

Supporting Information to „Highly Efficient Chiral-Pool Synthesis of (2*S*,4*R*)-4-hydroxyornithine“ (J. Rudolph, F. Hannig, H. Theis, R. Wischnat)

Experimental

General

Melting points were obtained on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter. ¹H NMR spectra were recorded on Bruker DPX400 spectrometers using the solvent signals as internal references. The chemical shifts are reported in δ (ppm) relative to TMS and the coupling constants (*J*) are given in Hertz (Hz). Electrospray-high resolution mass spectrometry (ESI-HRMS) was performed on an Finnigan-MAT 900 double focusing mass spectrometer. The ESI source was operated in the positive ion mode, spray needle voltage 3.5 kV, nitrogen sheath gas pressure 500 kPa, and the heated metal capillary held at 250°C.

All reactions were monitored by analytical TLC on aluminum backed TLC plates coated with silica gel (Merck Si 60 F₂₅₄) and visualization of spots was accomplished by UV detection. Column chromatography was carried out using Amicon silica gel (30-70 μm).

***tert*-Butyl *N*-(*tert*-butoxycarbonyl)-*L*-homoserinate (4):**

A solution of Boc-Asp-O-*t*Bu (4.97 g, 17.2 mmol) in 50 ml dry THF is cooled to –10°C. *N*-methylmorpholine (1.74 g, 17.2 mmol) is added. After 1 min ClCO₂Et (1.64 mL, 17.2 mmol) is added dropwise. The reaction mixture is stirred for additional 15 min at –5°C. The precipitated *N*-methylmorpholine hydrochloride is filtered off, and the filtrate is added to a solution of NaBH₄ (1.46 g, 38.7 mmol) in 20 mL water at 5-10°C within 10 min. The mixture is stirred at room temperature for 3.5 h and then cooled to 5 °C. 3 M HCl is added to give a pH of 2 and the mixture is extracted twice with ethyl acetate. The combined organic phases are washed twice with water and then dried with Na₂SO₄. The product is dried *in vacuo* and purified via silica gel flash chromatography using ethyl acetate:cyclohexane (2:3) as eluent (*R*_f = 0.16). ¹H-NMR (400 MHz, CDCl₃): δ=5.37 (d, *J*=7.4 Hz, 1H, NH), 4.36 (m, 1H, CH), 3.69 (m, 2H, CH₂OH), 3.48 (m, 1H, OH), 2.15 (m, 1H, CHCH₂), 1.45 (m, 19H, CHCH₂, *t*-Bu). MS (ESI): *m/z* = 276 [M+H]⁺.

***tert*-Butyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-oxobutanoate (**5**):**

In an inert atmosphere, a mixture of compound **4** (4.8 g, 17.4 mmol) and 30 g bromite resin (prepared according to Kirschning et al.)¹⁶ is given in 250 mL dry dichloromethane and a trace of TEMPO is added. After shaking for 16 h the solution is filtered off and the resin is subsequently washed with dichloromethane, and cyclohexane (3x). The combined organic solutions are dried *in vacuo* and the product is obtained as a white-yellow oil. ¹H-NMR (400 MHz, CDCl₃): δ=9.73 (s, 1H, CHO), 5.34 (d, *J* = 7.3 Hz, 1H, NH), 4.48 (m, 1H, CH), 3.03 (dd, *J* = 5 Hz, 18 Hz, 1H, CHCH₂), 2.95 (dd, *J* = 5 Hz, 18 Hz, 1H, CHCH₂), 1.46 (s, 9H, *t*-Bu), 1.45 (s, 9H, *t*-Bu). MS (ESI): *m/z* = 274 [M+H]⁺.

