Supplementary Material (ESI) for Chemical Communications

Small Peptides as Modular Catalysts for the Direct Asymmetric Aldol Reactions: Ancient Peptides with Aldolase Enzyme Activity

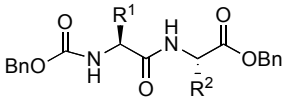
Webiao Zou, Ismail Ibrahem, Pawel Sziedzic, Henrik Sundén and Armando Córdova*

The Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE-10691Stockholm, Sweden

Supporting Information

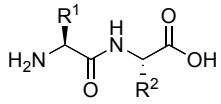
General. Chemicals and solvents were either purchased *puriss p.A.* from commercial suppliers or purified by standard techniques. The amino acids used for the synthesis of peptides were purchased from BACHEM. For thin-layer chromatography (TLC), silica gel plates Merck 60 F254 were used and compounds were visualized by irradiation with UV light and/or by treatment with a solution of phosphomolybdic acid (25 g), $Ce(SO_4)_2 \cdot H_2O$ (10 g), conc. H_2SO_4 (60 mL), and H_2O (940 mL) followed by heating or by treatment with a solution of *p*-anisaldehyde (23 mL), conc. H_2SO_4 (35 mL), acetic acid (10 mL), and ethanol (900 mL) followed by heating. Flash chromatography was performed using silica gel Merck 60 (particle size 0.040-0.063 mm), ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian AS 400. Chemical shifts are given in δ relative to tetramethylsilane (TMS), the coupling constants *J* are given in Hz. The spectra were recorded in $CDCl_3$ as solvent at room temperature, TMS served as internal standard (δ = 0 ppm) for ^{1}H NMR, and $CDCl_3$ was used as internal standard (δ = 77.0 ppm) for ^{13}C NMR. HPLC was carried out using a Waters 2690 Millennium with photodiode array detector. GC was carried out using a Varian 3800 GC Instrument. Chiral GC-column used: CP-Chirasil-Dex CB 25m x 0.32mm. Optical rotations were recorded on a Perkin Elemer 241 Polarimeter (λ = 589 nm, 1 dm cell). High-resolution mass spectra were recorded on an IonSpec FTMS mass spectrometer with a DHB-matrix.

General procedure for the preparation of di-peptides:



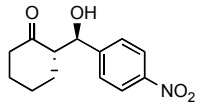
A stirred solution of Cbz-protected α-amino acid (10mmol) in 30 mL dichlomethane is cooled down to -15 °C and neutralized with NMM (N-methylmorpholine, 10 mmol). Next, isobutyl chlorocarbornate (10 mmol) was added. After 20 minutes of stirring a solution of the salt of amino acid ester (10 mmol) and NMM (10 mmol) in 30 mL dichlomethane was added. The mixture is stirred at -15 °C for 1 hour and is next allowed to warm up to room temperature. TLC using AMC stain monitored the reaction progress. At the completion, wash the reaction mixture was extracted with 1N HCl (3×20 mL), 1N Na_2CO_3 (3×20 mL), and brine (30 mL). The organic layer was dried with sodium sulphate. The dipeptide product was checked with TLC and NMR, in most case it was

pure enough for next step. If not, the product was purified by silica-gel column chromatography.

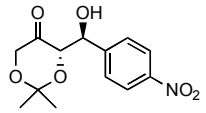


To a solution of protected dipeptide 1g in 20mL methanol, palladium on activated carbon (100 mg, 10wt.%) was added under Argon atmosphere. The reaction mixture was stirred under hydrogen (90 psi) for one day. The reaction was checked by TLC and NMR analyses. At the completion of hydrogenolysis, the Pd/C catalyst was removed by filtration on celite and washed with methanol and water. The combined filtrates were evaporated under reduced pressure. The di-peptide product was checked by NMR analyses and if necessary recrystallization was made in proper solvent.

