Design and Synthesis of Helical N-Terminal L-Prolyl Oligopeptides Possessing Hydrocarbon Stapling

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Abstract: We designed and synthesized helical short oligopeptides with an L-proline on the N-terminus and hydrocarbon stapling on the side chain. Side-chain stapling can stabilize the secondary structures of peptides, and, therefore, stapled peptides may be applicable to peptide-based organocatalysts. Olefin-tethered cis-4-hydroxy-L-proline 1 and L-serine 2 and 8, and (R)-α-allyl-proline 18 were used as cross-linking motifs and incorporated into helical peptide sequences. The Z- and E-selectivities were observed for the ring-closing metathesis reactions of peptides 3 and 11 (i,i+1 series), respectively, while no E/Z-selectivity was observed for that of 19 (i,i+3 series). The stapled peptide B' catalyzed the Michael addition reaction of 1-methylindole to α,β-unsaturated aldehyde, which was seven times faster than that of unstapled peptide B. Furthermore, the high catalytic activity was retained even at lower catalyst loadings (5 mol %) and lower temperatures (0 °C). The circular dichroism spectra of stapled peptide B' showed a right-handed helix with a higher intensity than that of unstapled peptide B. These results indicate that the introduction of side-chain stapling is beneficial for enhancing the catalytic activity of short oligopeptide catalysts.

Keywords: peptide; helix; hydrocarbon stapling; ring-closing metathesis; organocatalyst; L-proline; Michael addition

1. Introduction

Hydrocarbon stapling is one of the most commonly used methods to stabilize the secondary structure of peptides as a way to provide enhanced functionality [1–3]. This powerful tool is especially important for short oligopeptides due to their flexible secondary structure. Grubbs et al. reported the synthesis of 310-helical heptapeptides stabilized by hydrocarbon stapling at the i,i+4 positions using ring-closing metathesis [4,5]. In 2000, Verdine’s group reported all-hydrocarbon stapling using α-olefin-tethered alanine at i,i+4 as well as at i,i+7 positions, and the introduction of these staples highly induced α-helicities as well as the metabolic stabilities of the peptides [6]. Nowadays, the all-hydrocarbon staplings at the i,i+4 and i,i+7 positions are widely used in the medicinal chemistry of peptides [7–9].

Short peptides are also attractive compounds in the field of organocatalysis [10–13]. These peptide catalysts can be categorized into several classes based on their secondary structure, those including β-turn [14,15], helix [16–20], turn-helix types [21–23], and so forth. Therefore, hydrocarbon stapling can be an effective tool for the development of peptide-based organocatalysts by controlling their secondary structure. However, there are few examples of stapled peptide-catalyzed asymmetric organocatalytic reactions. Demizu and co-workers reported an enantioselective Juliá–Colonna epoxidation of chalcone.
catalyzed by a helical peptide-based primary amino catalyst possessing a crosslink between two L-homoserines at the i,i+4 positions (Figure 1) [24,25]. Likewise, secondary amino catalysts are powerful catalysts with a broad range of applicable reactions [26,27]. Moreover, the peptide hydrocarbon staplings at the i,i+1 and i,i+3 positions are rarely examined compared to i,i+4 series but have potential as constrained cyclic peptides for organocatalysis, material science, drug discovery, and so forth [30]. From this point of view, the introduction of allyl tethered cis-4-hydroxy-L-proline or (R)-α-allyl-proline can be suitable for this purpose. Secondary structures, as well as helical screw directions, can be controlled by introducing 1-aminocycloalkane-1-carboxylic acid in homopeptides [31–40] and heteropeptides [41–43], and these constrained peptides catalyze asymmetric 1,4-addition reactions [44–46]. Therefore, poly L-leucine-incorporating 1-aminocyclopentane-1-carboxylic acid was used as an α-helix-inducing motif. The stapling efficiency was evaluated by comparing the catalytic activities of stapled and unstapled peptides in Friedel–Crafts type 1,4-addition reactions [47,48]. Herein, we report the synthesis of helical N-terminal prolyl oligopeptides with hydrocarbon stapling at i,i+1 as well as i,i+3 positions and the enhancements of their catalytic activity for the Michael addition of 1-methylindole to α,β-unsaturated aldehyde.

![Figure 1. Hydrocarbon-stapled peptides used in the organocatalytic reaction. (a) Helical peptide-based primary amino catalyst with stapling at i,i+4 and (b) helical peptide-based secondary amino catalysts with stapling at i,i+1 and i,i+3 (This work).](image)

2. Results and Discussion

The synthesis of the unstapled peptides A and B and stapled peptides of the i,i+1 series A’ and B’ began from allyl tethered cis-4-hydroxy-L-proline 1 [49,50] and L-serine 2, as illustrated in Scheme 1. The coupling of 1 and 2 produced dipeptide 3, which was successfully introduced to a helix-inducing motif, H-(L-Leu-L-Leu-Ac5c)2-OMe [41,44]. The deprotection of the Boc-protecting groups of 3 and 4 produced N-terminal free peptides A and B in quantitative yields. On the other hand, the ring-closing metathesis of dipeptide 3 was performed with 20 mol % of the second-generation Grubbs catalyst to give i,i+1-stapled dipeptide 5. In this reaction, the Z-configured product was obtained as a major product (E/Z = 1.0:5.6), possibly due to the medium ring size of 5 (13-membered ring) along with the rigid 4-hydroxyproline part. The hydrogenation of 5 provided dipeptide 6, which was coupled with H-(L-Leu-L-Leu-Ac5c)2-OMe to afford stapled octapeptide 7. The N-Terminal free peptides A’ and B’ were obtained by the Boc-deprotection of 6 and 7, respectively. It should be noted that neither the ring-closing metathesis of octapeptide 4 nor that of the trans-4-hydroxy-L-proline derivatives of 3 produced the desired cyclization products. This poor reactivity may be caused by a ring strain of the 13-membered ring product, which resulted in a preference for the Z-configured isomer of 5.

Stapled peptide C’ possessing tethered side chains at the i,i+1 positions with a 15-membered macrocyclic ring was also synthesized from 4-pentenyl tethered L-Ser 8 [51] by a similar manner as described in Scheme 1 (Scheme 2). In contrast to the reaction of 4 to 7, unstapled peptide 11 underwent the ring-closing metathesis reaction smoothly to provide stapled peptide 12 in a 93% yield with a
preference for E-isomer over Z-isomer ($E/Z = 5.5:1.0$). This smooth reaction implies the released macrocyclic ring strain of the product, which resulted in a preference for thermodynamically favored $E$-olefin isomer.

![Scheme 1. Synthesis of unstapled peptides A and B and stapled peptides A' and B'.](image)

The peptide stapling at the $i,i+3$ positions could be another choice to restrict the conformational freedom of the N-terminus. We designed spirocyclic-stapled peptide D' possessing tethered crosslinks at the $i,i+3$ positions by introducing ($R$)-$\alpha$-allyl-proline 18 [52,53] (Scheme 3). The synthesis of D' began from $O$-(4-penteny)-L-serine 8, which was sequentially coupled with $H$-L-Leu-L-Leu-Ac$_3$-OMe [44] on C-terminus and with Boc-L-Leu-L-Leu-OMe followed by 18 on N-terminus to produce heptapeptide 19. The ring-closing metathesis of 19 proceeded at high yield, but no $E/Z$-selectivity was observed ($E/Z = 1.1:1.0$). The poor selectivity may be caused by sterically congested ($R$)-$\alpha$-allyl-proline. N-terminal-free heptapeptides D and D' were synthesized by the hydrogenolysis of 19 and 20, respectively.

Next, we examined the Michael addition reaction of 1-methylindole (22) and $\alpha,\beta$-unsaturated aldehyde 21 using 20 mol % of unstapled peptides A-D and stapled peptides A'–D' to compare their catalytic activities (Table 1). The reaction with stapled octapeptide B' showed a faster reaction rate than the reaction with stapled dipepptide A' (entries 2 and 4, 46% conversion after 6 d vs. 83% conversion after 1 d). This result suggests that the helical motif [-L-Leu-L-Leu-Ac$_3$]- of stapled peptide B' is important to catalytic activity. Furthermore, stapled peptide B' is more active than unstapled peptide B (entries 3 and 4, 83% conversion with a 76% isolated yield vs. 12% conversion). Similar trends were observed for other stapled peptides A', C', and D'. Therefore, the introduction of side-chain stapling plays a key role in enhancing catalytic activity. Moderate ee values could be improved by peptide...
sequence screening. The absolute configuration of 23 was determined by comparisons of the chiral HPLC chart and the specific rotation with those in the literature [47,48].

Scheme 2. Synthesis of unstapled peptide C and stapled peptide C' (tethering at $i, i+1$ positions with a 15-membered ring).

Scheme 3. Synthesis of unstapled peptide D and stapled peptide D' (tethering at $i, i+3$ positions with an 18-membered ring).
Table 1. Catalytic activities of unstapled peptides A–D and stapled peptides A’–D’ in the Michael addition reaction of 21 and 22.

| Entry | Peptide | Time (d) | Conv. (%) ¹ | Ee (%) ² |
|-------|---------|----------|-------------|----------|
| 1     | A       | 6        | 27          | −1       |
| 2     | A’      | 6        | 46          | 6        |
| 3     | B       | 1        | 12          | −29      |
| 4     | B’      | 1        | 83          | 36       |
| 5     | C       | 4        | 51          | −11      |
| 6     | C’      | 4        | 81          | −5       |
| 7     | D       | 2        | 50          | −47      |
| 8     | D’      | 2        | 69          | −56      |
| 9     | none    | 1        | 9           | N.D. ³   |

¹ Conversion was determined by ¹H NMR analysis. ² Ee was determined by HPLC. ³ Not determined.

The reaction with reduced catalyst loading (10 or 5 mol %) of stapled peptide B’ displayed almost the same results as the reaction with 20 mol % (Table 2, entries 4 and 6), while that of unstapled peptide B resulted in further decreases in both the conversion yield and ee value (entries 3 and 5). Lowering the reaction temperature to 0 °C contributed to the deactivation of unstapled peptide B (entry 7), but the reaction with stapled peptide B’ retained a high conversion, with increased ee values (entry 8). These results also support that side-chain hydrocarbon stapling enhances the catalytic activities in the reaction.

