1). Moreover, considerable amounts of dimer byproducts were formed in the reactions of **1c** and **1d**, possibly due to the mismatched configurations of the side chains. The octapeptide **3** incorporated with commonly used acyclic dAAs, Ala(4-Pte) was also synthesized. RCM of (*R*)- or (*S*)-Ala(4-Pte)-based peptide **3a**–**3d** under the optimized conditions (A), as well as under the standard conditions for all-hydrocarbon stapling (B)[7,23] gave a mixture of *E*- and *Z*-isomers with *E*/*Z* = 2.1:1–1:2.2 and with moderate yields.

Scheme 1. RCM reaction of octapeptides **1** and **3**[a]

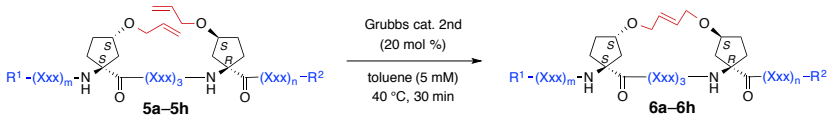


[a]Isolated yields (0.05 mmol scale) are provided with the *E*/*Z* ratio determined by ^{1}H NMR. [b]Including inseparable dimer byproducts. [c]NMR yield.

Next, we investigated the impact of the substrate on the *E*-selectivity. In contrast to the decrease of *E*-selectivity (*E*/*Z* = ca. 7–12:1) with shorter peptides reported by Grubbs et al.,[8] our Ac_5c-based peptides retained high *E*/*Z*-selectivity even at a pentapeptide level (*E*/*Z* = 23–26:1, Table 1, entries 1–3). The octapeptide **5d** having L-leucine tripeptide on the C-terminus ensured better *E*-selectivity (*E*/*Z* = 40:1, entry 4). The optimized conditions were inappropriate for Val-containing peptide **5e**, with a formation of an enormous amount of dimer byproducts and the decreased *E*/*Z* of 16:1 (entry 5). The heptapeptide **5f** containing Ac_5c^{3OAll} instead of *O*-allyl-L-homoserines in Karle and Balaram's sequence shown in Figure 1A (*E*/*Z* = ca. 5:1) improved the *E*-selectivity up to 35:1 (entry 6). Our method is also compatible with the solid-phase synthesis, as we did for peptides **5g** and **5h**.[22,23] The best *E*-selectivity was achieved with the **6h** peptide (*E*/*Z* = 59:1, entry 8).

Since Ac_5c^{3OAll} in compound **1a** provided a better RCM reaction rate with improved *E*-selectivity than that with commonly used (*S*)-Ala(4-Pte) (**3a**), we compared their crystallographic structures in stapled peptides. The crystallographic structures were successfully obtained for carbocyclic-dAAs-containing peptides **1a** and **2a**, and for acyclic-dAAs-containing peptides **3a**, (*E*)-**4a**, and (*Z*)-**4a** (Figure 2). A superimposed structure of stapled peptides **2a** and **4a** shows that **2a** has larger helical radius than that in **4a**. The crystalline peptides **1a** and **2a** involve four consecutive intramolecular hydrogen bonds [N(n + 4)H···O=C(n)] of the *i* ← *i* + 4 type in **1a**, and five consecutive intramolecular hydrogen bonds of the *i* ← *i* + 4 type in **2a** (Table S19). These values indicate the existence of an α-helical

