Electronic Supporting Information for:

N-hydroxy and *N*-acyloxy peptides: synthesis and chemical modifications

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Synthesis of α-keto-amides 1

(2*S*)-3-Methyl-2-(3-methyl-2-oxo-butyrylamino)-butyric acid benzyl ester (1a)

A solution of 3-methyl-2-oxo-butyric acid (2.40 g, 20.1 mmol; prepared from diethyl oxalate and isopropylmagnesium chloride)[1] in CH_2Cl_2 (27 mL) was cooled to 0°C and α,α-dichloromethylmethylether (1.83 mL, 2.32 g, 20.1 mmol) was slowly added[2] The solution was stirred for 15 minutes at 0°C, 45 minutes at room temperature and 1.5h at 50°C. The resulting solution of acyl chloride was cooled to room temperature, then added at 0°C to a solution of L-valine benzylester *p*-toluenesulfonate salt (7.62 g, 20.1 mmol) and NMM (*N*-methylmorpholine; 4.84 mL, 4.45 g, 44 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred for 15 minutes at 0°C, then overnight at room temperature. The solution was washed with brine and the organic layer dried over Na_2SO_4. Removal of the solvent under reduced pressure followed by purification by flash chromatography (Silica gel, CH_2Cl_2) of the oily residue gave **1a** (4.16 g, 13.6 mmol, 68%) as a colourless oil. R_f 0.34 (CH_2Cl_2). $[\alpha]_D^{25}$ +2.1° (*c* 2.79 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.48-7.27 (6H, m, CH_{ar}, NH), 5.18 (2H, ABq, *J* 12.2, δ_A-δ_B 14.9, CH_2-OBn), 4.54 (1H, dd, *J* 4.9 and 9.2, CH_α Val), 3.58 (1H, h, *J* 6.9, CH ^{i}Pr), 2.33-2.16 (1H, m, CH_{iPr} Val), 1.16 (3H, d, *J* 6.9, CH_3 ^{i}Pr), 1.13 (3H, d, *J* 6.9, CH_3 ^{i}Pr), 0.93 (3H, d, *J* 6.9, CH_3 Val), 0.89 (3H, d, *J* 6.9, CH_3 Val). δ_C (75 MHz; $CDCl_3$) 201.7, 171.0, 159.9 (C=O), 135.3 (C_{ar}), 128.8, 128.7, 128.6 (CH_{ar}), 67.4 (CH_2-OBn), 57.4 (CH_α Val), 34.4 (CH ^{i}Pr), 31.6 (CH_{iPr} Val), 19.2, 17.9, 17.8, 17.7 (CH_3). IR: 3404, 2969, 1742,

1689, 1514, 1147, 752, 698. LRMS (ESI +) *m/z*: 306.1 $(M+H)^+$, 328.1 $(M+Na)^+$, 344.0 $(M+K)^+$. Anal.: found, C66.83; H7.72; N4.60. $C_{17}H_{23}NO_4$ requires C66.86; H7.59; N4.59%.

(2*S*)-3-Methyl-2-(2-oxo-propionylamino)-butyric acid ethyl ester (1b)
To a solution of L-valine ethyl ester hydrochloride (2.63 g, 14.5 mmol) in CH_2Cl_2 (23 mL) at 0°C was added NMM (3.5 mL, 31.8 mmol) followed by a solution of 2-oxo-propanoyl chloride[3] (1.70 g, 15.9 mmol) in CH_2Cl_2 (11 mL). An orange solid precipitated and the mixture was stirred at room temperature overnight. The precipitate was removed by filtration over Celite® 535 and the solvent was evaporated under reduced pressure. Purification of the orange oil by flash chromatography (Silica gel, CH_2Cl_2-MeOH 97:3) gave **1b** as a yellow oil (2.73 g, 12.7 mmol, 88%). R_f 0.91 (CH_2Cl_2-MeOH 95 : 5). $[a]_D^{25}$ -1.88 (*c* 3.67 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 7.40 (1H, br s, NH), 4.46 (1H, dd, *J* 4.9 and 9.2, CH_a Val), 4.22 (2H, q, *J* 7.1, CH_2 -OEt), 2.48 (3H, s, CH_3CO), 2.32-2.16 (1H, m, CH_{iPr} Val), 1.29 (3H, t, *J* 7.1, CH_3 -OEt), 0.96 (3H, d, *J* 6.8, CH_3 Val), 0.94 (3H, d, *J* 6.8, CH_3 Val). δ_C (75 MHz; $CDCl_3$) 196.4 (CH_3C=O), 170.9 (NHC=O), 160.1 (OC=O), 61.6 (CH_2 -OEt), 57.4 (CH_a Val), 31.5 (CH_{iPr} Val), 24.5 (CH_3CO), 19.1, 17.8 (CH_3 Val), 14.3 (CH_3 -OEt). IR: 3397, 2962, 1741, 1682, 1505, 1196, 1019. HRMS (ESI +) *m/z*: found, 238.1055; $C_{10}H_{17}NO_4Na$ requires 238.1055.