***tert*-Butyl (2*S*,4*R*)-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxy-5-nitro-butanoate (**7a**) / *tert*-Butyl (2*S*,4*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxy-5-nitro-butanoate (**7b**)**

NaOEt (20 mg, 0.29 mmol) are dissolved in 75 mL of ethanol (abs.), and nitromethane (2.6 mL, 47.6 mmol) is then added. In a different flask, the aldehyde **5** (1.3 g, 4.76 mmol) are dissolved in 75 mL of ethanol (abs.) and added dropwise to the nitromethane solution. The reaction mixture is stirred at room temperature for 12 h. After completion of the reaction, citric acid (90 mg, 0.48 mmol) is added and the solvent is removed *in vacuo* with portions of nitromethane constantly added to prevent the retro reaction. The residue is separated via silica gel chromatography using toluene/THF/ethyl acetate (90:5:5) as eluent. *R_f* (**7a**) [2*S*,4*R*] = 0.18; *R_f* (**7b**) [2*S*,4*S*] = 0.24. Stereochemistry was assigned via X-ray analysis of the spot at *R_f* = 0.24 which proved to be the (2*S*,4*S*)-diastereomer **7b**.

7a [2*S*,4*R*]: mp = 132.5-134.5 °C. ¹H-NMR (400 MHz, CDCl₃): δ=5.43 (d, *J* = 7.5 Hz, 1H, NH), 4.59-4.25 (m, 4H, CH-OH, CH-NH, CH₂-NO₂), 3.38 (d, *J* = 3.7 Hz, 1H, OH), 2.09 (m, 1H, CH₂), 1.89 (m, 1H, CH₂), 1.47 (s, 9H, *t*-Bu), 1.46 (s, 9H, *t*-Bu). ¹³C-NMR (100 MHz, CDCl₃): δ=171.0 (C=O), 83.0 (CH₂-NO₂), 80.6 + 80.1 (O-C(CH₃)₃), 66.1 (CH-OH), 51.3 (CH-NH), 36.9 (CH₂), 28.3 + 28.1 (C(CH₃)₃). MS (ESI): *m/z* = 335 [M + H]⁺. FT-ICR-MS: *m/z* ([M + Na]⁺) = 357.16322 (calcd.), 357.16329 (found). [α]_D²⁵ = -5.025 (ethanol/water = 95:5 (v/v), *c* = 1).

7b [2*S*,4*S*]: mp = 94.0-95.0 °C. ¹H-NMR (400 MHz, CDCl₃): δ=5.42 (d, *J* = 7.5 Hz, 1H, NH), 4.77 (d, *J* = 3.7 Hz, 1H, OH), 4.55-4.28 (m, 4H, CHOH, CHNH, CH₂NO₂), 1.97 (m, 1H, CH₂), 1.52 (m, 1H, CH₂), 1.46 (s, 18H, *t*-Bu). ¹³C-NMR (100 MHz, CDCl₃): δ=171.0 + 157.2 (C=O), 83.1 (CH₂-NO₂), 81.2 + 79.8 (*t*-Bu), 65.0 (CH-OH), 50.5 (CH-NH), 38.6 (CH₂), 28.2,

27.9 (CH₃). MS (ESI): $m/z = 335$ [M + H]⁺. FT-ICR-MS: m/z ([M + Na]⁺) = 357.16322 (calcd.), 357.16327 (found). $[\alpha]_D^{25} = -48.21$ (ethanol:water = 95:5 (v/v), c = 1).