Typical experimental procedure for the peptide-catalyzed direct asymmetric aldol reactions: A catalytic amount of di-peptide or tri-peptide (0.15 mmol, 30 mol%) was added to a vial containing acceptor aldehyde (0.5 mmol), donor ketone **2** (1.5 mmol), H_2O (5 mmol, 90mL) in DMSO (2 mL). After 1-4 days of vigorously stirring at room temperature the reaction mixture was poured into an extraction funnel that contained brine (5. 0 mL), which was diluted with distilled H_2O (5.0 mL) and EtOAc (15 mL). The reaction vial was also washed with 2 mL of EtOAc, which was poured into the extraction funnel. The aqueous phase was extracted with EtOAc (2x15.0 mL). The combined organic phases were dried with Na_2SO_4 and the solvent removed under reduced pressure. The reaction can also be quenched by directly putting the reaction mixture on a silica-gel column. The crude aldol product was purified by silica-gel column chromatography (EtOAc:pentane-mixtures) to furnish the desired aldol product **2**. The ee of the aldol products **2** were determined by chiral-phase HPLC analysis or chiral-phase GC analyses.

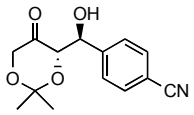


2a:[1] ^{1}H NMR ($CDCl_3$, 400 MHz): 1.52-2.14 (m, 6H), 2.33-2.52 (m, 2H), 2.59 (m, 1H), 3.15 (bs, 1H), 4.90 (d, J = 8.6 Hz, 1H), 7.49 (d, J = 8.7 Hz, 2H), 8.20 (d, J = 8.7 Hz, 2H); ^{13}C NMR (100 MHz): δ =24.6, 27.6, 30.7, 42.6, 57.1, 73.9, 123.5, 127.8, 147.5. 148.4, 214.7; HPLC (Daicel Chiralpak AD, *iso*-hexanes/*i*-PrOH = 80:20, flow rate 0.5 mL/min, λ = 254 nm): major isomer: t_R = 31.12 min; minor isomer: t_R = 24.14 min; $[\alpha]_D$ = +12.8 (c = 1.1, $CHCl_3$); MALDI-TOF MS: 272.0897; $C_{13}H_{15}NO_4$ (M+Na^+: calcd 272.0899).

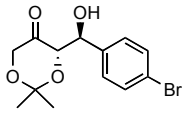


1. A. Córdova, W. Zou, I. Ibrahem, E. Reyes, M. Engqvist, W. W. Liao, *Chem. Commun*. 2005, 3586.

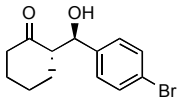
2b:[1] ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.30 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 3.9 (m, 1H), 4.13-4.50 (m, 3H), 5.01 (d, *J* = 7.8 Hz, 1H, C*H*OH), 7.70 (d, *J* = 8.4, 2H, ArH), 8.22 (d, *J* = 8.4, 2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 23.3, 23.4, 66.6, 71.7, 75.8, 101.4, 123.2, 127.9, 138.3, 146.5, 210.6; HPLC (Daicel Chiralpak AD, *iso*-hexanes/*i*-PrOH = 96:4, flow rate 0.5 mL/min, λ = 254 nm): major isomer: t_R = 52.12 min; minor isomer: t_R = 57.02 min; $[\alpha]_D^{25}$ = -131.1 (*c* = 1.2, $CHCl_3$).



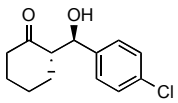
2c:[1] ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.20 (s, 3H, CH_3), 1.37 (s, 3H, CH_3) 3.78 (bs, 1H), 4.06 (d, *J* = 17.3, 1H), 4.19-4.29 (m, 2H), 4.93 (d, *J* = 7.8 Hz, 1H, C*H*OH), 7.51 (d, *J* = 8.4, 2H, ArH), 7.63 (d, *J* = 8.4, 2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 23.2, 23.5, 66.6, 72.0, 76.0, 101.2, 128.2, 128.4, 133.7, 137.8, 210.9; HPLC (Daicel Chiralpak OJ, *iso*-hexanes/*i*-PrOH = 90:10, flow rate 0.5 mL/min, λ = 254 nm): major isomer: t_R = 34.50 min; minor isomer: t_R = 36.52 min; $[\alpha]_D^{25}$ = -101.3 (*c* = 1.0, $CHCl_3$).