(2*S*)-4-Methyl-2-(2-oxo-propionylamino)-pentanoic acid ethyl ester (1c)
The title compound was prepared as described for **1b** using L-leucine ethyl ester hydrochloride (4.65 g, 23.7 mmol) and was obtained as a pale yellow oil (4,73 g, 20,6 mmol, 87%). R_f 0.76 (CH_2Cl_2-MeOH 97:3). δ_H (200 MHz; $CDCl_3$) 7.39 (1H, d, *J* 8.9, NH), 4.62-4.51 (1H, m, CH_a Leu), 4.17 (2H, q, *J* 7.2, CH_2 -OEt), 2.47 (3H, s, CH_3CO), 1.70-1.66 (3H, m, CH_{iBu} Leu, CH_2 Leu), 1.29 (3H, t, *J* 7.2, CH_3 -OEt), 0.95 (6H, d, *J* 6.5, CH_3 Leu). δ_C (75 MHz; $CDCl_3$) 196.3 (CH_3C=O), 171.8 (NHC=O), 159.8 (OC=O), 61.5 (CH_2 -OEt), 50.8 (CH_a Leu), 41.3 (CH_2 Leu), 24.8 (CH_{iBu} Leu), 24.3 (CH_3CO), 22.7, 21.7 (CH_3 Leu), 14.1 (CH_3 -OEt).

(2*S*)-2-(3-Methyl-2-oxo-butyrylamino)-3-phenyl-propionic acid benzyl ester (1d)
The title compound was prepared as described for **1a** using L-phenylalanine benzylester *p*-toluenesulfonate salt (6.49 g, 15.2 mmol) and was obtained as a colourless oil (4.16 g, 13.7 mmol, 50%). R_f 0.61 (CH_2Cl_2). $[\alpha]_D^{25}$ –0.9° (*c* 1.14 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) δ 7.28-7.21 (9H, m, CH_{ar}), 7.02 (2H, m, CH_{ar}, NH), 5.15 (2H, ABq, *J* 12.0, δ_A-δ_B 8.0, CH_2 -OBn), 4.87 (1H, dt, *J* 6.2 and 8.2, CH_α Phe), 3.52 (1H, h, *J* 6.9, CH ^{i}Pr), 3.14 (2H, ABX, ^3J 6.2, ^2J 13.9, δ_A-δ_B 12.3, CH_2 Phe), 1.10 (3H, d, *J* 7.2, CH_3), 1.09 (3H, d, *J* 6.9, CH_3). δ_C (75 MHz; $CDCl_3$) 201.5, 170.6, 159.5 (C=O), 135.4, 135.1 (C_{ar}), 129.4, 128.9, 128.8 (2 peaks), 128.7, 127.4 (CH_{ar}), 67.6 (CH_2 -OBn), 53.4 (CH_α Phe), 38.1 (CH_2 Phe), 34.3 (CH_{iPr}), 17.8, 17.7 (CH_3). IR: 3394, 2969, 1744, 1688, 1519, 1175. LRMS (DCI) *m/z*: 354.1 $(M+H)^+$, 371.8 $(M+NH_4)^+$. HRMS (ESI +) *m/z*: found, 376.1523; $C_{21}H_{23}NO_4Na$ requires 376.1525.

