Electronic Supplementary Information

PHIP-Label: Parahydrogen-Induced Polarization in Propargylglycine-Containing Synthetic Oligopeptides

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General Information

Methanol-d₄ was purchased from Euriso-Top, the catalyst Rh(dppb)(COD)BF₄ (CAS 79255-71-3) was purchased from Strem Chemicals Inc.. Solid phase resins, Fmoc-protected amino acids, and coupling reagents were purchased from IRIS Biotech, Bachem, and Orpegen Pharma. N,N'-dimethylformamide (99.8 %) and piperidine were purchased from Carl Roth.

If not state otherwise, analytical and preparative reversed-phase HPLC were done in a Waters modular system consistent of a Waters 600 controller, Waters 996 photodiode array detector, and a Waters 717 autosampler unit. Analytical HPLC was conducted using a Multospher 120 RP-3µ column (125 mm x 2 mm), preparative HPLC was carried out using an Eurospher II 102-5 C18A (250 mm x 16 mm) column. The eluent system was consistent of eluent A (0.1 % TFA in acetonitrile) and eluent B (0.1 % TFA in water).

ESI mass spectra were recorded in a Bruker Esquire-LC device.

Para-hydrogen was enriched at liquid nitrogen temperature in a home-build apparatus which was described previously.¹

NMR experiments were performed in a 7 T Bruker AVANCE III 300 spectrometer.

Peptide Syntheses

Tripeptides (3-13), general procedure. Pra-containing tripeptides were manually synthesized on a Rink-amide MBHA resin using the Fmoc strategy (i.e. the N-Fmoc protecting group was removed from the peptide chain with a solution of piperidine in DMF after each formation of the respective peptide bond; Fmoc = 9-fluorenylmethoxycarbonyl; DMF = N,N-dimethylformamide).

Attachment of the amino acids was done in DMF by double-coupling of 4 eq. of the respective N-Fmoc-protected amino acid, 4 eq. of HBTU (O-benzotriazole-N,N,N',N'-tetramethyl-uronium hexafluoro-phosphate) and HOBT (N-hydroxybenzotriazole), and activated with 8 eq. DIEA (N,N-diisopropylethylamine).

Fmoc-propargylglycine instead was attached by double-coupling using 1.95 eq. of HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), 2 eq. of HOBT and 8 eq. DIEA.

H-Gly-Pra-Gly-NH₂ (3). The tripeptide was synthesized according to the general procedure on a 0.7 mmol scale. Acidolytic cleavage, ether precipitation and subsequent purification by HPLC resulted in 76.6 mg (0.339 mmol, 48.4 % overall yield) of peptide 3.

RP-HPLC (conducted on an Agilent 1200 System, using a ZORBAX 300SB-C8, 4.6x150 mm, 5 µm column): Rt = 1.6 min, 20 % → 80 % A in B over 10 min at a flow rate of 1.0 mL/min. ESI-MS: m/z: [M+Na]⁺ obsd. = 249.1 (calc. = 249.10).
H-Ala-Pra-Ala-NH₂ (4). The tripeptide was synthesized according to the general procedure on a 0.147 mmol scale. Acidolytic cleavage, ether precipitation and subsequent purification by HPLC resulted in 4.0 mg (15.7 µmol, 10.7 % overall yield) of peptide 4.

RP-HPLC: \( R_t = 7.6 \text{ min}, 5 \% \to 40 \% \text{ A in B over 10 min at a flow rate of 2.0 mL/min.} \) ESI-MS: \( m/z: [M+Na]^+ \text{ obsd.} = 277.4 \) (calc. = 277.1).

H-Phe-Pra-Phe-NH₂ (5). The tripeptide was synthesized according to the general procedure on a 0.295 mmol scale. Acidolytic cleavage, ether precipitation and subsequent purification by HPLC resulted in 71.2 mg (0.175 mmol, 59.4 % overall yield) of peptide 5.

RP-HPLC: \( R_t = 7.8 \text{ min}, 5 \% \to 40 \% \text{ A in B over 10 min at a flow rate of 2.0 mL/min.} \) ESI-MS: \( m/z: [M+H]^+ \text{ obsd.} = 407.4 \) (calc. = 407.2).

