A Stereoselective Tripeptide Catalyst for Conjugate Addition Reactions of Acetophenones to Dicyanoolefins

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1. General Aspects and Materials

Reagents and materials 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 aluminium sheets. Visualization of the compounds was achieved by UV or KMnO₄. Flash chromatography and plug filtrations were performed using Fluka silica gel 60 (particle size 0.040 – 0.063 mm, 200 – 400 mesh). Solvents for extraction and chromatography were of technical quality and distilled before use. ¹H and ¹³C NMR spectra were recorded on a Varian Mercury-VX 300, a Bruker AV 300 (300 MHz/75 MHz), a Bruker DRX 400, a Bruker AV III 400 (400 MHz/100 MHz) or a Bruker AV III 600 (600 MHz/150 MHz). 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 tetramethylsilane (TMS) using the residual solvent signals. SFC analyses were performed on an analytical SFC with a diode array detector ACQUITY-UPLC-PDA from Waters using chiral columns (Trefoil, AS, AD, IA, Whelk, IC, OD, OJ) (150 mm x 30 mm) from Daicel or Waters under the reported conditions. High-resolution electron ionization (HR-EI) mass spectra were measured on a Waters Micromass AutoSpec Ultima spectrometer. A Bruker maXis (UHR-TOF) was used for high-resolution electrospray ionisation (HR-ESI) mass spectrometry. High-resolution MALDI spectra were acquired on a Bruker solariX 94 (ESI/MALDI-FT-ICR) and a Bruker Ultra-Flex II (MALDI-TOF) spectrometer.
2. Synthesis and Analytical Data of Peptides A-K

2.1 General Protocols for Solid Phase Peptide Synthesis

Peptides were prepared on solid phase using Rink Amide resin. The general protocol for Fmoc/tBu peptide synthesis was followed according to the general procedures below.

*General procedure for peptide couplings:* $i$Pr$_2$NEt (6 equiv.) was added to a solution of Fmoc-Xxx-OH (3 equiv.) and HATU (3 equiv.) in DMF. The solution of the activated amino acid ($\approx$ 100 mM) was added to the amino-functionalized resin, preswollen in CH$_2$Cl$_2$ and the mixture was agitated for 1 h before washing with DMF (3 ×) and CH$_2$Cl$_2$ (3 ×).

*General procedure for Fmoc-deprotections:* A solution of 20% piperidine in DMF was added to the resin (preswollen in CH$_2$Cl$_2$) and 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$_2$Cl$_2$ (3 ×). The couplings as well as the deprotections were monitored by qualitative Kaiser (primary amines),[1] and chloranil tests (secondary amines).[2]

*General procedure for side chain deprotection and cleavage of the peptides from the solid support:* The peptides were deprotected and cleaved from the resin by stirring in a mixture of TFA/TIS/H$_2$O (95:2.5:2.5) 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$_2$O afforded the peptides as their TFA salts. The peptides were redissolved in MeCN/H$_2$O 1:1, dried by lyophilisation and used without further purification.

2.2 Analytical Data of Peptides A–K

*TFA·H-D-Pro-Pro-Glu-NH$_2$ (A):* The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[3]

*TFA·H-D-Pro-Pro-Aad-NH$_2$ (B):* The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[3]

*TFA·H-D-Pro-Pro-Asp-NH$_2$ (C):* The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[3]
**TFA-H-D-Pro-Pro-Gln-NH₂ (D):** The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.⁴

