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Functional group requirements within the peptide H-Pro-Pro-Asp-NH_2 as a catalyst for aldol reactions

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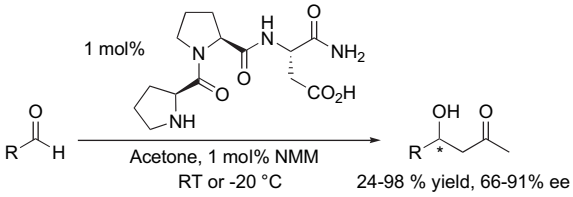
Dedicated to Professor Hisashi Yamamoto

Abstract—H-Pro-Pro-Asp-NH_2 **1** is a versatile catalyst for asymmetric aldol reactions. In this work, the functional group tolerance within the catalyst structure has been examined. Several analogs of H-Pro-Pro-Asp-NH_2 in which the N-terminal secondary amine or the carboxylic acid in the side chain of the aspartic acid residue is replaced by different functional groups were prepared. Evaluation of their catalytic properties revealed that both the N-terminal secondary amine and the carboxylic acid are important for catalysis. The implications for the reaction mechanism are discussed.

1. Introduction

The aldol reaction is one of the most important carbon–carbon bond forming reactions. Many different protocols have been established with which the aldol reaction can be performed and likewise numerous excellent asymmetric catalysts have been developed.[1] Over recent years, proline and other secondary amine based catalysts have become increasingly popular organocatalysts for aldol and related reactions.[2] For many substrates, excellent selectivities are achieved, however, often poor reactivity renders the use of large catalyst loadings necessary. Since rigid small organocatalysts bear only a limited number of sites for modifications, peptides have been envisioned as useful alternative catalysts.[3,4] Short chain peptides of intermediate complexity offer many sites for functional and structural modifications that can be used to develop optimal catalysts.[3,4] Work by List, Reymond, Gong and others showed that peptides can catalyze aldol reactions but also demonstrated that the purely rational design of peptidic catalysts is not trivial.[5] The many degrees of freedom of even short chain peptides render a prediction of their structures and catalytic properties challenging. Smart combinatorial screening methods that allow for the identification of catalysts within libraries of different peptides are therefore attractive to aid the discovery process.[3,4] One such method is 'catalyst–substrate coimmobilization', which allows for the identification of catalysts for bimolecular reactions among the members of split-and-mix libraries.[6] Using this method we identified the peptides H-Pro-Pro-Asp-NH_2 **1** and H-Pro-D-Ala-D-Asp-NH_2 **2** as catalysts for aldol reactions.[7] Particularly, **1** is a highly active and selective catalyst, 1 mol % suffices to catalyze aldol reactions between acetone and several aldehydes in high yields and enantioselectivities (Scheme 1).



Scheme 1. Aldol reactions catalyzed by H-Pro-Pro-Asp-NH_2 **1**.

These results represent a significant increase in catalytic activity over proline, which, for the same reactions is required at 30 mol % to obtain comparable product yields and selectivities within the same time. Thus, the higher complexity of the peptidic catalyst proved to be an excellent trade-off for its higher activity.[7,8] A further enhancement of the catalytic activity of H-Pro-Pro-Asp-NH_2 **1** was achieved by attachment of a triethylene glycol chain to its C-terminus.[9] H-Pro-Pro-Asp-NH$(CH_2CH_2O)_3CH_3$ is significantly more soluble compared to **1**, thereby only requiring the use of 0.5 mol % for efficient catalysis. Furthermore, immobilization of **1** on TentaGel or PEGA furnished solid supported catalysts that can be reused multiple times.[9]

With these results in hand we wish to gain insight into the mechanism by which H-Pro-Pro-Asp-NH_2 catalyzes aldol

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reactions. We started by examining, which functional groups are necessary for efficient catalysis and disclose the results of these initial mechanistic studies here.