H-Tyr-Pra-Tyr-NH₂ (6). The tripeptide was synthesized according to the general procedure on a 0.59 mmol scale. Acidolytic cleavage, ether precipitation and subsequent purification by HPLC resulted in 25.6 mg (0.058 mmol, 9.8 % overall yield) of peptide 6.

RP-HPLC: \( R_t = 0.7 \text{ min}, 5 \% \to 40 \% \text{ A in B over 10 min at a flow rate of 2.0 mL/min.} \) ESI-MS: \( m/z: [M+H]^+ \text{ obsd.} = 493.4 \) (calc. = 493.2).

H-Lys-Pra-Lys-NH₂ (7). The tripeptide was synthesized according to the general procedure on a 0.59 mmol scale. Acidolytic cleavage, ether precipitation and washing resulting in 2.8 mg (8.18 µmol, 1.4 % overall yield) of peptide 8.

RP-HPLC: \( R_t = 4.3 \text{ min}, 5 \% \to 40 \% \text{ A in B over 10 min at a flow rate of 1.25 mL/min.} \) ESI-MS: \( m/z: [M+Na]^+ \text{ obsd.} = 365.3 \) (calc. = 365.1).

H-Asp-Pra-Asp-NH₂ (8). The tripeptide was synthesized according to the general procedure on a 0.59 mmol scale. Acidolytic cleavage, ether precipitation and washing resulting in 2.8 mg (8.18 µmol, 1.4 % overall yield) of peptide 8.

RP-HPLC: \( R_t = 4.3 \text{ min}, 5 \% \to 40 \% \text{ A in B over 10 min at a flow rate of 1.25 mL/min.} \) ESI-MS: \( m/z: [M+Na]^+ \text{ obsd.} = 365.3 \) (calc. = 365.1).

H-Ser-Pra-Ser-NH₂ (9). The tripeptide was synthesized according to the general procedure on a 0.59 mmol scale. Acidolytic cleavage, ether precipitation and subsequent purification by HPLC resulted in 53.6 mg (0.187 mmol, 31.7 % overall yield) of peptide 9.

RP-HPLC: \( R_t = 0.7 \text{ min}, 5 \% \to 40 \% \text{ A in B over 10 min at a flow rate of 2.0 mL/min.} \) ESI-MS: \( m/z: [M+H]^+ \text{ obsd.} = 287.2 \) (calc. = 287.1).

H-Cys-Pra-Cys-NH₂ (10). The tripeptide was synthesized according to the general procedure on a 0.147 mmol scale. Acidolytic cleavage, ether precipitation and subsequent purification by HPLC resulted in 39.2 mg (123.1 µmol, 83.7 % overall yield) of peptide 10.

RP-HPLC: \( R_t = 8.5 \text{ min}, 5 \% \to 40 \% \text{ A in B over 10 min at a flow rate of 1.25 mL/min.} \) ESI-MS: \( m/z: [M+H]^+ \text{ obsd.} = 319.3 \) (calc. = 319.09), \( [M+Na]^+ \text{ obsd.} = 341.3 \) (calc. = 341.07).
H-Cys(Me)-Pra-Cys(Me)-NH₂ (11). The tripeptide was synthesized according to the general procedure on a 0.5 mmol scale. Acidolytic cleavage, ether precipitation and subsequent purification by HPLC resulted in 128.8 mg (0.372 mmol, 74.3 % overall yield) of peptide 11.

RP-HPLC (conducted on an Agilent 1200 System, using a ZORBAX Eclipse XDB-C18, 4.6x150 mm, 5 µm column): Rt = 6.1 min, 5 % → 40 % A in B over 25 min at a flow rate of 1.00 mL/min. ESI-MS: m/z: [M+Na]+ obsd. = 369.1 (calc. = 369.1).