**TFA-H-D-Pro-Pro-Arg-NH₂ (E):** The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Pro-Pro containing peptides, coexistence of the trans and cis configured amide bond was observed in the NMR spectra. Only the signals of the trans conformer are given. ¹H NMR (400 MHz, D₂O) δ = 4.67 (dd, J = 8.9, 6.8 Hz, 1H), 4.49 (dd, J = 9.0, 3.7 Hz, 1H), 4.29 (dd, J = 8.9, 5.5 Hz, 1H), 3.76 (ddd, J = 12.3, 6.7, 3.9 Hz, 1H), 3.62 (dt, J = 10.0, 6.9 Hz, 1H), 3.53 – 3.33 (m, 2H), 3.32 – 3.19 (m, 2H), 2.66 – 2.50 (m, 1H), 2.47 – 2.27 (m, 1H), 2.21 – 1.57 (m, 7H). ¹³C NMR (101 MHz, D₂O) δ = 176.57, 174.36, 168.53, 157.00, 61.03, 59.52, 47.95, 46.89, 40.70, 29.82, 28.31, 28.17, 24.74, 24.52, 24.15. HRMS (MALDI): m/z calcd for C₁₆H₃₀N₂O₅⁺: 368.2405 [M + H]⁺; found: 368.2404.

**TFA-H-D-Pro-Pro-Ser-NH₂ (F):** The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Pro-Pro containing peptides, coexistence of the trans and cis configured amide bond was observed in the NMR spectra. Only the signals of the trans conformer are given. ¹H NMR (400 MHz, D₂O) δ = 4.62 (dd, J = 8.9, 6.9 Hz, 1H), 4.50 (dd, J = 8.6, 3.8 Hz, 1H), 4.41 (t, J = 5.2 Hz, 1H), 3.87 (dd, J = 5.3, 1.7 Hz, 2H), 3.80 – 3.66 (m, 1H), 3.67 – 3.54 (m, 1H), 3.51 – 3.28 (m, 2H), 2.64 – 2.42 (m, 1H), 2.42 – 2.24 (m, 1H), 2.05 (m, 6H). ¹³C NMR (101 MHz, D₂O) δ = 174.10, 174.08, 168.15, 60.98, 60.79, 59.24, 55.41, 47.66, 46.57, 29.49, 28.00, 24.20, 23.84. HRMS (MALDI): m/z calcd for C₁₃H₂₃N₄O₄⁺: 299.1714 [M + H]⁺; found: 299.1714.

**TFA-H-D-Pro-Pro-Thr-NH₂ (G):** The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Pro-Pro containing peptides, coexistence of the trans and cis configured amide bond was observed in the NMR spectra. Only the signals of the trans conformer are given. ¹H NMR (400 MHz, D₂O) δ = 4.64 (dd, J = 8.9, 6.8 Hz, 1H), 4.59 – 4.52 (m, 1H), 4.32 (d, J = 4.2 Hz, 1H), 4.30 – 4.18 (m, 1H), 3.75 (dt, J = 10.2, 6.4 Hz, 1H), 3.62 (dt, J = 10.2, 6.9 Hz, 1H), 3.54 – 3.33 (m, 2H), 2.63 – 2.49 (m, 1H), 2.34 (tt, J = 8.7, 3.2 Hz, 1H), 2.19 – 1.96 (m, 6H), 1.23 (d, J = 6.5 Hz, 3H). ¹³C NMR (101 MHz, D₂O) δ = 174.33, 174.31, 168.15, 67.00, 60.82, 59.24, 58.85, 47.66, 46.60, 29.53, 28.03, 24.25, 23.86, 18.77. HRMS (MALDI): m/z calcd for C₁₄H₂₅N₄O₄⁺: 313.1870 [M + H]⁺; found: 313.1871.