(2*S*,3*S*)-3-Methyl-2-(3-methyl-2-oxo-butyrylamino)-pentanoic acid benzyl ester (1e)
The title compound was prepared as described for **1a** using L-isoleucine benzylester *p*-toluenesulfonate salt (7.87 g, 20.1 mmol) and was obtained as a slightly yellow oil (2.11 g, 6.6 mmol, 33%). R_f 0.56 (CH_2Cl_2). $[\alpha]_D^{25}$ +3.6° (*c* 2.94 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.40-7.25 (6H, m, CH_{ar}, NH), 5.18 (2H, ABq, *J* 12.2, δ_A-δ_B 18.5, CH_2 -OBn), 4.58 (1H, dd, *J* 4.9 and 9.1, CH_α Ile), 3.55 (1H, h, *J* 6.9, CH ^{i}Pr), 2.00-1.95 (1H, m, CH_{sBu} Ile), 1.45-1.30 (1H, m, CH*H* Ile), 1.25-1.05 (1H, m, C*H*H Ile), 1.14 (3H, d, *J* 6.9, CH_3 ^{i}Pr), 1.13 (3H, d, *J* 6.9, CH_3 ^{i}Pr), 0.90 (3H, d, *J* 7.0, CH_3 Ile), 0.88 (3H, t, *J* 7.5, CH_3 Ile). δ_C (75 MHz; $CDCl_3$) 201.8, 171.0, 159.8 (C=O), 135.4 (C_{ar}), 128.8, 128.7, 128.6 (CH_{ar}), 67.4 (CH_2 -OBn), 56.8 (CH_α Ile), 38.2 (CH ^{i}Pr), 34.4 (CH_{sBu} Ile), 25.2 (CH_2 Ile), 17.9, 17.8 (CH_3 ^{i}Pr), 15.7, 11.7 (CH_3 Ile). IR: 3399, 2970, 1740, 1720, 1685, 1516, 1146, 911, 734. LRMS (DCI) *m/z*: 320.2 $(M+H)^+$, 336.7 $(M+NH_4)^+$. HRMS (ESI +) *m/z*: found, 342.1677; $C_{18}H_{25}NO_4Na$ requires 342.1681.

Synthesis of α-hydroxyimino-amides 2

(2*S*)-2-(2-Hydroxyimino-3-methyl-butyrylamino)-3-methyl-butyric acid benzyl ester (2a)
To a solution of **1a** (1.93 g, 6.3 mmol) in THF (30 mL) was added hydroxylamine hydrochloride (0.91 g, 13.1 mmol) and NaOAc•3H_2O (1.23 g, 9.0 mmol). The mixture was stirred for 18h at reflux. The suspension was then filtered and the solvent evaporated under reduced pressure. Flash chromatography (Silica gel, CH_2Cl_2) gave **2a** (1.80 g, 5.6 mmol, 89%) as a colourless oil. R_f 0.26 (CH_2Cl_2). $[\alpha]_D^{25}$ +4.1° (*c* 2.99 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.50-7.23 (5H, m, CH_{ar}), 7.17 (1H, d, *J* 8.9, NH), 5.19 (2H, ABq, *J* 12.2, δ_A-δ_B 25.4, CH_2 -OBn), 4.63 (1H, dd, *J* 4.9 and 8.9, CH_α Val), 3.45 (1H, h, *J* 7.0, CH ^{i}Pr), 2.30-2.15 (1H, m, CH_{iPr} Val), 1.26 (3H, d, *J* 7.0, CH_3 ^{i}Pr), 1.24 (3H, d, *J* 7.1, CH_3 ^{i}Pr), 0.93 (3H, d, *J* 6.9, CH_3 Val), 0.88 (3H, d, *J* 6.9, CH_3 Val). δ_C (75 MHz; $CDCl_3$) 172.7, 163.8 (C=O), 159.1 (C=N), 135.4 (C_{ar}), 128.8, 128.7, 128.6 (CH_{ar}), 67.5 (CH_2 -OBn), 57.2 (CH_α Val), 31.5 (CH ^{i}Pr), 25.6 (CH_{iPr} Val), 19.2, 18.7, 18.5, 18.0 (CH_3). IR: 3333, 2966, 1739, 1659, 1520, 1194, 1003, 698. LRMS (DCI) *m/z*: 321.0 $(M+H)^+$, 337.7 $(M+NH_4)^+$. HRMS (ESI +) *m/z*: found, 343.1640; $C_{17}H_{24}N_2O_4Na$ requires 343.1634.