2. Results and discussion

In common with proline, H-Pro-Pro-Asp-NH_2 **1** and H-Pro-D-Ala-D-Asp-NH_2 **2** contain a secondary amine and a carboxylic acid. This suggests that the mechanism by which the peptides catalyze intermolecular aldol reactions may be similar to that proposed for proline by List and Houk.[10–12] This mechanism involves enamine formation followed by nucleophilic addition of the enamine to the aldehyde with simultaneous proton transfer from the carboxylic acid (Fig. 1).

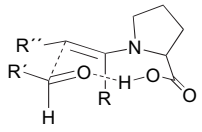


Figure 1. Transition state proposed by List and Houk for aldol reactions catalyzed by proline.[10,11]

To test the importance of these two functional groups we prepared analogs of H-Pro-Pro-Asp-NH_2 **1** in which the secondary amine is acetylated (Ac-Pro-Pro-Asp-NH_2 **3**) or methylated (Me-Pro-Pro-Asp-NH_2 **4**) and the carboxylic acid replaced by an amide (H-Pro-Pro-Asn-NH_2 **5**) or a sulfonic acid (H-Pro-Pro-Cys(SO_3H)-NH_2 **6**) (Fig. 2).

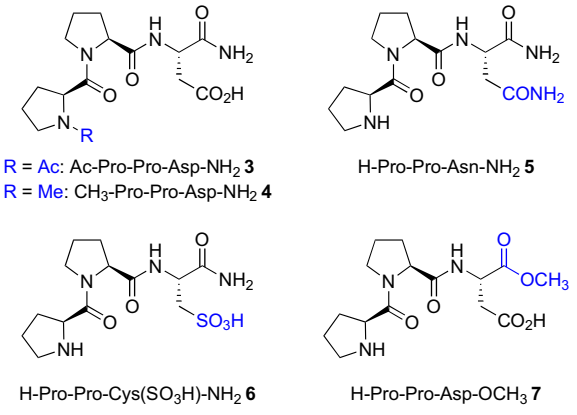


Figure 2. Peptides **3**–**7**.

Peptides **5** and **6** were chosen to bear functionalities in place of the carboxylic acid that either lack an acidic proton (peptide **5**) or are more acidic (peptide **6**). In peptide **3** the secondary amine is replaced by an amide, whereas in peptide **4** the basic functionality is retained. In addition, we prepared H-Pro-Pro-Asp-OCH_3 **7** to investigate the role of the C-terminal amide during catalysis.

2.1. Synthesis of peptides 3–7

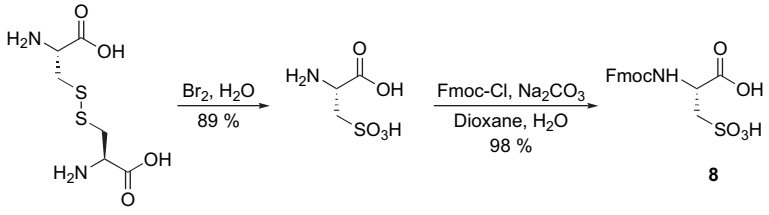
Peptides **3**–**7** were either prepared on Wang resin (peptide **7**) or Rink-amide resin (peptides **3**–**6**) following the general Fmoc/^{t}Bu protocol for peptide synthesis. For the preparation of peptides **3**, **5**, and **7**, commercially available α-*N*-Fmoc protected amino acids with acid sensitive protecting groups (^{t}Bu for Asp and trityl for Asn) on the side chain functional groups were used. *N*-Methylproline for the synthesis of peptide **4** was prepared by reductive amination in quantitative yield.[13] For the synthesis of peptide **6**, Fmoc-Cys(SO_3H)-OH was prepared starting from cystine (Scheme 2). Oxidation of L-cystine with an aqueous bromine solution yielded L-cysteic acid, which was isolated in 89% yield after recrystallization.[14] Protection of the amine was accomplished under standard Schotten–Baumann conditions using Fmoc-Cl in a mixture of aqueous Na_2CO_3 solution and dioxane following established procedures.[14]