Table 1. Effect of peptide sequence in substrate on *E*-selective stapling



Entry	Sequence [Y = allyl or (*E*)-2-butenyl tether]	Yield (%)	*E*/*Z*[a]
1	Boc-Leu-Leu-(1*S*,3*S*)-Ac$_5$c^{3OY}-Leu-Leu-Leu-(1*R*,3*S*)-Ac$_5$c^{3OY}-OMe (**5a**/**6a**)	68	23:1
2	Boc-Leu-(1*S*,3*S*)-Ac$_5$c^{3OY}-Leu-Leu-Leu-(1*R*,3*S*)-Ac$_5$c^{3OY}-OMe (**5b**/**6b**)	85	24:1
3	Boc-(1*S*,3*S*)-Ac$_5$c^{3OY}-Leu-Leu-Leu-(1*R*,3*S*)-Ac$_5$c^{3OY}-OMe (**5c**/**6c**)	75	26:1
4	Boc-(1*S*,3*S*)-Ac$_5$c^{3OY}-Leu-Leu-Leu-(1*R*,3*S*)-Ac$_5$c^{3OY}-Leu-Leu-Leu-OMe (**5d**/**6d**)	98	40:1
5[b]	Boc-(1*S*,3*S*)-Ac$_5$c^{3OY}-Val-Val-Val-(1*R*,3*S*)-Ac$_5$c^{3OY}-OMe (**5e**/**6e**)	99	16:1
6	Boc-Val-(1*S*,3*S*)-Ac$_5$c^{3OY}-Leu-Aib-Val-(1*R*,3*S*)-Ac$_5$c^{3OY}-Leu-OMe (**5f**/**6f**)	78	35:1
7[c,d]	Bz-(1*S*,3*S*)-Ac$_5$c^{3OY}-Leu-Leu-Leu-(1*R*,3*S*)-Ac$_5$c^{3OY}-NH$_2$ (**5g**/**6g**)	65	23:1
8[c,d]	Ac-Thr-Phe-(1*S*,3*S*)-Ac$_5$c^{3OY}-Asp-Leu-Leu-(1*R*,3*S*)-Ac$_5$c^{3OY}-Tyr-Tyr-Gly-Pro-NH$_2$ (**5h**/**6h**)	99[e,f]	59:1[f]

[a]Determined by ^{1}H NMR. [b]In CH_2Cl_2 at 25 °C. [c]The result was obtained after cleavage from the resin. [d]The first-generation Grubbs catalyst was used in CH_2Cl_2 and the reaction was repeated once. [e]Conversion. [f]Determined by HPLC.

secondary structure in **1a** and **2a**. Conversely, crystalline peptide **3a** involves four consecutive intramolecular hydrogen bonds of the $i \leftarrow i + 4$ type and one intramolecular hydrogen bond [N(n + 3)H···O=C(n)] of the $i \leftarrow i + 3$ type. Two consecutive intramolecular hydrogen bonds of both the $i \leftarrow i + 4$ type and $i \leftarrow i + 3$ type were observed in (*E*)-**4a** and (*Z*)-**4a**. These values indicate the coexistence of the α- and 3_{10}-helical structures. Based on types and numbers of these intramolecular hydrogen bonds, the stapling in **2a** increased α-helicity compared to **1a**, while the stapling in (*E*)-**4a** and (*Z*)-**4a** increased