***tert*-Butyl *N*-(*tert*-butoxycarbonyl)-5-nitro-4-oxo-L-norvalinate (9)**

Boc-Asp-O-*t*Bu (1.0 g, 3.46 mmol) is added to a solution of 1,1'-carbonyldiimidazole (0.59 g, 3.63 mmol) in 50 ml dry THF at room temperature. This solution is stirred for 5 h. In a different flask, a solution of nitromethane (1.87 ml, 34.6 mmol) in 20 ml dry THF is added dropwise to a solution of *t*-BuOK (0.43 g, 3.80 mmol) in 50 ml dry THF at 0°C and stirred for 1 h. The solution with activated Boc-Asp-O-*t*Bu is then added to this solution and the reaction mixture is stirred for 15 h. 1 M HCl is then added, and it is subsequently extracted with ethyl acetate (2x), washed with aqueous NaCl solution and then dried with MgSO₄. The solvent is removed in vacuo to give 1.10 g (96%) of pure compound **9** as a white solid. Mp= 78-82°C. ¹H-NMR (400 MHz, CDCl₃): δ=5.35 (d, *J* = 6.6 Hz, 1H, NH), 5.23 (s, 2H, CH₂NO₂), 4.39 (m, 1H, CHNH), 3.03 (m, 2H, CH₂), 1.38 (s, 18H, *t*-Bu). ¹³C-NMR (100 MHz, CDCl₃): δ=194.3, 169.3, 155.5 (C=O), 83.3 (CH₂-NO₂), 83.0 + 82.5 (*t*-Bu), 50.2 (CH-NH), 42.9 (CH₂), 28.31, 28.27 (CH₃). MS (ESI): $m/z = 333.2$ [M + H]⁺. FT-ICR-MS: m/z ([M + Na]⁺) = 355.14757 (calcd.), 355.14750 (found). $[\alpha]_D^{25} = -16$ (ethanol:water = 95:5 (v/v), c = 1).

***tert*-Butyl (2*S*,4*R*)-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxy-5-nitro-butanoate (7a):**

The nitroketone **1** (0.67 g, 2.00 mmol) is dissolved in 30 ml dry THF and cooled to -78°C. 2 mL of a 1 M solution of L-Selectride® in THF is added dropwise and the temperature kept at -78°C. After 3 h the reaction is completed and the mixture is quenched with saturated aqueous ammonium chloride solution. The aqueous phase is extracted with ethyl acetate (3x). The crude product shows a diastereoisomer ratio of 85:15 (HPLC, correlated with ¹H-NMR) in favor of the (2*S*,4*R*)-isomer. The crude product is purified via silica gel chromatography using toluene:THF:ethyl acetate (90:5:5) (*R*_f = 0.18). At a diastereoisomer ratio of >90:10, purification can also be done through recrystallization from hexane. The product is obtained as a white powder in a yield of 0.35 g (52 %). The analytical data are given before.

***tert*-Butyl-(2*S*,4*R*)-*N*^α-(*tert*-butoxycarbonyl)-4-hydroxyornithinate (8):**

The nitroalcohol **7a** (5 g, 14.9 mmol) is dissolved in 50 ml of methanol. The reaction mixture is cooled to -10°C, and palladium on carbon (10 %, purissimum, Fluka) (2.5 g) and dry ammonium formate (9.43 g, 150 mmol, 10 eq) are added while maintaining the reaction

temperature at -10°C. After stirring the reaction for 2 h, the catalyst is filtered off. The solvent is removed and ethyl acetate and saturated aqueous sodium bicarbonate solution are added (pH \geq 7). The phases are separated and, after two additional washings with ethyl acetate, the combined organic phases are washed with brine, dried over Na₂SO₄, and the solvent is removed *in vacuo*. The product is obtained as a colorless-yellow oil in a crude yield of 4.55 g (100 %). Due to its instability, the product **9** is not further purified and directly further transformed to compound **10**. ¹H-NMR (400 MHz, CDCl₃): δ =7.94 (s (br), 2H, NH₂), 5.56 (m, 1H, NH), 4.29 (m, 1H, CHOH), 4.25 (m, 1H, OH), 4.11 (m, 1H, CHNH), 3.02 (m, 1H, CH₂-NH₂), 2.85 (m, 1H, CH₂-NH₂), 1.92 (m, 1H, CH₂), 1.85 (m, 1H, CH₂), 1.46 (s, 9H, *t*-Bu), 1.44 (s, 9H, *t*-Bu). ¹³C-NMR (100.58 MHz, CDCl₃): δ =66.5 (CHOH), 59.5 (CH₂NH₂), 52.7 (CHNH), 38.2 (CH₂), 28.7 (CH₃). MS (ESI): *m/z* = 305 [M + H]⁺. FT-ICR-MS: *m/z* ([M + H]⁺) = 305.20710 (calcd.), 305.20709 (found).