2.2. Catalytic properties of peptides 3–7

The catalytic properties of peptides **3**–**7** were evaluated using the aldol reaction between acetone and *p*-nitrobenzaldehyde as a test reaction. When using H-Pro-Pro-Asp-NH_2 **1** as a catalyst, 1 mol % is sufficient to obtain the aldol product in quantitative yield and 80% ee at room temperature within 4 h.[7] At −20 °C, the product forms with an enantiomeric excess of 91% in 98% yield using 5 mol % of **1** within 24 h.[7] Peptides **3**–**7** were evaluated under identical conditions to those used with the parent catalyst **1**. However, since only peptide **7** provided the aldol product in good quantities with a catalyst loading of 1 mol %, amounts of 5 or 10 mol % were employed in the reactions with peptides **3**–**6** (Table 1).

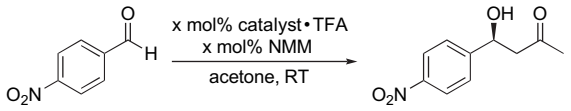
Neither the N-terminally acetylated peptide **3** nor peptide **4** with the basic tertiary amine at the N-terminus catalyzed the aldol reaction within 72 h (entry 3 and 4). Furthermore, when the TFA salt of H-Pro-Pro-Asp-NH_2 **1** was used as catalyst in the absence of a base such as *N*-methylmorpholine (NMM), the reaction proceeded only slowly, demonstrating that the protonation state of the secondary amine is crucial (entry 5). These findings demonstrate the importance of the secondary amine for catalysis and suggest that the formation of an enamine as an intermediate is crucial for catalysis.

Evaluation of peptide **5** with an amide functionality in place of the carboxylic acid allowed for analysis of the role of the



Scheme 2. Synthesis of Fmoc-Cys(SO_3H)-OH (**8**).

Table 1. Aldol reaction between *p*-nitrobenzaldehyde and acetone catalyzed by peptides **3–7**



Entry	Catalyst	mol %	Time (h)	Yield[a]	ee[b]
1 (Ref. 7)	**1**	1	4	99	80
2[c] (Ref. 7)	**1**	5	24	98	90
3	**3**	10	72	≤5	nd[d]
4	**4**	10	72	≤5	nd[d]
5[e]	**1**	5	24	67	77
6	**5**	10	24	39	54
7[f]	**6**	10	24 (72)	26 (49)	53 (52)
8	**7**	1	12	87	73

[a] Yields are listed in %; in entries **3–7** unreacted aldehyde could be reisolated, essentially no side products were observed.
[b] The ee was determined by chiral stationary HPLC analysis and is listed in %.
[c] Reaction was performed at −20 °C.
[d] Not determined.
[e] Reaction was performed in the absence of NMM.
[f] Data in brackets after 72 h of reaction time.

acidic proton. Peptide **5** is significantly less active compared to **1**, however, catalytic turn over is still observed. This is remarkable since the respective proline derivative H-Pro-NH_2 mediates aldol reactions only very slowly. With 40 mol % of H-Pro-NH_2, the aldol product was obtained in ≤10% yield in DMSO after 24 h.[15] Peptide **6** with a sulfonic acid was designed to test the effect on catalysis of a functionality with higher acidity in place of the carboxylic acid.[16] Peptide **6** proved to be a less competent catalyst compared to **1** both in terms of catalytic activity and selectivity. However, like **5**, peptide **6** allowed still for catalytic turn over. These results demonstrate that peptides **1** and **7** bearing a carboxylic acid functionality are optimal compared with those bearing an amide or sulfonic acid. However, the acidic functional group did not prove essential for catalytically active peptides.[17]

Finally we tested whether the C-terminal amide in catalyst **1** is crucial for catalysis, possibly by stabilizing the conformation of the peptide through H-bonding. Peptide **7** bearing a methyl ester in place of the amide performed almost as well as **1**. Thus, the N-terminal amide does not play a key role for catalysis thereby allowing for modifications at this site.