(2*S*)-2-(2-Hydroxyimino-propionylamino)-3-methyl-butyric acid ethyl ester (2b)
A solution of **1b** (3.53 g, 16.4 mmol), hydroxylamine hydrochloride (1.25 g, 18.0 mmol) and triethylamine (5 mL, 36.1 mmol) in EtOH (15 mL) was refluxed for 1.5h. The mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The orange oil was purified by flash chromatography (silica gel, pentane-EtOAc 2:1) to give **2b** (2.96 g, 9.5 mmol, 78%) as a colourless oil. R_f 0.63 (CH_2Cl_2-MeOH 95:5). $[a]_D^{25}$ +14.7 (*c* 2.60 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 9.94 (1H, br s, NOH), 7.46 (1H, d, *J* 9.2, NH), 4.59 (1H, dd, *J* 5.3 and 9.2, CH_a Val), 4.35-4.15 (2H, m, CH_2 -OEt), 2.35-2.10 (1H, m, CH_{iPr} Val), 2.08 (3H, s, CH_3C=N), 1.31 (3H, t, *J* 7.0, CH_3 -OEt), 0.98 (3H, d, *J* 6.9, CH_3 Val), 0.96 (3H, d, *J* 6.9, CH_3 Val). δ_C (75 MHz; $CDCl_3$) 173.1, 164.0 (C=O), 151.9 (C=N), 61.8 (CH_2 -OEt), 57.4 (CH_a Val), 31.3 (CH_{iPr} Val), 19.2, 18.1 (CH_3 Val), 14.3 (CH_3 -OEt), 9.5 (*C*H_3C=N). IR: 3304, 2965, 1736, 1663, 1520, 1186, 1019, 719. HRMS (ESI +) *m/z*: found, 253.1167; $C_{10}H_{18}N_2O_4Na$ requires 253.1164.

(2*S*)-2-(2-Hydroxyimino-propionylamino)-4-methyl-pentanoic acid ethyl ester (2c)
The title compound was prepared as described for **2b** using **1c** (3.25 g, 14.2 mmol). Removal of the solvent under reduced pressure gave a yellow/brown solid, which was dissolved in CH_2Cl_2-MeOH 90:10 and filtered through a 10cm plug of Silica gel. Crystallization from CH_2Cl_2 gave **2c** (2.99 g, 12.2 mmol, 86%) as white needles. Mp 192°C. R_f 0.50 (CH_2Cl_2-MeOH 95:5). $[a]_D^{25}$ –12.6 (*c* 2.90 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 9.70 (1H, br s, NOH), 7.51 (1H, d, *J* 8.6, NH), 4.74-4.62 (1H, m, CH_a Leu), 4.23 (2H, q, *J* 7.2, CH_2 -OEt), 2.07 (3H, s, CH_3C=N), 1.74-1.52 (3H, m, CH_{iBu} Leu, CH_2 Leu), 1.30 (3H, t, *J* 7.2, CH_3 -OEt), 0.94 (6H, d, *J* 6.2, CH_3 Leu). δ_C (75 MHz ; $CDCl_3$) 174.9, 164.1 (C=O), 151.8 (C=N), 62.1 (CH_2 -OEt), 51.0 (CH_a Leu), 41.3 (CH_2 Leu), 25.1 (CH_{iBu} Leu), 22.9, 21.9 (CH_3 Leu), 14.2 (CH_3 -OEt), 9.6 (*C*H_3C=N). IR: 3354, 3266, 2965, 1691, 1529, 1303, 1018, 724. LRMS (DCI) *m/z*: 245.0 $(M+H^+)$. HRMS (ESI +) *m/z*: found, 267.1322; $C_{11}H_{20}N_2O_4Na$ requires 267.1321.

(2*S*)-2-(2-Hydroxyimino-3-methyl-butyrylamino)-3-phenyl-propionic acid benzylester (2d)
The title compound was prepared as described for **2a** using **1d** (2.48 g, 7.3 mmol) and, after flash chromatography (Silica gel, pentane-EtOAc 4:1), was obtained as a colourless oil (2.30 g, 6.2 mmol, 89%). R_f 0.16 (CH_2Cl_2). $[\alpha]_D^{25}$ –0.9° (*c* 1.03 in $CHCl_3$).δ_H (300 MHz; $CDCl_3$) 7.64 (s, 1H, NOH), 7.37-7.17 (m, 11H, CH_{ar}, NH), 5.13 (2H, ABq, *J* 12.3, δ_A-δ_B 15.9, CH_2 -OBn), 4.92 (1H, dt, *J* 4.1 and 6.1, CH_α Phe), 3.44 (1H, h, *J* 7.1, CH ^{i}Pr), 3.11 (2H, ABX, ^3J 5.7, ^2J 13.5, δ_A-δ_B 13.6, CH_2 Phe), 1.23 (3H, d, *J* 7.1, CH_3), 1.22 (3H, d, *J* 7.1, CH_3). δ_C (75 MHz; $CDCl_3$) 171.9, 163.5 (C=O), 158.5 (C=N), 135.8, 135.1 (C_{ar}), 129.4, 128.6 (2 peaks), 127.2 (CH_{ar}), 67.5 (CH_2 -OBn), 53.4 (CH_α

Phe), 38.1 (CH_2 Phe), 25.5 (CH ^{i}Pr), 18.5 (CH_3). IR: 3330, 2965, 1741, 1660, 1516, 1192, 1003, 740, 699. LRMS (DCI) *m/z*: 368.9 $(M+H)^+$, 386.1 $(M+NH_4)^+$. HRMS (ESI +): found, 391.1634; $C_{21}H_{24}N_2O_4Na$ requires 391.1634.