**TFA-H-D-Pro-Pro-Tyr-NH₂ (H):** The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Pro-Pro containing peptides, coexistence of the trans and cis configured amide bond was observed in the NMR spectra. Only the signals of the trans conformer are given. ¹H NMR (400 MHz, D₂O) δ = 7.21 – 7.13 (m, 2H), 6.91 – 6.82 (m, 2H), 4.67 – 4.60 (m, 1H), 4.57 (dd, J = 9.0, 6.5 Hz, 1H), 4.40 (dd, J = 9.0, 3.7 Hz, 1H), 3.67 (ddd, J = 10.0, 7.3, 4.5 Hz, 1H), 3.62 – 3.51 (m, 1H), 3.51 – 3.31 (m, 2H), 3.19 – 3.06 (m, 1H), 2.96 (dd, J = 14.0, 9.1 Hz, 1H), 2.55 (ddt, J = 13.0, 9.1, 6.5 Hz, 1H), 2.35 – 1.66 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ = 175.68, 173.60, 168.46, 154.36, 130.44, 128.34, 115.40, 60.86, 59.30, 54.67, 47.55, 46.65, 35.75, 29.34, 27.99, 23.94, 23.89. Isolated signals of a minor conformer: ¹H NMR (400 MHz, D₂O) δ = 7.27 – 7.21 (m, 2H), 4.15 (dd, J = 9.1, 7.6 Hz, 1H), 3.31 – 3.19 (m, 1H), 2.86 (dd, J = 14.2, 11.3 Hz, 1H), 1.49 (m, 1H), 1.34 – 1.20 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ = 175.56, 173.42, 168.15, 154.44,
128.59, 117.76, 114.86, 59.96, 58.66, 54.96, 47.81, 46.39, 36.14, 31.74, 27.89, 24.25, 21.39. HRMS (MALDI): $m/z$ calcd for $C_{19}H_{27}N_4O_4^+$: 375.2027 $[M + H]^+$; found: 375.2026.

**TFA-H-Pro-D-Pro-D-(4-NO$_2$)Phe-NH$_2$ (I):** The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Pro-Pro containing peptides, coexistence of the trans and cis configured amide bond was observed in the NMR spectra. Only the signals of the trans conformer are given. $^1H$ NMR (400 MHz, D$_2$O) $\delta = 8.37 – 8.07$ (m, 2H), 7.63 – 7.47 (m, 2H), 4.71 (dd, $J = 9.3$, 6.3 Hz, 1H), 4.63 (dd, $J = 8.9$, 7.0 Hz, 1H), 4.39 (dd, $J = 9.0$, 3.9 Hz, 1H), 3.76 – 3.63 (m, 1H), 3.62 – 3.51 (m, 1H), 3.50 – 3.30 (m, 3H), 3.18 (dd, $J = 14.0$, 9.4 Hz, 1H), 2.55 (ddd, $J = 12.9$, 8.9, 6.6 Hz, 1H), 2.29 – 2.16 (m, 1H), 2.16 – 1.68 (m, 6H). $^{13}$C NMR (101 MHz, D$_2$O) $\delta = 174.95$, 173.71, 168.38, 146.72, 144.80, 130.13, 123.75, 60.82, 59.29, 54.01, 47.55, 46.65, 46.32, 29.43, 27.98, 24.04, 23.89. HRMS (MALDI): $m/z$ calcd for $C_{21}H_{25}F_3N_3O_7^+$: 516.1712 $[M + TFA + H]^+$; found: 516.1708.

**TFA-H-D-Pro-Pro-Trp-NH$_2$ (J):** The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Pro-Pro containing peptides, coexistence of the trans and cis configured amide bond was observed in the NMR spectra. Only the signals of the trans conformer are given. $^1H$ NMR (400 MHz, D$_2$O) $\delta = 7.70$ (dt, $J = 7.9$, 1.0 Hz, 1H), 7.51 (dt, $J = 8.3$, 1.0 Hz, 1H), 7.33 – 7.22 (m, 2H), 7.17 (ddd, $J = 8.0$, 7.0, 1.1 Hz, 1H), 4.69 (dd, $J = 8.3$, 6.8 Hz, 1H), 4.55 (dd, $J = 8.8$, 7.0 Hz, 1H), 4.36 (dd, $J = 8.9$, 3.8 Hz, 1H), 3.65 – 3.03 (m, 6H), 2.52 (ddt, $J = 13.1$, 9.0, 6.5 Hz, 1H), 2.22 – 1.84 (m, 4H), 1.80 – 1.59 (m, 2H). $^{13}$C NMR (101 MHz, D$_2$O) $\delta = 176.06$, 173.55, 168.39, 136.12, 126.80, 124.44, 121.91, 119.27, 118.36, 111.81, 109.02, 60.88, 59.25, 53.95, 47.52, 46.65, 9.20, 27.97, 26.67, 23.90, 23.86. HRMS (MALDI): $m/z$ calcd for $C_{23}H_{27}F_3N_3O_5^+$: 510.1970 $[M + TFA + H]^+$; found: 510.1965.

