Supporting Information for *Organic Letters*

Increased Structural Complexity Leads to Higher Activity: Peptides as Efficient and Versatile Catalysts for Asymmetric Aldol Reactions

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General aspects: 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 F₂₅₄ plates. Compounds were visualized by UV, ceric ammonium molybdate (CAM), KMnO₄ and ninhydrin. Flash chromatographies were performed using Merck silica gel 60, particle size 40 - 63 µm. For ion exchange chromatographies Dowex[®] 1x2-400 from Sigma-Aldrich was used. ¹H and ¹³C NMR spectra were recorded on a Bruker DPX 500 spectrometer. Chemical shifts are reported in ppm 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 spectrometry. HPLC analysis were carried out on LiChrospher 100 RP-18e 5 µm (250 mm x 4.6 mm) from Merck, Nucleosil 100-5, (250 mm x 4.6 mm) from Macherey-Nagel and Chiralcel AS, (250 mm x 4.6 mm) from Daicel. Chiral GC analysis were carried out on β-Cyclodextrin (SE54) from Brechbühler AG and FS-HYDRODEX[®] β-3P-Heptakis-(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin from Macherey-Nagel. TentaGel S NH₂ was purchased from Rapp Polymere, Tübingen (Germany).

Solid-phase synthesis

General procedure for peptide couplings: Fmoc-amino acid (3 eq) and 1-hydroxybenzotriazole (3 eq) dissolved in the minimum amount of DMF necessary were added to the suspension of amino-functionalized resin in CH_2Cl_2 (200 mM). The mixture was agitated for 2 min before adding diisopropylcarbodiimide (3 eq) and agitating for another 2 h. The suspension was washed with DMF (3x) and CH_2Cl_2 (5x).

General procedure for Fmoc-deprotections: To the resin (preswollen in DMF) was added 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 (7x) and CH_2Cl_2 (5x).

General procedure for Alloc-deprotections: $\text{Pd}(\text{PPh}_3)_4$ (3 mol%) followed by pyrrolidine (3 eq) was added to the suspension of the Alloc-protected resin in CH_2Cl_2 under Argon. The mixture was agitated for 1 h, drained and washed with CH_2Cl_2 (5x). The process was repeated to ensure complete deprotection.

General procedure for acid-labile protecting group deprotections on Tentagel resin: The acidic “deprotection cocktail” consisting of trifluoroacetic acid (TFA) (84% v/v), phenol (5%), H_2O (5%), thioanisole (5%) and triisopropylsilane (1%) was added to the resin. The resin was agitated for 5 min, drained and treated again under the acidic conditions for further 1.5 h before washing with CH_2Cl_2 (5x), $\text{CH}_2\text{Cl}_2:\text{NEt}_3$ (9:1) (5x) and CH_2Cl_2 (5x). The Boc-group was removed by a mixture of TFA: CH_2Cl_2 (1:2) within 30 min.

Both the couplings and the deprotections were monitored by the qualitative Kaiser test.¹⁴ Quantitative Fmoc-monitoring was carried out as spot checks.

Combinatorial Experiments

Tripeptide library: The library was prepared by the split-and-mix protocol after Fmoc-deprotection of the bifunctional linker **A** (for the preparation of **A** see ref. 5). The ketone was introduced after Alloc-deprotection by coupling of 4-oxo-pentanoic acid (levulinic acid) following the standard protocol for peptide couplings. The side chain protecting groups were removed following the standard protocol.

Synthesis of the dye-marked benzaldehyde **1**

4-Carboxybenzaldehyde **B** (240 mg, 1.60 mmol) and the amine **C**¹⁵ (250mg, 0.80 mmol) were suspended in CH_2Cl_2 (10 ml). After the addition of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (457 mg, 2.40 mmol) the reaction mixture was stirred for 4 h at room temperature and then extracted twice with phosphate buffer (0.5 M, pH 5.5, 50 ml each). The aqueous layers were extracted three times with CH_2Cl_2 (50 ml each), washed with brine (50 ml) and dried over Na_2SO_4 . Filtration and evaporation of the solvent at reduced

pressure yielded the crude product which was purified by flash chromatography on silica gel (gradient of CH₂Cl₂:CH₃OH from 100:0.5 to 100:2) to yield the dye-marked benzaldehyde **1** (200 mg, 56 %) as a red solid.