(2*S*,3*S*)-2-(2-Hydroxyimino-3-methyl-butyrylamino)-3-methyl-pentanoic acid benzylester (2e)
The title compound was prepared as described for **2a** using **1e** (0.94 g, 2.9 mmol) and, after flash chromatography (Silica gel, pentane-EtOAc 4:1), was obtained as a colourless oil (0.84 g, 2.5 mmol, 85%). R_f 0.12 (CH_2Cl_2). $[\alpha]_D^{25}$ +7.9° (*c* 3.40 in $CHCl_3$). δ_H (300MHz; $CDCl_3$) 9.34 (1H, s, NOH), 7.39-7.16 (6H, m, CH_{ar}, NH), 5.09 (2H, ABq, *J* 12.2, δ_A-δ_B 33.6, CH_2 -OBn), 4.62 (1H, dd, *J* 4.7 and 9.1, CH_α Ile), 3.37 (1H, h, *J* 7.0, CH ^{i}Pr), 1.92-1.79 (1H, m, CH_{sBu} Ile), 1.41-1.23 (1H, m, CH*H* Ile), 1.18 (3H, d, *J* 7.2, CH_3 ^{i}Pr), 1.15 (3H, d, *J* 7.0, CH_3 ^{i}Pr), 1.10-0.99 (1H, m, C*H*H Ile), 0.82 (3H, d, *J* 6.8, CH_3 Ile), 0.77 (3H, t, *J* 7.4, CH_3 Ile). δ_C (75 MHz; $CDCl_3$) 173.1, 164.2 (C=O), 158.6 (C=N), 135.2 (C_{ar}), 128.7 (2 peaks), 128.5, 128.4 (CH_{ar}), 67.5 (CH_2 -OBn), 56.5 (CH_α Ile), 37.9 (CH_{sBu} Ile), 25.6 (CH ^{i}Pr), 25.3 (CH_2 Ile), 18.7, 18.3 (CH_3 ^{i}Pr), 15.7, 11.6 (CH_3 Ile). IR: 3334, 2966, 1739, 1662, 1516, 1192, 1003, 738, 698. LRMS (DCI) *m/z*: 335.1 $(M+H)^+$, 351.7 $(M+NH_4)^+$. HRMS (ESI +) *m/z*: found, 357.1794; $C_{18}H_{26}N_2O_4Na$ requires 357.1790.

Assignment of configuration for compounds 3

Reduction of N–O bond for benzyl ester derivatives
To a solution of *N*-hydroxy dipeptide **3a,d,e** (single diastereomer; 150 μmol) in THF (1 mL) was added a 0.1M solution of SmI_2 in THF[4] (3.8 mL). The mixture was stirred at room temperature for 1.5h. A saturated aqueous solution of $Na_2S_2O_3$ (10 mL) was added. The suspension was filtered over Celite® 535 and the filtrate dried over Na_2SO_4. Removal of the solvent under reduced pressure followed by purification by flash chromatography gave pure dipeptide H-AA_1-AA_2-OBn.

Reduction of N–O bond for ethyl ester derivatives
A solution of *N*-hydroxy dipeptide **3b,c** (single diastereomer; 300 μmol) in EtOH (3 mL) was transfered over Raney Nickel.[5] The suspension was placed under an atmosphere of hydrogen and stirred at room temperature for 3h. The ethanolic solution was then pumped out. Removal of the solvent under reduced pressure followed by purification by flash chromatography gave pure dipeptide H-AA_1-AA_2-OEt.

Comparison with authentic (*S*,*S*)-H-AA_1-AA_2-OR samples
Authentic (*S*,*S*) samples are prepared by DCC- or EDCI-mediated coupling of L-Fmoc-AA_1-OH with L-H-AA_2-OR followed by piperidine-induced cleavage of the Fmoc group.
Roughly equimolar amounts of authentic (*S*,*S*) dipeptide and unknown diastereomer were mixed in $CDCl_3$ and ^{1}H and ^{13}C NMR spectra were recorded. Matching (respectively mismatching) of the signals indicated the configuration of the diastereomer was (*S*,*S*) (respectively (*R*,*S*)).

References

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