**TFA-H-D-Pro-Pro-His-NH$_2$ (K):** The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Pro-Pro containing peptides, coexistence of the trans and cis configured amide bond was observed in the NMR spectra. Only the signals of the trans conformer are given. $^1H$ NMR (400 MHz, D$_2$O) $\delta = 8.63$ (s, 1H), 7.34 (s, 1H), 4.70 (dd, $J = 8.7$, 5.8 Hz, 1H), 4.62 (t, $J = 8.0$ Hz, 1H), 4.43 (dd, $J = 8.8$, 3.9 Hz, 1H), 3.70 (dt, $J = 11.7$, 4.7 Hz, 1H), 3.64 – 3.52 (m, 1H), 3.53 – 3.10 (m, 4H), 2.63 – 2.49 (m, 1H), 2.27 (dt, $J = 13.0$, 8.2 Hz, 1H), 2.20 – 1.82 (m, 4H). $^{13}$C NMR (101 MHz, D$_2$O) $\delta = 174.30$, 174.25, 168.54, 133.85, 128.77, 117.61, 61.13, 59.62, 52.55, 47.99, 47.01, 29.86, 28.37, 26.55, 24.55, 24.24. HRMS (MALDI): $m/z$ calcd for $C_{18}H_{24}F_3N_6O_5^+$: 461.1763 $[M + TFA + H]^+$; found: 461.1763.

3. **Analytical Data of the γ,γ'-Dicyanoacetophenones**

**General Procedure**

H-D-Pro-Pro-Glu-NH$_2$ (0.2 equiv., 20 µmol) was added to a solution of acetophenone (5 equiv., 500 µmol, 58.6 µl) and dicyanostyrene (1 equiv., 100 µmol, 15.4 mg) in methanol (400 µl). The reaction mixture was stirred for 3 d and the product was purified by flash column chromatography (hexane/ethyl acetate).
(S)-2-(3-Oxo-1,3-diphenylpropyl)malononitrile

Yield = 85%. The analytical data are in accordance with previously published data.[5] 
1H NMR (300 MHz, CDCl3) δ = 8.01 – 7.92 (m, 2H), 7.71 – 7.58 (m, 1H), 7.51 (dd, J = 8.3, 6.9 Hz, 2H), 7.41 (s, 3H), 4.63 (d, J = 5.0 Hz, 1H), 3.95 (dt, J = 8.2, 5.3 Hz, 1H), 3.75 – 3.56 (m, 2H).
The enantiomeric excess was determined by chiral stationary phase SFC using the AD column (10% MeOH, 40 °C) at 2 mLmin⁻¹, UV-detection at λ = 254 nm: tR (minor) = 1.76 min, tR (major) = 2.06 min, e.r. = 87:13.

(S)-2-(1-(4-Nitrophenyl)-3-oxo-3-phenylpropyl)malononitrile

Yield = 90%. The analytical data are in accordance with previously published data.[5] 
1H NMR (300 MHz, CDCl3) δ = 8.10 – 7.85 (m, 2H), 7.72 – 7.57 (m, 1H), 7.55– 7.37 (m, 7H), 4.66 (d, J = 5.1 Hz, 1H), 3.96 (dt, J = 8.1, 5.4 Hz, 1H), 3.83 – 3.52 (m, 2H).
The enantiomeric excess was determined by chiral stationary phase SFC using the OD column (15% iPrOH, 40 °C) at 2 mLmin⁻¹, UV-detection at λ = 254 nm: tR (minor) = 2.82 min, tR (major) = 3.20 min, e.r. = 87:13.

