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# Effect of γ-Substituted Proline Derivatives on the Performance of the Peptidic Catalyst H-DPro-Pro-Glu-$NH_2$

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Dedicated to Professor Scott E. Denmark

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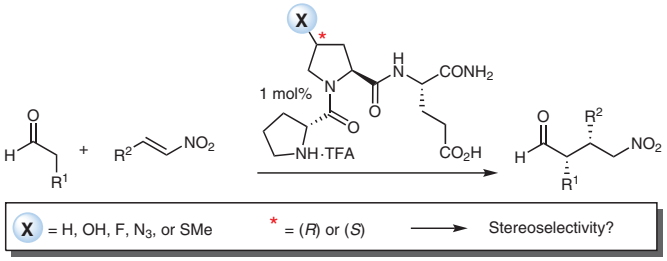


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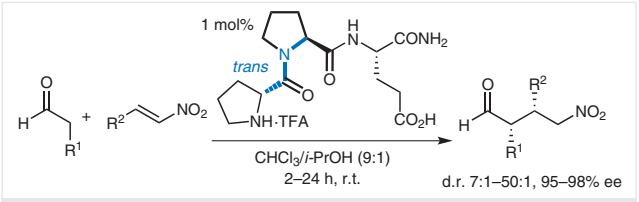
**Abstract** Substituents at Cγ of proline are valuable probes to tune the *trans*/*cis* ratio of Xaa–Pro bonds. We investigated the effect of Cγ-substituents on the reactivity and stereoselectivity of the peptidic catalyst H-DPro-Pro-Glu-$NH_2$. Derivatives that bear electron-withdrawing and -donating substituents (OH, F, $N_3$, and SMe) at Cγ of the middle Pro-residue were examined. The results show that substituents at a 4*R*-configured Cγ hardly affect the stereoselectivity of the peptidic catalyst whereas substituents at a 4*S*-configured Cγ can be used to tune and improve the catalytic performance.

**Key words** proline, peptides, *trans*/*cis* isomerization, organocatalysis, conjugate addition reactions

Peptides have become valuable as stereoselective catalysts for many different reactions, including acylations, epoxidations, transfer hydrogenations, and C–C bond formations.[1,2] Our group contributed peptidic catalysts of the H-Pro-Pro-Xaa type (Xaa = any amino acid) for aldol and conjugate addition reactions.[3–6] These tripeptides are so reactive that catalyst loadings of ≤1 mol% typically suffice to enable the C–C bond formation with very good stereoselectivities and yields. For example, as little as 0.1 mol% of H-DPro-Pro-Glu-$NH_2$ will suffice to obtain the products of addition reactions of aldehydes to nitroolefins with excellent enantio- and diastereoselectivity (Scheme 1).[4] Recently, we showed that the *trans*/*cis* isomer ratio of the tertiary amide bond of the DPro-Pro moiety has a significant effect on the reactivity and stereoselectivity of the peptidic catalyst.[7] We accessed different *trans*/*cis* ratios by varying the solvent and incorporating ring-size analogues of proline in the second position. These experiments showed that the higher the population of the *trans*-isomer the higher is the reactivity and stereoselectivity of the peptidic catalyst.[7]




**Scheme 1** Conjugate addition reactions catalyzed by H-DPro-Pro-Glu-$NH_2$

Substituents at the pyrrolidine ring of proline also affect the *trans*/*cis* ratio of Xaa–Pro bonds.[8–13] Among the most investigated derivatives are proline residues that bear substituents at Cγ.