3. Conclusions

In conclusion, the peptidic catalyst H-Pro-Pro-Asp-NH_2 relies on both the N-terminal secondary amine and the carboxylic acid in the side chain of the aspartic acid residue for efficient catalysis. Acetylation, alkylation, or protonation of the secondary amine strongly diminishes or prevents catalytic activity. Modifications at the site of the carboxylic acid with stronger acids or functionalities lacking an acidic proton reduce both the catalytic activity and selectivity but are not entirely detrimental to catalysis. These results support a mechanism relying on enamine catalysis. (Note: oxazolidinones, which have recently been proposed as key intermediates in proline catalysis,[18] cannot form in the peptidic catalysts discussed here.) Given that the carboxylic acid can be replaced by amide and sulfonic acid functional groups without breakdown of catalysis raises the question of whether nucleophilic addition of the enamine to the aldehyde is accompanied by simultaneous proton transfer as proposed for proline catalysis. One intriguing alternative could be that the proton is transferred via the base, which is held in place by coordination to the carboxylic acid or related functional group.

4. Experimental

4.1. General

Materials and reagents were of the highest commercially available grade and used without further purification. Reactions were monitored by thin layer chromatography using Merck silica gel 60 F_{254} plates. Compounds were visualized by UV, $KMnO_4$ and ninhydrin. Flash chromatography was performed using Merck silica gel 60, particle size 40–63 μm. Ion exchange chromatography was performed using Dowex® 1×2-400 from Sigma—Aldrich. ^1H and ^{13}C NMR spectra were recorded on Bruker DPX 400 and 600 spectrometers. Chemical shifts are reported in parts per million using the solvent residue signals as reference. Finnigan MAT LCQ and Finnigan MAT 312 instruments were used for electrospray ionization (ESI) and fast atom bombardment (FAB) mass spectrometries. HPLC analyses were carried out using a Chiralcel AS (250 mm×4.6 mm) from Daicel.

4.2. General procedures for peptide synthesis on solid support

General procedure for peptide couplings: Fmoc-amino acid (3 equiv) and 1-hydroxybenzotriazole (3 equiv) dissolved in the minimum amount of DMF necessary were added to the suspension of amino-functionalized resin in CH_2Cl_2. The mixture was agitated for 2 min before adding diisopropylcarbodiimide (3 equiv) and agitating for another 2 h. The suspension was washed with DMF (3×) and CH_2Cl_2 (5×). *General procedure for Fmoc-deprotections*: to the resin (pre-swollen in DMF) was added a solution of 20% piperidine in DMF and the reaction mixture was agitated for 5 min, drained, and the piperidine treatment repeated for 10 min. Finally the resin was washed with DMF (7×) and CH_2Cl_2 (5×). Both the couplings and the deprotections were monitored by the qualitative Kaiser test. Quantitative Fmoc-monitoring was carried out as spot checks.

Peptides **3–6** were prepared on Rink-amide resin following the general protocol for solid phase synthesis and were cleaved off the resin by stirring in a mixture of TFA/CH_2Cl_2 (1:1) for 2 h. Filtration and removal of all volatiles under reduced pressure followed by precipitation with Et_2O afforded the TFA salts of the peptides. Peptide **7** was prepared on Wang resin following the general protocol for peptide synthesis and cleaved off the resin by agitation in a mixture of CH_3OH/NEt_3 4:1 for 3 days. Filtration and removal of all volatiles under reduced pressure was followed by purification of H-Pro-Pro-Asp(^{t}Bu)-OCH_3 over a size exclusion column (LH20, 1% MeOH in CH_2Cl_2). The

^{t}Bu-protecting group was removed as described for peptides **3**–**6** to afford the TFA salt of **7**.