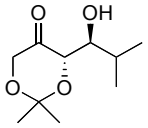
2d:[1] ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.20 (s, 3H, CH_3), 1.37 (s, 3H, CH_3) 3.78 (bs, 1H), 4.03 (d, *J* = 17.3, 1H), 4.19-4.29 (m, 2H), 4.93 (d, *J* = 7.8 Hz, 1H, C*H*OH), 7.52 (d, *J* = 8.4, 2H, ArH), 7.63 (d, *J* = 8.4, 2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 23.2, 23.5, 66.6, 72.0, 76.0, 101.2, 128.2, 128.4, 133.7, 137.8, 210.9; HPLC (Daicel Chiralpak AS, *iso*-hexanes/*i*-PrOH = 97:3, flow rate 0.5 mL/min, λ = 254 nm): major isomer: t_R = 24.10 min; minor isomer: t_R = 30.32 min; $[\alpha]_D^{25}$ = -110.9 (*c* = 1.0, $CHCl_3$).



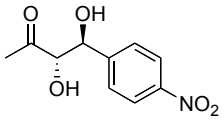
2e:[1] ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.23-1.32 (m, 1H), 1.49-1.58 (m, 2H), 1.61-1.69 (m, 1H), 1.75-1.81 (m, 1H), 2.03-2.10 (m, 1H), 2.30-2.41 (m, 1H), 2.43-2.48 (m, 1H), 2.50-2.59 (m, 1H), 3.99 (bs, 1H), 4.74 (d, *J* = 8.6 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 2H), 8.45 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (100 MHz): δ = 25.0, 28.0, 30.9, 42.8, 57.5, 74.3, 121.9, 128.9, 131.6, 140.3, 215.4; HPLC (Daicel Chiralpak AS, *iso*-hexanes/*i*-PrOH = 90:10, flow rate 1.0 mL/min, λ = 254 nm): major isomer: t_R = 23.04 min; minor isomer: t_R = 21.22 min; $[\alpha]_D$ = +20.5 (c = 1.7, $CHCl_3$).



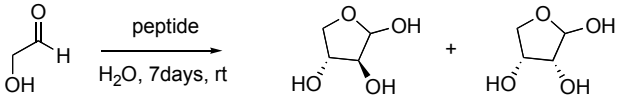
2f:[1] ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.23-1.32 (m, 1H), 1.50-1.58 (m, 2H), 1.60-1.66 (m, 1H), 1.73-1.81 (m, 1H), 2.03-2.10 (m, 1H), 2.29-2.37 (m, 1H), 2.43-2.48 (m, 1H), 2.51-2.57 (m, 1H), 3.99 (d, *J* = 2.9 Hz, 1H), 4.74 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (100 MHz): δ = 24.9, 27.9, 31.0, 42.8, 57.5, 74.2, 128.6, 128.7, 133.7, 139.8, 215.4; HPLC (Daicel Chiralpak AS, *iso*-hexanes/*i*-PrOH = 90:10, flow rate 1.0 mL/min, λ = 254 nm): major isomer: t_R = 22.04 min; minor isomer: t_R = 20.36 min; $[\alpha]_D$ = +21.6 (c = 1.0, $CHCl_3$).



2g:[2] ^{1}H NMR ($CDCl_3$, 400 MHz): 0.88 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H); 1.38 (s, 3H), 1.43 (s, 3H), 1.92-2.01 (m, 1H), 3.07 (d, *J* = 3.2 Hz, 1H), 3.64-3.67 (m, 1H), 3.97 (d, *J* = 17.3 Hz, 1H), 4.22 (dd, *J* = 1.5, 17.3 Hz, 1H); ^{13}C NMR (100 MHz): δ = 15.5, 19.4, 23.8, 24.0, 28.7, 66.7, 74.1, 74.3, 101.1, 212.4; HPLC (Daicel Chiralpak AD, *iso*-hexanes/*i*-PrOH = 95:5, flow rate 0.5 mL/min, λ = 254 nm): major isomer: t_R = 15.21 min; minor isomer: t_R = 12.81 min; $[\alpha]_D$ = -27.74 (c = 1.1, $CHCl_3$).