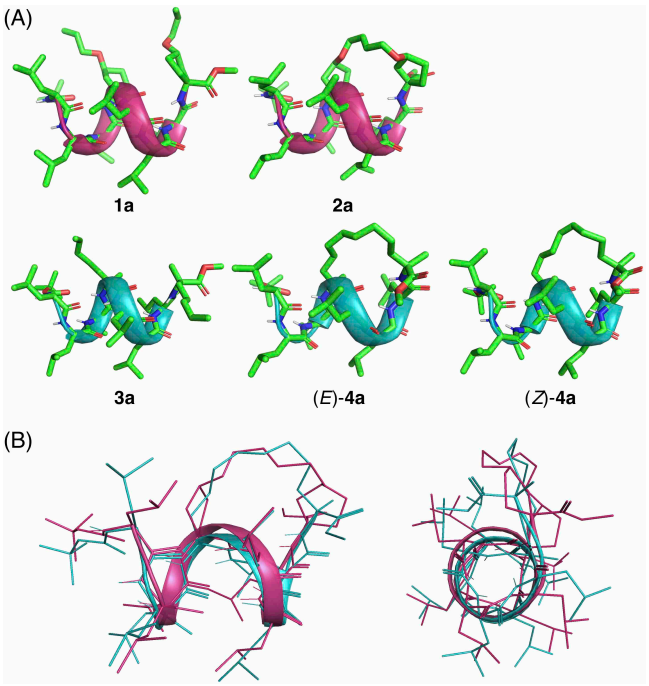


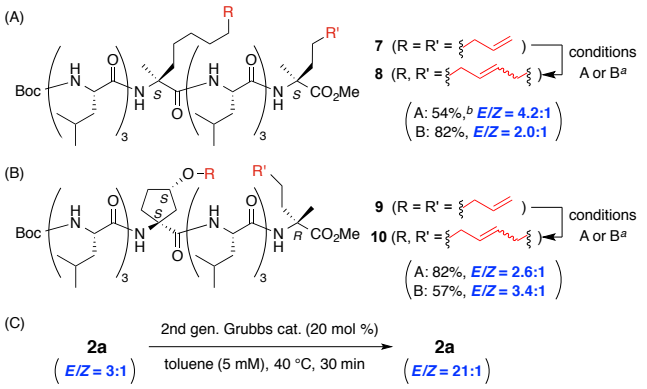
Figure 2. (A) Crystallographic structures of unstapled peptides (**1a**, **3a**) and stapled peptides [**2a**, (*E*)-**4a**, (*Z*)-**4a**]. (B) Superimposed structure of **2a** (magenta) and (*E*)-**4a** (cyan).

3_{10}-helicity compared to **3a**. Similar trends were observed with the torsion angles, where the average torsion angles of **2a** [avg.(ϕ1–ϕ7) = −66.8° and avg.(ψ1–ψ7) = −42.0°] are much closer to the ideal values of the right-handed α-helix [ϕ = −63° and ψ = −42°][24] than that of **1a** [avg.(ϕ1–ϕ7) = −68.0° and avg.(ψ1–ψ7) = −38.8°].[22] Conversely, the average torsion angles of peptides **3a**, (*E*)-**4a**, and (*Z*)-**4a** [avg.(ϕ1–ϕ7) = −68.0°, −74.5°, and −73.1°; avg.(ψ1–ψ7) = −34.7°, −32.3°, and −33.5°, respectively] are much closer to the ideal values of the right-handed 3_{10}-helix [ϕ = −57° and ψ = −30°] rather than those of the α-helix. These results suggest that α-helicity of stapled peptides is better enhanced by cyclic dAAs (Ac$_5$c^{3OAll}) than by commonly used acyclic-dAAs [Ala(4-Pte)] in the crystalline state.

Although the detailed reaction mechanism of the *E*-selective side-chain formation is unclear so far, we performed additional experiments illustrated in Scheme 2 to reveal the conditions providing a high *E*-selectivity. The poor *E*/*Z*-selectivities of **4a** was not caused by a macro-ring strain, since the poor *E*-selectivity of 4.2−2.0:1 was also observed for the RCM of **7**, which involves 7-octenyl side chain. Combination of a cyclic dAA, (1*S*,3*S*)-Ac$_5$c^{3OAll}, and an acyclic dAA, (*R*)-Ala(4-Pte), in peptide **9** was not adapted for *E*-selective RCM reaction that resulted in a poor *E*/*Z*-selectivity (*E*/*Z* = 3.4−2.6:1). It was possible to isomerize the olefin geometry of **2a** (*E*/*Z* = 3:1) to a 21:1 ratio under the optimized conditions. These results suggest that high *E*-selectivity is due to the thermodynamic preference of *E*-isomer by the combination of (1*S*,3*S*)-Ac$_5$c^{3OAll} and (1*R*,3*S*)-Ac$_5$c^{3OAll}.

In conclusion, we developed an *E*-selective RCM reaction in α-helical stapled peptide at *i*,*i* + 4 positions. The combination of carbocyclic dAAs, (1*S*,3*S*)-Ac$_5$c^{3OAll}, and (1*R*,3*S*)-Ac$_5$c^{3OAll} provided the maximum *E*-selectivity of up to 59:1, while commonly used acyclic (*S*)- and (*R*)-Ala(4-Pte), produced a mixture of *E*- and *Z*-isomers with the *E*/*Z* of 2.1−0.5:1. A variety of peptide sequences with a different amount of residues was detected in the solution-phase and by the solid-phase synthesis. X-ray crystallographic analysis suggested that peptide **2a**, composed

Scheme 2. Further RCM reactions



[a]Conditions A: The second-generation Grubbs catalyst (20 mol %), toluene (5 mM), 40 °C, 30 min; Conditions B: the first-generation Grubbs catalyst (20 mol %), 1,2-dichloroethane (5 mM), 25 °C, 2 h. [b]Including inseparable dimer byproducts.