Table 2. Effect of the catalyst loading and temperature in the Michael addition reaction.

| Entry | Peptide (mol %) | Temp. (°C) | Conv. (%) ¹ | Ee (%) ² |
|-------|-----------------|------------|-------------|----------|
| 1     | B (20)          | rt¹        | 12          | −29      |
| 2     | B’ (20)         | rt¹        | 83          | 36       |
| 3     | B (10)          | rt¹        | 6           | −13      |
| 4     | B’ (10)         | rt¹        | 81          | −5       |
| 5     | B (5)           | rt¹        | trace       | N.D. ⁴   |
| 6     | B’ (5)          | rt¹        | 66          | 30       |
| 7     | B (20)          | 0          | trace       | N.D. ⁴   |
| 8     | B’ (20)         | 0          | 78          | 47       |

¹ Conversion was determined by ¹H NMR analysis. ² Ee was determined by HPLC. ³ Room temperature. ⁴ Not determined.

Circular dichroism (CD) spectra were measured for all peptide catalysts to obtain their secondary structure information (Figure 2). The CD spectra of octapeptides B, B’, C, and C’ and heptapeptides D and D’ showed right-handed helical structures, while those of dipeptides A and A’ showed β-turn structures. The helicity of stapled peptide B’, which gave the best conversion in Table 1, was higher than that of unstapled peptide B. These results suggest that reinforcement of helicity via side-chain stapling...
presumably increased the catalytic activity of the stapled peptide B'. Interestingly, the Michael reactions catalyzed by unstapled peptide B and stapled peptide B' produced opposite ee values, whereas both peptides showed right-handed helical structures. Therefore, the introduction of side-chain stapling to peptide catalysts can possibly reverse the enantioselectivities after the fine-tuning of the peptide sequence.

![Figure 2. Circular dichroism (CD) spectra of peptide catalysts A–D and A’–D’. (0.5 mM in 2,2,2-trifluoroethanol).](image)

Based on the right-handed helical structure, the plausible reaction mechanism catalyzed by stapled peptide B' and unstapled peptide B is shown in Figure 3. For the reaction catalyzed by stapled peptide B', the reactive iminium ion was formed inside the helical pipe with a rigid conformation caused by the hydrocarbon stapling, which accelerated the Friedel–Crafts type attack of 1-methylindole from the si face. On the other hand, the iminium species formed by unstapled peptide B exists outside the helical pipe with a flexible conformation, which decreased the reaction rate and enabled 1-methylindole to be accessed from both faces. Although the pioneering organocatalyst in this transformation, MacMillan’s imidazolidinone catalyst showed high enantioselectivities [54]; its derivatization as such a covalent immobilization to polymer supports as a recyclable catalyst is difficult and resulted in decreasing yield and enantioselectivities [35]. On the other hand, peptide catalysts are easier to modify and can be reused by immobilization in the resin at C-terminus, which does not affect the reactive site of N-terminus [21–23].

In summary, we have developed synthetic routes to the N-terminal l-prolyl oligopeptides A’–D’ possessing side-chain hydrocarbon stapling. The ring-closing metathesis reactions of peptides A and C selectively produced Z- and E-configured stapled peptides, respectively, while no E/Z-selectivity was observed for the ring-closing metathesis of D. The stapled peptide B' catalyzed the Michael addition of 1-methylindole to α,β-unsaturated aldehyde, which was seven times faster than that of unstapled peptide B. Since the reactions with B' at lower catalyst loadings or lower temperatures retained conversion yields comparable to those of B, the introduction of side-chain hydrocarbon stapling is effective in enhancing the catalytic activity of peptides. These results provide useful information related to the recent progress of the E/Z-selective ring-closing metathesis of peptides [56,57], l-prolyl catalysts [58–60], and peptide foldamer [61–63]. Further studies including enantioselectivity...
improvement, an expansion of the reaction scope using catalyst B’, as well as applications to cell-penetrating peptides [64–66] are ongoing in our laboratory.

Figure 3. A plausible reaction mechanism catalyzed by (a) stapled peptide B’ and (b) unstapled peptide B.

3. Materials and Methods

3.1. General Procedure and Method

Melting points were taken on an AS ONE melting point apparatus ATM-01 (AS ONE Corporation, Osaka, Japan) and were uncorrected. Optical rotations were measured on a JASCO DIP-370 polarimeter (JASCO Corporation, Tokyo, Japan) using CHCl₃ as a solvent. ¹H NMR and ¹³C NMR spectra were recorded on the JEOL JNM-AL-400 (400 MHz), a Varian NMR System 500PS SN (500 MHz and 125 MHz) spectrometer (Agilent Inc., Santa Clara, CA, USA). Chemical shifts (δ) are reported in parts per million (ppm). For the ¹H NMR spectra (CDCl₃), tetramethylsilane was used as the internal reference (0.00 ppm), while the central solvent peak was used as the reference (77.0 ppm in CDCl₃) for the ¹³C NMR spectra. The IR spectra were recorded on a Shimadzu IRAffinity-1 FT-IR spectrophotometer (Shimadzu Corporation, Kyoto, Japan). High-resolution mass spectra (HRMS) were obtained on a JEOL JMS-T100TD using electrospray ionization (ESI) (JEOL Ltd., Tokyo, Japan) or direct analysis in the real-time (DART) ionization in time-of-flight (TOF) mode. Circular dichroism (CD) spectra were measured with a JASCO J-725N spectropolarimeter (JASCO Corporation, Tokyo, Japan) using a 1.0 mm path length cell. Analytical and semi-preparative thin layer chromatography (TLC) was performed with Merck Millipore pre-coated TLC plates (MilliporeSigma, Burlington, NJ, USA), silica gel 60 F₂₅₄, and layer thicknesses of 0.25 and 0.50 mm, respectively. Compounds were observed in UV light at 254 nm and then visualized by staining with iodine, p-anisaldehyde, or phosphomolybdic acid stain. Flash and gravity column chromatography separations were performed on Kanto Chemical silica gel 60N, spherical neutral, with particle sizes of 63–210 µm, respectively. Analytical high-performance liquid chromatography (HPLC) was carried out with JASCO PU-2089 on a UV spectrophotometric detector (254 nm, JASCO UV-2075, JASCO Corporation, Tokyo, Japan), to which a 4.6 × 250 mm size chiral column (Daicel Chiralpak AD-H, Daicel Corporation, Osaka, Japan) was attached. All moisture-sensitive reactions were conducted under an inert atmosphere. Reagents and solvents were of commercial grade and were used as supplied, unless otherwise noted. Compounds 1 [49,50], 2 [24,25], 8 [51], 18 [52,53], H-((1-Leu-1-Leu-Ac₅c)₂-OMe [44], and H-1-Leu-1-Leu-Ac₅c-OMe [44] were prepared according to the reported procedures. Copies of NMR Spectra are given in the Supplementary Materials.

3.2. Synthesis of Unstapled Peptides A and B and Stapled Peptides A’ and B’

Boc-1-Hyp⁰All₁-Ser⁰All₂-OMe (3): To a solution of Boc-1-Hyp⁰All₁-OH (1 [49,50]; 4.22 g, 15.5 mmol) in CH₂Cl₂ (52 mL) were added 3-[bis(dimethylamino)methylimiumyl]-3H-benzotriazol-1-oxide hexafluorophosphate (HBTU; 6.48 g, 17.1 mmol) and 1-hydroxybenzotriazole hydrate (HOBt·H₂O; 2.62 g, 17.1 mmol) at 0 °C, and the solution was stirred for 30 min. Then, a solution of HCl-H₁-Ser⁰All₂-OMe (2 [24,25]; 2.47 g, 15.5 mmol) in CH₂Cl₂ (52 mL) and N,N-diisopropylethylamine
(DIPEA; 5.41 mL, 31.1 mmol) was added to the reaction mixture at the same temperature, and the resultant mixture was gradually warmed to room temperature. After stirring overnight, the CH₂Cl₂ was removed and the residue was diluted with EtOAc. The solution was washed successively with 1 M of HCl, water, sat. aq NaHCO₃, and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (40% EtOAc in n-hexane) to give 3 (3.89 g, 61%) as a yellow oil. [α]D⁰⁻3.2 (c 1.02, CHCl₃). ¹H NMR (500 MHz, CDCl₃, VT = 50 °C): δ: 5.98–5.77 (m, 2H), 5.28–5.11 (m, 4H), 4.77–4.66 (m, 1H), 4.33 (d, J = 8.8 Hz, 1H), 4.07–4.04 (m, 1H), 4.00–3.86 (m, 4H), 3.82 (dd, J = 9.7, 3.5, Hz, 1H), 3.78–3.70 (m, 1H), 3.74 (s, 3H), 3.61–3.49 (m, 3H), 2.56–2.44 (m, 1H), 2.23–2.09 (m, 1H), 1.48 (s, 9H). ¹³C NMR (125 MHz, CDCl₃, VT = 50 °C): δ: 172.0, 170.5, 156.0, 134.6, 134.3, 117.0, 116.9, 81.0, 76.4, 72.2, 69.9, 69.6, 58.6, 53.0, 52.8, 52.2, 37.0, 28.3 (3C). IR (film): 3304, 2978, 2933, 1751, 1701 cm⁻¹. HRMS (DART) m/z: [M + H]+ calc for C₁₉H₃₇N₂O₇, 413.2288; found, 413.2282.

