Electronic Supplementary Material for Organic & Biomolecular Chemistry

The small peptide-catalyzed direct asymmetric aldol reaction in water

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Supporting Information

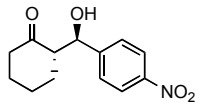
General. Chemicals and solvents were either purchased *puriss p.A.* from commercial suppliers or purified by standard techniques. For thin-layer chromatography (TLC), silica gel plates Merck 60 F254 were used and compounds were visualized by irradiation with UV light and/or by treatment with a solution 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. 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.

Typical experimental procedure for the peptide- and amino acid-catalyzed direct asymmetric aldol reactions in water (Table 1). A catalytic amount of L-amino acid, dipeptide or tripeptide (0.075 mmol, 30 mol%) was added to a vial containing acceptor aldehyde (0.25 mmol, 38mg), cyclohexanone **1a** (0.75 mmol, 80 μL), SDS (72 mg, 0.25 mmol) in H_2O (1 mL). After vigorously stirring the reaction mixture for the time shown in Table 1 at room temperature it was directly loaded on a silica-gel column or the reaction was quenched by extraction with EtOAc (3x15 mL). In the latter, case the combined organic phases were dried with Na_2SO_4, filtred and concentrated under reduced pressure. The crude aldol product was purified by silica-gel column chromatography (EtOAc:pentane-mixtures) to furnish the desired aldol product **2a**. The ee of the aldol products **2a** was determined by chiral-phase HPLC analyses.

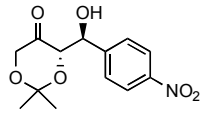
Typical experimental procedure for the peptide- and amino acid-catalyzed direct asymmetric aldol reactions in aqueous media (Table 2). A catalytic amount of L-amino acid, dipeptide or tripeptide (0.075 mmol, 30 mol%) was added to a vial containing acceptor aldehyde (0.25 mmol) and cyclohexanone **1a** (0.75 mmol, 80 μL) in H_2O (0.25 mmol α-cyclodextrin, 1 mL), aqueous media (1 mL) or aqueous buffer (1

equiv. SDS, 1 mL). After 1-4 days of vigorously stirring at room temperature the reaction mixture was directly loaded on a silica-gel column or the reaction was quenched by extraction with EtOAc (3x15 mL). In the latter, case the combined organic phases were dried with Na_2SO_4, filtred and concentrated under reduced pressure. The crude aldol product was purified by silica-gel column chromatography (EtOAc:pentane-mixtures) to furnish the desired aldol product **2a**. The ee of the aldol products **2a** was determined by chiral-phase HPLC analyses.

Typical experimental procedure for the peptide- and amino acid-catalyzed direct asymmetric aldol reactions in aqueous media (Table 3). A catalytic amount of L-amino acid, dipeptide or tripeptide (0.075 mmol, 30 mol%) was added to a vial containing acceptor aldehyde (0.25 mmol, 38mg) and donor ketone **1a**-**1b** (0.75 mmol), **1c** (1.36 mmol, 100 mL) or 1d (dimeric form, 500mg) in H_2O (1 equiv SDS, 1 mL) or aqueous media (1 mL). After 1-4 days of vigorously stirring at room temperature the reaction mixture was directly loaded on a silica-gel column. The crude aldol products were purified by silica-gel column chromatography (EtOAc:pentane-mixtures) to furnish the desired aldol products **2**. The ees of the aldol products **2** were determined by chiral-phase HPLC analyses. Compounds **2a**-**2e** are known.[1]



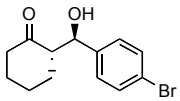
2a: ^{1}H NMR ($CDCl_3$, 400 MHz): 1.52-2.14 (m, 6H), 2.33-2.52 (m, 2H), 2.59 (m, 1H), 3.15 (bs, 1H), 4.90 (d, J = 8.6 Hz, 1H), 7.49 (d, J = 8.7 Hz, 2H), 8.20 (d, J = 8.7 Hz, 2H); ^{13}C NMR (100 MHz): δ =24.6, 27.6, 30.7, 42.6, 57.1, 73.9, 123.5, 127.8, 147.5. 148.4, 214.7; HPLC (Daicel Chiralpak AD, *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; $[\alpha]_D$ = +9.9 (c = 0.9, $CHCl_3$); MALDI-TOF MS: 272.0897; $C_{13}H_{15}NO_4$ ($M+Na^+$: calcd 272.0899).