The substituent influences the pucker of the pyrrolidine ring and the *trans*/*cis* ratio of Xaa–Pro bonds either by steric effects,[8] stereoelectronic gauche effects,[9] repulsive interactions, and/or transannular hydrogen bonding.[10] For example, electron-withdrawing groups such as OH, F, or $N_3$ exert a gauche effect and favor a Cγ-*exo* pucker when the substituent is installed at a 4*R*-configured Cγ and a Cγ-*endo* pucker when Cγ is 4*S*-configured (Figure 1).[9]

In the case of 4*R*-configured proline derivatives, the *trans*/*cis* ratio is higher compared to that of unsubstituted proline but lower in the case of 4*S*-configured derivatives (Figure 1c). The *trans*-conformer is in both diastereoisomers stabilized by an $n\rightarrow\pi^*$ interaction between adjacent amide groups (Figure 1a).[14] This interaction is weakened in the case of the 4*S*-configured derivatives since a repulsion between the substituent at Cγ and the carbonyl group leads to an unfavorable Ψ-angle for the $n\rightarrow\pi^*$ interaction and hence a higher population of the *cis* conformer (Figure 1b and 1c, right). Conversely, the Cγ-*exo* pucker of 4*R*-configured derivatives favors the $n\rightarrow\pi^*$ interaction (Figure 1b and 1c, left). Nature takes advantage of these effects and uses, for example, (4*R*)-hydroxyproline (Hyp) to stabilize the col-

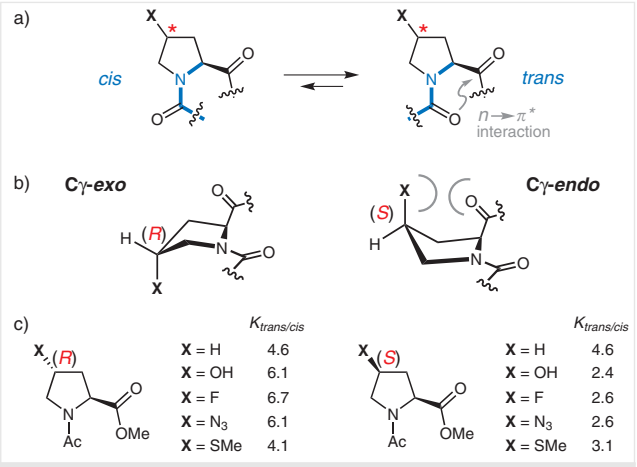



**Figure 1** a) *trans*/*cis*-Isomers of Pro amide bonds; b) Cγ-*exo* and Cγ-*endo* conformers; c) $K_{trans/cis}$ of model compounds Ac-Xaa-OMe in $D_2O$; data taken from ref. 12, 13

lagen triple helix with all-*trans* amide bonds.[15] Hyp and other substituted proline derivatives have also been used by us and others as tools to control the *trans*/*cis* ratio of Xaa–Pro bonds within peptides and proteins.[15–17] We therefore envisioned that Cγ-substituted proline derivatives might be valuable to tune the *trans*/*cis* ratio in Pro-Pro-Xaa type catalysts and thereby their stereoselectivity.

Herein, we implemented different substituents at Cγ of the middle Pro residue within H-DPro-Pro-Glu-$NH_2$ (**1**) and investigated their effect on the performance of the peptidic catalysts in conjugate addition reactions. We show that 4*R*-configured proline residues decrease the stereoselectivity of the peptidic catalyst to a small extent whereas 4*S*-configured proline residues affect the catalytic properties significantly. The study resulted in a catalyst with even higher enantioselectivity compared to the parent compound.

We started by preparing analogues of **1** bearing different substituents at either 4*R*- or 4*S*-configured Cγ-carbons in the middle position. Proline derivatives with hydroxy (Hyp), fluorine (Flp), azido (Azp), and methyl thioether (Mtp) moieties were chosen as groups with different steric and stereoelectronic effects (Figure 2).

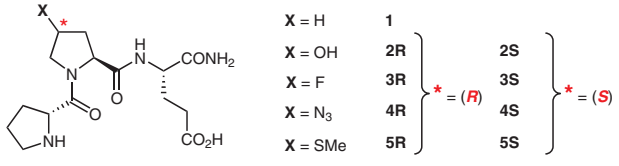
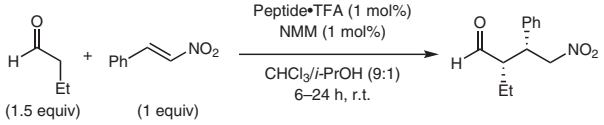