4.2.1. Ac-Pro-Pro-Asp-NH_2 (3). *s-cis*/*s-trans* Conformers around the Pro–Pro amide bond are observed in a ratio of 3:1 in the ^{1}H NMR spectrum in DMSO-d_6. Major isomer: ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 10.50 (br s, 1H; Asp-OH), 7.89 (d, J=8.2 Hz, 1H; Pro-CONH), 7.15 (s, 1H; Asp-$CONH_2$), 6.99 (s, 1H; Asp-$CONH_2$), 4.51 (dd, J=8.3, 3.2 Hz, 1H; Pro-Hα), 4.40 (m, 1H; Asp-Hα), 4.20 (dd, J=8.3, 4.8 Hz, 1H; Pro-Hα), 3.68 (m, 1H; Pro-Hδ), 3.52 (m, 1H; Pro-Hδ, 1H; Pro-Hδ), 3.34 (m, 1H; Pro-Hδ), 2.68 (dd, J=16.4, 5.5 Hz, 1H; Asp-Hβ), 2.57 (dd, J=16.4, 7.3 Hz, 1H; Asp-Hβ), 2.30–1.55 (m, 8H; Pro-Hβ, Pro-Hγ), 1.94 (s, 3H; CH_3). ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C): δ 173.3, 173.1, 172.1, 171.7, 168.9, 61.0, 58.2, 50.1, 48.4, 47.7, 36.5, 29.5, 29.2, 25.5, 25.1, 22.9. Minor isomer: ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): 10.50 (br s, 1H; Asp-OH), 8.36 (d, J=8.2 Hz, 1H; Pro-CONH), 7.37 (s, 1H; Asp-$CONH_2$), 7.22 (s, 1H; Asp-$CONH_2$), 4.72 (dd, J=8.6, 2.5 Hz, 1H; Asp-Hα), 4.47 (m, 1H; Pro-Hα), 4.30 (dd, J=8.1, 4.8 Hz, 1H; Pro-Hα), 3.68 (m, 1H; Pro-Hδ), 3.52 (m, 2H; Pro-Hδ), 3.34 (m, 1H; Pro-Hδ), 2.76, (dd, J=16.4, 4.9 Hz, 1H; Asp-Hβ), 2.68 (dd, J=16.4, 5.5 Hz, 1H; Asp-Hβ), 2.30–1.55 (m, 8H; Pro-Hβ, Pro-Hγ), 1.76 (s, 3H; CH_3). ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C): δ 173.3, 173.2, 172.7, 172.7, 169.3, 59.5, 59.2, 50.2, 47.5, 46.9, 36.5, 29.4, 29.2, 25.5, 25.2, 23.1. HRMS (ESI) m/z: calcd for $C_{16}H_{24}N_4O_6Na$ 391.1593; found, 391.1589.

4.2.2. TFA·Me-Pro-Pro-Asp-NH_2 (4). *s-cis*/*s-trans* Conformers around the Pro–Pro amide bond were observed in a ratio of 1.5:1 in the ^{1}H NMR spectrum in DMSO-d_6. Major isomer: ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 9.68 (br s, 1H; $^+$NH), 8.22 (d, J=8.0 Hz, 1H; NH), 7.16 (s, 1H; $CONH_2$), 7.11 (s, 1H; $CONH_2$), 4.48 (m, 3H; Pro/Pro/Asp-Hα), 3.59 (m, 2H; Pro-Hδ), 3.41 (m, 1H; Pro-Hδ), 3.11 (m, 1H; Pro-Hδ), 2.79 (s, 3H; Me), 2.59 (m, 2H; Asp-Hβ), 2.49 (m, 1H; Pro-Hβ), 2.18–1.70 (m, 7H; Pro-Hβ, Pro-Hγ). ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C): δ 173.2, 173.0, 172.7, 171.5, 67.8, 60.8, 56.6, 50.3, 47.6, 36.9, 32.4, 29.8, 28.1, 25.2, 23.0. Minor isomer: δ 9.74 (br s, 1H; $^+$NH), 8.71 (d, J=8.0 Hz, 1H; NH), 7.46 (s, 1H; NH_2), 7.16 (s, 1H; NH_2), 4.53 (m, 1H; Hα), 4.33 (m, 1H; Hα), 4.85 (m, 1H; Hα), 3.59 (m, 2H; Pro-Hδ), 3.41 (m, 1H; Pro-Hδ), 3.11 (m, 1H; Pro-Hδ), 2.73 (s, 3H; Me), 2.59 (m, 2H; Asp-Hβ), 2.25 (m, 1H; Pro-Hβ), 2.18–1.70 (m, 7H; Pro-Hβ, Pro-Hγ). ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C): δ 173.2, 173.1, 172.7, 171.7, 67.8, 59.9, 56.1, 50.4, 48.1, 36.9, 32.4, 29.8, 28.3, 25.2, 22.9. HRMS (ESI) m/z: calcd for $C_{15}H_{25}N_4O_5$ 341.1824; found, 341.1823.