H-L-HypOAll-L-SerOAll-OMe (A): To a solution of Boc-protected dipeptide 3 (100 mg, 0.242 mmol) in CH₂Cl₂ (2.4 mL) was added trifluoroacetic acid (0.24 mL) dropwise at room temperature, and the reaction mixture was stirred for 2 days at the same temperature. The reaction mixture was neutralized by adding sat. aq NaHCO₃ and the aqueous phase was extracted with CHCl₃ three times. The combined organic extracts were dried over anhydrous MgSO₄ and concentrated under vacuum to give amine A (75.2 mg, quant) as an amorphous solid. [α]D⁰⁻0.29 (EtOAc). Mp 81–83 °C. [α]D⁰⁻39.4 (c 1.00, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ: 8.27 (d, J = 8.6 Hz, 1H), 5.93–5.78 (m, 2H), 5.29–5.21 (m, 2H), 5.21–5.11 (m, 2H), 4.72 (dt, J = 8.3, 3.5 Hz, 1H), 4.07–4.00 (m, 1H), 3.94 (dt, J = 5.4, 1.5 Hz, 1H), 3.92–3.85 (m, 2H), 3.82 (dd, J = 8.3, 4.9 Hz, 1H), 3.76 (s, 3H), 3.58 (dd, J = 9.5, 3.7 Hz, 1H), 3.17 (dd, J = 11.2, 5.4 Hz, 1H), 3.05 (dd, J = 11.2, 2.9 Hz, 1H), 2.28–2.16 (m, 2H). ¹³C NMR (125 MHz, CDCl₃): δ: 168.4, 163.9, 134.1, 133.6, 118.0, 117.4, 72.6, 72.3, 70.1, 68.5, 57.2, 54.1, 52.5, 50.7, 33.6. IR (film): 3304, 2978, 2933, 1751, 1701 cm⁻¹. HRMS (ESI) m/z: [M + Na]+ calc for C₁₉H₃₇N₂NaO₇, 335.1583; found, 335.1573.

Boc-L-HypOAll-L-SerOAll-[(l-Leu)₂-Ac₅Cl₂]-OMe (4): To a solution of dipeptide 3 (50.0 mg, 0.121 mmol) in MeOH (1.2 mL) was added 1 M of aqueous NaOH (0.121 mL, 0.121 mmol) at room temperature, and the mixture was stirred overnight at the same temperature. The solution was acidified with 1 M of aqueous HCl and the MeOH was removed in vacuo. The resulting aqueous solution was extracted with EtOAc three times. The combined organic extracts were washed with brine, dried over Na₂SO₄, and concentrated in vacuo to give a carboxylic acid (44.7 mg, 93%). To a solution of the acid (206 mg, 0.500 mmol) in CH₂Cl₂ (2.5 mL) was added EDCI-HCl (96.0 mg, 0.500 mmol) and HOBT·H₂O (92.0 mg, 0.600 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. Then, a solution of H-[l-Leu]-Ac₅Cl₂-OMe (354 mg, 0.500 mmol) in CH₂Cl₂ (2.5 mL) was added dropwise to the reaction mixture at 0 °C. The reaction was gradually warmed to room temperature and stirred overnight. After the removal of CH₂Cl₂, the residue was diluted with EtOAc. The solution was washed successively with 1 M of HCl, water, sat. aq NaHCO₃, and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated in vacuo to give crude product, which was purified by flash column chromatography on silica gel (70% EtOAc in n-hexane) to give 4 (269 mg, 49%) as a white solid. [α]D⁰⁻0.20 (60% EtOAc in n-hexane). Mp 76–79 °C. [α]D⁰⁺2.2 (c 1.02, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ: 7.49 (d, J = 5.6 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.39 (d, J = 4.9 Hz, 1H), 7.28–0.23 (m, 4H), 5.96–5.74 (m, 2H), 5.37–5.16 (m, 4H), 4.34 (t, J = 8.6 Hz, 1H), 4.25–4.12 (m, 5H), 4.05–3.90 (m, 5H), 3.84–3.73 (m, 2H), 3.72–3.65 (m, 1H), 3.67 (s, 3H), 3.48 (dd, J = 12.0, 3.4 Hz, 1H), 2.70–2.60 (m, 1H), 2.39–2.22 (m, 3H), 2.22–2.11 (m, 3H), 2.11–2.02 (m, 1H), 1.96–1.57 (m, 22H), 1.50 (s, 9H), 1.02–0.83 (m, 24H). ¹³C NMR (125 MHz, CDCl₃): δ: 175.6, 175.2, 174.6, 174.2, 174.1, 173.2, 173.1, 171.8, 155.7, 133.9, 133.5, 117.8, 117.5, 81.7, 72.2, 69.6, 67.6, 66.7, 65.7, 60.1, 56.2, 54.8, 54.1, 54.0, 53.3, 52.3, 52.2, 52.1, 39.6, 39.4, 39.1, 38.3, 37.3, 36.74, 36.70, 35.5, 34.4, 28.32, 28.26 (3C), 25.2, 25.0, 24.73, 24.65, 24.54, 24.53, 24.46, 23.5, 23.4, 22.99, 22.97, 21.1, 21.0, 20.90, 20.87. IR (CDCl₃): 3325, 2961, 1732, 1661, 1530 cm⁻¹. HRMS (ESI) m/z: [M + Na]+ calc for C₉₆H₉₄N₉O₁₃Na, 1109.6838; found, 1109.6808.
\(H\text{-}l\text{-Hyp}^{\text{OAll}}l\text{-Ser}^{\text{OAll}}\text{-}l\text{-Leu}2\text{-Ac}\text{C}2\text{-OMe} (\text{B})\): To a solution of Boc-protected peptide 4 (135 mg, 0.124 mmol) in \(\text{CH}_2\text{Cl}_2\) (1 mL) was added trifluoroacetic acid (0.12 mL) dropwise at room temperature, and the reaction mixture was stirred overnight at the same temperature. The reaction mixture was neutralized by adding sat. aq \(\text{NaHCO}_3\) and the aqueous phase was extracted with \(\text{CHCl}_3\) four times. The combined organic extracts were dried over anhydrous \(\text{MgSO}_4\) and concentrated under vacuum to give amine product B (124 mg, quant). \(R_t = 0.10\) (80% EtOAc in \(n\)-hexane). Mp 107–108 °C. \(\delta_{\text{H}}(100)\) 8.06 (br s, 1H), 7.64 (d, \(J = 6.8\) Hz, 1H), 7.57 (br s, 1H), 7.52–7.42 (m, 2H), 7.35 (d, \(J = 4.9\) Hz, 1H), 5.92–5.75 (m, 2H), 5.32–5.14 (m, 4H), 4.60 (br s, 1H), 4.35 (d, \(J = 4.2\) Hz, 1H), 4.29 (br s, 1H), 4.21–4.04 (m, 3H), 4.04–3.95 (m, 4H), 3.95–3.88 (m, 1H), 3.86–3.74 (m, 2H), 3.68 (s, 3H), 3.60 (d, \(J = 11.7\) Hz, 1H), 3.47 (d, \(J = 8.8\) Hz, 1H), 2.65–2.44 (m, 3H), 2.29–2.16 (m, 2H), 2.12 (br s, 2H), 2.03 (dd, \(J = 11.7, 6.1\) Hz, 1H), 1.92–1.53 (m, 22H), 1.03–0.78 (m, 24H).\n
\(\text{C NMR} (125\ MHz, \text{CDCl}_3) \delta: 175.7, 175.5, 175.4, 174.7, 174.4, 174.3, 173.9, 173.1, 133.8, 133.5, 118.0, 117.9, 76.1, 72.3, 70.0, 68.1, 66.7, 66.0, 59.3, 56.4, 55.0, 54.9, 54.4, 53.3, 52.4, 51.3, 40.1, 39.7, 39.4, 39.2, 37.9, 36.89, 36.85, 35.3, 35.0, 29.7, 25.0, 24.78, 24.76, 24.58, 24.56, 24.43, 24.23, 24.17, 23.2, 23.1, 22.5, 21.8, 21.6, 21.2. IR (KBr): 3329, 2959, 1736, 1655, 1535 cm\(^{-1}\). HRMS (ESI) \(m/z\): [M + Na]\(^{+}\) calc'd for C\(_{51}\)H\(_{80}\)N\(_{5}\)O\(_{11}\)Na, 1009.6314; found, 1009.6288.

**Stapled Boc-l-Hyp-l-Ser-OMe (6):** Under an argon atmosphere, to a solution of 3 (90.0 mg, 0.218 mmol) in \(\text{CH}_2\text{Cl}_2\) (11 mL) was added second-generation Grubbs catalyst (37.0 mg, 0.0436 mmol) at room temperature, and the reaction mixture was stirred for 2 h at the same temperature. The reaction mixture was filtered through a short pad of silica gel (60% EtOAc in \(n\)-hexane) and concentrated. The crude material was purified by flash chromatography on silica gel (60% EtOAc in \(n\)-hexane) to provide a stapled peptide 5 (46.2 mg, 55%) as a mixture of \(E\) - and \(Z\) -isomers (\(E/Z = 1.0:5.6\)). \(R_t = 0.30\) (EtOAc). Next, to a solution of stapled peptides 5 (46.2 mg, 0.120 mmol) in MeOH (12 mL) was added 10% Pd-C (23 mg, 50 wt %) under a nitrogen atmosphere. After being vigorously stirred under a hydrogen atmosphere for 19 h at room temperature, the reaction mixture was passed through a short plug of Celite. The filtrate was concentrated under vacuum to give a crude product, which was purified by flash column chromatography on silica gel (70% EtOAc in \(n\)-hexane) to give 6 (35.5 mg, 77%) as an amber oil. \(R_t = 0.29\) (EtOAc). \(\delta_{\text{H}}(100)\) 8.52 (d, \(J = 7.6\) Hz, 1H), 4.69–4.63 (m, 1H), 4.39–4.29 (m, 1H), 3.97 (br s, 2H), 3.15 (m, 2H), 3.02 (d, \(J = 11.7\) Hz, 1H), 2.40–2.17 (m, 2H), 1.92–1.77 (m, 2H), 1.77–1.54 (m, 2H), 1.54–1.38 (m, 9H).\n
\(\text{C NMR} (125\ MHz, \text{CDCl}_3) \delta: 172.5, 170.3, 154.9, 80.8, 154.9, 69.7, 69.5, 69.1, 60.6, 53.0, 52.4, 52.0, 37.2, 28.1 (3C), 26.9, 25.5. IR (film): 3422, 2934, 1751, 1697 cm\(^{-1}\). HRMS (DART) \(m/z\): [M + H]\(^{+}\) calc'd for C\(_{18}\)H\(_{31}\)N\(_{2}\)O\(_{7}\), 387.2131; found, 387.2130.