of (1*S*,3*S*)-Ac$_5$c^{3OAll} and (1*R*,3*S*)-Ac$_5$c^{3OAll}, possessed a right-handed α-helical structure, while peptide **4a**, composed of (*S*)-Ala(4-Pte), comprises a mixture of right-handed 3_{10}- and α-helical segment conformations. Further studies including mechanistic investigation and application to helical foldamer organocatalysts,[25] as well as biologically active peptides[26] are in the progress in our laboratory.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental details, characterization data for all new compounds, and crystallographic analyses. (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Gellman, S. H. Foldamers: a manifesto. *Acc. Chem. Res.* **1998**, *31*, 173–180. (b) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. A field guide to foldamers. *Chem. Rev.* **2001**, *101*, 3893–4012. (c) Huc, I. Aromatic oligoamide foldamers. *Eur. J. Org. Chem.* **2004**, 17–29. (d) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. Foldamers as versatile frameworks for the design and evolution of function. *Nat. Chem. Biol.* **2007**, *3*, 252–262. (e) Horne, W. S.; Gellman, S. H. Foldamers with heterogeneous backbones. *Acc. Chem. Res.* **2008**, *41*, 1399–1408. (f) Guichard, G.; Huc, I. Synthetic foldamers. *Chem. Commun.* **2011**, *47*, 5933–5941. (g) Martinek, T. A.; Fülöp, F. Peptidic foldamers: ramping up diversity. *Chem. Soc. Rev.* **2012**, *41*, 687–702. (h) Girvin, Z. C.; Gellman, S. H. Foldamer catalysis. *J. Am. Chem. Soc.* **2020**, *142*, 17211–17223.

(2) (a) Berkessel, A.; Koch, B.; Toniolo, C.; Rainaldi, M.; Broxterman, Q. B.; Kaptein, B. Asymmetric enone epoxidation by short solid-phase bound peptides: further evidence for catalyst helicity and catalytic activity of individual peptide strands. *Biopolymers (Pept. Sci.)* **2006**, *84*, 90–96. (b) Akagawa, K.; Suzuki, R.; Kudo, K. Development of a peptide-based primary aminocatalyst with a helical structure. *Asian J. Org. Chem.* **2014**, *3*, 514–522. (c) Oba, M. Cell-penetrating peptide foldamers: drug-delivery tools. *ChemBioChem* **2019**, *20*, 2041–2045. (d) Mir, F. M.; Crisma, M.; Toniolo, C.; Lubell, W. D. Isolated α-turn and incipient γ-helix. *Chem. Sci.* **2019**, *10*, 6908–6914. (e) Girvin, Z. C.; Andrews, M. K.; Liu, X.; Gellman, S. H. Foldamer-templated catalysis of macrocycle formation. *Science* **2019**, *366*, 1528–1531.

(3) (a) Violette, A.; Averlant-Petit, M. C.; Semetey, V.; Hemmerlin, C.; Casimir, R.; Graff, R.; Marraud, M.; Briand, J.-P.; Rognan, D.; Guichard, G. *N,N'*-Linked oligoureas as foldamers: chain length requirements for helix formation in protic solvent investigated by circular dichroism, NMR spectroscopy, and molecular dynamics. *J. Am. Chem. Soc.* **2005**, *127*, 2156–2164. (b) Bećart, D.; Diemer, V.; Salaün, A.; Oiarbide, M.; Nelli, Y. R.; Kauffmann, B.; Fischer, L.; Palomo, C.; Guichard, G. Helical oligourea foldamers as powerful hydrogen bonding catalysts for enantioselective C–C bond-forming reactions. *J. Am. Chem. Soc.* **2017**, *139*, 12524–12532. (c) Cussol, L.; Mauran-Ambrosino, L.; Buratto, J.; Belorusova, A. Y.; Neuville, M.; Osz, J.; Fribourg, S.; Fremaux, J.; Dolain, C.; Goudreau, S. R.; Rochel, N.; Guichard, G. Structural basis for α-helix mimicry and inhibition of protein–protein interactions with oligourea foldamers. *Angew. Chem., Int. Ed.* **2021**, *60*, 2296–2303.

(4) (a) Yamamoto, T.; Suginome, M. Helical poly(quinoxaline-2,3-diyl)s bearing metal-binding sites as polymer-based chiral ligands for asymmetric catalysis. *Angew. Chem., Int. Ed.* **2009**, *48*, 539–542. (b) Tang, Z.; Iida, H.; Hu, H.-Y.; Yashima, E. Remarkable enhancement of the enantioselectivity of an organocatalyzed asymmetric Henry reaction assisted by helical poly(phenylacetylene)s bearing cinchona alkaloid pendants via an amide linkage. *ACS Macro Lett.* **2012**, *1*, 261. (c) Nakanishi, K.; Fukatsu, D.; Takaishi, K.; Tsuji, T.; Uenaka, K.; Kuramochi, K.; Kawabata, T.; Tsubaki, K. Oligonaphthofurans: fan-shaped and three-dimensional π-compounds. *J. Am. Chem. Soc.* **2014**, *136*, 7101–7109.