2h:[3] ^{1}H NMR ($CDCl_3$, 400 MHz): (*anti*:*syn* = 1:1 mixture) 2.01 (s, 3H), 2.35 (s, 3H); 1.38 (s, 3H), 3.10 (bs, 1H, OH), 3.78 (bs, 1H, OH), 4.39 (m, 1H), 4.45 (d, *J* = 4.6 Hz, 1H), 5.07 (d, *J* = 4.5 Hz, 1H), 5.20 (m, 1H), 7.59 (d, *J* = 8.8, 4H), 8.20 (d, *J* = 8.8, 1H). ^{13}C NMR (100 MHz): δ = 26.2, 28.0, 73.1, 74.6, 80.3, 80.9, 123.8, 123.9, 127.3, 127.5, 146.8, 147.7, 207.4, 208.0; HPLC (Daicel Chiralpak OD-H, *iso*-hexanes/*i*-PrOH = 90:10, flow rate 0.5 mL/min, λ = 254 nm): major isomer: t_{Ranti} = 38.5 min; minor isomer: t_{Ranti} = 36.1 min; major isomer: t_{Rsyn} = 32.1 min; minor isomer: t_{Ranti} = 34.0 min.



Typical experimental procedure for peptide-catalyzed asymmetric synthesis of sugars in water: A solution of glycol aldehyde (2 mmol) and di-peptide (30 mol%) in water (2 mL) was stirred at room temperature. The reaction was quenched by removal of the water by lyophilization. Next, methanol (3 mL) was added to the lyophilized powder and the crude sugar mixture was reduced by addition of excess $NaBH_4$ at 0 °C. The reduction was quenched by addition of 2 M HCl at 0 °C and the methanol removed under

2. D. Enders, O. F. Prokopenko, G. Raabe, J. Runsink, *Synthesis* 1996, 1095.
3. Z. Tang, Z. -H. Yang, L. -F. Chun, L -Z. Gong, A. Q. Mi, Y.-Z. Jiang, *Org. Lett.* 2004, **6**, 2285.

reduced pressure. Next, CH_2Cl_2 (5 mL) and pyridine (1 mL), acetic anhydride (2 mL) and DMAP (0.2 mmol) were added to the crude tetrol and hexitol mixture. After 16h water was added (3 mL) and the reaction mixture extracted with CH_2Cl_2 (3x15 mL). The combined organic extract was subsequently washed with 1N HCl, brine, water and the organic phase was dried over anhydrous $NaSO_4$, which was subsequently removed by filtration. Next, the solvent was removed under reduced pressure and the crude peracetylated tetrol and hexitol products were dissolved in EtOAc and analyzed by chiral-phase GC analyses.[4] GC tetra-acetylated erythrol: (CP-Chirasil-Dex CB); T_{inj} = 250 °C, T_{det} = 275 °C, flow = 1.5 mL/min, t_i = 100 °C (10 min), (1.5 °C/ min) t_f = 180 °C (10.0 °C/ min) t_f = 200 °C (10 min): t_R = 36.71 min; GC tetra-acetylated threotol: (CP-Chirasil-Dex CB); T_{inj} = 250 °C, T_{det} = 275 °C, flow = 1.5 mL/min, t_i = 100 °C (10 min), (180 °C/ min) t_f = 200°C (10 min): L-isomer: t_R = 38.56 min; D-isomer: t_R = 38.70 min. GC peracetylated hexitols: (CP-Chirasil-Dex CB); T_{inj} = 250 °C, T_{det} = 275 °C, flow = 1.8 mL/min, t_i = 100 °C (10 min), (1.5 °C/ min) t_f = 180 °C (10.0 °C/ min) t_f = 200 °C (10 min): (The hexitols appeared in the range 63-66 min) major glucitol t_R = 64.70 min; mannitol t_R = 64.30 min; gulitol = 64.78 min; allitol t_R = 62.71 min. The tetra-acetylated tetrols were also isolated by silica-gel column chromatography (EtOAc:pentane-mixtures). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): (*erythro*:*threo*-2:1) 2.06-2.10 (m, 12H, 4xOAc), 4.08 (m, 1H, CH_2), 4.16 (m, 1H, CH_2), 4.30 (m, 2H, CH_2), 5.26 (m, 1H, CH), 5.32 (m, 1H, CH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 20.88, 20.89, 21.0, 62.0, 69.3, 69.4, 170.0, 170.1, 170.6, 170.7.

4. Peracetylated tetrol and hexitol standards were prepared from authentic samples of the commercially available D-tetroses and D-hexoses.