**Stapled H-l-Hyp-l-Ser-OMe (A'):** To a solution of Boc-protected dipeptide 6 (45.0 mg, 0.116 mmol) in \(\text{CH}_2\text{Cl}_2\) (1 mL) was added trifluoroacetic acid (0.2 mL) dropwise at room temperature, and the reaction mixture was stirred for 24 h at the same temperature. The reaction mixture was neutralized by adding sat. aq \(\text{NaHCO}_3\), and the aqueous phase was extracted with \(\text{CHCl}_3\) three times. The combined organic extracts were dried over anhydrous \(\text{MgSO}_4\) and concentrated under vacuum to give crude product A' (20.5 mg, 62%) as an amber oil, which was used for the next step without further purification. \(R_t = 0.30\) (EtOAc). \(\delta_{\text{H}}(100)\) 8.32 (d, \(J = 7.6\) Hz, 1H), 1.03–0.78 (m, 24H). IR (film): 3422, 2934, 1751, 1736, 1535 cm\(^{-1}\). HRMS (ESI) \(m/z\): [M + Na]\(^{+}\) calc'd for C\(_{18}\)H\(_{31}\)N\(_{2}\)O\(_{7}\)Na, 309.1426; found, 309.1428.

**Stapled Boc-l-Hyp-l-Ser-[(l-Leu)\text{-}2-\text{Ac}\text{C}2\text{-OMe} (7):** To a solution of stapled dipeptide 6 (104 mg, 0.269 mmol) in MeOH (3 mL) was added 1 M of aqueous \(\text{NaOH}\) (0.270 mL, 0.270 mmol) at room temperature, and the reaction mixture was stirred overnight at the same temperature. The solution was acidified
with 1 M of aqueous HCl and the MeOH was removed in vacuo. The resulting aqueous solution was extracted with EtOAc three times. The combined organic extracts were washed with brine, dried over Na₂SO₄, and concentrated in vacuo to give a crude product (95.0 mg, 95%), which was used for the next step without further purification. Rᵣ = 0.27 (EtOAc). To a solution of the crude acid (85.6 mg, 0.230 mmol) in CH₂Cl₂ (2.3 mL) was added N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDCI·HCl; 44.0 mg, 0.230 mmol) and HOBt·H₂O (42.0 mg, 0.276 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. Then, a solution of H-[l-Leu]₂-AC₅-C₈-OMe [44] (163 mg, 0.230 mmol) in CH₂Cl₂ (1 mL) was added dropwise to the reaction mixture at 0 °C. The reaction mixture was gradually warmed to room temperature and stirred for 2 days. After the removal of CH₂Cl₂, the residue was diluted with EtOAc. The solution was washed successively with 1 M of HCl, water, sat. aq NaHCO₃, and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (80% EtOAc in n-hexane) to give 7 (178 mg, 73%) as a yellow oil. Rᵣ = 0.46 (EtOAc). [α]2⁰ᵣD = 4.1 (c 1.07, CHCl₃). \(^1\)H NMR (500 MHz, CDCl₃) δ: 7.75 (br s, 1H), 7.54–7.41 (m, 2H), 7.31 (s, 1H), 7.28–7.25 (m, 2H), 7.22 (d, J = 6.1 Hz, 1H), 4.37–4.30 (m, 1H), 4.28 (dd, J = 10.5, 4.2 Hz, 1H), 4.24–4.16 (m, 2H), 4.14 (d, J = 10.3 Hz, 1H), 4.08–4.03 (m, 1H), 3.98 (dd, J = 11.0, 4.9 Hz, 1H), 3.96–3.90 (m, 1H), 3.84 (d, J = 11.7 Hz, 1H), 3.75–3.69 (m, 1H), 3.67 (s, 3H), 3.65–3.50 (m, 3H), 3.46–3.36 (m, 2H), 2.70–2.60 (m, 1H), 2.39 (dd, J = 14.6, 10.9, 4.0 Hz, 1H), 2.31–2.03 (m, 8H), 1.94–1.59 (m, 24H), 1.52 (s, 9H), 1.01–0.84 (m, 24H). \(^13\)C NMR (125 MHz, CDCl₃) δ: 175.4, 175.2 (2C), 174.4, 174.2, 173.2, 173.1, 171.0, 155.4, 81.4, 78.9, 70.8, 68.5, 68.4, 66.7, 65.7, 60.4, 54.9, 54.7, 54.0, 53.9, 53.4, 52.2, 52.0, 40.0, 39.6, 39.4, 39.2, 38.2, 37.2, 36.7, 35.4, 35.3, 28.3 (3C), 27.4, 26.3, 25.0, 24.82, 24.81, 24.6, 24.50 (2C), 24.48, 24.4, 23.5, 23.4, 22.9, 22.6, 21.5, 21.2, 21.0, 20.9. IR (KBr): 3329, 2958, 1655, 1526 cm⁻¹. HRMS (ESI) m/z: [M + Na]⁺ calcd for C₅₄H₆₉N₈O₁₃Na, 1083.6682; found, 1083.6685.

**3.3. Synthesis of Unstapled Peptide C and Stapled Peptide C’**

*Boc-l-Ser^{Ope}-l-Leu]-₅-AC₅-C₈-OMe (9):* To a solution of Boc-l-Ser^{Ope}-OH 8 [51] (193 mg, 0.707 mmol) in CH₂Cl₂ (2.5 mL) were added EDCI·HCl (136 mg, 0.707 mmol) and HOBt·H₂O (130 mg, 0.848 mmol) at 0 °C, and the solution was stirred for 30 min. Then, a solution of H-[l-Leu]₂-AC₅-C₈-OMe [44] (500 mg, 0.707 mmol) in CH₂Cl₂ (2.5 mL) was added to the reaction mixture at the same temperature, and the resultant mixture was gradually warmed to room temperature. After stirring overnight, CH₂Cl₂ was removed, and the residue was diluted with EtOAc. The solution was washed successively with 1 M of HCl, water, sat. aq NaHCO₃, and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (80% EtOAc in n-hexane) to give 9 (193 mg, 0.707 mmol) as a yellow oil. Rᵣ = 0.44 (EtOAc). [α]2⁰ᵣD = −3.1 (c 1.07, CHCl₃). \(^1\)H NMR (500 MHz, CDCl₃) δ: 7.84 (d, J = 5.4 Hz, 1H), 7.58 (d, J = 4.6 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.34 (s, 1H), 7.32–7.24 (m, 3H), 4.37–4.27 (m, 2H), 4.23–4.16 (m, 1H), 4.11 (br s, 2H), 4.02 (dd, J = 10.9, 5.0 Hz, 1H), 3.94 (dd, J = 9.7, 4.8 Hz, 1H), 3.89 (dd, J = 8.8, 3.4 Hz, 1H), 3.78 (dd, J = 10.9, 3.1 Hz, 1H), 3.67 (s, 3H), 3.66–3.61 (m, 1H), 3.60–3.50 (m, 3H), 3.34 (d, J = 10.5 Hz, 1H), 3.06 (dd, J = 10.5, 2.4 Hz, 1H), 2.69–2.60 (m, 1H), 2.36 (br s, 3H), 2.30–2.21 (m, 4H), 2.20–2.03 (m, 3H), 1.97–1.55 (m, 24H), 1.03–0.80 (m, 24H). \(^13\)C NMR (125 MHz, CDCl₃) δ: 178.1, 175.6, 175.1, 174.2, 173.9, 173.2, 173.1, 172.4, 80.0, 70.3, 69.8, 68.7, 66.8, 65.7, 59.8, 55.2, 54.8, 54.1, 54.0, 52.3, 52.1, 51.5, 39.6, 39.3, 38.2, 37.2, 36.7, 36.3, 35.4, 29.6, 28.4, 27.2, 26.5, 25.2, 25.12, 25.07, 24.7, 24.50, 24.49, 24.4 (2C), 23.5, 23.4, 23.1, 22.8, 21.3, 21.1, 21.0, 20.8. IR (CDCl₃): 3325, 2958, 1655, 1526 cm⁻¹. HRMS (ESI) m/z: [M + Na]⁺ calcd for C₅₀H₄₈N₆O₁₅Na, 983.6157; found, 983.6142.
on silica gel (60% EtOAc in n-hexane) to give 9 (478 mg, 70%) as a white solid. \( R_f = 0.66 \) (EtOAc). Mp 109–115 °C. \([\delta^\text{H}] = -4.3 \) (c 1.00, CHCl₃). \(^1\)H NMR (500 MHz, CDCl₃): \( \delta = 7.42 \) (d, \( J = 8.1 \) Hz, 1H), 7.33 (s, 1H), 7.29 (d, \( J = 4.4 \) Hz, 1H), 7.25–7.19 (m, 2H), 6.60 (d, \( J = 3.7 \) Hz, 1H), 5.80 (dtt, \( J = 17.0, 10.3, 6.6 \) Hz, 1H), 5.50 (d, \( J = 2.4 \) Hz, 1H), 5.08–4.96 (m, 2H), 4.39–4.32 (m, 1H), 4.20 (dd, \( J = 11.2, 6.1 \) Hz, 1H), 4.14–4.07 (m, 1H), 4.04 (q, \( J = 3.7 \) Hz, 1H), 3.98 (dt, \( J = 9.7, 5.0 \) Hz, 1H), 3.77–3.70 (m, 2H), 3.67 (s, 3H), 3.55–3.44 (m, 2H), 2.70–2.60 (m, 1H), 2.32–2.23 (m, 1H), 2.23–2.02 (m, 6H), 1.96–1.54 (m, 24H), 1.50 (s, 9H), 1.05–0.82 (m, 24H). \(^{13}\)C NMR (125 MHz, CDCl₃): \( \delta = 175.5, 175.1, 173.6, 173.4, 173.0, 172.9, 172.0, 156.9, 137.7, 115.1, 81.7, 71.0, 68.8, 66.5, 65.7, 56.9, 54.7, 54.1, 54.0, 52.2, 52.1, 40.1, 39.8, 39.6, 38.1, 37.3, 36.7, 35.5, 30.2, 28.5, 28.1 (3C), 25.3, 25.1, 24.8, 24.7, 24.53, 24.51, 24.43, 24.40, 23.5, 23.4, 23.0, 22.9, 21.4, 21.3, 21.1, 20.9. IR (KBr): 3329, 2959, 1701, 1632, 1524 cm⁻¹.