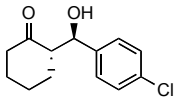
2b: ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.30 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 3.9 (m, 1H), 4.13-4.50 (m, 3H), 5.01 (d, J = 7.8 Hz, 1H, C*H*OH), 7.70 (d, J = 8.4, 2H, ArH), 8.22 (d, J = 8.4, 2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 23.3, 23.4, 66.6, 71.7, 75.8, 101.4, 123.2, 127.9, 138.3, 146.5, 210.6; HPLC (Daicel Chiralpak AD, *iso*-

1. B. List, R. A. Lerner, C. F. Barbas III, *J. Am. Chem. Soc.* 2000, **122**, 2395.; K. S. Sakthivel, W. Notz, T. Bui, C. F. Barbas III, *J. Am. Chem. Soc.* 2001, **123**, 5260.; A. J. A. Cobb, D. M. Shaw, D. A. Longbottom, J. B. Gold, S. V. Ley, *Org. Biomol. Chem.* 2005, **3**, 84.; D. Enders, C. Grondal, *Angew. Chem. Int. Ed.* 2005, **44**, 1210.; I. Ibrahem, A. Córdova, *Tetrahedron Lett.* 2005, **46**, 3363.; A. Córdova, W. Notz, C. F. Barbas III, *Chem. Commun.* 2002, 3024.; A. Córdova, W. Zou, I. Ibrahem, E. Reyes, M. Engqvist, W.-W. Liao, *Chem. Commun.* 2005, 3586.

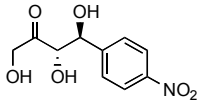
hexanes/*i*-PrOH = 96:4, flow rate 0.5 mL/min, λ = 254 nm): major isomer: t_R = 52.12 min; minor isomer: t_R = 57.02 min; $[\alpha]_D^{25}$ = -131.1 (*c* = 1.2, $CHCl_3$).



2c: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = (*anti*:*syn**-1:1) 1.21-1.34 (m, 1H), 1.42-1.87 (m, 4H+6H*), 2.03-2.12 (m, 1H+1H*), 2.23-2.59 (m, 3H+2H*), 3.10 (bs, 1H*), 3.99 (bs, 1H), 4.74 (d, *J* = 8.6 Hz, 1H), 5.34 (d, *J* = 2.4 Hz, 1H*), 7.18-7.35(m, 4H+4H*); ^{13}C NMR (100 MHz): δ = 24.9, 25.0, 26.1, 28.0 (2C), 28.1, 30.9, 42.8 (2C), 57.2, 57.5, 70.4, 74.3, 127.4, 128.9, 131.5, 131.70, 131.8, 132.0. 134.5, 134.7, 140.3, 140.8, 214.8, 215.4; HPLC (Daicel Chiralpak AS, *iso*-hexanes/*i*-PrOH = 90:10, flow rate 0.5 mL/min, λ = 254 nm): major$_{anti}$ isomer: t_R = 23.04 min; minor$_{anti}$ isomer: t_R = 21.74 min; major$_{syn}$ isomer: t_R = 19.89 min; minor$_{syn}$ isomer: t_R = 18.81 min.