4.2.3. TFA·H-Pro-Pro-Asn-NH_2 (5). *s-cis*/*s-trans* Conformers around the Pro–Pro amide bond are observed in a ratio of 3:1 in the ^{1}H NMR spectrum in DMSO-d_6. Major isomer: ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 8.56 (br s, 1H; Pro-NH), 8.16 (d, J=8.3 Hz, 1H; CONH), 7.43 (s, 1H; $CONH_2$), 7.08 (s, 1H; $CONH_2$), 7.00 (s, 1H; $CONH_2$), 6.91 (s, 1H; $CONH_2$), 4.50 (m, 1H; Asn-Hα), 4.39 (m, 1H; Pro-Hα), 4.32 (m, 1H; Pro-Hα), 3.62 (m, 1H; Pro-Hδ), 3.45 (m, 1H; Pro-Hδ), 3.18 (m, 2H; Pro-Hδ), 2.44 (m, 2H; Asn-Hβ), 2.38 (m, 1H; Pro-Hβ), 2.09 (m, 1H; Pro-Hβ), 1.87 (m, 6H; Pro-Hβ, Pro-Hγ). ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C): δ 173.7, 172.8, 171.5, 168.2, 61.0, 59.0, 50.5, 47.7, 46.6, 37.5, 29.8, 28.8, 25.3, 24.4. Minor isomer: ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 8.63 (d, J=8.3 Hz, 1H; CONH), 8.56 (br s, 1H; Pro-NH), 7.49 (s, 1H; $CONH_2$), 7.37 (s, 1H; $CONH_2$), 7.12 (s, 1H; $CONH_2$), 6.93 (s, 1H; $CONH_2$), 4.55 (m, 1H; Asn-Hα), 4.39 (m, 1H; Pro-Hα), 3.98 (m, 1H; Pro-Hα), 3.62 (m, 1H; Pro-Hδ), 3.45 (m, 1H; Pro-Hδ), 3.18 (m, 2H; Pro-Hδ), 2.58 (m, 1H; Asn-Hβ), 2.44 (m, 1H; Asn-Hβ), 2.38 (m, 1H; Pro-Hβ), 2.21 (m, 1H; Pro-Hβ), 1.87 (m, 6H; 2H; Pro-Hβ, 4H; Pro-Hγ). ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C): δ 173.6, 172.7, 171.5, 167.6, 59.7, 59.2, 50.7, 48.3, 46.3, 38.1, 32.4, 28.7, 24.3, 22.9. HRMS (ESI) m/z: calcd for $C_{14}H_{24}N_5O_4$ 326.1828; found, 326.1819.