\( \text{Boc-l-Hyp}^{\text{OAll}}-\text{L-Ser}^{\text{OPre}}-[(\text{l-Leu})_2-\text{Ac5cl}_2]-\text{OME} (11) \): To a solution of Boc-protected peptide 9 (480 mg, 0.499 mmol) in CH₂Cl₂ (5 mL) was added trifluoroacetic acid (0.03 mL) dropwise at room temperature, and the reaction mixture was stirred overnight at the same temperature. The combined organic extracts were dried over anhydrous MgSO₄ and concentrated under a vacuum to give crude product 10 (391 mg, 91%), which was used for the next step without further purification. \( R_f = 0.20 \) (60% EtOAc in n-hexane). Mp 109–115 °C. HRMS (ESI) \([\alpha] = m/z: [M + Na]^+ \) calcd for C₅₀H₇₇N₂O₁₁Na, 984.6361; found, 984.6386.

**H-L-Ser-OPre-[(l-Leu)₂-AC₅C₇₂]-OMe (C):** To a solution of Boc-protected peptide 11 (30.0 mg, 0.0269 mmol) in CH₂Cl₂ (1 mL) were added EDCI (0.20 mmol) in CH₂Cl₂ (30.0 mg, 0.0269 mmol) in CH₂Cl₂ (1 mL). The solution was stirred for 30 min. Then, a solution of amine 10 (391 mg, 0.454 mmol) in CH₂Cl₂ (2.5 mL) was added to the reaction mixture at the same temperature, and the resultant mixture was gradually warmed to room temperature. After stirring overnight, the CH₂Cl₂ was removed and the residue was diluted with EtOAc. The solution was washed successively with 1 M of HCl, water, sat. aq NaHCO₃, and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated in vacuo to give crude product C (23.5 mg, 86%), which was used for the next step without further purification. \( R_f = 0.26 \) (EtOAc). Mp 75–85 °C. HRMS (ESI) \([\alpha] = m/z: [M + Na]^+ \) calcd for C₅₈H₆₈N₇O₁₃Na, 1137.7151; found, 1137.7201.

\( \text{Molecules 2020, 25, 4667} \)
Stapled Boc-l-Hyp-l-Ser-[l-Leu]$_2$-Ac$_5$C$_2$-OMe (13): Under an argon atmosphere, to a solution of 11 (70.0 mg, 0.0628 mmol) in CH$_2$Cl$_2$ (3 mL) was added second-generation Grubbs catalyst (10.7 mg, 0.0126 mmol) at room temperature, and the mixture was stirred for 2 h at the same temperature. The reaction mixture was filtered through short pad of silica gel (EtOAc) and concentrated. The crude material was purified by flash chromatography on silica gel (70% EtOAc in n-hexane) to provide a stapled peptide 12 (63.2 mg, 93%) as a mixture of E- and Z-isomers (E/Z = 5.5:1). R$_f$ = 0.43 (EtOAc). Next, to a solution of stapled peptides 12 (52.9 mg, 0.0486 mmol) in MeOH (4 mL) was added 10% Pd-C (26 mg, 50 wt %) under a nitrogen atmosphere. After being vigorously stirred under a hydrogen atmosphere for 23 h at room temperature, the reaction mixture was passed through a short plug of Celite. The filtrate was concentrated under vacuum to give a crude product, which was purified by flash column chromatography on silica gel (4% MeOH in CHCl$_3$) to give 13 (46.9 mg, 89%) as a colorless oil. R$_f$ = 0.13 (3% MeOH in CHCl$_3$). [a]$_D^{25}$ = –7.8 (c 1.00, CHCl$_3$). $^1$H NMR (500 MHz, CDCl$_3$): 7.58 (d, $J$ = 5.9 Hz, 1H), 7.50 (d, $J$ = 4.9 Hz, 1H), 7.45 (d, $J$ = 7.8 Hz, 1H), 7.31 (s, 1H), 7.27–7.22 (m, 2H), 7.17 (d, $J$ = 2.0 Hz, 1H), 4.37–4.30 (m, 2H), 4.23–4.13 (m, 3H), 4.02 (t, $J$ = 3.4 Hz, 1H), 3.97–3.91 (m, 1H), 3.83–3.76 (m, 2H), 3.67 (s, 3H), 3.63–3.51 (m, 1H), 3.50–3.44 (m, 1H), 3.33 (dd, $J$ = 12.0, 2.9 Hz, 1H), 2.65 (dt, $J$ = 13.6, 8.2 Hz, 1H), 2.46–2.37 (m, 1H), 2.31–2.03 (m, 6H), 2.00 (br s, 1H), 1.95–1.54 (m, 26H), 1.52 (s, 9H), 1.51–1.42 (m, 3H), 1.01–0.82 (m, 24H). $^{13}$C NMR (125 MHz, CDCl$_3$): 175.6, 175.2, 174.5, 174.4, 174.1, 173.1, 173.0, 171.1, 155.8, 81.6, 78.0, 71.8, 69.7, 69.4, 66.7, 65.7, 60.5, 56.2, 54.8, 54.2, 52.7, 52.2, 52.0, 39.7, 39.6, 39.4, 39.1, 38.3, 37.3, 36.7, 35.6, 35.4, 29.1, 28.2 (3C), 27.0, 26.9, 25.4, 25.1, 25.0, 24.8, 24.7, 24.54, 24.52 (2C), 24.4, 23.5, 23.4, 23.0, 22.8, 21.14, 21.05, 21.0, 20.9. IR (KBr): 3343, 2957, 1639, 1547 cm$^{-1}$. HRMS (ESI) m/z: [M + Na]$^+$ calcd for C$_{55}$H$_{86}$N$_{8}$O$_{13}$Na, 1111.6995; found, 1111.7016.

Stapled H-l-Hyp-l-Ser-[l-Leu]$_2$-Ac$_5$C$_2$-OMe (C'): To a solution of Boc-protected peptide 13 (11.5 mg, 0.0110 mmol) in CH$_2$Cl$_2$ (1 mL) was added trifluoroacetic acid (0.0110 mL) dropwise at room temperature, and the reaction mixture was stirred for 2 days at the same temperature. The reaction mixture was neutralized by adding sat. aq NaHCO$_3$, and the aqueous phase was extracted with CHCl$_3$ three times. The combined organic extracts were dried over anhydrous MgSO$_4$ and concentrated under vacuum to give crude product C' (11.6 mg, quant), which was used for the next step without further purification. R$_f$ = 0.20 (EtOAc). Mp 105–107 °C. [a]$_D^{27}$ = −8.7 (c 1.00, CHCl$_3$). $^1$H NMR (500 MHz, CDCl$_3$): 8.27 (d, $J$ = 5.1 Hz, 1H), 7.44 (d, $J$ = 7.8 Hz, 1H), 7.33 (d, $J$ = 4.6 Hz, 1H), 7.26–7.21 (m, 3H), 7.00 (d, $J$ = 4.2 Hz, 1H), 4.37–4.27 (m, 2H), 4.19 (dd, $J$ = 10.8, 5.9 Hz, 1H), 4.13–4.04 (m, 2H), 3.97–3.86 (m, 2H), 3.84 (dd, $J$ = 10.5, 5.9 Hz, 1H), 3.70 (dd, $J$ = 10.6, 2.8 Hz, 1H), 3.67 (s, 3H), 3.64–3.59 (m, 1H), 3.59–3.53 (m, 1H), 3.48 (t, $J$ = 8.9 Hz, 1H), 3.43–3.37 (m, 1H), 3.35 (dd, $J$ = 10.5, 2.9 Hz, 1H), 2.69–2.60 (m, 1H), 2.37–2.03 (m, 7H), 1.97–1.49 (m, 30H), 1.03–0.78 (m, 24H). $^{13}$C NMR (125 MHz, CDCl$_3$): 175.7, 175.3, 174.2, 174.0, 173.23, 173.20 (2C), 172.0, 79.5, 71.5, 69.3, 69.0, 66.8, 65.7, 59.6, 55.9, 54.8, 54.1 (2C), 52.4, 52.2, 51.0, 39.6, 39.5, 39.4, 39.2, 38.3, 37.2, 36.7, 36.0, 35.4, 28.9, 28.3, 27.5, 26.4, 25.23, 25.20, 25.1, 24.8, 24.6, 24.52, 24.49, 24.40, 23.5, 23.4, 23.1, 22.9, 21.3, 21.1, 21.0, 20.9. IR (KBr): 3337, 2957, 1736, 1655, 1535 cm$^{-1}$. HRMS (ESI) m/z: [M + Na]$^+$ calcd for C$_{51}$H$_{86}$N$_{8}$O$_{11}$Na, 1011.6470; found, 1011.6467.