***tert*-Butyl-(2*S*,4*R*)-*N* ^{α} -(*tert*-butoxycarbonyl)-*N* ^{δ} -(benzyloxycarbonyl)-4-hydroxy-ornithinate (**10**)**

The amino alcohol **9** (4.55 g crude product, ~ 15 mmol) is dissolved in 50 mL DMF and *N,N*-diisopropylamine (3.8 g, 30 mmol, 2 eq) and benzyl *N*-succinimidyl carbonate (Z-OSu) (3.74 g, 15 mmol) are added. After stirring for 16 h, the solvent is removed *in vacuo*, and the residue is taken up in dichloromethane, and washed subsequently with 5% citric acid, sodium bicarbonate solution and brine. After drying with sodium sulfate and removal of the solvent *in vacuo*, the product is purified via silica gel chromatography using hexane/ethyl acetate (10:1 \rightarrow 3:1) The product is obtained as a white solid in a yield (from two steps) of 3.3 g (51 %). Mp = 79-81°C. ¹H-NMR (400 MHz, CDCl₃): δ =7.32 (m, 5 H, arom), 5.49 (m, 2H, NH, OH), 5.09 (s, 2H, Ph-CH₂), 4.22 (m, 1H, CH-OH), 3.89 (m, 1H, CH-NHBoc), 3.35 (m, 1H, CH₂N), 3.11 (m, 1H, CH₂N), 1.92 (m, 1H, C-CH₂-C), 1.78 (m, 1H, C-CH₂-C), 1.45 (s, 9H, *t*-Bu), 1.42 (s, 9H, *t*-Bu). ¹³C-NMR (100.58 MHz, CDCl₃): δ =172.1, 157.5, 156.2 (C=O), 136.8, 128.9, 128.7, 128.5 (ar.), 82.7, 80.5 (*t*Bu), 69.1 (C-OH), 67.2 (CH₂Ph), 52.5 (CHN), 47.2 (CH₂N), 38.0 (C-CH₂-C), 28.7, 28.3 (CH₃). FT-ICR-MS: *m/z* ([M + H]⁺) = 439.24388 (calcd.), 439.24427 (found).

Table 1. Crystal data and structure refinement for **7b**.

Empirical formula	C ₁₄ H ₂₆ N ₂ O ₇
Formula weight	334.37
Temperature	153(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit cell dimensions	<i>a</i> = 6.0845(5) Å $\alpha = 90^\circ$. <i>b</i> = 10.7116(11) Å $\beta = 90^\circ$. <i>c</i> = 28.293(3) Å $\gamma = 90^\circ$.
Volume	1844.0(3) Å ³
Z	4
Density (calculated)	1.204 Mg/m ³
Absorption coefficient	0.096 mm ⁻¹
F(000)	720
Crystal size	0.03 x 0.10 x 0.30 mm ³
Theta range for data collection	1.44 to 29.00°.
Index ranges	-8 ≤ <i>h</i> ≤ 8, -14 ≤ <i>k</i> ≤ 14, -38 ≤ <i>l</i> ≤ 38
Reflections collected	25872
Independent reflections	4896 [R(int) = 0.0892]
Completeness to theta = 29.00°	99.9 %
Absorption correction	SADABS (Bruker-AXS)
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	4896 / 0 / 312
Goodness-of-fit on F ²	0.930
Final R indices [I>2sigma(I)]	R1 = 0.0399, wR2 = 0.0892
R indices (all data)	R1 = 0.0791, wR2 = 0.1023
Absolute structure parameter	0.8(9)
Largest diff. peak and hole	0.255 and -0.187 e.Å ⁻³

Table 2. Bond lengths [Å] and angles [°] for **7b**.