(S)-2-(1-(4-Chlorophenyl)-3-oxo-3-phenylpropyl)malononitrile

Yield = 78%. The analytical data are in accordance with previously published data.[6] 
1H NMR (300 MHz, CDCl3) δ = 8.05 – 7.86 (m, 2H), 7.70 – 7.57 (m, 1H), 7.58 – 7.46 (m, 2H), 7.46 – 7.35 (m, 4H), 4.63 (d, J = 5.1 Hz, 1H), 3.95 (dt, J = 8.1, 5.4 Hz, 1H), 3.77 – 3.48 (m, 2H).
The enantiomeric excess was determined by chiral stationary phase SFC using the AD column (15% MeOH, 40 °C) at 2 mLmin⁻¹, UV-detection at λ = 254 nm: tR (minor) = 1.22 min, tR (major) = 1.39 min, e.r. = 69:31.
4. $^1$H and $^{13}$C NMR Spectra of Peptides E–K
5. $^1$H NMR Spectra of the $\gamma,\gamma$-Dicyanoacetophenones
6. Chiral Stationary Phase SFC Chromatograms

(S)-2-(3-Oxo-1,3-diphenylpropyl)malononitrile

AD column (10% MeOH, 40 °C) at 2 mLmin⁻¹, UV-detection at λ = 254 nm: \( t_R \) (minor) = 1.76 min, \( t_R \) (major) = 2.06 min, e.r. = 87:13.

(S)-2-((1-(4-Nitrophenyl)-3-oxo-3-phenylpropyl)malononitrile

AD column (15% iPrOH, 40 °C) at 2 mLmin⁻¹, UV-detection at λ = 254 nm: \( t_R \) (minor) = 2.82 min, \( t_R \) (major) = 3.20 min, e.r. = 87:13.
(S)-2-(1-(4-Chlorophenyl)-3-oxo-3-phenylpropyl)malononitrile

AD column (15% MeOH, 40 °C) at 2 mLmin⁻¹, UV-detection at λ = 254 nm: \(t_R\) (minor) = 1.22 min, \(t_R\) (major) = 1.39 min, e.r. = 68:31.

(S)-2-(3-Oxo-3-phenyl-1-(p-tolyl)propyl)malononitrile

OD column (15% iPrOH, 40 °C) at 2 mLmin⁻¹, UV-detection at λ = 254 nm: \(t_R\) (minor) = 1.10 min, \(t_R\) (major) = 1.33 min, e.r. = 87:13.
(S)-2-(1-(4-Methoxyphenyl)-3-oxo-3-phenylpropyl)malononitrile

OD column (15% PrOH, 40 °C) at 2 mL min⁻¹, UV-detection at λ = 254 nm: tᵣ (major) = 1.71 min, 
tᵣ (minor) = 1.80 min, e.r. = 87:13.

(S)-2-(3-(4-Methoxyphenyl)-3-oxo-1-phenylpropyl)malononitrile

OD column (15% PrOH, 40 °C) at 2 mL min⁻¹, UV-detection at λ = 254 nm: tᵣ (major) = 1.60 min, 
tᵣ (minor) = 1.84 min, e.r. = 87:13.
(S)-2-(3-(3,5-Bis(trifluoromethyl)phenyl)-3-oxo-1-phenylpropyl)malononitrile

OD column (15% PrOH, 40 °C) at 2 mL min⁻¹, UV-detection at λ = 254 nm: tᵣ (major) = 0.98 min, tᵣ (minor) = 1.21 min, e.r. = 53:47.

7. References

2. T. Vojkovsky, Peptide Res. 1995, 8, 236.