3.4. Synthesis of Unstapled Peptide D and Stapled Peptide D'

Boc-l-Ser$_{Ophe}$-[l-Leu]$_2$-Ac$_5$C$_2$-OMe (14): To a solution of Boc-l-Ser$_{Ophe}$-OH 8 [51] (200 mg, 0.732 mmol) in CH$_2$Cl$_2$ (2.5 mL) were added EDCI·HCl (140 mg, 0.732 mmol) and HOBT·H$_2$O (135 g, 0.878 mmol) at 0 °C, and the solution was stirred for 30 min. Then, a solution of H-(l-Leu)$_2$-Ac$_5$C$_2$-OMe [44] (270 mg, 0.732 mmol) in CH$_2$Cl$_2$ (2.5 mL) was added to the reaction mixture at the same temperature, and the
resultant mixture was gradually warmed to room temperature. After stirring overnight, the CH$_2$Cl$_2$ was removed and the residue was diluted with EtOAc. The solution was washed successively with 1 M of HCl, water, sat. aq NaHCO$_3$ and brine. The organic layer was dried over anhydrous MgSO$_4$ and concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (50% EtOAc in n-hexane) to give 14 (307 mg, 67%) as a white solid. R$_f$ = 0.52 (60% EtOAc in n-hexane). Mp 109–115 °C. $\delta$ = 174.9, 174.3, 173.4, 172.4, 172.47, 170.9, 156.6, 137.8, 114.9, 81.5, 70.5, 68.7, 65.7, 56.3, 54.3, 54.0, 53.4, 52.2, 52.0, 40.2, 39.62 (2C), 39.59, 39.4, 37.2, 36.8, 30.1, 28.7, 28.22 (3C), 28.17 (2C), 25.0, 24.9, 24.8, 24.7, 24.4, 24.3, 23.4, 22.9, 21.5, 21.2, 20.7. IR (KBr): 3277, 2957, 1719, 1670, 1560 cm$^{-1}$. HRMS (ESI) $m/z$: [M + Na]$^+$ calc'd for C$_{32}$H$_{56}$N$_4$O$_8$Na, 647.3996; found, 647.3991.

Boc-(l-Leu)$_2$-l-Ser$^{Opp}$-(l-Leu)$_2$-Ac$_5$-OMe (16): To a solution of Boc-protected peptide 14 (307 mg, 0.491 mmol) in CH$_2$Cl$_2$ (5 mL) was added trifluoroacetic acid (0.982 mL) dropwise at room temperature, and the reaction mixture was stirred overnight at the same temperature. The reaction mixture was neutralized by adding sat. aq NaHCO$_3$, and the aqueous phase was extracted with CHCl$_3$ four times. The combined organic extracts were dried over anhydrous MgSO$_4$ and concentrated under a vacuum to give crude product 15 (295 mg, quant), which was used for the next step without further purification. R$_f$ = 0.20 (60% EtOAc in n-hexane). To a solution of Boc-(l-Leu)$_2$-OH (194 mg, 0.562 mmol) in CH$_2$Cl$_2$ (2 mL) were added EDCI·HCl (108 mg, 0.562 mmol) and HOBt·H$_2$O (103 mg, 0.674 mmol) at 0 °C, and the solution was stirred for 30 min. Then, a solution of amine 15 (295 mg, 0.562 mmol) in CH$_2$Cl$_2$ (2 mL) was added to the reaction mixture at the same temperature, and the resultant mixture was gradually warmed to room temperature. After stirring for 5 days, the CH$_2$Cl$_2$ was removed and the residue was diluted with EtOAc. The solution was washed successively with 1 M of HCl, water, sat. aq NaHCO$_3$, and brine. The organic layer was dried over anhydrous MgSO$_4$ and concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (60% EtOAc in n-hexane) to give 16 (300 mg, 63%) as a white solid. R$_f$ = 0.30 (60% EtOAc in n-hexane). Mp 243–246 °C. $\delta$ = 174.6, 171.6, 171.5, 171.1, 156.3, 137.8, 115.1, 81.1, 71.0, 69.4, 65.9, 55.6, 53.0, 52.3, 51.5, 40.4, 39.9, 37.2, 36.9, 30.2, 28.6, 28.2 (3C), 25.1, 24.8, 24.5, 24.4, 23.1, 23.0, 21.6, 21.4. IR (KBr): 3277, 2957, 1719, 1670, 1560 cm$^{-1}$. HRMS (ESI) $m/z$: [M + Na]$^+$ calc'd for C$_{32}$H$_{56}$N$_4$O$_8$Na, 647.3996; found, 647.3991.

Cbz-l-Pro$^{αAll}$-(l-Leu)$_2$-l-Ser$^{Opp}$-(l-Leu)$_2$-Ac$_5$-OMe (19): To a solution of Boc-protected peptide 16 (112 mg, 0.132 mmol) in CH$_2$Cl$_2$ (1.3 mL) was added trifluoroacetic acid (0.264 mL) dropwise at room temperature, and the reaction mixture was stirred for 2 days at the same temperature. The reaction mixture was neutralized by adding sat. aq NaHCO$_3$, and the aqueous phase was extracted with CHCl$_3$ three times. The combined organic extracts were dried over anhydrous MgSO$_4$ and concentrated under a vacuum to give crude product 17 (85.8 mg, 87%), which was used for the next step without further purification. R$_f$ = 0.37 (EtOAc). To a solution of Cbz-l-Pro$^{αAll}$-OH (18 [52,53]; 27.2 mg, 0.0939 mmol) in CH$_2$Cl$_2$ (1 mL) were added EDCI·HCl (18.0 mg, 0.0939 mmol) and HOBt·H$_2$O (17.3 mg, 0.113 mmol) at 0 °C, and the solution was stirred for 30 min. Then, a solution of amine 17 (70.5 mg, 0.0939 mmol) in CH$_2$Cl$_2$ (1 mL) was added to the reaction mixture at the same temperature, and the resultant mixture
was gradually warmed to room temperature. After stirring for 23 h, the CH₂Cl₂ was removed and the residue was diluted with EtOAc. The solution was washed successively with 1 M of HCl, water, sat. aq NaHCO₃, and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated in vacuo to give a crude product, which was purified by flash column chromatography on silica gel (50% EtOAc in n-hexane) to give 19 (77.0 mg, 80%) as a colorless oil. [α]D²⁵ = −8.6 (c 2.13, CHCl₃). "H NMR (500 MHz, CDCl₃): δ: 7.65 (br s, 1H), 7.51 (br s, 1H), 7.42–7.35 (m, 3H), 7.35–7.31 (m, 2H), 7.29 (d, J = 6.6 Hz, 1H), 7.19 (d, J = 7.8 Hz, 1H), 7.08 (s, 1H), 6.33 (br s, 1H), 5.82–5.63 (m, 2H), 5.18 (s, 2H), 5.17–5.05 (m, 2H), 5.00–4.89 (m, 2H), 4.37 (q, J = 7.5 Hz, 1H), 4.32–4.24 (m, 2H), 4.10–3.98 (m, 2H), 3.92–3.85 (m, 1H), 3.83–3.71 (m, 2H), 3.67 (s, 3H), 3.54–3.47 (m, 1H), 3.45 (t, J = 6.6 Hz, 2H), 2.96 (dd, J = 14.2, 7.3 Hz, 1H), 2.76 (dd, J = 14.2, 7.6 Hz, 1H), 2.28–2.11 (m, 5H), 2.11–1.99 (m, 5H), 1.91–1.54 (m, 17H), 1.46–1.38 (m, 1H), 1.05–0.81 (m, 24H). "C NMR (125 MHz, CDCl₃): δ: 175.3, 175.0, 174.6, 174.1, 172.62, 172.60, 171.0, 155.2, 138.1, 136.0, 132.0, 128.7 (2C), 128.5, 127.4 (2C), 120.5, 114.6, 70.2, 69.0, 68.9, 67.5, 65.7, 56.7, 54.6, 54.2, 53.5, 52.12, 52.11, 48.6, 39.7 (2C), 39.4, 39.3, 37.6, 37.1, 36.8, 35.7, 30.2, 28.7, 25.3, 25.0, 24.9, 24.8, 24.4 (2C), 24.3, 23.4 (2C), 23.3 (2C), 23.1, 22.9, 21.3, 20.9. IR (CDCl₃): 3323, 2959, 1663, 1531 cm⁻¹. HRMS (ESI) m/z: [M + Na]⁺ calcd for C₅₅H₆₇N₇O₁₁Na, 1044.6361; found, 1044.6360.

_H-Pro^(all)-1-{Leu}-1-Ser-0_{Pro-(1-Leu)-2-ACS-c-OMe (D):} To a solution of Cbz-protected peptide 19 (21.4 mg, 0.0209 mmol) in MeOH (2 mL) was added 10% Pd-C (10 mg, 50 wt %) under a nitrogen atmosphere. After being vigorously stirred under a hydrogen atmosphere for 2 days at room temperature, the reaction mixture was passed through a short plug of Celite. The filtrate was concentrated under a vacuum to give a crude product, which was purified by flash column chromatography on silica gel (70% EtOAc in n-hexane) to give D (14.5 mg, 78%) as a white solid. [α]D²⁵ = +54.3 (c 1.60, CHCl₃). "H NMR (500 MHz, CDCl₃): δ: 8.45 (d, J = 4.4 Hz, 1H), 7.32 (d, J = 5.1 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.19–7.10 (m, 2H), 6.99 (s, 1H), 4.43–4.29 (m, 2H), 4.22–4.14 (m, 2H), 4.00–3.92 (m, 1H), 3.85 (dd, J = 9.9, 4.8 Hz, 1H), 3.70 (dd, J = 9.8, 3.4 Hz, 1H), 3.67 (s, 3H), 3.48–3.37 (m, 2H), 3.16–3.07 (m, 1H), 2.84 (dt, J = 10.5, 5.4 Hz, 1H), 2.27–2.12 (m, 3H), 2.12–1.94 (m, 3H), 1.86 (d, J = 12.5 Hz, 2H), 1.83–1.61 (m, 18H), 1.59–1.46 (m, 4H), 1.41–1.22 (m, 4H), 1.04–0.81 (m, 30H). "C NMR (125 MHz, CDCl₃): δ: 179.4, 174.9, 173.9, 173.4, 172.5, 172.4, 171.0, 75.1, 75.0, 68.6, 65.7, 56.2, 54.5, 53.3, 52.3, 52.2, 52.0, 47.4, 41.3, 40.4, 39.6, 39.3, 38.5, 37.2, 37.0, 36.8, 29.3, 28.0, 26.3, 24.9, 24.8 (3C), 24.4, 24.3, 23.6, 23.4, 23.2, 22.9, 22.5, 21.3, 21.2, 21.1, 20.7, 18.6, 14.4, 14.0. IR (CDCl₃): 3327, 2961, 1734, 1663, 1530 cm⁻¹. HRMS (ESI) m/z: [M + Na]⁺ calcd for C₄₇H₅₈N₇O₃Na, 914.6306; found, 914.6267.