**Figure 2** Derivatives of the tripeptidic catalyst H-DPro-Pro-Glu-$NH_2$

Peptides **2R**–**5R** and **2S**–**5S** were readily obtained as their trifluoroacetic acid (TFA)-salts by standard solid-phase peptide synthesis following the Fmoc/*t*-Bu protocol. As expected, NMR spectra of all peptides show two spin systems that were assigned by nuclear Overhauser effects (NOEs) as *trans* and *cis* conformers. We determined the *trans*/*cis* ratios by $^1$H NMR spectroscopy in solutions of $CDCl_3/CD_3OH$ (9:1) to be as close as possible to the $CDCl_3$/*i*-PrOH (9:1) solvent mixture that is optimal for conjugate addition reactions of aldehydes to nitroolefins catalyzed by **1**.[4,7] The conjugate addition reaction of butanal to nitrostyrene was then used as model reaction to evaluate the catalytic performance of the peptides. The reaction was performed in $CHCl_3$/*i*-PrOH (9:1) with 1 mol% of the peptide TFA-salts and an equimolar amount of *N*-methylmorpholine (NMM) to liberate the secondary amine. All peptides provided the desired conjugate addition product in a yield of ≥95%, but their stereoselectivities differed significantly (Tables 1 and 2).

First, we focused on the properties of the tripeptides bearing a 4*R*-configured proline residue. Peptides **2R**–**5R** have *trans*/*cis* ratios between 30 and 38 (Table 1, entries 2–5). These values are smaller compared to that of the parent tripeptide **1** ($K_{trans/cis}$ = 46, entry 1). In agreement with our previous finding that lower *trans*/*cis* ratios go hand in hand with lower stereoselectivity, the dia- and enantioselectivities of peptides **2R**–**5R** are lower (d.r. ~25:1, 95% ee) compared to those of peptide **1** (d.r. 35:1, 97% ee).

**Table 1** Conjugate Addition Reaction of Butanal to Nitrostyrene Catalyzed by Peptides **1** and **2R**–**5R**




| Entry | Peptide | X | $K_{t/c}$[a] | Yield (%)[b] | d.r.[c] | ee (%)[d] |
|---|---|---|---|---|---|---|
| 1[e] | **1** | H | 46 | >95 | 35:1 | 97 |
| 2 | **2R** | OH | 36 | >95 | 25:1 | 95 |
| 3 | **3R** | F | 34 | >95 | 26:1 | 95 |
| 4 | **4R** | $N_3$ | 30 | >95 | 24:1 | 95 |
| 5 | **5R** | SMe | 38 | >95 | 24:1 | 96 |

[a] Determined by $^1$H NMR spectroscopic analysis in $CDCl_3/CD_3OH$ (9:1).
[b] Yield of the isolated product. Of note, most reactions were complete after 6–8 h.
[c] Determined by $^1$H NMR spectroscopic analysis.
[d] Determined by chiral stationary phase SFC analysis.
[e] Data taken from ref. 7.

The smaller *trans*/*cis* ratios of **2R**–**5R** compared to **1** are at first glance surprising as 4*R*-configured Hyp, Flp, Azp, and Mtp derivatives have in general higher *trans*/*cis* ratios compared to unsubstituted proline (Figure 1c, left).[9,11–13] The reasons become apparent by comparing the conformation of the 4*R*-configured proline derivatives with that of peptide **1**. A recent crystal structure of the TFA-salt of peptide **1**, which is in agreement with NMR spectroscopic studies in solution, shows that the middle proline residue adopts a Cγ-*endo* pucker (Figure 3).[7] In contrast, the pucker

of 4*R*-configured proline derivatives with electron-withdrawing groups is preferentially Cγ-*exo*. In addition, peptide **1** adopts a type I β-turn in which the adjacent carbonyl groups of the DPro and Pro residues are not engaged in an $n\rightarrow\pi^*$ interaction.[7,18] The enhanced $n\rightarrow\pi^*$ interaction of 4*R*-configured proline derivatives is therefore overwritten by the H-bonded β-turn structure of the peptide and cannot stabilize the *trans* conformer of peptide **1**.[19]

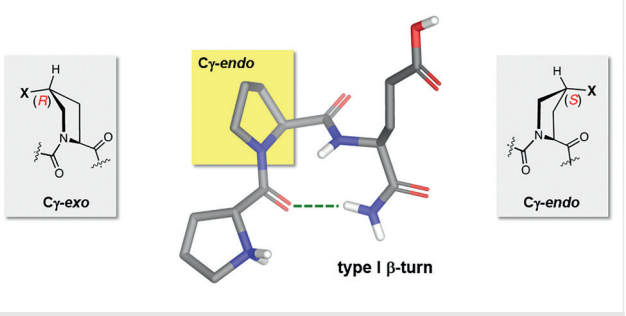


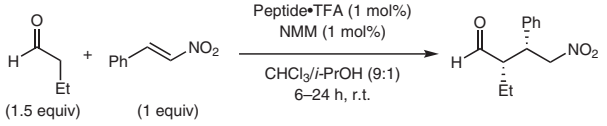

**Figure 3** Crystal structure of the TFA salt of H-DPro-Pro-Glu-$NH_2$ (TFA anion not shown for clarity). Structure taken from ref. 7.

Thus, the combination of the mismatched pucker and the lack of an $n\rightarrow\pi^*$ interaction explain the lower *trans*/*cis* ratio of peptides **2R**–**5R** compared to that of peptide **1**. Noteworthy is also the identical enantioselectivity of peptides **2R**–**5R**. This finding is also easily understandable when taking the structure of peptide **1** into account: the substituent at a 4*R*-configured Cγ points away from the cavity of the peptide and does therefore not interfere with the C–C bond formation (Figure 3, left).

Next, the catalytic performance of tripeptides **2S**–**5S** with 4*S*-configured proline residues was analyzed. In contrast to the 4*R*-configured diastereoisomers, the substituent points in **2S**–**5S** towards the cavity of the peptidic catalyst (Figure 3, right). We therefore expected a larger effect of the substituent on the stereoselectivity than for **2R**–**5R**. Indeed, their enantioselectivities vary between 93–99% ee and their *trans*/*cis* ratios between 20 and >50 (Table 2 entries 2–5).

The hydroxy functionalized peptide **2S** has the highest $K_{trans/cis}$ (>50) but the lowest enantioselectivity (93% ee). Conversely, the azido-functionalized peptide **4S** has the lowest $K_{trans/cis}$ (20) and the highest enantioselectivity (99% ee). Thus, the *trans*/*cis* ratios of the peptidic catalysts **2S**–**5S** do not correlate with their stereoselectivity. Of note, the enantioselectivity of the azido-functionalized peptide **4S** is even higher than that of the parent catalyst **1** (97% ee). This finding shows that the effect of a substituent that points towards the cavity of the catalyst overwrites that of the *trans*/*cis* ratio. The choice of the substituent therefore allows for fine-tuning of the peptide structure and the catalytic performance.

**Table 2** Conjugate Addition Reaction of Butanal to Nitrostyrene Catalyzed by Peptides **1** and **2S**–**5S**




| Entry | Peptide | X | $K_{t/c}$[a] | Yield (%)[b] | d.r.[c] | ee (%)[d] |
|---|---|---|---|---|---|---|
| 1[e] | **1** | H | 46 | >95 | 35:1 | 97 |
| 2 | **2S** | OH | >50 | >95 | 17:1 | 93 |
| 3 | **3S** | F | 30 | >95 | 26:1 | 98 |
| 4 | **4S** | $N_3$ | 20 | >95 | 29:1 | 99 |
| 5 | **5S** | SMe | 44 | >95 | 30:1 | 97 |

[a] Determined by $^1$H NMR spectroscopic analysis in $CDCl_3/CD_3OH$ (9:1).
[b] Yield of the isolated product. Of note, most reactions were complete after 6–8 h.
[c] Determined by $^1$H NMR spectroscopic analysis.
[d] Determined by chiral stationary phase SFC analysis.
[e] Data taken from ref. 7

In conclusion, our study showed that substituents in the Cγ-position of proline residues are valuable tools to tune the stereoselectivity of Pro-Pro-Xaa type catalysts. Substituents at a 4*S*-configured Cγ in the middle position point towards the active site and have a significant effect on the catalytic performance. Their effect on the catalytic performance can even overcome unfavorable *trans*/*cis* ratios of the DPro–Pro bond. The study allowed for the development of peptide H-DPro-(4*S*)Azp-Glu-$NH_2$ (**4S**) as a catalyst with higher enantioselectivity compared to the parent catalyst H-DPro-Pro-Glu-$NH_2$ (**1**).

Reagents and materials were of the highest commercially available grade and used without further purification. Reactions were monitored by TLC using Merck silica gel 60 F254 aluminum sheets. Visualization of the compounds was achieved by UV or $KMnO_4$. Flash chromatography and plug filtrations were performed using Fluka silica gel 60 (particle size 0.040–0.063 mm, 200–400 mesh). Solvents for chromatography were of technical quality and distilled before use. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker DRX 400, a Bruker AV III 400 (400 MHz/100 MHz) or a Bruker AV III 600 (600 MHz/150 MHz) spectrometer. All spectra were recorded at 25 °C, unless stated otherwise. Chemical shifts (δ) are reported in parts per million (ppm) relative to the signal of TMS. SFC analyses were performed on an analytical SFC with a diode array detector ACQUITY-UPLC-PDA from Waters using the chiral AD column (150 mm × 30 mm) from Daicel under the reported conditions. A Bruker maXis (UHR-TOF) was used for high-resolution electrospray ionization (HR-ESI) mass spectrometry.

**Peptide Coupling; General Procedure**

*i*-$Pr_2$NEt (6 equiv) was added to a solution of Fmoc-Xaa-OH (3 equiv) and HATU (3 equiv) in a minimal amount of DMF necessary to obtain a solution. The solution of the activated amino acid (≈100 mM) was

added to the amino-functionalized resin (pre-swollen in $CH_2Cl_2$) and the mixture was agitated for 1 h before washing with DMF (3 ×) and $CH_2Cl_2$ (3 ×).

### Fmoc-Deprotection; General Procedure

A solution of 20% piperidine in DMF was added to the resin pre-swollen in $CH_2Cl_2$, the reaction mixture was agitated for 10 min, drained, and the piperidine treatment was repeated for 10 min. Finally, the resin was washed with DMF (3 ×) and $CH_2Cl_2$ (3 ×).

The amino acid coupling steps and the Fmoc-deprotections were monitored by qualitative Kaiser (primary amines) and chloranil tests (secondary amines).

### Deprotection of Side Chain Functional Groups and Cleavage of the Peptide from the Solid Support; General Procedure

A mixture of TFA/TIS/$H_2O$ (95:2.5:2.5) was added to the immobilized peptide and the suspension was agitated for 1 h and a second time for 30 min. Pooling of the filtrates and removal of all volatiles under reduced pressure followed by precipitation and thorough washing with $Et_2O$ afforded the peptide as a TFA salt. The peptide was redissolved in MeCN/$H_2O$ (1:1), dried by lyophilization, and used without further purification.

### Analytical Data

The peptides were synthesized following the general procedures and obtained as white solids. Only the signals of the *trans*-isomer are reported.

#### H-D-Pro-(4*R*)Hyp-Glu-$NH_2$·TFA (2R)

$^1$H NMR ($CDCl_3$/$CD_3OH$ 9:1, 600 MHz): δ = 8.75 (d, *J* = 7.3 Hz, 1 H), 7.14 (d, *J* = 29.7 Hz, 1 H), 6.21 (s, 1 H), 4.62 (dd, *J* = 8.9, 6.2 Hz, 1 H), 4.55 (t, *J* = 8.3 Hz, 1 H), 4.51 (dq, *J* = 7.0, 4.3, 3.4 Hz, 1 H), 4.37 (td, *J* = 7.6, 3.2 Hz, 1 H), 3.78 (dd, *J* = 11.1, 4.0 Hz, 1 H), 3.56 (dt, *J* = 11.1, 1.9 Hz, 1 H), 3.48 (dt, *J* = 11.3, 7.0 Hz, 1 H), 3.44–3.37 (m, 1 H), 2.57–2.37 (m, 4 H), 2.22–1.96 (m, 6 H).