O(1)-C(2)	1.224(2)	N(2)-O(4)	1.217(2)	O(7)-C(11)	1.487(2)
C(1)-N(1)	1.445(2)	N(2)-C(9)	1.494(3)	C(7)-C(8)	1.524(2)
C(1)-C(10)	1.521(2)	O(3)-C(8)	1.416(2)	C(8)-C(9)	1.510(3)
C(1)-C(7)	1.534(3)	C(3)-C(6)	1.509(3)	C(11)-C(13)	1.498(3)
N(1)-C(2)	1.345(2)	C(3)-C(4)	1.511(3)	C(11)-C(12)	1.502(3)
O(2)-C(2)	1.335(2)	C(3)-C(5)	1.511(3)	C(11)-C(14)	1.515(3)
O(2)-C(3)	1.472(2)	O(6)-C(10)	1.194(2)		
N(2)-O(5)	1.208(2)	O(7)-C(10)	1.320(2)		
N(1)-C(1)-C(10)	108.36(14)	C(4)-C(3)-C(5)	112.46(19)		
N(1)-C(1)-C(7)	114.90(15)	C(10)-O(7)-C(11)	121.36(14)		
C(10)-C(1)-C(7)	107.56(15)	C(8)-C(7)-C(1)	114.96(15)		
C(2)-N(1)-C(1)	121.95(15)	O(3)-C(8)-C(9)	106.29(15)		
C(2)-O(2)-C(3)	120.92(14)	O(3)-C(8)-C(7)	113.40(15)		
O(1)-C(2)-O(2)	125.17(15)	C(9)-C(8)-C(7)	108.13(15)		
O(1)-C(2)-N(1)	124.18(16)	N(2)-C(9)-C(8)	110.76(15)		
O(2)-C(2)-N(1)	110.65(15)	O(6)-C(10)-O(7)	125.85(17)		
O(5)-N(2)-O(4)	123.8(2)	O(6)-C(10)-C(1)	123.45(17)		
O(5)-N(2)-C(9)	119.11(19)	O(7)-C(10)-C(1)	110.62(15)		
O(4)-N(2)-C(9)	117.06(18)	O(7)-C(11)-C(13)	109.92(17)		
O(2)-C(3)-C(6)	101.46(16)	O(7)-C(11)-C(12)	102.64(17)		
O(2)-C(3)-C(4)	109.49(17)	C(13)-C(11)-C(12)	113.0(3)		
C(6)-C(3)-C(4)	111.3(2)	O(7)-C(11)-C(14)	109.43(16)		
O(2)-C(3)-C(5)	110.14(17)	C(13)-C(11)-C(14)	111.7(2)		
C(6)-C(3)-C(5)	111.4(2)	C(12)-C(11)-C(14)	109.7(3)		

Table 3. Torsion angles [°] for **7b**.

C(10)-C(1)-N(1)-C(2)	-139.97(17)	N(1)-C(1)-C(10)-O(6)	-20.9(3)
C(7)-C(1)-N(1)-C(2)	99.7(2)	C(7)-C(1)-C(10)-O(6)	103.9(2)
C(3)-O(2)-C(2)-O(1)	-6.1(3)	N(1)-C(1)-C(10)-O(7)	162.27(15)
C(3)-O(2)-C(2)-N(1)	174.09(16)	C(7)-C(1)-C(10)-O(7)	-72.96(19)
C(1)-N(1)-C(2)-O(1)	-14.6(3)	C(10)-O(7)-C(11)-C(13)	-62.1(3)
C(1)-N(1)-C(2)-O(2)	165.25(15)	C(10)-O(7)-C(11)-C(12)	177.3(3)
C(2)-O(2)-C(3)-C(6)	-179.17(19)	C(10)-O(7)-C(11)-C(14)	60.9(2)
C(2)-O(2)-C(3)-C(4)	-61.5(2)		
C(2)-O(2)-C(3)-C(5)	62.7(2)		
N(1)-C(1)-C(7)-C(8)	-54.5(2)		
C(10)-C(1)-C(7)-C(8)	-175.25(15)		
C(1)-C(7)-C(8)-O(3)	-50.5(2)		
C(1)-C(7)-C(8)-C(9)	-168.08(16)		
O(5)-N(2)-C(9)-C(8)	-111.75(19)		
O(4)-N(2)-C(9)-C(8)	69.8(2)		
O(3)-C(8)-C(9)-N(2)	62.97(19)		
C(7)-C(8)-C(9)-N(2)	-174.96(16)		
C(11)-O(7)-C(10)-O(6)	0.6(3)		
C(11)-O(7)-C(10)-C(1)	177.41(15)		