(5) For reviews of stapled peptides, see the following: (a) Moretto, A.; Crisma, M.; Formaggio, F.; Toniolo, C. Building a bridge between peptide chemistry and organic chemistry: intramolecular macrocyclization reactions and supramolecular chemistry with helical peptide substrates. *Biopolymers (Pept. Sci.)* **2010**, *94*, 721–732. (b) Verdine, G. L.; Hilinski, G. J. Stapled peptides for intracellular drug targets. *Methods Enzymol.* **2012**, *503*, 3–33. (c) Cromm, P. M.; Spiegel, J.; Grossmann, T. N. Hydrocarbon stapled peptides as modulators of biological function. *ACS Chem. Biol.* **2015**, *10*, 1362–1375. (d) Pérez de Vega, M. J.;

García-Aranda, M. I.; González-Muñiz, R. A role for ring-closing metathesis in medicinal chemistry: mimicking secondary architectures in bioactive peptides. *Med. Res. Rev.* **2011**, *31*, 677–715. (e) Brik, A. Metathesis in peptides and peptidomimetics. *Adv. Synth. Catal.* **2008**, *350*, 1661–1675.

(6) (a) Blackwell, H. E.; Grubbs, R. H. Highly efficient synthesis of covalently cross-linked peptide helices by ring-closing metathesis. *Angew. Chem., Int. Ed.* **1998**, *37*, 3281–3284. (b) Blackwell, H. E.; Sadowsky, J. D.; Howard, R. J.; Sampson, J. N.; Chao, J. A.; Steinmetz, W. E.; O'Leary, D. J.; Grubbs, R. H. Ring-closing metathesis of olefinic peptides: design, synthesis, and structural characterization of macrocyclic helical peptides *J. Org. Chem.* **2001**, *66*, 5291–5302.

(7) Schafmeister, C. E.; Po, J.; Verdine, G. L. An all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. *J. Am. Chem. Soc.* **2000**, *122*, 5891–5892.

(8) Boal, A. K.; Guryanov, I.; Moretto, A.; Crisma, M.; Lanni, E. L.; Toniolo, C.; Grubbs, R. H.; O'Leary, D. J. Facile and *E*-selective intramolecular ring-closing metathesis reactions in 3_{10}-helical peptides: a 3D structural study. *J. Am. Chem. Soc.* **2007**, *129*, 6986–6987.

(9) (a) Karle, I. L.; Balaram, P. Structural characteristics of α-helical peptide molecules containing Aib residues. *Biochemistry* **1990**, *29*, 6747–6756. (b) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. Control of peptide conformation by the Thorpe–Ingold effect (C^{α}-tetrasubstitution). *Biopolymers (Pept. Sci.)* **2001**, *60*, 396–419.

(10) Mangold, S. L.; O'Leary, D. J.; Grubbs, R. H. *Z*-Selective olefin metathesis on peptides: investigation of side-chain influence, preorganization, and guidelines in substrate selection. *J. Am. Chem. Soc.* **2014**, *136*, 12469–12478.

(11) (a) Shen, X.; Nguyen, T. T.; Koh, M. J.; Xu, D.; Speed, A. W. H.; Schrock, R. R.; Hoveyda, A. H. Kinetically *E*-selective macrocyclic ring-closing metathesis. *Nature* **2017**, *19*, 380–385. (b) Marx, V. M.; Herbert, M. B.; Keitz, B. K.; Grubbs, R. H. Stereoselective access to *Z* and *E* macrocycles by ruthenium-catalyzed *Z*-selective ring-closing metathesis and ethenolysis. *J. Am. Chem. Soc.* **2013**, *135*, 94–97.

(12) Mangold, S. L.; Grubbs, R. H. Stereoselective synthesis of macrocyclic peptides via a dual olefin metathesis and ethenolysis approach. *Chem. Sci.* **2015**, *6*, 4561–4569.