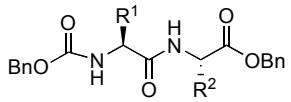


2d: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = (*anti*:*syn**-1:1) 1.23-1.32 (m, 1H), 1.45-1.90 (m, 4H+6H*), 2.03-2.15 (m, 1H+1H*), 2.29-2.62 (m, 3H+2H*), 3.07 (bs, 1H*), 3.99 (bs, 1H), 4.74 (d, *J* = 8.8Hz, 1H), 5.35 (d, *J* = 2.3 Hz, 1H*), 7.20-7.31 (m, 4H+4H*); ^{13}C NMR (100 MHz): δ = 24.9, 25.1, 26.2, 27.9, 28.1, 31.0, 42.8, 57.2, 57.5, 70.3, 74.2, 127.4, 128.5, 128.6, 128.7, 132.9, 133.8, 139.7, 140.2, 214.8, 215.5; HPLC (Daicel Chiralpak AS, *iso*-hexanes/*i*-PrOH = 90:10, flow rate 0.5 mL/min, λ = 254 nm): major$_{anti}$ isomer: t_R = 22.04 min; minor$_{anti}$ isomer: t_R = 20.36 min; major$_{syn}$ isomer: t_R = 19.50 min; minor$_{syn}$ isomer: t_R = 18.00 min.

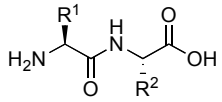


2e: ^{1}H NMR ($CDCl_3$, 400 MHz): (1:1 mixture of diastereoisomers) δ = 4.12 (d, *J* = 19.4 Hz, 1H), 4.25 (d, *J* = 5.8 Hz, 1H), 4.30 (d, *J* = 2.6 Hz, 1H), 4.44 (d, *J* = 19.4 Hz, 1H), 4.48 (d, *J* = 4.0 Hz, 2H), 4.86 (d, *J* = 5.9 Hz, 1H), 5.15 (d, *J* = 2.2 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 2H, ArH), 7.60 (d, *J* = 8.4 Hz, 2H, ArH), 8.12 (m, 4H, ArH); ^{13}C NMR (100 MHz): δ = 68.2, 68.3, 74.7, 75.4, 79.7, 80.7, 124.0, 124.1, 128.7, 129.3, 148.7, 148.9, 150.2, 150.9, 212.1, 212.6; HPLC (Daicel Chiralpak AD, *iso*-hexanes/*i*-PrOH = 90:10, flow rate 0.5 mL/min, λ = 254 nm): major isomer: t_R = 77.52 min; minor isomer: t_R = 80.23; major isomer: t_R = 88.52 min; minor isomer: t_R = 83.23 min. MALDI-TOF MS: 264.0491; $C_{10}H_{11}NO_6$ ($M+Na^+$: calcd 264.0484).

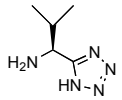
General procedure for the preparation of di-peptides:



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-methyl morpholine, 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.



Synthesis of valine-tetrazole **3**: To a solution of Boc_2O (2.5 g, 1.3 equiv.) and Cbz-L-Valine (2.0 g) in MeCN (14 mL) and Py (2.2 mL, 3 equiv.) was added NH_4HCO_3 (0.89 g, 1.26 equiv.) and the reaction mixture stirred over night at rt. The solvents were removed by evaporation and the residue dissolved in EtOAc and washed with 2x15 ml water. The water was reextracted with EtOAc and the combined volume of EtOAc dried over $MgSO_4$, filtered and concentrated. The crude product was weakly UV active and had an R_f=0.42 (MeOH/DCM 1:9). ^{1}H NMR (400MHz, $CDCl_3$) of the crude Cbz-Alanine amide: δ = 0.99(dd, *J* = 6.8 Hz, 6H), 2.15 (m, 1H), 4.02 (m, 1H), 5.12 (s, 2H), 5.29(bs, 1H), 5.39 (bs, 1H) 5.78 (bs, 1H), 7.30 (m, 5H). To a solution of Cbz-L-valine amide (1.1 g) in Py (10 mL) at -10°C was added drop wise $POCl_3$ (0.55 ml, 1.2 equiv. in DCM (5 mL)) and the resulting mixture stirred for 3 h. When the starting material was "depleted" by TLC (MeOH/DCM 1:9) the mixture was poured onto ice (~30 g). The organic phase was separated and pyridine removed by repeated washing with a not concentrated $CuSO_4$ solution. The organic phase was then pre-dried with brine and later dried over $MgSO_4$. Filtration and concentration afforded the crude product as an oil, TLC R_f=