_Stapled H-Pro^{all-1-Leu)}-2-L-Ser^{all-1-Leu)}-2-ACS-c-OMe (D):_ Under an argon atmosphere, to a solution of 19 (22.3 mg, 0.0218 mmol) in CH₂Cl₂ (1 mL) was added second-generation Grubbs catalyst (3.7 mg, 4.4 μmol) at room temperature, and the mixture was stirred for 2 h at the same temperature. The reaction mixture was filtered through a short pad of silica gel (EtOAc) and concentrated. The crude material was purified by flash chromatography on silica gel (80% EtOAc in n-hexane) to provide stapled peptide 20 (21.0 mg, 96%) as a mixture of E- and Z-isomers (E/Z = 1:1.1). [α]D²⁵ = −30.2 (c 1.38, CHCl₃). "H NMR (500 MHz, CDCl₃): δ: 8.49 (br s, 1H), 7.27 (d, J = 7.3 Hz, 1H), 7.23 (d, J = 6.1 Hz, 1H), 7.09 (d, J = 7.8 Hz, 1H), 6.96 (s, 1H), 6.38 (br s, 1H), 4.48 (ddd, J = 10.0, 6.1, 3.4 Hz, 1H), 4.40–4.27 (m, 2H), 4.03–3.93 (m, 2H), 3.90 (dd, J = 9.8, 3.4 Hz, 1H), 3.77–3.68 (m, 1H), 3.68 (s, 3H), 3.54 (dt, J = 9.2, 4.5 Hz, 1H), 3.50–3.43 (m, 1H), 3.14–3.06 (m, 1H), 2.86–2.78 (m, 1H), 2.31–2.20 (m, 2H), 2.19–2.12 (m, 1H), 2.11–1.99 (m, 2H), 1.99–1.91 (m, 1H), 1.90–1.58 (m, 21H), 1.57–1.36 (m, 7H), 1.05–0.82 (m, 24H). "C NMR (125 MHz, CDCl₃): δ: 174.9, 174.2, 173.39, 173.38, 172.5, 172.4, 170.4, 69.0, 68.8,
3.5. General Procedure for Peptide-Catalyzed Michael Addition of 1-Methylindole to a,β-Unsaturated Aldehyde

Michael adduct 23: To a mixture of (E)-4-nitrocinnamaldehyde (21; 8.9 mg, 0.050 mmol), peptide catalyst (0.010 mmol), and benzoic acid (1.2 mg, 0.010 mmol) in THF (0.25 mL) was added 1-methylindole (0.0187 mL, 0.150 mmol) at room temperature, and the mixture was stirred for the given time. To the reaction mixture were added NaBH₄ (9.5 mg, 0.25 mmol) and EtOH (0.25 mL) at the same temperature, and additionally stirred for 30 min. After filtration via a short plug of silica gel (60% EtOAc in n-hexane), the filtrate was concentrated under a vacuum to give crude product 23 as a white solid. Rf = 0.14 (50% EtOAc in n-hexane). ¹H NMR (500 MHz, CDCl₃) δ: 8.12 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 7.8 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.96 (s, 1H), 4.55 (t, J = 7.7 Hz, 1H), 3.79 (s, 3H), 3.74–3.61 (m, 2H), 2.52–2.45 (m, 1H), 2.31–2.24 (m, 1H). HPLC (Chiralpak AD-H, 10% i-propanol in n-hexane, flow rate = 1.0 mL/min): t_R = 26.0 min (minor), t_R = 35.2 min (major), ee = 47%. HPLC chart is given in the Supplementary Materials.

Supplementary Materials: The following are available online, ¹H and ¹³C NMR spectra of compounds 3, 4, 5, 6, A, 7, B, 9, 11, C, 13, C’, 14, 16, 19, D, and D’; HPLC chart of compound 23.

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References

1. 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 2010, 94, 721–732. [CrossRef]

2. Verdine, G.L.; Hilinski, G.J. Stapled peptides for intracellular drug targets. Methods Enzymol. 2012, 503, 3–33.

3. Cromm, P.M.; Spiegel, J.; Grossmann, T.N. Hydrocarbon stapled peptides as modulators of biological function. ACS Chem. Biol. 2015, 10, 1362–1375. [CrossRef]

4. 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. [CrossRef]

5. 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. [CrossRef] [PubMed]

6. 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. [CrossRef]