H-Met-Pra-Met-NH₂ (12). The tripeptide was synthesized according to the general procedure on a 0.59 mmol scale. Acidolytic cleavage, ether precipitation and subsequent purification by HPLC resulted in 29.0 mg (0.775 mmol, 43.7 % overall yield) of peptide 12.

RP-HPLC: Rt = 2.2 min, 5 % → 40 % A in B over 10 min at a flow rate of 2.0 mL/min. ESI-MS: m/z: [M+H]+ obsd. = 375.3 (calc. = 375.1).

Pra-Cys-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly (head-to-tail cyclized, 13) and Pra-Lys-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly (head-to-tail cyclized, 14). The linear precursor peptide was synthesized via microwave-assisted Fmoc-SPPS on 0.2 g of 2-chloro-chlorotrityl resin with a binding capacity of 0.62 mmol/g preloaded with H-Gly (124 µmol, 1 eq.). 496 µmol (4 eq.) of the Fmoc protected amino acids, 183 mg HBTU (484 µmol, 3.9 eq.) and 169 µL DIEA (992 µmol, 8 eq.) were employed in each single coupling step according to the standard protocol, except for the first proline residue (position 9 from the N-terminus), which was introduced in a 5 eq. excess (620 µmol) and activated by 230 mg HBTU (608 µmol, 4.9 eq.) and 230 µL DIEA (1.24 mmol, 10 eq.) employing triple coupling. The alanine, the second proline, the respective lysine or the cysteine residue were attached by double coupling. The assembled peptide was cleaved from the resin using a cleavage cocktail containing 50 % acetic acid, 40 % DCM and 10 % MeOH. The solvent mixture was removed under reduced pressure, which was used directly for the head-to-tail cyclization without further purification.

For head-to-tail cyclization, 96.4 µmol (1 eq.) of the crude linear precursor peptide were dissolved in 100 mL DMF in a 250 mL flask and treated with 36.5 mg HBTU (96.4 µmol, 1 eq.) and 33 µL DIEA (193 µmol, 2 eq.). The reaction mixture was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the resulted solid was dissolved in water-acetonitrile 5:1 and injected into the semi-preparative RP-HPLC column (18 % isocratic acetonitrile over 6 min followed by 18 → 90 % acetonitrile over 30 minutes in 0.1 % aq. TFA at flow rate 10 mL/min). The lyophilisation of the collected product fraction gave the cyclic decapeptide 16 (14.2 µmol, 14.7 %) as a white powder.

To remove the protection of the side chain group at position 2, 14.2 µmol (1 eq.) were treated with 3 mL of 95 % aq. TFA for 2 h at ambient temperature. The deprotected peptide was precipitated and washed with MTBE. The crude cyclic decapeptide was dissolved in water-acetonitrile 5:1 and lyophilized to yield the repsectively desired product as a white powder.

Pra-Cys-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly (head-to-tail cyclized, 13): RP-HPLC: Rt = 7.6 min, 5 % → 40 % A in B over 10 min at a flow rate of 2.0 mL/min. ESI-MS: m/z [M+H]+ obsd. 863.3 (calc. 863.55), [M+Na]+ obsd. 885.4 (calc. 885.3).

Pra-Lys-Pra-Pro-Gly-Pra-Ala-Pra-Pro-Gly (head-to-tail cyclized, 14): RP-HPLC: Rt = 6.5 min, 5 % → 40 % A in B over 10 min at a flow rate of 2.0 mL/min. ESI-MS: m/z [M+H]+ obsd. 888.5 (calc. 888.44), [M+Na]+ obsd. 910.4 (calc. 910.4).
NMR Experiments

PHIP Experiments. Hydrogenation reactions were performed in the terrestrial magnetic field at room temperature in 5 mm “screw cap” NMR tubes equipped with a silicon rubber septum. 2.5 µmol of the catalyst and 50 µmol of the substrate (respective to the amount of C-C triple bonds in the sample) were dissolved in 600 µL methanol-d4. The NMR tube was sealed and para-enriched hydrogen gas was filled into the sample tube through the septum with a pressure of 2 bar. Subsequently the sample was shooked for 10 s. The sample was then inserted into the spectrometer, and a standard PHIP sequence (i.e. signal acquisition after a 45° pulse) was applied for irradiation. All spectra have been recorded at room temperature as “single-shot experiments”, performing one scan. Full relaxation of the polarized protons products was observed at least 2 minutes after the hydrogenation.