(13) (a) Walensky, L. D.; Kung, A. L.; Escher, I.; Malia, T. J.; Barbuto, S.; Wright, R. D.; Wagner, G.; Verdine, G. L.; Korsmeyer, S. J. Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. *Science* **2004**, *305*, 1466–1470. (b) Ali, A. M.; Atmaj, J.; Oosterwijk, N. V.; Groves, M. R.; Dömling, A. Stapled peptides inhibitors: a new window for target drug discovery. *Comput. Struct. Biotechnol. J.* **2019**, *17*, 263–281.

(14) Yuen, T. Y.; Brown, C. J.; Xue, Y.; Tan, Y. S.; Ferrer Gago, F. J.; Lee, X. E.; Neo, J. Y.; Thean, D.; Kaan, H. Y. K.; Partridge, A. W.; Verma, C. S.; Lane, D. P.; Johannes, C. W. Stereoisomerism of stapled peptide inhibitors of the p53-Mdm2 interaction: an assessment of synthetic strategies and activity profiles. *Chem. Sci.* **2019**, *10*, 6457–6466.

(15) For the examples of *E*- or *Z*-selective RCM reaction in nonhelical peptides, see the following: (a) Miller, S. J.; Grubbs, R. H. Synthesis of conformationally restricted amino acids and peptides employing olefin metathesis. *J. Am. Chem. Soc.* **1995**, *117*, 5855–5856. (b) Kaul, R.; Surprenant, S.; Lubell, W. D. Systematic study of the synthesis of macrocyclic dipeptide β-turn mimics possessing 8-, 9-, and 10- membered rings by ring-closing metathesis. *J. Org. Chem.* **2005**, *70*, 3838–3844. (c) Makura, Y.; Ueda, A.; Kato, T.; Iyoshi, A.; Higuchi, M.; Doi, M.; Tanaka, M. X-ray crystallographic structure of α-helical peptide stabilized by hydrocarbon stapling at *i*,*i* + 1 positions. *Int. J. Mol. Sci.* **2021**, *22*, 5364.

(16) For reviews of synthesis of cyclic dAAs, see the following: (a) Cativiela, C.; Díaz-de-Villegas, M. D. Stereoselective synthesis of quaternary α-amino acids. Part 2: cyclic compounds. *Tetrahedron: Asymmetry* **2000**, *11*, 645−732. (b) Cativiela, C.; Ordoñez, M. Recent progress on the stereoselective synthesis of cyclic quaternary α-amino acids. *Tetrahedron: Asymmetry* **2009**, *20*, 1−63.

(17) For examples of dAAs-based peptide foldamers, see the following: (a) Toniolo, C.; Polese, A.; Formaggio, F.; Crisma, M.; Kamphuis, J. Circular dichroism spectrum of a peptide 3_{10}-helix. *J. Am. Chem. Soc.* **1996**, *118*, 2744–2745. (b) Tanaka, M.; Anan, K.; Demizu, Y.; Kurihara, M.; Doi, M.; Suemune, H. Side-chain chiral centers of amino acid and helical-screw handedness of its peptides. *J. Am. Chem. Soc.* **2005**, *127*, 11570–11571. (c) Royo, S.; De Borggraeve, W. M.; Peggion, C.; Formaggio, F.; Crisma, M.; Jimeńez, A. I.; Cativiela, C.; Toniolo, C. Turn and helical peptide handedness governed exclusively by side-chain chiral centers. *J. Am. Chem. Soc.* **2005**, *127*, 2036–2037. (d) Tanaka, M. Design and synthesis of chiral α,α-disubstituted amino acids and conformational study of their oligopeptides. *Chem. Pharm. Bull.* **2007**, *55*, 349–358. (e) Crisma, M.; Toniolo, C. Helical screw-sense preferences of peptides based on chiral, C^{α}-tetrasubstituted α-amino acids. *Biopolymers (Pept. Sci.)* **2015**, *104*, 46–64.

(18) De Zotti, M.; Clayden, J. Extended diethylglycine homopeptides formed by desulfurization of their tetrahydrothiopyran analogues. *Org. Lett.* **2019**, *21*, 2209–2212.