$^{13}$C NMR ($CDCl_3$/$CD_3OH$ 9:1, 151 MHz): δ =178.33, 174.43, 171.29, 169.43, 69.21, 60.35, 59.19, 55.29, 53.15, 46.82, 37.72, 30.43, 28.95, 25.34, 24.74.

HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{15}H_{25}N_4O_6Na$: 357.1769; found: 357.1774.

#### H-D-Pro-(4*S*)Hyp-Glu-$NH_2$·TFA (2S)

$^1$H NMR ($CDCl_3$/$CD_3OH$ 9:1, 600 MHz): δ = 9.38 (d, *J* = 5.2 Hz, 1 H), 7.16–6.98 (m, 1 H), 6.46–6.33 (m, 1 H), 4.66–4.60 (m, 1 H), 4.53 (qd, *J* = 3.5, 3.0, 1.2 Hz, 1 H), 4.47 (dd, *J* = 7.0, 5.1 Hz, 1 H), 4.23 (q, *J* = 4.8 Hz, 1 H), 3.89 (dt, *J* = 11.0, 1.1 Hz, 1 H), 3.65–3.57 (m, 1 H), 3.47 (ddd, *J* = 11.5, 7.5, 6.4 Hz, 1 H), 3.30 (ddd, *J* = 11.5, 8.4, 7.1 Hz, 1 H), 2.49–2.25 (m, 5 H), 2.24–2.12 (m, 2 H), 2.08 (ddd, *J* = 8.5, 5.5, 4.2 Hz, 2 H), 1.89 (dtd, *J* = 12.9, 8.9, 7.7 Hz, 1 H).

$^{13}$C NMR ($CDCl_3$/$CD_3OH$ 9:1, 151 MHz): δ = 181.73, 175.33, 171.57, 168.97, 69.66, 60.99, 60.00, 55.64, 54.77, 44.95, 37.29, 32.87, 27.24, 25.35, 24.33.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{25}N_4O_6$: 357.1769; found: 357.1764.

#### H-D-Pro-(4*R*)Flp-Glu-$NH_2$·TFA (3R)

$^1$H NMR ($CDCl_3$/$CD_3OH$ 9:1, 600 MHz): δ = 8.68 (d, *J* = 7.4 Hz, 1 H), 7.10 (s, 1 H), 6.23 (s, 1 H), 5.35 (dt, *J* = 51.7, 3.3 Hz, 1 H), 4.74–4.61 (m, 2 H), 4.38 (td, *J* = 7.7, 3.4 Hz, 1 H), 4.02–3.80 (m, 2 H), 3.56–3.36 (m, 2 H), 2.86–2.68 (m, 1 H), 2.63–1.87 (m, 9 H).

$^{13}$C NMR ($CDCl_3$/$CD_3OH$ 9:1, 151 MHz): δ = 178.09, 174.31, 170.33, 169.39, 91.36 (d, $J_{C,F}$ = 179.8 Hz), 59.86, 59.05, 53.77 (d, $J_{C,F}$ = 22.5 Hz), 53.11, 46.84, 35.84 (d, $J_{C,F}$ = 22.0 Hz), 30.44, 29.08, 25.54, 24.72.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{24}FN_4O_5$: 359.1725; found: 359.1719.