Enhancement. Enhancement factors were calculated from the normalized integrals of the respective signal after deconvolution using the implemented line fitting function in MestReNova 8. The enhancement factors ef were calculated as quotient of the integral Int (PHIP) for the polarized signal and the integral Int (therm.) for the thermal product signal, following equation 1:

\[ ef = \frac{\text{Int (PHIP)}}{\text{Int (therm.)}} \]  

(1)

Since the integral sum of the antiphase signals is equal to zero, Int (PHIP) was determined as sum of the absolute values each of the emission and absorption area of the respective antiphase signal. ef (H^a/H^b) represents the average enhancement of the H^a-H^b spin system (see figure S1 for the nomenclature of protons). Due to the strong J-coupling the NMR spectra showed superimposed signals for H^a and H^b. Therefore it was not possible to distinguish between the proton signals. Signal enhancements for H^c were not calculated due to minor PHIP signal intensities for H^c.

Scheme S1. Hydrogenation of a C-C triple bond. Protons are labeled according to the nomenclature applied in this ESI.

T1 Relaxation Times. T1 times of the hydrogenation products were determined using the inversion recovery method and are listed in table S1. T1 times of Fmoc-L-allyglycine (H^b, 2.2 s; H^c 2.9 s) and Fmoc-L-norvaline (CH3 1.2 s; CH2 0.9 s) were determined using commercial available substances. T1 (H^a/H^b) represents the average relaxation time of the H^a-H^b spin system. It was not possible to distinguish between the protons, as previously mentioned.

Table S1. T1 Relaxation times of the hydrogenation products.

<table>
<thead>
<tr>
<th>entry</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (H^a/H^b)/s *</td>
<td>2.2</td>
<td>n.h.</td>
<td>2.2</td>
<td>1.7</td>
<td>1.5</td>
<td>–</td>
<td>1.5</td>
<td>–</td>
<td>1.4</td>
<td>n.h.</td>
<td>n.h.</td>
<td>1.5</td>
<td>1.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* n.h.: no hydrogenation observed. Values for 6 and 8 were not determined due to interference of the solvent signal.
Figure S1. $^1$H NMR spectrum of Rh(dppb)(COD)BF$_4$ in methanol-$d_4$.

Figure S2. $^1$H NMR spectrum of Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 30 s after the hydrogenation with para-enriched hydrogen was started.
Figure S3. $^1$H NMR spectrum of Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 60 s after the hydrogenation with para-enriched hydrogen was started.

Figure S4. $^1$H NMR spectrum of 1 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$. 

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Figure S5. $^1$H NMR spectrum of 1 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 38 s after the hydrogenation with para-enriched hydrogen was started.

Figure S6. $^1$H NMR spectrum of 1 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 70 s after hydrogenation with parahydrogen.
Figure S7. $^1$H NMR spectrum of 3 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$.

Figure S8. $^1$H NMR spectrum of 3 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 57 s after the hydrogenation with para-enriched hydrogen was started.
Enriched hydrogen was started.

Figure S9. $^1$H NMR spectrum of 3 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$. 192 s after the hydrogenation with para-enriched hydrogen was started.

Figure S10. $^1$H NMR spectrum of 4 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$. 

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enriched hydrogen was started.

Figure S11. $^1$H NMR spectrum of 4 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 45 s after the hydrogenation with para-enriched hydrogen was started.

Figure S12. $^1$H NMR spectrum of 4 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 182 s after the hydrogenation with para-enriched hydrogen was started.
Figure S13. $^1$H NMR spectrum of 5 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$.

Figure S14. $^1$H NMR spectrum of 5 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 30 s after the hydrogenation with para-enriched hydrogen was started.
Figure S15. $^1$H NMR spectrum of 5 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 60 s after the hydrogenation with para-enriched hydrogen was started.

Figure S16. $^1$H NMR spectrum of 5 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 180 s after the hydrogenation with para-enriched hydrogen was started.
Figure S17. $^1$H NMR spectrum of 5 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 240 s after the hydrogenation with para-enriched hydrogen was started.

Figure S18. $^1$H NMR spectrum of 6 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$. 
Figure S19. $^1$H NMR spectrum of 6 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 30 s after the hydrogenation with para-enriched hydrogen was started.

Figure S20. $^1$H NMR spectrum of 6 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 60 s after the hydrogenation with para-enriched hydrogen was started.
Figure S21. $^1$H NMR spectrum of 6 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 180 s after the hydrogenation with para-enriched hydrogen was started.

Figure S22. $^1$H NMR spectrum of 6 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 360 s after the hydrogenation with para-enriched hydrogen was started.
Figure S23. $^1$H NMR spectrum of 7 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$.

Figure S24. $^1$H NMR spectrum of 7 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 60 s after the hydrogenation with para-enriched hydrogen was started.
Figure S25. $^1$H NMR spectrum of 7 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 120 s after the hydrogenation with para-enriched hydrogen was started.

Figure S26. $^1$H NMR spectrum of 8 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$. 
Figure S27. $^1$H NMR spectrum of 8 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 30 s after the hydrogenation with para-enriched hydrogen was started.

Figure S28. $^1$H NMR spectrum of 8 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 60 s after the hydrogenation with para-enriched hydrogen was started.
Figure S29. $^1$H NMR spectrum of 8 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 180 s after the hydrogenation with para-enriched hydrogen was started.

Figure S30. $^1$H NMR spectrum of 9 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$. 
Figure S31. $^1$H NMR spectrum of 9 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 30 s after the hydrogenation with para-enriched hydrogen was started.

Figure S32. $^1$H NMR spectrum of 9 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 90 s after the hydrogenation with para-enriched hydrogen was started.
Figure S33. $^1$H NMR spectrum of 9 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 330 s after the hydrogenation with para-enriched hydrogen was started.

Figure S34. $^1$H NMR spectrum of pure 10 in methanol-$d_4$. 

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Figure S35. $^1$H NMR spectrum of 10 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 30 s after the hydrogenation with para-enriched hydrogen was started.

Figure S36. $^1$H NMR spectrum of 10 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 210 s after the hydrogenation with para-enriched hydrogen was started.
Figure S37. $^1$H NMR spectrum of pure 11 in methanol-$d_4$.

Figure S38. $^1$H NMR spectrum of 11 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 30 s after the hydrogenation with para-enriched hydrogen was started.
Figure S39. $^1$H NMR spectrum of 11 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$, 240 s after the hydrogenation with para-enriched hydrogen was started.

Figure S40. $^1$H NMR spectrum of 12 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$. 

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Figure S41. $^1$H NMR spectrum of 12 and Rh(dppb)(COD)BF$_4$ in methanol-$_d$$_6$, 60 s after the hydrogenation with para-enriched hydrogen was started.

Figure S42. $^1$H NMR spectrum of 12 and Rh(dppb)(COD)BF$_4$ in methanol-$_d$$_6$, 180 s after the hydrogenation with para-enriched hydrogen was started.
Figure S43. $^1$H NMR spectrum of 13 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$. 60 s after the hydrogenation with para-enriched hydrogen was started.

Figure S44. $^1$H NMR spectrum of 13 and Rh(dppb)(COD)BF$_4$ in methanol-$d_4$. 60 s after the hydrogenation with para-enriched hydrogen was started.
Figure S45. $^1$H NMR spectrum of 13 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 150 s after the hydrogenation with para-enriched hydrogen was started.

Figure S46. $^1$H NMR spectrum of 14 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$. 
Figure S47. $^1$H NMR spectrum of 14 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 60 s after the hydrogenation with para-enriched hydrogen was started.

Figure S48. $^1$H NMR spectrum of 14 and Rh(dppb)(COD)BF$_4$ in methanol-$d_6$, 150 s after the hydrogenation with para-enriched hydrogen was started.
References.