(19) (a) Tanaka, M.; Demizu, Y.; Doi, M.; Kurihara, M.; Suemune, H. Chiral centers in the side chains of α-amino acids control the helical screw sense of peptides. *Angew. Chem., Int. Ed.* **2004**, *43*, 5360–5363. (b) Eto, R.; Oba, M.; Ueda, A.; Uku, T.; Doi, M.; Matsuo, Y.; Tanaka, T.; Demizu, Y.; Kurihara, M.; Tanaka, M. Diastereomeric right- and left-handed helical structures with fourteen (*R*)-chiral centers. *Chem.—Eur. J.* **2017**, *23*, 18120–18124. (c) Koba, Y.; Ueda, A.; Oba, M.; Doi, M.; Kato, T.; Demizu, Y.; Tanaka, M. Left-handed helix of three-membered ring amino acid homopeptide interrupted by an N–H···ethereal O-type hydrogen bond. *Org. Lett.* **2018**, *20*, 7830–7834.

(20) Oba, M.; Kunitake, M.; Kato, T.; Ueda, A.; Tanaka, M. Enhanced and prolonged cell-penetrating abilities of arginine-rich peptides by introducing cyclic α,α-disubstituted α-amino acids with stapling. *Bioconjugate Chem.* **2017**, *28*, 1801–1806.

(21) Koba, Y.; Hirata, Y.; Ueda, A.; Oba, M.; Doi, M.; Demizu, Y.; Kurihara, M.; Tanaka, M. Synthesis of chiral five-membered carbocyclic ring amino acids with an acetal moiety and helical conformations of its homo-chiral homopeptides. *Biopolymers (Pept. Sci.)* **2016**, *106*, 555–562.

(22) For details, see the Supporting Information.

(23) Kim, Y.-W.; Grossmann, T. N.; Verdine, G. L. Synthesis of all-hydrocarbon stapled α-helical peptides by ring-closing olefin metathesis. *Nat. Protoc.* **2011**, *6*, 761–771.

(24) (a) Toniolo, C.; Benedetti, E. The polypeptide 3_{10}-helix. *Trends Biochem. Sci.* **1991**, *16*, 350–353; (b) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C.; Broxterman, Q. B.; Kaptein, B. Molecular spacers for physicochemical investigations based on novel helical and extended peptide structures. *Biopolymers (Pept. Sci.)* **2004**, *76*, 162–176.

(25) (a) Ueda, A.; Umeno, T.; Doi, M.; Akagawa, K.; Kudo, K.; Tanaka, M. Helical-peptide-catalyzed enantioselective Michael addition reactions and their mechanistic insights. *J. Org. Chem.* **2016**, *81*, 5864–5871. (b) Ueda, A.; Higuchi, M.; Umeno, T.; Tanaka, M. Enantioselective synthesis of 2,4,5-trisubstituted tetrahydropyrans via peptide-catalyzed Michael addition followed by Kishi's reductive cyclization. *Heterocycles* **2019**, *99*, 989–1002. (c) Umeno, T.; Ueda, A.; Doi, M.; Kato, T.; Oba, M.; Tanaka, M. Helical foldamer-catalyzed enantioselective 1,4-addition reaction of dialkyl malonates to cyclic enones. *Tetrahedron Lett.* **2019**, *60*, 151301. (d) Sato, K.; Umeno, T.; Ueda, A.; Kato, T.; Doi, M.; Tanaka, M. Asymmetric 1,4-addition reactions catalyzed by N-terminal thiourea-modified helical L-Leu-peptide with cyclic amino acids. *Chem.—Eur. J.* **2021**, *27*, 11216–11220.

(26) (a) Kato, T.; Oba, M.; Nishida, K.; Tanaka, M. Cell-penetrating helical peptides having L-arginines and five-membered ring α,α-disubstituted α-amino acids. *Bioconjugate Chem.* **2014**, *25*, 1761–1768. (b) Kato, T.; Oba, M.; Nishida, K.; Tanaka, M. Cell-penetrating peptides using cyclic α,α-disubstituted α-amino acids with basic functional groups *ACS Biomater. Sci. Eng.* **2018**, *4*, 1368–1376. (c) Kato, T.; Kita, Y.; Iwanari, K.; Asano, A.; Oba, M.; Tanaka, M.; Doi, M. Synthesis of six-membered carbocyclic ring α,α-disubstituted amino acids and arginine-rich peptides to investigate the effect of ring size on the properties of the peptide. *Bioorg. Med. Chem.* **2021**, *38*, 116111.