4.2.4. H-Pro-Pro-Cys(SO_3H)-NH_2 (6). *s-cis*/*s-trans* Conformers around the Pro–Pro amide bond are observed in a ratio of 1:1 in the ^{1}H NMR spectrum in DMSO-d_6. Isomer 1: ^{1}H NMR (600 MHz, DMSO-d_6, 25 °C): δ 9.29 (1H; Pro-NH_2), 8.61 (1H; Pro-NH_2), 8.52 (d, J=8.2 Hz, 1H; Cys-NH), 7.04 (1H; Cys-$CONH_2$), 6.89 (1H; Cys-$CONH_2$), 4.65 (m, 1H; Cys(SO_3H), Hα), 4.49 (m, 1H; Pro-Hα), 4.48 (m, 1H; Pro′-Hα), 3.64 (m, 1H; Pro′-Hδ), 3.53 (m, 1H; Pro′-Hδ), 3.24 (m, 1H; Pro-Hδ), 3.17 (m, 1H; Pro-Hδ), 2.98 (dd, J=13.9, 3.1 Hz, 1H; Cys(SO_3H), Hβ), 2.83 (dd, J=13.9, 11.0 Hz, 1H; Cys(SO_3H), Hβ), 2.35 (m, 1H; Pro-Hβ), 2.18 (m, 1H; Pro-Hβ), 2.26 (m, 1H; Pro′-Hβ), 2.02 (m, 1H; Pro′-Hβ), 1.97 (m, 1H; Pro′-Hγ), 1.89 (m, 1H; Pro′-Hγ), 1.92 (m, 2H; Pro-Hγ). ^{13}C NMR (151 MHz, DMSO-d_6, 25 °C): δ 172.1, 171.0, 167.5, 59.6, 58.3, 51.6, 50.3, 46.7, 45.6, 31.0, 24.2, 23.2. Isomer 2: ^{1}H NMR (600 MHz, DMSO-d_6, 25 °C): δ 8.99 (1H; Pro-NH_2), 8.68 (1H; Pro-NH_2), 8.35 (d, J=6.4 Hz, 1H; Cys-NH), 7.41 (1H; Cys-$CONH_2$), 7.13 (1H; Cys-$CONH_2$), 4.35 (m, 1H; Pro-Hα), 4.29 (m, 1H; Pro′-Hα), 4.18 (m, 1H; Cys(SO_3H), Hα), 3.51 (m, 1H; Pro′-Hδ), 3.39 (m, 1H; Pro′-Hδ), 3.17 (m, 2H; Pro-Hδ), 2.85 (dd, J=13.7, 6 Hz, 1H; Cys(SO_3H), Hβ), 2.78 (dd, J=13.7, 6 Hz, 1H; Cys(SO_3H), Hβ), 2.31 (m, 1H; Pro-Hβ), 2.14 (m, 1H; Pro′-Hβ), 1.94 (m, 2H; Pro-Hγ), 1.89 (m, 1H; Pro′-Hβ), 1.81 (m, 1H; Pro-Hβ), 1.81 (m, 2H; Pro′-Hγ). ^{13}C NMR (151 MHz, DMSO-d_6, 25 °C): δ 172.1, 170.1, 167.3, 60.6, 57.4, 50.6, 50.0, 46.9, 45.8, 27.6, 22.3, 22.0. HRMS (ESI) m/z: calcd for $C_{13}H_{23}N_4O_6S$ 363.1338; found, 363.1331.

4.2.5. TFA·H-Pro-Pro-Asp-OCH_3 (7). *s-cis*/*s-trans* Conformers around the Pro–Pro amide bond were observed in a ratio of 4:1 in the ^{1}H NMR spectrum in DMSO-d_6. Major isomer: ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 9.89 (s, 1H; Asp-OH), 8.45 (br s, 1H; Pro-NH), 8.42 (d, J=8.2 Hz, 1H; Pro-CONH), 4.55 (dd, J=13.9, 6.4 Hz, 1H; Pro-Hα), 4.46 (m, 1H; Asp-Hα), 4.11 (dd, J=8.3, 4.8 Hz, 1H; Pro-Hα), 3.60 (m, 1H; Pro-Hδ), 3.58 (s, 3H; Asp-OCH_3), 3.42 (m, 1H; Pro-Hδ), 3.23 (m, 1H; Pro-Hδ), 3.16 (m, 1H; Pro-Hδ), 2.75 (m, 1H; Asp-Hβ), 2.65 (m, 1H; Asp-Hβ), 2.40 (m, 1H; Pro-Hβ), 2.12 (m, 1H; Pro-Hβ), 2.94–1.72 (m, 6H; Pro-Hβ, Pro-Hγ). ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C): δ 172.4, 172.3, 172.2, 171.8, 60.2, 59.1, 52.9, 49.4, 47.6, 46.6, 36.7, 29.9, 28.7, 25.2, 24.4. Minor conformer: ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 9.80 (s, 1H; Asp-OH), 8.55 (br s, 1H; Pro-NH), 8.88 (d, J=7.6 Hz, 1H; Pro-CONH), 4.61 (m, 1H; Asp-Hα), 4.46 (m, 1H; Pro-Hα), 4.11 (dd, J=8.3, 4.7 Hz, 1H; Pro-Hα), 3.87 (m, 1H;

Pro-Hδ), 3.61 (s, 3H; Asp-OCH_3), 3.60 (m, 1H; Pro-Hδ), 3.45 (m, 1H; Pro-Hδ), 3.23 (m, 1H; Pro-Hδ), 2.78, (m, 1H; Asp-Hβ), 2.60 (m, 1H; Asp-Hβ), 2.40 (m, 1H; Pro-Hβ), 2.26 (m, 1H; Pro-Hβ), 2.94–1.72 (m, 6H; Pro-Hβ, Pro-Hγ). ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C): δ 172.36, 171.91, 171.67, 171.56, 60.37, 59.16, 53.12, 49.51, 47.15, 46.37, 36.38, 29.81, 28.85, 25.13, 24.41. HRMS (ESI) *m*/*z*: calcd for $C_{15}H_{24}N_3O_6$ 342.1665; found, 342.1654.

4.2.6. Fmoc-Cys(SO_3H)-OH (8). Fmoc-Cys(SO_3H)-OH **8** was prepared as described in Refs. 13 and 14. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 12.69 (br s, 1H; CO_2H), 7.86 (d, *J*=7.7 Hz, 2H; ArH), 7.68 (d, *J*=7.1 Hz, 2H; ArH), 7.49 (d, *J*=7.0 Hz, 1H; OCONH), 7.38 (t, *J*=7.3 Hz, 2H; ArH), 7.31 (t, *J*=7.0 Hz, 2H; ArH), 4.28 (m, 1H; Hα), 4.24 (m, 3H; Fmoc CH and CH_2), 2.92 (d, *J*=5.5 Hz, 2H; Hβ). ^{13}C NMR (100.6 MHz, DMSO-d_6, 25 °C): δ 173.2, 156.5, 144.7, 144.6, 141.5, 128.5, 128.0, 126.1, 121.0, 66.7, 52.2, 47.4.

4.3. General procedure for aldol reactions

The peptide (0.004 mmol, 1 mol % or 0.04 mmol, 10 mol %) was suspended in a solution of *N*-methylmorpholine (0.5 μl, 0.004 mmol or 4.6 μl, 0.04 mmol (standard solution in acetone)) in acetone (2 ml, 27.2 mmol) and 4-nitrobenzaldehyde (60 mg, 0.40 mmol) was added. The reaction mixture was stirred for 4–24 h, then quenched with half saturated NH_4Cl solution (2 ml). The mixture was extracted with Et_2O (4×5 ml), and the combined extracts were washed with brine and dried over $MgSO_4$. The filtered solution was concentrated in vacuo and purified by flash chromatography on silica gel (15 g, 40% EtOAc in pentanes) to afford the aldol product. Enantiomeric excess was determined by chiral HPLC. For a full characterization of the aldol product see Refs. 7 and 15.

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