#### H-D-Pro-(4*S*)Flp-Glu-$NH_2$·TFA (3S)

$^1$H NMR ($CDCl_3$/$CD_3OH$ 9:1, 600 MHz): δ = 8.94 (d, *J* = 6.9 Hz, 1 H), 6.95 (s, 1 H), 6.21 (s, 1 H), 5.36 (dt, *J* = 51.3, 3.4 Hz, 1 H), 4.78 (dd, *J* = 8.8, 6.7 Hz, 1 H), 4.63 (d, *J* = 10.2 Hz, 1 H), 4.43–4.28 (m, 1 H), 4.18 (ddd, *J* = 22.7, 12.4, 1.9 Hz, 1 H), 3.74 (ddd, *J* = 36.5, 12.4, 3.5 Hz, 1 H), 3.55 (dt, *J* = 11.2, 6.7 Hz, 1 H), 2.74–2.59 (m, 1 H), 2.58–2.28 (m, 4 H), 2.26–2.07 (m, 3 H), 2.00–1.90 (m, 2 H).

$^{13}$C NMR ($CDCl_3$/$CD_3OH$ 9:1, 151 MHz): δ = 179.09, 174.52, 170.19, 169.58, 91.77 (d, $J_{C,F}$ = 179.1 Hz), 60.52, 59.17, 53.90 (d, $J_{C,F}$ = 24.5 Hz), 53.34, 46.71, 36.03 (d, $J_{C,F}$ = 21.4 Hz), 30.30, 28.68, 24.94, 24.90.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{24}FN_4O_5$: 359.1725; found: 359.1725.

#### H-D-Pro-(4*R*)Azp-Glu-$NH_2$·TFA (4R)

$^1$H NMR ($CDCl_3$/$CD_3OH$ 9:1, 600 MHz): δ = 8.77 (d, *J* = 7.3 Hz, 1 H), 7.05 (s, 1 H), 6.21 (s, 1 H), 4.67 (dd, *J* = 8.8, 6.6 Hz, 1 H), 4.56 (t, *J* = 7.9 Hz, 1 H), 4.38 (td, *J* = 7.3, 3.6 Hz, 2 H), 3.91 (dd, *J* = 11.4, 4.9 Hz, 1 H), 3.57 (ddd, *J* = 11.3, 3.2, 1.4 Hz, 1 H), 3.49 (dt, *J* = 11.3, 6.9 Hz, 1 H), 3.45–3.39 (m, 1 H), 2.50 (ddt, *J* = 13.6, 8.5, 5.6 Hz, 4 H), 2.37 (ddd, *J* = 13.9, 7.4, 5.3 Hz, 1 H), 2.24–1.93 (m, 5 H).

$^{13}$C NMR ($CDCl_3$/$CD_3OH$ 9:1, 151 MHz): δ = 178.35, 174.25, 170.08, 169.32, 59.94, 59.43, 59.07, 53.19, 52.37, 46.75, 34.60, 30.51, 28.98, 25.48, 24.77.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{24}N_7O_5$: 382.1833; found: 382.1830.

#### H-D-Pro-(4*S*)Azp-Glu-$NH_2$·TFA (4S)

$^1$H NMR ($CDCl_3$/$CD_3OH$ 9:1, 600 MHz): δ = 8.97 (s, 1 H), 6.89 (s, 1 H), 6.09 (s, 1 H), 4.65 (dd, *J* = 8.7, 7.1 Hz, 1 H), 4.49 (dd, *J* = 9.5, 2.2 Hz, 1 H), 4.34 (tt, *J* = 5.1, 1.8 Hz, 1 H), 4.29 (td, *J* = 7.1, 3.0 Hz, 1 H), 3.86–3.77 (m, 1 H), 3.64 (dd, *J* = 11.2, 5.1 Hz, 1 H), 3.50–3.39 (m, 1 H), 3.32 (pent, *J* = 1.7 Hz, 1 H), 2.54–2.30 (m, 4 H), 2.16–1.80 (m, 6 H).

$^{13}$C NMR ($CDCl_3$/$CD_3OH$ 9:1, 151 MHz): δ = 179.63, 174.34, 169.82, 169.28, 60.34, 59.56, 59.08, 53.54, 52.49, 46.22, 34.30, 30.63, 28.27, 24.90, 24.65.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{24}N_7O_5$: 382.1833; found: 382.1831.