7. Moiola, M.; Memeo, M.G.; Quadrrelli, P. Stapled peptides—A useful improvement for peptide-based drugs. Molecules 2019, 24, 3654. [CrossRef] [PubMed]
1. Sawyer, T.K.; Partridge, A.W.; Kaan, H.Y.K.; Juang, Y.C.; Lim, S.; Johannes, C.; Yuen, T.Y.; Verma, C.; Kannan, S.; Aronica, P.; et al. Macrocyclic alpha helical peptide therapeutic modality: A perspective of learnings and challenges. *Bioorg. Med. Chem.* 2018, 26, 2807–2815. [CrossRef]
2. 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. [CrossRef]
3. Jarvo, E.R.; Miller, S.J. Amino acids and peptides as asymmetric organocatalysts. *Tetrahedron* 2007, 63, 4241–4259. [CrossRef]
4. Sawyer, T.K.; Partridge, A.W.; Kaan, H.Y.K.; Juang, Y.C.; Lim, S.; Johannes, C.; Yuen, T.Y.; Verma, C.; Kannan, S.; Aronica, P.; et al. Macrocyclic alpha helical peptide therapeutic modality: A perspective of learnings and challenges. *Bioorg. Med. Chem.* 2018, 26, 2807–2815. [CrossRef]
5. Girvin, Z.C.; Andrews, M.K.; Liu, X.; Gellman, S.H. Foldamer-templated catalysis of macrocycle formation. *Angew. Chem. Int. Ed.* 2008, 47, 3564–3566. [CrossRef]
6. Rossi, P.; Felluga, F.; Tecilla, P.; Formaggio, F.; Crisma, M.; Toniolo, C.; Scrimin, P. A bimetallic helical heptapeptide as a transphosphorylation catalyst in water. *J. Am. Chem. Soc.* 1999, 121, 6948–6949. [CrossRef]
7. Nagano, M.; Doi, M.; Kurihara, M.; Suemune, H.; Tanaka, M. Stabilized α-helix-catalyzed enantioselective epoxidation of α,β-unsaturated ketones. *Org. Lett.* 2010, 12, 3564–3566. [CrossRef]
8. Müller, M.M.; Windsor, M.A.; Pomerantz, W.C.; Sellman, S.H.; Hilvert, D. A rationally designed aldolase foldamer. *Angew. Chem. Int. Ed.* 2009, 48, 922–925. [CrossRef]
9. Girvin, Z.C.; Andrews, M.K.; Liu, X.; Sellman, S.H. Foldamer-templated catalysis of macrocycle formation. *Science* 2009, 326, 1528–1531. [CrossRef]
10. Akagawa, K.; Akabane, H.; Sakamoto, S.; Kudo, K. Organocatalytic asymmetric transfer hydrogenation in aqueous media using resin-supported peptide having a polyleucine tether. *Org. Lett.* 2008, 10, 2035–2037. [CrossRef]
11. Metrano, A.J.; Miller, S.J. Peptide-based catalysts reach the outer sphere through remote desymmetrization and atroposelectivity. *Acc. Chem. Res.* 2019, 52, 199–215. [CrossRef]
12. Julias, S.; Masana, J.; Vega, J.C. “Synthetic enzymes”. Highly stereoselective epoxidation of chalcone in a triphasic toluene-water-poly[(S)-alanine] system. *Angew. Chem. Int. Ed. Engl.* 1980, 19, 929–931. [CrossRef]
13. Rossi, P.; Felluga, F.; Tecilla, P.; Formaggio, F.; Crisma, M.; Toniolo, C.; Scrimin, P. A bimetallic helical heptapeptide as a transphosphorylation catalyst in water. *J. Am. Chem. Soc.* 1999, 121, 6948–6949. [CrossRef]
14. Nagano, M.; Doi, M.; Kurihara, M.; Suemune, H.; Tanaka, M. Stabilized α-helix-catalyzed enantioselective epoxidation of α,β-unsaturated ketones. *Org. Lett.* 2010, 12, 3564–3566. [CrossRef]
15. Müller, M.M.; Windsor, M.A.; Pomerantz, W.C.; Sellman, S.H.; Hilvert, D. A rationally designed aldolase foldamer. *Angew. Chem. Int. Ed.* 2009, 48, 922–925. [CrossRef]
16. Metrano, A.J.; Miller, S.J. Peptide-based catalysts reach the outer sphere through remote desymmetrization and atroposelectivity. *Acc. Chem. Res.* 2019, 52, 199–215. [CrossRef]
17. Julias, S.; Masana, J.; Vega, J.C. “Synthetic enzymes”. Highly stereoselective epoxidation of chalcone in a triphasic toluene-water-poly[(S)-alanine] system. *Angew. Chem. Int. Ed. Engl.* 1980, 19, 929–931. [CrossRef]
18. Rossi, P.; Felluga, F.; Tecilla, P.; Formaggio, F.; Crisma, M.; Toniolo, C.; Scrimin, P. A bimetallic helical heptapeptide as a transphosphorylation catalyst in water. *J. Am. Chem. Soc.* 1999, 121, 6948–6949. [CrossRef]
19. Nagano, M.; Doi, M.; Kurihara, M.; Suemune, H.; Tanaka, M. Stabilized α-helix-catalyzed enantioselective epoxidation of α,β-unsaturated ketones. *Org. Lett.* 2010, 12, 3564–3566. [CrossRef]
20. Müller, M.M.; Windsor, M.A.; Pomerantz, W.C.; Sellman, S.H.; Hilvert, D. A rationally designed aldolase foldamer. *Angew. Chem. Int. Ed.* 2009, 48, 922–925. [CrossRef]
21. Akagawa, K.; Akabane, H.; Sakamoto, S.; Kudo, K. Organocatalytic asymmetric transfer hydrogenation in aqueous media using resin-supported peptide having a polyleucine tether. *Org. Lett.* 2008, 10, 2035–2037. [CrossRef]
22. Akagawa, K.; Kudo, K. Construction of an all-carbon quaternary stereocenter by the peptide-catalyzed asymmetric Michael addition of nitromethane to β-disubstituted α,β-unsaturated aldehydes. *Angew. Chem. Int. Ed.* 2012, 51, 12786–12789. [CrossRef] [PubMed]
23. Akagawa, K.; Sakai, N.; Kudo, K. Histidine-containing peptide catalysts developed by a facile library screening method. *Angew. Chem. Int. Ed.* 2015, 54, 1822–1826. [CrossRef]
24. Yamagata, N.; Demizu, Y.; Sato, Y.; Doi, M.; Tanaka, M.; Nagasawa, K.; Okuda, H.; Kurihara, M. Design of a stabilized short helical peptide and its application to catalytic enantioselective epoxidation of (E)-chalcone. *Tetrahedron Lett.* 2011, 52, 798–801. [CrossRef]
25. Demizu, Y.; Yamagata, N.; Nagoya, S.; Sato, Y.; Doi, M.; Tanaka, M.; Nagasawa, K.; Okuda, H.; Kurihara, M. Enantioselective epoxidation of α,β-unsaturated ketones catalyzed by stapled helical i-Leu-based peptides. *Tetrahedron* 2011, 67, 6155–6165. [CrossRef]
26. Erkkiä, L.; Majander, I.; Pihko, P.M. Iminium catalysis. *Chem. Rev.* 2007, 107, 5416–5470. [CrossRef]
27. Mukherjee, S.; Yang, J.W.; Hoffmann, S.; List, B. Asymmetric enamine catalysis. *Chem. Rev.* 2007, 107, 5471–5569. [CrossRef]
28. 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 3i0-helical peptides: A 3D structural study. *J. Am. Chem. Soc.* 2007, 129, 6986–6987. [CrossRef]
29. Kim, Y.-W.; Kutchukian, P.S.; Verdone, G.L. Introduction of all-hydrocarbon i,i+3 staples into α-helices via ring-closing olefin metathesis. *Org. Lett.* 2010, 12, 3046–3049. [CrossRef]
30. Hill, T.A.; Shepherd, N.E.; Diness, F.; Fairlie, D.P. Constraining cyclic peptides to mimic protein structure motifs. *Angew. Chem. Int. Ed.* 2014, 53, 13020–13041. [CrossRef]
31. Paul, P.K.C.; Sukumar, M.; Bardi, R.; Piazzesi, A.M.; Valle, G.; Toniolo, C.; Balaram, P. Stereoelectronically constrained peptides. Theoretical and experimental studies on the conformations of peptides containing 1-aminocyclohexanecarboxylic acid. J. Am. Chem. Soc. 1986, 108, 6363–6370. [CrossRef]
32. Royo, S.; De Borggave, W.M.; Peggion, C.; Formaggio, F.; Crisma, M.; Jiménez, 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. [CrossRef] [PubMed]
33. 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. [CrossRef]
34. Nagano, M.; Tanaka, M.; Doi, M.; Demizu, Y.; Kurihara, M.; Suemune, H. Helical-screw directions of diastereoisomeric cyclic α-amino acid oligomers. Org. Lett. 2009, 11, 1135–1137. [CrossRef] [PubMed]
35. Oba, M.; Ishikawa, N.; Doi, M.; Kurihara, M.; Tanaka, M. Helical oligomers with a changeable chiral acetal moiety. Eur. J. Org. Chem. 2013, 7679–7682. [CrossRef]
36. Hirata, T.; Ueda, A.; Oba, M.; Doi, M.; Demizu, Y.; Kurihara, M.; Nagano, M.; Suemune, H.; Tanaka, M. Amino equatorial effect of a six-membered ring amino acid on its peptide 310- and α-helices. Tetrahedron 2015, 71, 2409–2420. [CrossRef]
37. Crisma, M.; Toniolo, C. Helical screw-sense preferences of peptides based on chiral, Cα-tetrasubstituted α-amino acids. Biopolymers 2015, 104, 46–64. [CrossRef]
38. 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 2016, 106, 555–562. [CrossRef]
39. 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. [CrossRef]
40. 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···etherole O-type hydrogen bond. Org. Lett. 2018, 20, 7830–7834. [CrossRef]
41. Demizu, Y.; Tanaka, M.; Nagano, M.; Kurihara, M.; Doi, M.; Maruyama, T.; Suemune, H. Controlling 310-helix and α-helix of short peptides in the solid state. Chem. Pharm. Bull. 2007, 55, 840–842. [CrossRef] [PubMed]
42. Umeno, T.; Ueda, A.; Oba, M.; Doi, M.; Hirata, T.; Suemune, H.; Tanaka, M. Helical structures of l-Leu-based peptides having chiral six-membered ring amino acids. Tetrahedron 2016, 72, 3124–3131. [CrossRef]
43. Koba, Y.; Ueda, A.; Oba, M.; Doi, M.; Demizu, Y.; Kurihara, M.; Tanaka, M. Helical l-Leu-based peptides having chiral five-membered carbocyclic ring amino acids with an ethylene acetal moiety. ChemistrySelect 2017, 2, 8108–8114. [CrossRef]
44. Ueda, A.; Umeno, T.; Doi, M.; Akaqawa, K.; Kudo, K.; Tanaka, M. Helical-peptide-catalyzed enantioselective Michael addition reactions and their mechanistic insights. J. Org. Chem. 2016, 81, 5864–5871. [CrossRef] [PubMed]
45. 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. [CrossRef]
46. 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. [CrossRef]
47. Akagawa, K.; Yamashita, T.; Sakamoto, S.; Kudo, K. Friedel–Crafts-type alkylation in aqueous media using resin-supported peptide catalyst having polyoleucine. Tetrahedron Lett. 2009, 50, 5602–5604. [CrossRef]
48. Akagawa, K.; Suzuki, R.; Kudo, K. Effect of the helical tether of a resin-supported peptide catalyst for Friedel–Crafts-type alkylation in water. Adv. Synth. Catal. 2012, 354, 1280–1286. [CrossRef]
49. Peters, C.; Bacher, M.; Buenemann, C.L.; Kricke, F.; Rondeau, J.-M.; Weigand, K. Conformationally constrained mimics of the membrane-proximal domain of FccRlx. ChemBioChem 2007, 8, 1785–1789. [CrossRef]
50. Bortolini, O.; Cavazzini, A.; Giovannini, P.P.; Greco, R.; Marchetti, N.; Massi, A.; Pasti, L. A combined kinetic and thermodynamic approach for the interpretation of continuous-flow heterogeneous catalytic processes. Chem. Eur. J. 2013, 19, 7802–7808. [CrossRef]
51. Tang, H.; Yin, L.; Lu, H.; Cheng, J. Water-soluble poly(t-serine)s with elongated and charged side-chains: Synthesis, conformations, and cell-penetrating properties. Biomacromolecules 2012, 13, 2609–2615. [CrossRef] [PubMed]
52. Vartak, A.P.; Skoblenick, K.; Thomas, N.; Mishra, R.K.; Johnson, R.L. Allosteric modulation of the dopamine receptor by conformationally constrained type VI β-turn peptidomimetics of Pro-Leu-Gly-NH$_2$. *J. Med. Chem.* 2007, 50, 6725–6729. [CrossRef] [PubMed]

53. McGrath, S.; Tortorici, M.; Drouin, L.; Solanki, S.; Vidler, L.; Westwood, I.; Gimeson, P.; Montfort, R.V.; Hoelder, S. Structure-enabled discovery of a stapled peptide inhibitor to target the oncogenic transcriptional repressor TLE1. *Chem. Eur. J.* 2017, 23, 9577–9584. [CrossRef] [PubMed]

54. Austin, J.F.; MacMillan, D.W.C. Enantioselective organocatalytic indole alkylations. Design of a new and highly effective chiral amine for iminium catalysis. *J. Am. Chem. Soc.* 2002, 124, 1172–1173. [CrossRef]

55. Ranjbar, S.; Riente, P.; Rodriguez-Escrich, C.; Yadav, J.; Ramineni, K.; Pericas, M.A. Polystyrene or magnetic nanoparticles as support in enantioselective organocatalysis? A case study in Friedel–Crafts chemistry. *Org. Lett.* 2016, 18, 1602–1605. [CrossRef]

56. 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. [CrossRef]

57. 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. [CrossRef]

58. Akagawa, K.; Kudo, K. Development of selective peptide catalysts with secondary structural frameworks. *Acc. Chem. Res.* 2017, 50, 2429–2439. [CrossRef]

59. Wang, Z. Advances in the asymmetric total synthesis of natural products using chiral secondary amine catalyzed reactions of α,β-unsaturated aldehydes. *Molecules* 2019, 24, 3412. [CrossRef]

60. Arlegui, A.; Torres, P.; Cuesta, V.; Crusats, J.; Moyano, A. Chiral amphiphilic secondary amine-porphyrin hybrids for aqueous organocatalysis. *Molecules* 2020, 25, 3420. [CrossRef]

61. Gellman, S.H. Foldamers: A manifesto. *Acc. Chem. Res.* 1998, 31, 173–180. [CrossRef]

62. Guichard, G.; Huc, I. Synthetic foldamers. *Chem. Commun.* 2011, 47, 5933–5941. [CrossRef] [PubMed]

63. Oba, M. Cell-penetrating peptide foldamers: Drug-delivery tools. *ChemBioChem* 2019, 20, 2041–2045. [CrossRef] [PubMed]

64. 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. [CrossRef] [PubMed]

65. 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.

66. Furukawa, K.; Tanaka, M.; Oba, M. siRNA delivery using amphipathic cell-penetrating peptides into human hepatoma cells. *Bioorg. Med. Chem.* 2020, 28, 115402. [CrossRef] [PubMed]

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