¹H NMR (500 MHz, CDCl₃, 25°C): δ = 10.06 (s, 1H), 8.29 (m, 2H), 7.9 (m, 8H), 6.85 (m, 2H), 6.65 (t, J = 5.0 Hz, 1H), 3.74 (m, 4H), 3.56 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H); ¹³C NMR (125.8 MHz, CDCl₃, 25°C): δ = 191.4, 166.9, 156.6, 151.3, 147.3, 143.8, 139.1, 138.3, 129.9, 127.6, 126.3, 124.7, 122.6, 111.4, 49.1, 45.8, 38.2, 12.3; MS (FAB): m/z (%): 446 (72%) [$M+H$]⁺, 283 (100%) [dye-CH₂], 133 (65%) [M -dyeNH]⁺.

Combinatorial assay: For each screening a total of at least three theoretical copies of the library was used in order to ensure the presence of each library member.¹⁶

The tripeptide library (5 mg) was placed in a 2 ml syringe equipped with a filter and washed with CH₂Cl₂ (5x). A solution of the dye-marked benzaldehyde **1** (2.5 mM) was added and the mixture was agitated for 2 h before washing with CH₂Cl₂ (6x), DMF (3x), 1 M HCl (3x), DMF (3x), CH₂Cl₂:TFA (3:1) (3x), CH₂Cl₂ (3x), ⁱPrOH:NEt₃ (3:1) (3x), ⁱPrOH (3x); 3ml each for 1-2 min. Active red beads were visualized and isolated under a low power microscope (magnification: 40 times).

Peptides found in the combinatorial assay of the tripeptide library with the dye-marked benzaldehyde 1 using a) DMF and b) CH₃OH as solvent

Note that the enantiomeric tripeptides could not be selected in the combinatorial screening since in each position of the library both L-Pro and D-Pro but only either L-Asp or D-Asp and either L-Ala or D-Ala were used.⁸

a) DMF

AA3	AA2	AA1
L-Pro	L-Asp	L-Ala
L-Pro	D-Glu	D-Asp
L-Pro	D-Gln	D-Asp
L-Pro	D-Pro	D-Asp
L-Pro	D-Pro	D-Asp
D-Pro	D-Pro	D-Asp
D-Pro	D-Pro	D-Asp
D-Pro	L-Val	D-Asp
L-Pro	D-Leu	D-Asp
L-Pro	D-Leu	D-Asp

b) CH₃OH

AA3	AA2	AA1
D-Pro	L-Asp	L-Glu
L-Pro	D-Ala	D-Asp
L-Pro	D-Ala	D-Asp
L-Pro	D-Ala	D-Asp
L-Pro	D-Ala	D-Asp
L-Pro	D-Tyr	L-Glu
L-Pro	D-Tyr	D-Asp
L-Pro	D-Tyr	D-Asp
L-Pro	D-His	D-Asp
D-Pro	L-Arg	L-Glu
D-Pro	L-Arg	L-Glu
L-Pro	D-Gln	Gly

Solution phase aldol reactions

General procedure: Peptide **3** (0.066 mmol, 1 mol%) was suspended in acetone (25 ml, 0.34 mol) and the aldehyde (6.62 mmol) was added. The reaction mixture was stirred for between 4-72 h, then quenched with half saturated NH_4Cl -solution (25 ml). The mixture was extracted with Et_2O (4 x 20 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 (90 g, $\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$, 1:3) to afford the aldol product. The obtained aldol products were identical to the ones described by List.¹¹ Ee's were determined either by chiral HPLC ($\text{R} = 4\text{-NO}_2\text{Ph}$) or chiral GC ($\text{R} = \text{Ph}$, *i*-Pr, *c*-hex, *n*-Pent).

The use of peptides like H-Pro-D-Ala-OH **4** or H-Pro-Pro-OH **5** as catalysts resulted in low yields ($\leq 40\%$) and low enantioselectivities ($< 30\%$), indicating that the relative position of the carboxylic acid and the secondary amine functionalities to each other is crucial. No catalysis was observed, when acetylated peptides were tested as catalysts.

Peptides 2 and 3: The peptides 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 $\text{TFA}:\text{CH}_2\text{Cl}_2$ (1:1) for 2 hours. Filtration and removal of all volatiles under reduced pressure followed by precipitation with $\text{Et}_2\text{O}:\text{pentane}$ (1:1) afforded the TFA-salts of peptides **2** and **3** that were eluted over an ion exchange resin (Dowex[®] 1x2-400 – acetate form) and lyophilized.