0.51(MeOH/DCM 1:9). To a solution of crude Cbz-alanine nitrile (850 mg) in DMF (13 ml) was simultaneously NaN_3 (300 mg, 1.1 equiv.) and NH_4Cl (256 mg, 1.15 equiv.) added. The reaction was heated to 90-95°C and kept at that temperature (3h) until the TLC (HOAc/EtOAc 1:99) spot at R_f=0.63 did not increase in strength. The reaction mixture was poured onto ice (30 g), acidified to pH close to 2 with 2M HCl and extracted with $CHCl_3$ (3x20 mL). The organic phase was washed with water (20 mL), then predried with brine (20 mL) and finally dried over $MgSO_4$ before filtration and removal of solvent by evaporation. Traces of DMF was removed under reduced pressure. The crude product is a yellowish solid. ^{1}H NMR (400MHz, $CDCl_3$) of the crude Cbz-Valine tetrazole: The Cbz-alanine tetrazole (825 mg) was dissolved in MeOH (10 mL) and a catalytic amount of Pd/C was added. After 17h the catalyst was filtered off using celite and the solvent removed under reduced pressure to quantitatively give alanine tetrazole **3** as a white solid. ^{1}H NMR (400MHz, D_2O): δ = 0.70(d, J = 6.8 Hz, 3H), 0.93(d, J = 6.8 Hz, 3H), 2.25(m, 1H), 4.38 (d, J =7.6,1H).

Collecting enzyme-free cytosolic peptides from cells:

The human melanoma cell line MelJuSo was cultured in Iscove's modified Dulbecco's medium (Sigma-Aldrich), with 10% fetal calf serum (Sigma-Aldrich), 10 U/ml penicillin and 10 mg/ml streptomycin (Sigma-Aldrich) added. Harvested cells were washed three times in phosphate-buffered saline prior cell lysis using repeated freeze-thawed cycles followed by mechanical disruption of the cell membrane. Cytosolic content of the cell lysates was obtained from the supernatant after centrifugation (1500 g, 5 min). The solution was passed through an Ultrafree-CL spin column (Millipore) during 5 h at 4000 g (cut off 10 kDa) for removal of enzymes, and thereafter the enzyme-free solution was de-salted using a PepClean C-18 spin column (Pierce).

Amino acid analysis (Table S1):

Portions of cell extract (500 μl) were adjusted to 6 M in HCl by addition of 1 ml 9 M HCl. Then, 2 ml 6 M HCl containing 1mg/ml reagent grade phenol and 20 nmol norleucine was added. The samples were hydrolyzed for 24 h at 110 ºC in thoroughly evacuated and sealed Pyrex tubes. Thereafter, the hydrolyzates were evaporated to dryness and the residues were dissolved in 400 μl of pH 2.2 sample application buffer and aliquots (100 μl) were analyzed with a Biochrom 20 Amino Acid Analyzer using a sodium citrate buffer and ninhydrine detection. The results were normalized on the basis of the recovery of the internal standard and the volume of sample taken for analysis.

Table S1. Amount and composition of amino acids per mL cytosol extract.

Amino acid	Amount amino acid (μg/ml)
Aspartic acid	7.01
Threonine	6.15

Serine	3.02
Glutamic acid	29.31
Proline	12.64
Glycine	14.96
Alanine	2.60
Half-cystine	16.89
Valine	4.39
Methionine	1.92
Isoleucine	4.06
Leucine	4.17
Tyrosine	3.53
Phenylalanine	3.60
Histidine	1.36
Lysine	1.80
Arginine	1.46
Tryptophan	Not determined
Total amount	118.88