#### H-D-Pro-(4*R*)Mtp-Glu-$NH_2$·TFA (5R)

$^1$H NMR ($CDCl_3$/$CD_3OH$ 9:1, 600 MHz): δ = 8.84 (d, *J* = 7.3 Hz, 1 H), 7.09–6.92 (m, 1 H), 6.25 (s, 1 H), 4.70 (dd, *J* = 8.9, 6.5 Hz, 1 H), 4.58 (dd, *J* = 8.9, 4.5 Hz, 1 H), 4.39 (td, *J* = 7.5, 3.1 Hz, 1 H), 4.12 (dd, *J* = 10.1, 6.0 Hz, 1 H), 3.55–3.43 (m, 3 H), 3.42–3.37 (m, 1 H), 3.43–3.37 (m, 1 H), 2.60–2.42 (m, 4 H), 2.35–2.25 (m, 1 H), 2.25–1.94 (m, 8 H).

$^{13}$C NMR ($CDCl_3$/$CD_3OH$ 9:1, 151 MHz): δ = 178.60, 174.30, 170.36, 169.22, 61.10, 59.10, 53.23, 53.18, 46.73, 42.94, 35.38, 30.42, 28.90, 25.33, 24.82, 14.81.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{16}H_{27}N_4O_5S$: 387.1697; found: 387.1702.

**H-D-Pro-(4*S*)Mtp-Glu-$NH_2$·TFA (5S)**

$^1$H NMR ($CDCl_3$/$CD_3OH$ 9:1, 600 MHz): δ = 8.80 (d, *J* = 6.7 Hz, 1 H), 7.07–6.90 (m, 1 H), 6.21 (s, 1 H), 4.72 (dd, *J* = 8.8, 6.7 Hz, 1 H), 4.52 (dd, *J* = 9.1, 4.2 Hz, 1 H), 4.33 (ddd, *J* = 8.1, 6.7, 3.1 Hz, 1 H), 3.90 (dd, *J* = 11.0, 6.5 Hz, 1 H), 3.74 (dd, *J* = 11.0, 4.4 Hz, 1 H), 3.59–3.52 (m, 1 H), 3.46 (tt, *J* = 6.5, 4.5 Hz, 1 H), 3.41–3.38 (m, 1 H), 2.62 (ddd, *J* = 13.8, 9.2, 6.5 Hz, 1 H), 2.57–2.53 (m, 2 H), 2.53–2.45 (m, 1 H), 2.34 (dt, *J* = 13.8, 4.4 Hz, 1 H), 2.23–2.07 (m, 5 H), 2.03 (s, 2 H), 1.95 (dq, *J* = 12.7, 7.2 Hz, 1 H).

$^{13}$C NMR ($CDCl_3$/$CD_3OH$ 9:1, 151 MHz): δ = 177.19, 172.85, 168.71, 167.57, 59.39, 57.35, 51.87, 51.34, 44.98, 41.48, 33.00, 28.69, 27.06, 23.11, 23.03, 13.12.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{16}H_{27}N_4O_5S$: 387.1697; found: 387.1690.

**Peptide-Catalyzed Conjugate Addition Reaction of Butanal to (*E*)-Nitrostyrene; General Procedure**

The peptide TFA salt (10 µmol, 1 mol%) was added to a solution of *N*-methylmorpholine (10 µmol, 1 mol%), (*E*)-nitrostyrene (149.2 mg, 1 mmol, 1 equiv), and butanal (135.4 µL, 1.5 mmol, 1.5 equiv) in $CHCl_3$/*i*-PrOH (9:1, 2 mL). The reaction mixture was stirred for 24 h. The solvent was removed under reduced pressure and the crude mixture was subjected to flash chromatography (1% → 50% EtOAc in hexane, silica gel). The γ-nitroaldehyde was isolated as a light yellow solid (Tables 1 and 2).

The enantiomeric excess was determined by chiral stationary phase SFC [AD-3, 5% MeOH, 2.0 mL/min, 40 °C, 214 nm, 1.00 min (*syn*, minor), 1.21 min (*syn*, major)].

The analytical data are in agreement with the previously published data.[4]

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## Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0037-1609547.

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