H-L-Pro-D-Ala-D-Asp-NH₂ (2): ¹H NMR (500 MHz, D_2O , 22°C): $\delta = 4.40$ (dd, $J = 7.9$ Hz, 5.3 Hz, 1H; Asp-H α), 4.27 (dd, $J = 8.6$ Hz, 6.7 Hz, 1H; Pro-H α), 4.22 (q, $J = 7.2$ Hz, 1H; Ala-H α), 3.27 (m, 2H; Pro-H δ), 2.54 (dd, $J = 15.9$ Hz, 5.3 Hz; 1H, Asp-H β), 2.48 (dd, $J = 15.9$ Hz, 7.9 Hz, 1H; Asp-H β'), 2.31 (m, 1H; Pro-H β), 1.92 (m, 3H; Pro-H β'),

Pro-H γ), 1.26 (d, $J = 7.2$ Hz, 3H; Ala-H β); ^{13}C NMR (125.8 MHz, D_2O , 22°C): $\delta = 177.6, 176.0, 174.3, 169.7, 59.7, 51.3, 50.0, 46.4, 38.4, 29.5, 23.7, 16.2$; MS (ESI): m/z (%): 301.4 (100) $[M+H]^+$.

H-L-Pro-L-Pro-L-Asp-NH₂ (3): The ^1H and ^{13}C NMR spectra show a double set of peaks in a ratio of 5:1 due to *s-trans* and *s-cis* conformers around the tertiary amide. major conformer: ^1H -NMR (500 MHz, D_2O , 22°C): $\delta = 4.63$ (dd, $J = 8.3$ Hz, 6.3 Hz, 1H; Pro¹-H α), 4.53 (dd, $J = 7.3$ Hz, 5.9 Hz, 1H; Asp-H α), 4.48 (dd, $J = 8.1$ Hz, 6.1 Hz, 1H; Pro²-H α), 3.70 (m, 1H; Pro²-H δ), 3.60 (m, 1H; Pro²-H δ), 3.44 (m, 1H; Pro¹-H δ), 3.39 (m, 1H; Pro¹-H δ), 2.68 (dd, $J = 5.8$ Hz, 3.9 Hz, 1H; Asp-H β), 2.57 (m 1H; Asp-H β), 2.35 (m, 1H; Pro¹-H β), 2.31 (m, 1H; Pro²-H β), 2.11-1.91 (m, 6H; Pro¹-H β , Pro²-H β , Pro¹-2H γ , Pro²-2H γ); ^{13}C -NMR (125.8 MHz, D_2O , 22°C): $\delta = 177.0, 175.7, 173.4, 168.3, 60.6, 59.1, 51.1, 47.7, 46.5, 38.0, 29.2, 28.3, 24.5, 23.8$; minor conformer: ^1H -NMR (500 MHz, D_2O , 22°C): $\delta = 4.67$ (dd, $J = 10.1$ Hz, 4.2 Hz, 1H; Asp-H α), 4.56 (dd, $J = 8.5$ Hz, 2.8 Hz, 1H; Pro²-H α), 4.16 (dd, $J = 8.5$ Hz, 7.4 Hz, 1H; Pro¹-H α), 3.65 (m, 1H; Pro²-H δ), 3.53 (m, 1H; Pro²-H δ), 3.44 (m, 1H; Pro¹-H δ), 3.39 (m, 1H; Pro¹-H δ), 2.74, (dd, $J = 15.8$ Hz, 4.3 Hz, 1H; Asp-H β), 2.48 (m, 1H; Asp-H β), 2.41 (m, 1H; Pro¹-H β), 2.18 (m, 1H; Pro²-H β), 2.01 (m, 1H; Pro²-H β), 2.11-1.91 (m, 5H; Pro¹-H β , Pro¹-2H γ , Pro²-2H γ); ^{13}C -NMR (125.8 MHz, D_2O , 22°C): $\delta = 177.1, 175.5, 173.0, 168.3, 59.8, 58.9, 51.5, 47.9, 46.6, 38.7, 31.7, 28.5, 23.9, 21.9$; MS (ESI): m/z (%): 327.4 (100) $[M+H]^+$, 349.5 (24) $[M+Na]^+$.

Conformational searches using MacroModel 8.0

The calculations used the OPLS-AA¹⁷ and the GB/SA model for chloroform.¹⁸ Searching was performed using the MCMM method in blocks of 20000 steps.

References

- (14) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595-598.
- (15) Zhang, J.; Drugeon, G.; L'hermite, N. *Tetrahedron Lett.* **2001**, *41*, 3599-3601.
- (16) (a) Burgess, K.; Liaw, A. I.; Wang, N. Y. *J. Med. Chem.* **1994**, *37*, 2985-2987. (b) Zhao, P.-L.; Zambias, R.; Bolognese, J. A.; Boulton, D.; Chapman, K. T. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 10212-10216.
- (17) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225-11236.
- (18) Qiu, D.; Shenkin, P. S.; Hollinger, F. P.; Still, W. C. *J. Phys. Chem. A* **1997**, *101*, 3005-3014.

^1H and ^{13}C NMR spectra of the dye-marked benzaldehyde **1** and the peptides **2** and **3**:

dye-marked benzaldehyde **1**:

peptide 2:

peptide 3: