Supporting information

An investigation of nitrile transforming in the chemo-enzymatic synthesis of the taxol sidechain

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Synthesis of substrates and reference materials

3-Phenyloxirane-2-carbonitrile

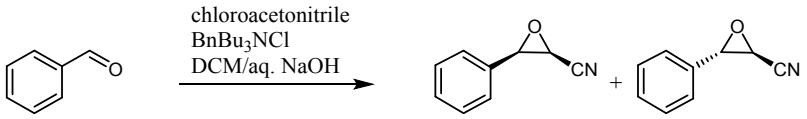

Figure S1: Synthesis of *(±)-trans*- and *(±)-cis*-3-phenylioxirane-2-carbonitrile

A solution of benzaldehyde (20.3mL, 0.199mmol) and chloroacetonitrile (12.6mL, 0.199mol) in dichloromethane was added dropwise to aqueous NaOH (50%m/m) containing $BnBu_3NCl$ (3.14g, 10.0mmol) as phase transfer catalyst. The reaction mixture was stirred at room temperature for two hours. Subsequently, the reaction mixture was diluted with dichloromethane and washed twice with ice water and once with brine. The organic phase was dried over Na_2SO_4 and purified by column chromatography. *(±)-trans*-phenyloxiriane-2-carbonitrile was isolated as white solid (5.57g, 19.3%). ^{1}H NMR ($CDCl_3$) δ 3.33 (1H, d, *J*=1.6Hz, H-2), 4.20 (1H, d, *J*=1.4Hz, H-3), 7.15-7.23 (2H, m, H-2', H-6'), 7.29-2.35 (3H, m, H-3', H-4', H-5'); ^{13}C NMR ($CDCl_3$) δ 44.64 (C-2), 58.51 (C-3), 116.02 (CN), 125.68 (C-2', C-6'), 129.00 (C-3', C-5'), 129.85 (C-4'), 132.78 (C-1'). **(±)-***cis*-phenyloxirane-2-carbonitrile: light yellow solid, yield 6.94g, 24.0%. ^{1}H NMR (DMSO-d_6) δ 3.68 (d, *J*=3.67Hz, 1H, H-2), 4.16 (d, *J*=3.65Hz, 1H, H-3), 7.35 (s, 5H, H-2'-H-6'); ^{13}C NMR ($CDCl_3$) δ 45.12 (C-2), 57.74 (C-3), 115.04 (CN), 126.34 (C-2', C-6'), 128.72 (C-3', C-5'), 129.75 (C-4'), 131.43 (C-1').

(±)-*trans*- and (±)-*cis*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxamide[1]

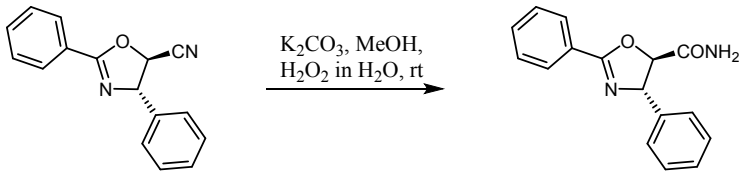

Figure S2: Synthesis of *(±)-trans*-2,4-diphenyl-4,5-dihydrooxazole-5-carboxamide

(±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxamide

(±)-trans-2,4-Diphenyl-4,5-dihydrooxazole-5-carbonitrile (1.00g, 4.03mmol) and potassium carbonate (2.78g, 20.1mmol) were suspended in 20mL of methanol. Hydrogen peroxide solution (30%wt. in water, 10mL) was added to the reaction mixture. The reaction was monitored by TLC. After TLC indicated complete conversion, the reaction was diluted with deionized water and extracted with ethyl acetate three times. The combined organic layers were dried over Na_2SO_4 and purified by column chromatography with cyclohexane/ ethyl acetate 1/1 as eluent. *(±)-trans*-2,4-Diphenyl-4,5-dihydrooxazole-5-carboxamide was isolated as white solid (460mg, 42.9%). m.p. 148-154°C; ; EI-HRMS m/z 221.0850 ($[M]^+$, C_{16} $H_{14}N_2O_2$ – C_1H_3NO, calc. 221.0841); ^{1}H NMR (DMSO-d_6) δ 4.84 (1H, d, *J*=6.8Hz, H-5), 5.35 (1H, d, *J*=6.8Hz, H-4), 7.30-7.44 (5H, m, H-2'', H-3'', H-4'', H-5'', H-6''), 7.50-7.64 (3H, m, H-3', H-4', H-5'), 7.83 (1H, s, $CONH_2$), 8.07 (2H, d, *J*=7.1Hz, H-2', H-6'); ^{13}C NMR (DMSO-d_6) δ 73.80 (C-4), 83.64 (C-5), 126.63 (C-4''), 126.80 (C-1'), 127.54, 128.41, 128.59 (C-2'', C-3'', C-5'', C-6'', C-3', C-5'), 129.07 (C-2', C-6'), 131.90 (C-4'), 141.97 (C-1''), 162.37 (C-2), 171.58 ($CONH_2$).

(±)-*cis*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxamide

(±)-cis-2,4-Diphenyl-4,5-dihydrooxazole-5-carboxamide was prepared analogously from *(±)-cis*-2,4-diphenyl-4,5-dihydrooxazole-5-carbonitrile (137mg, 0.552mmol). *(±)-cis*-2,4-Diphenyl-4,5-dihydrooxazole-5-carboxamide was isolated as white solid (78.0mg, 53.1%). m.p. 172-174°C; EI-HRMS m/z 221.0858 ($[M]^+$, C_{16} $H_{13}NO_3$ - $C_1H_2O_2$, calc. 221.0841); ^{1}H NMR (DMSO-d_6) δ 5.34 (1H, d, J=10.7Hz, H-5), 5.70 (1H, d, J=10.7Hz, H-4), 7.10 (1H, s, NH_2), 7.21-7.36 (5H, m, H-2'', H-3'', H-4'', H-5'', H-6''), 7.59 (2H, t, J=7.2Hz, H-3', H-5'), 7.67 (1H, t, J=7.2Hz, H-4'), 8.09 (2H, d, J=7.2Hz, H-2', H-6'); ^{13}C NMR (DMSO-d_6) δ 72.14 (C-4), 81.62 (C-5), 127.05 (C-1'), 127.36, 127.50, 128.16, 128.29 (C-2'', C-3'', C-4'', C-5'', C-6''), 128.29 (C-2', C-6'), 128.56 (C-3', C-5'), 131.90 (C-4'), 137.90 (C-1''), 163.14 (C-2), 168.69 (C-1).

[1] 2,4-Diphenyl-4,5-dihydrooxazole-5-carbonitrile, 2,4-diphenyl-4,5-dihydrooxazole-5-carboxamide and 2,4-diphenyl-4,5-dihydrooxazole-5-carboxylic acid were previously published by our group: M. Winkler, A. Glieder, N. Klempier, *Chem. Commun.* 2006, **12**, 1298-1300.

(±)-*trans*- and (±)-*cis*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxylic acid[2]

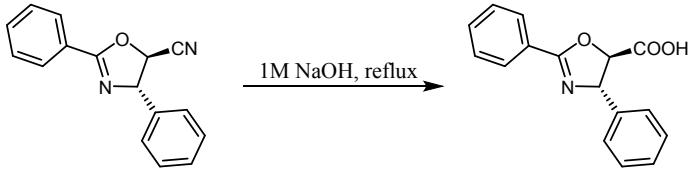

Figure S3: Synthesis of *(±)-trans*-2,4-diphenyl-4,5-dihydrooxazole-5-carboxylic acid

(±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxylic acid

(±)-trans-2,4-Diphenyl-4,5-dihydrooxazole-5-carbonitrile (503.1mg, 2.01mmol) was refluxed in aqueous sodium hydroxide solution (1M, 10mL). The reaction was monitored by TLC. After TLC indicated complete conversion (approximately 3.5 hours), the reaction mixture was allowed to cool to room temperature. The pH value of the reaction mixture was then adjusted to pH 7 by addition of aqueous HCl (5M). The product precipitated from the solution and was isolated by filtration. The product was purified by column chromatography with a gradient of chloroform/ methanol = 20/1 to chloroform/ methanol = 1/1. *(±)-trans*-2,4-Diphenyl-4,5-dihydrooxazole-5-carboxylic acid was isolated as white solid (292.9mg, 54.1%). m.p. 205°C; ^{1}H NMR (DMSO-d_6) δ 3.42 (bs, COOH), 5.01 (1H, d, *J*=6.3Hz, H-5), 5.42 (1H, d, *J*=6.3Hz, H-4), 7.30-7.45 (5H, m, H-2'', H-3'', H-4'', H-5'', H-6''), 7.56 (2H, t, *J*=7.5Hz, H-3', H-5'), 7.64 (1H, t, *J*=7.2Hz, H-4'), 8.01 (2H, d, *J*=7.1Hz, H-2', H-6'); ^{13}C NMR (DMSO-d_6) δ 73.76 (C-4), 82.40 (C-5), 126.63 (C-1'), 126.49, 127.76, 128.15, 128.68, 128.77 (C-2', C-3', C-5', C-6', C-2'', C-3'', C-4'', C-5'', C-6''), 132.06 (C-4'), 141.51 (C-1''), 162.83 (C-2), 171.39 (COOH).

(±)-*cis*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-caroxylic acid

(±)-cis-2,4-Diphenyl-4,5-dihydrooxazole-5-carboxylic acid was prepared analogously from *(±)-cis*-2,4-diphenyl-4,5-dihydrooxazole-5-carbonitrile (500.1mg, 2.01mmol). *(±)-cis*-2,4-Diphenyl-4,5-dihydrooxazole-5-carboxylic acid was isolated as white solid (59.0mg, 11.0%). m.p. 152°C; ^{1}H NMR (DMSO-d_6) δ 3.62 (bs, COOH), 4.66 (1H, d, J=6.3Hz, H-5), 5.36 (1H, d, J=6.3Hz, H-4), 7.28-7.44 (5H, m, H-2'', H-3'', H-4'', H-5'', H-6''), 7.56 (2H, t, J=7.3Hz, H-3', H-5'), 7.61 (1H, t, J=7.1Hz, H-4'), 8.04 (2H, d, J=7.1Hz, H-2', H-6'); ^{13}C NMR (DMSO-d_6) δ 73.81 (C-4), 85.24 (C-5), 127.69 (C-1'), 126.47, 127.08, 128.06, 128.35, 128.52 (C-2', C-3', C-5', C-6', C-2'', C-3'', C-4'', C-5'', C-6''), 131.51 (C-4'), 143.35 (C-1''), 162.99 (C-2), 174.06 (COOH).

(±)-*syn*- and (±)-*anti*-3-benzamido-2-hydroxy-3-phenylpropanamide[3]

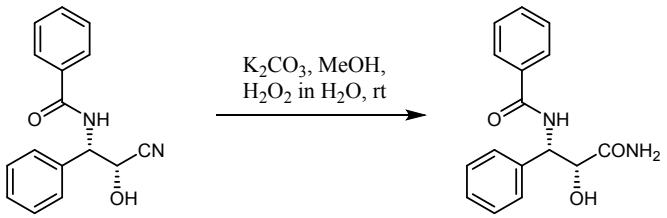

Figure S4: Synthesis of (±)-*syn*-3-benzamido-2-hydroxy-3-phenylpropanamide

(±)-*syn*-3-benzamido-2-hydroxy-3-phenylpropanamide

(±)-s*yn*-*N*-(2-Cyano-2-hydroxy-1-phenylethyl)benzamide (206 mg, 0.774mmol) was dissolved in methanol (6mL). Potassium carbonate (524 mg, 3.79mmol) and hydrogen peroxide solution (30%wt. in water, 2.2mL) were added. The reaction mixture was stirred for 1.5 hours at room temperature. Then, the reaction mixture was diluted with deionized water (20mL) and extracted with ethyl acetate three times. The combined organic phases were dried over Na_2SO_4. The crude product was purified by recrystallization from methanol. (±)-s*yn*-3-benzamido-2-hydroxy-3-phenylpropanamide was isolated was white solid (26.4mg, 11.8%). ^{1}H NMR (DMSO-d_6) δ 4.31 (1H, d, J=3.8Hz, H-2), 5.43 (1H, dd, H-3), 5.84 (1H, bs, OH), 7.20 (2H, d, J=12.4Hz, NH_2), 7.25-7.39 (3H, m, H-3'', H-4'', H-5''), 7.45 (2H, d, J=7.0Hz, H-2'', H-6''), 7.49-7.64 (3H, m, H-3', H-4', H-5'), 7.91 (2H, d, J=6.9Hz, H-2', H-6'), 8.68 (1H, d, J=8.4Hz, NH); ^{13}C NMR (DMSO-d_6) δ 55.85 (C-3), 73.63 (C-2), 126.84,

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[2] 2,4-Diphenyl-4,5-dihydrooxazole-5-carbonitrile, 2,4-diphenyl-4,5-dihydrooxazole-5-carboxamide and 2,4-diphenyl-4,5-dihydrooxazole-5-carboxylic acid were previously published by our group: M. Winkler, A. Glieder, N. Klempier, *Chem. Commun.* 2006, **12**, 1298-1300.

[3] *N*-(2-Cyano-2-hydroxy-1-phenylethyl)benzamide is found in the following reference: P. G. Wuts, J. M. Northuis, T. A. Kwan, *J. Org. Chem.* 2000, **65**, 9223-9225.

3-benzamido-2-hydroxy-3-phenylpropanamide is found in the following reference: Z. Zhou, X. Mei, *Synth. Commun.* 2003, **33**, 723-728.

127.30, 127.64, 128.08, 128.27 (C-2', C-3', C-5', C-6', C-2'', C-3'', C-4'', C-5'', C-6''), 131.26 (C-4'), 134.51 (C-1'), 139.53 (C-1''), 165.58 (CONH), 173.64 (C-1).

(±)-*anti*-3-benzamido-2-hydroxy-3-phenylpropanamide

(±)-A*nti*-3-benzamido-2-hydroxy-3-phenylpropanamide was prepared analogously from *(±)-anti-N*-(2-cyano-2-hydroxy-1-phenylethyl)benzamide (171mg, 0.642mmol). (±)-*anti*-3-benzamido-2-hydroxy-3-phenylpropanamide was isolated as white solid (15.0mg, 8.2%). ^{1}H NMR (DMSO-d_6) δ 4.28 (1H, t, J=5.5Hz, H-2), 5.39 (1H, dd, H-3), 5.80 (1H, d, J=5.8Hz, OH), 7.17 (d, J=12.9Hz, NH_2), 7.21-7.35 (3H, m, H-3'', H-4'', H-5''), 7.41 (2H, d, J=7.0Hz, H-2'', H-6''), 7.45-7.61 (3H, m, H-3', H-4', H-5'), 7.88 (2H, d, J=7.6Hz, H-2', H-6'), 8.66 (1H, s, J=8.4Hz, NH); ^{13}C NMR (DMSO-d_6) δ 55.84 (C-3), 73.63 (C-2), 126.84, 127.30, 127.64, 128.08, 128.27 (C-2', C-3', C-5', C-6', C-2'', C-3'', C-4'', C-5'', C-6''), 131.26 (C-4'), 134.51 (C-1'), 139.53 (C-1''), 165.58 (CONH), 173.64 (C-1).

***(±)-syn*-3-benzamido-2-hydroxy-3-phenylpropanoic acid[4]**

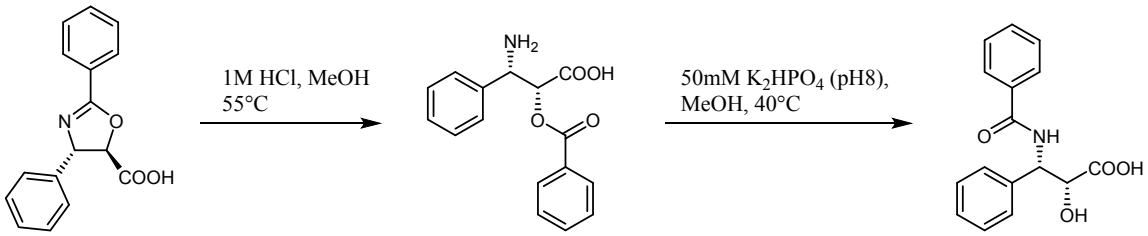


Figure S5: Synthesis of (±)-*syn*-3-benzamido-2-hydroxy-3-phenylpropanoic acid

***(±)-syn*-3-amino-2-benzyloxy-3-phenylpropanoic acid**

(±)-trans-2,4-Diphenyl-4,5-dihydrooxazole-5-carboxylic acid (175mg, 0.654mmol) was dissolved in methanol (7mL) and aqueous hydrochloric acid (1M, 3mL) was added. The reaction mixture was stirred at 56°C for five hours and subsequently at room temperature over night. The reaction mixture was then extracted with ethyl acetate three times. The combined organic layers were dried over Na_2SO_4. The pure product was purified by recrystallization from cyclohexane/ethyl acetate. (±)-*Syn*-3-amino-2-benzyloxy-3-phenylpropanoic acid was obtained as white solid (129.1mg, 69.2%). ^{1}H NMR (DMSO-d_6) δ 4.88 (1H, d, J=7.3Hz, H-2), 5.55 (1H, d, J=7.4Hz, H-1), 7.40-7.50 (3H, m, H-2'', H-4'', H-6''), 7.59 (2H, t, J=7.6Hz, H-3'', H-5''), 7.64-7.71 (2H, m, H-3', H-5'), 7.75 (1H, t, J=7.4Hz, H-4'), 8.26 (2H, d, J=7.3Hz, H-2', H-6'), 9.22 (2H, bs, NH_2); ^{13}C NMR (DMSO-d_6) δ 54.32 (C-2), 74.08 (C-1), 128.1 (C-1'), 128.66, 128.70, 129.30, 130.09 (C-2', C-3', C-5', C-6', C-2'', C-3'', C-4'', C-5'', C-6''), 133.47 (C-1''), 134.07 (C-4'), 164.84 (COOR), 167.72 (COOH).

***(±)-syn*-3-benzamido-2-hydroxy-3-phenylpropanoic acid**

(±)-*Syn*-3-amino-2-benzyloxy-3-phenylpropanoic acid (27.0mg, 0.0946mmol) was dissolved in methanol (1mL) and aqueous K_2HPO_4 buffer (50mM, pH 8, 9mL) was added. The solution was stirred at 40°C for six hours and subsequently at room temperature over night. Aqueous hydrochloric acid (1M, 1mL) was added and the reaction mixture was extracted with ethyl acetate three times. The organic layer was dried over Na_2SO_4. (±)-*Syn*-3-benzamino-2-benzyloxy-3-phenylpropanoic acid was obtained as white solid (23.3mg, 86.3%). ^{1}H NMR (DMSO-d_6) δ 4.44 (1H, d, J=4.0Hz, H-2), 5.53 (1H, dd, H-3), 7.30 (1H, t, J=7.0Hz, H-4''), 7.38 (2H, t, J=7.2Hz, H-3'', H-5''), 7.47 (2H, d, J=7.3Hz, H-2'', H-6''), 7.52-7.54 (3H, m, H-3', H-4', H-5'), 7.91 (2H,d, J=7.0Hz, H-2', H-6'), 8.62 (1H, d, J=8.8Hz, NH); ^{13}C NMR (DMSO-d_6) δ 55.81 (C-3), 73.58 (C-2), 126.94, 127.16, 127.33, 128.02, 128.31 (C-2', C-3', C-5', C-6', C-2'', C-3'', C-4'', C-5'', C-6''), 131.38 (C-4'), 134.35 (C-1'), 140.27 (C-1''), 166.05 (CONH), 173.42 (C-1).

[4] 3-amino-2-benzyloxy-3-phenylpropanoic acid was prepared in numerous synthetic procedures. Selected references: a) I. Ojima, I. Habus, M. Zhao, *J. Org. Chem.* 1991, **56**, 1681-1683; b) L. Deng, E. N. Jacobsen, *J. Org. Chem.* 1992, **57**, 4320-4323; c) G. I. Georg, Z. S. Cheruvallath, *J. Med. Chem.* 1992, **35**, 4230-4237; d) Y. Jiang, X. Chen, Y. Zheng, Z. Xue, C. Shu, W. Yuan, X. Zhang, *Angew. Chem. Int. Ed*. 2011, **50**, 7304-7307; e) C. Gennari, A. Vulpetti, M. Donghi, N. Mongelli, E. Vanotti, *Angew. Chem. Int. Ed.* 1996, **35**, 1723-1725; f) Z. Hu, P. W. Erhardt, *Org. Process Res. Dev.* 1997, **1**, 387-390.

***(±)-anti*-3-benzamido-2-hydroxy-3-phenylpropanoic acid**[5]

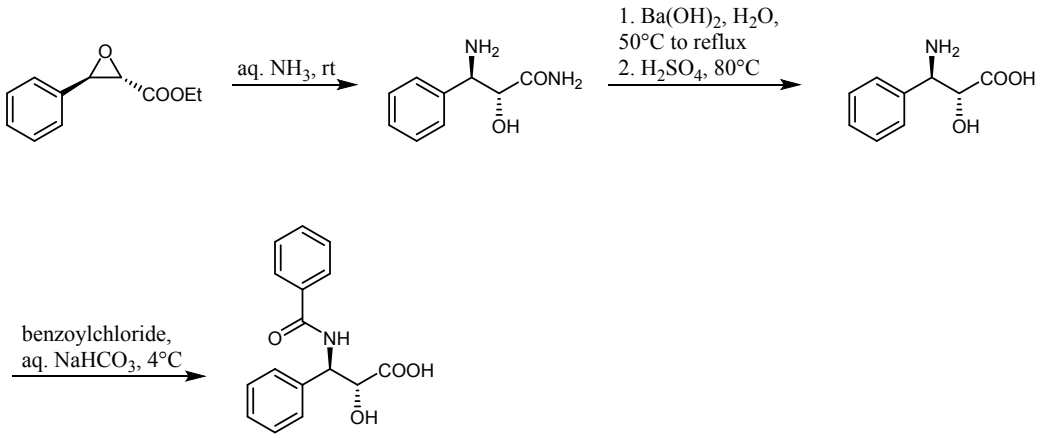


Figure S6: Synthesis of (±)-*anti*-3-benzamido-2-hydroxy-3-phenylpropanoic acid

***(±)-anti*-3-amino-2-hydroxy-3-phenylpropanamide**

(±)-Trans-ethyl 3-methyl-3-phenyloxirane-2-carboxylate (1.80mL, 9.49mmol) stirred in aqueous ammonia solution (28% wt., 25mL) at room temperature. The product precipitated from the solution. The solvent was removed under reduced pressure and the white product was used for the next synthetic step without further purification (1.57g, 91.8%). ^{1}H NMR (DMSO-d_6) δ 4.00 (1H,d, J=4.7Hz, H-3), 4.09 (1H, d, J=4.7Hz, H-2), 7.04 (2H, bs, NH_2), 7.15-7.35 (5H, m, H-2', H-3', H-4', H-5'); ^{13}C NMR (DMSO-d_6) δ 57.63 (C-3), 75.63 (C-2), 126.41, 127.38, 127.49, 127.80, 128.46 (C-2', C-3', C-4', C-5', C-6'), 142.73 (C-1'), 174.57 (C-1).

***(±)-anti*-3-amino-2-hydroxy-3-phenylpropanoic acid**

(±)-anti-3-amino-2-hydroxy-3-phenylpropanamide (344mg, 1.91mmol) was suspended in deionized water (6mL) and barium hydroxide ocathydrate (647mg, 2.05mmol) was added. The reaction mixture was refluxed for seven hours, stirred at 50°C over night, and refluxed for additional four hours. Subsequently, the reaction mixture was cooled to 80°C and diluted with deionized water (23mL). After stirring at 80°C for 20 minutes sulphuric acid (1.16M, 1.77mL, 2.05mmol) was added. The reaction was stirred for ten additional minutes at 80°C, and then cooled to room temperature. The barium sulphate was removed by filtration. The resulting filtrate was reduced in vacuum until dryness. *(±)-anti*-3-Amino-2-hydroxy-3-phenylpropanoic acid was obtained as white solid (342mg, 98.9%). ^{1}H NMR (D_2O) δ 4.21 (1H, d, J=4.3Hz, H-3), 4.26 (1H, d, J=4.3Hz, H-2), 7.34-7.47 (5H, m, C-2', C-3', C-4', C-5', C-6'); ^{13}C NMR (D_2O) δ 57.50 (C-3), 76.87 (C-2), 126.79, 127.45, 128.27 (C-2', C-3', C-4', C-5', C-6'), 140.58 (C-1'), 178.73 (C-1).

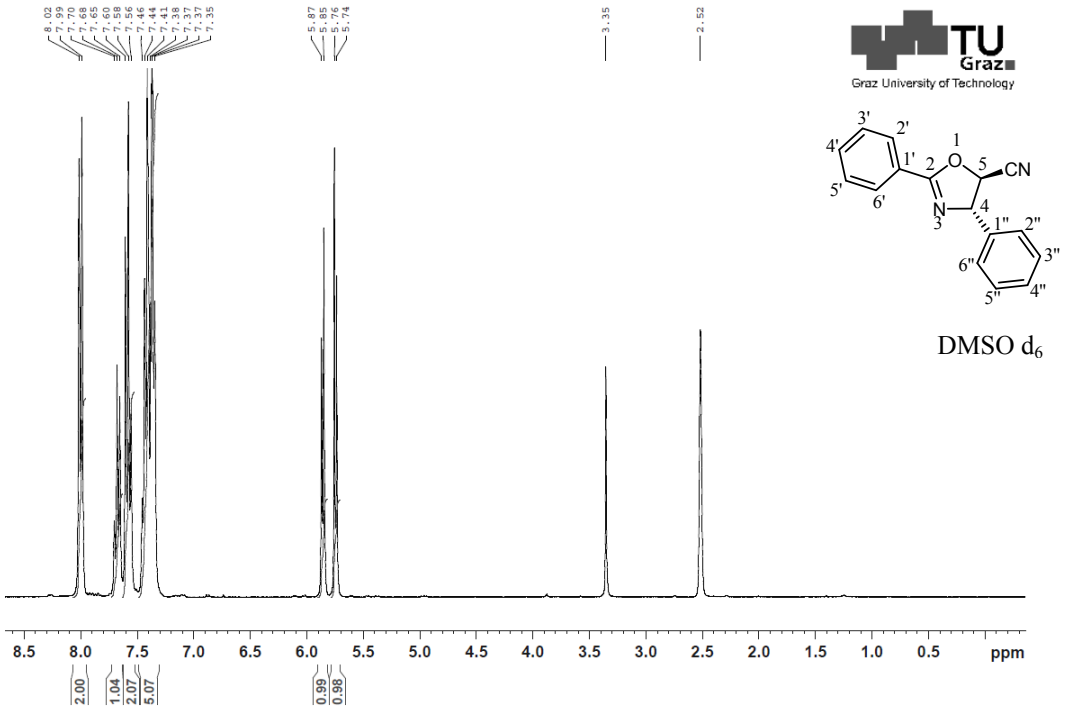
***(±)-anti*-3-benzamido-2-hydroxy-3-phenylpropanoic acid**

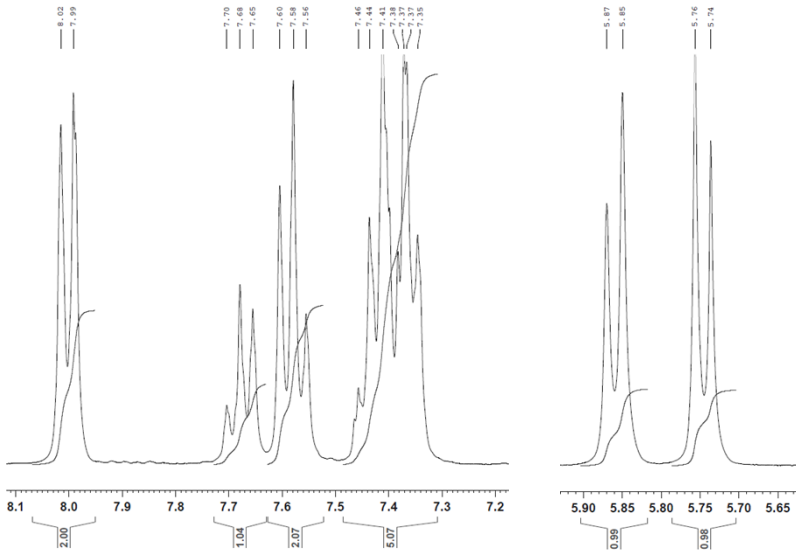
(±)-anti-3-amino-2-hydroxy-3-phenylpropanoic acid (241mg, 1.18mmol) in saturated, aqueous $NaHCO_3$-solution (33mL) was cooled to 4°C and benzoylchloride (0.49mL, 4.22mmol) was added. The reaction mixture was stirred for six hours at 4°C. Subsequently, the pH was adjusted to pH 1 by addition of aqueous hydrochloric acid (18.5% wt.). The solution was extracted with THF/dichloromethane 4/1 three times. The combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuum and the remaining residue was recrystallized from ethyl acetate/cyclohexane. The product was further purified by column chromatography. *(±)-anti*-3-Benzamino-2-hydroxy-3-phenylpropanoic acid was obtained as white solid (105.0mg, 31.2%). ^{1}H NMR (DMSO-d_6) δ 4.00 (1H, m, H-3), 5.23 (1H, m, H-2), 7.10-7.29 (3H, m, H-3'', H-4'', H-5''), 7.40 (2H, d, J=7.3Hz, H-2'', H-6''), 7.44-7.57 (3H, m, H-3', H-4', H-5'), 7.89 (2H, d, J=7.1Hz, H-2', H-6'), 9.02 (1H, d, J=5.5Hz, NH); ^{13}C NMR (DMSO-d_6) δ 56.81 (C-3), 74.75 (C-2), 126.22, 127.21, 127.39, 127.94, 128.26 (C-2', C-3', C-5', C-6', C-2'', C-3'', C-4'', C-5'', C-6''), 131.30 (C-4'), 134.68 (C-1'), 141.04 (C-1''), 165.44 (CONH), 174.52 (C-1).

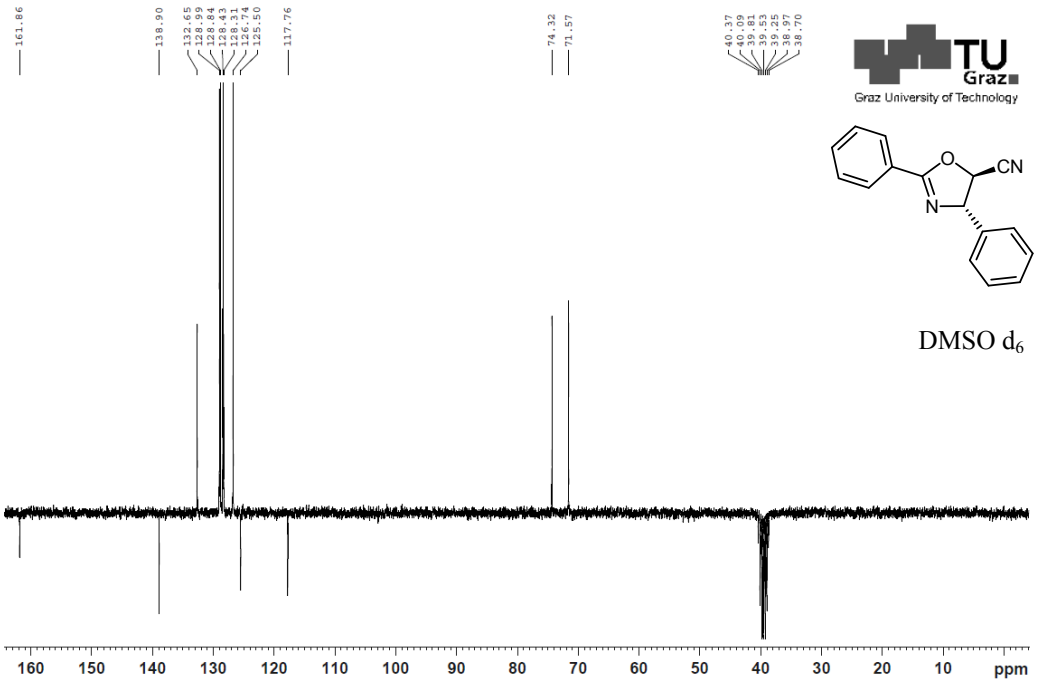
[5] 3-amino-2-benzyloxy-3-phenylpropanoic acid was prepared in numerous synthetic procedures. Selected references: a) I. Ojima, I. Habus, M. Zhao, *J. Org. Chem.* 1991, **56**, 1681-1683; b) L. Deng, E. N. Jacobsen, *J. Org. Chem.* 1992, **57**, 4320-4323; c) G. I. Georg, Z. S. Cheruvallath, *J. Med. Chem.* 1992, **35**, 4230-4237; d) Y. Jiang, X. Chen, Y. Zheng, Z. Xue, C. Shu, W. Yuan, X. Zhang, *Angew. Chem. Int. Ed.* 2011, **50**, 7304-7307; e) C. Gennari, A. Vulpetti, M. Donghi, N. Mongelli, E. Vanotti, *Angew. Chem. Int. Ed.* 1996, **35**, 1723-1725; f) Z. Hu, P. W. Erhardt, *Org. Process Res. Dev.* 1997, **1**, 387-390.

NMR spectra

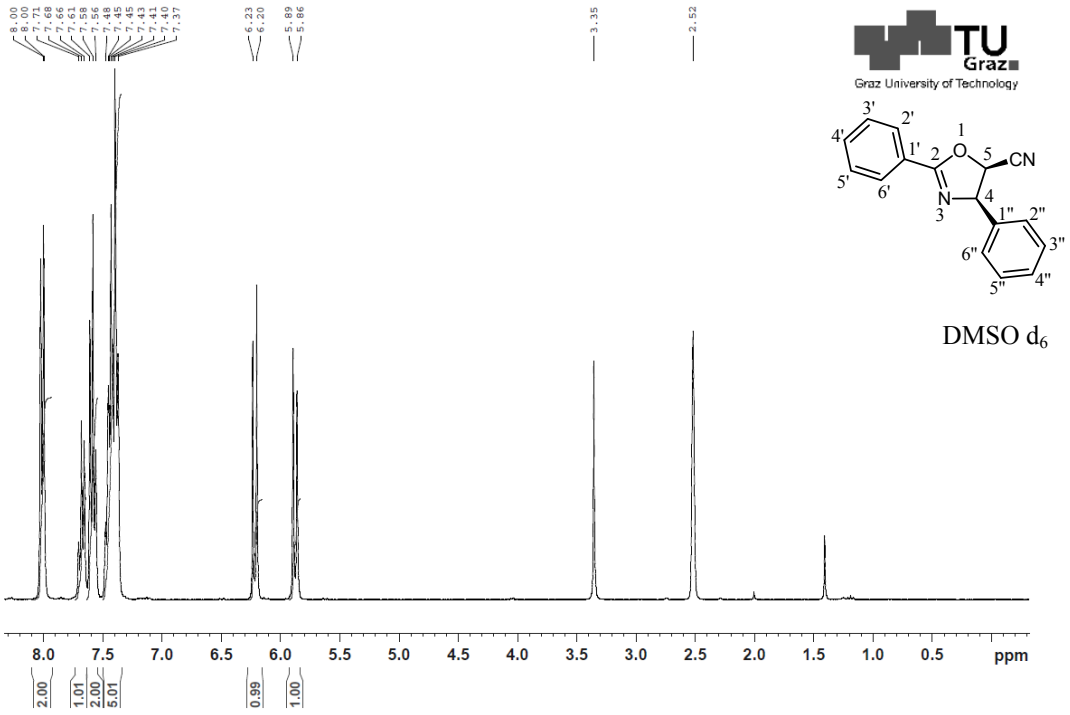
(±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile

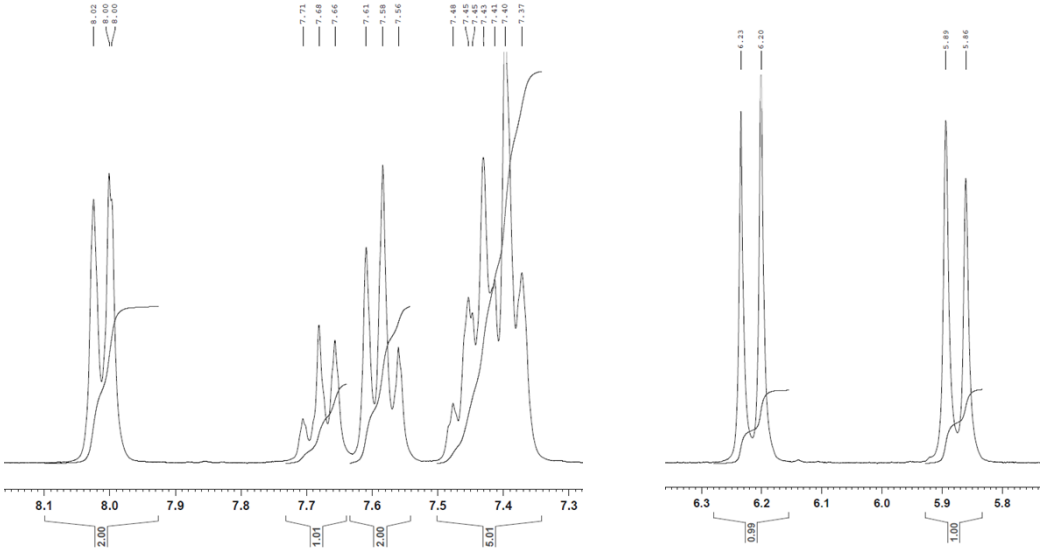


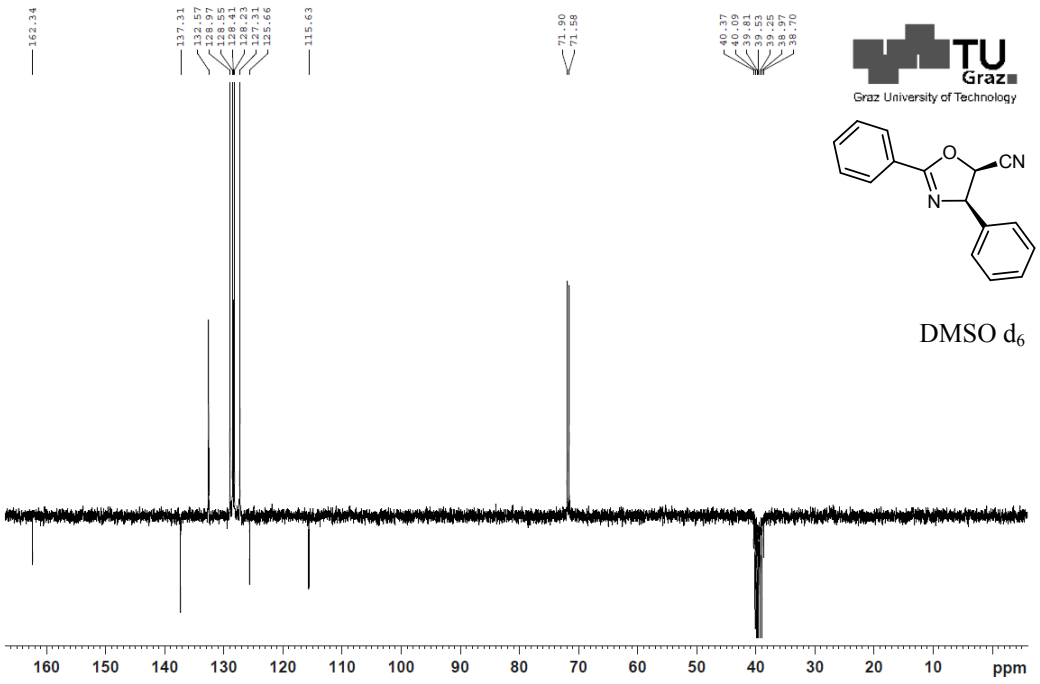




(±)-*cis*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile



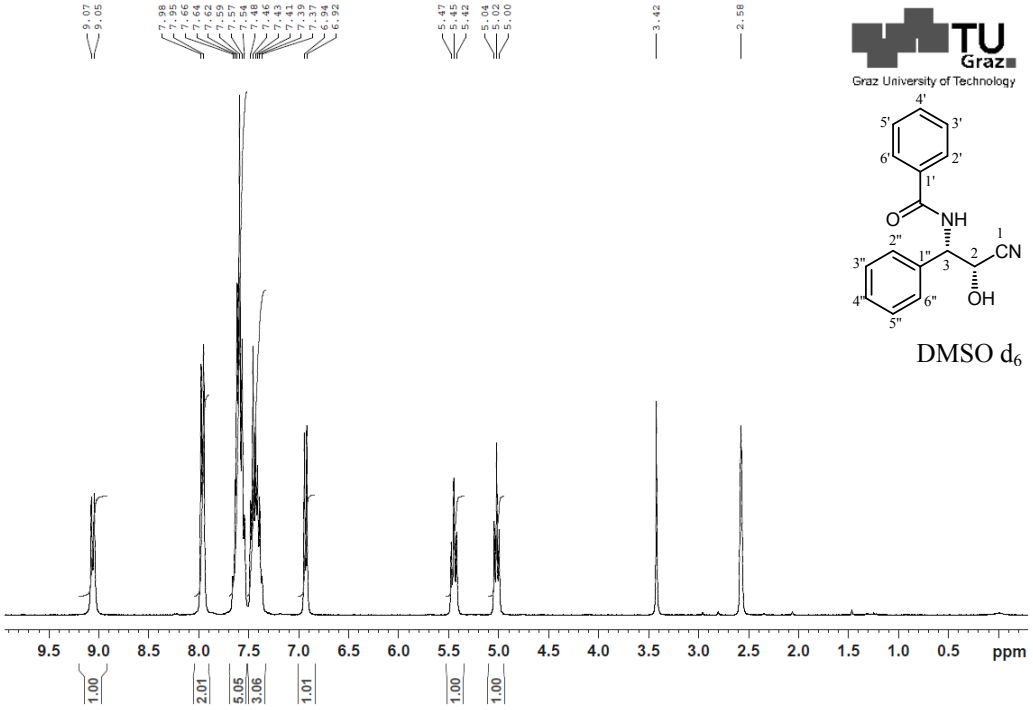


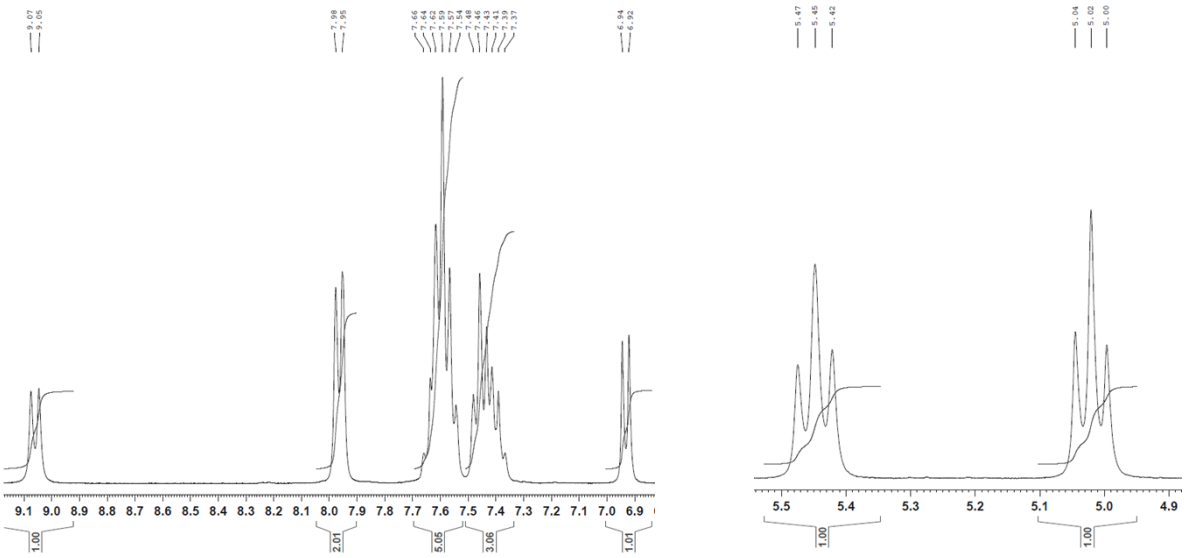


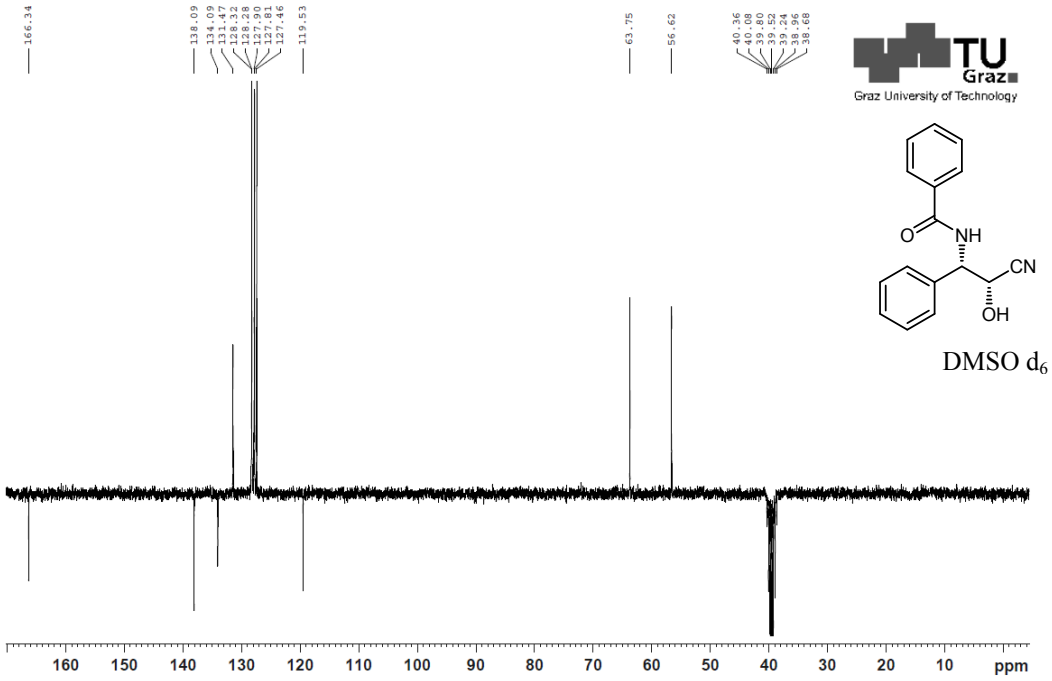
Graz University of Technology

DMSO d_6

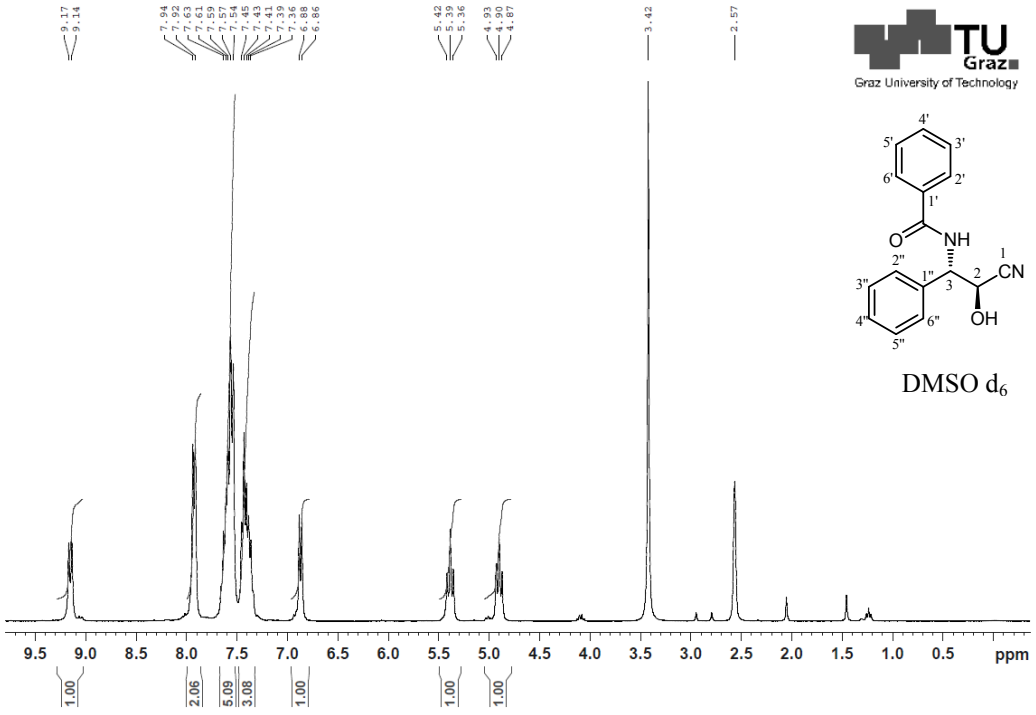
(±)-*syn*-*N*-(2-cyano-2-hydroxy-1- phenylethyl)benzamide

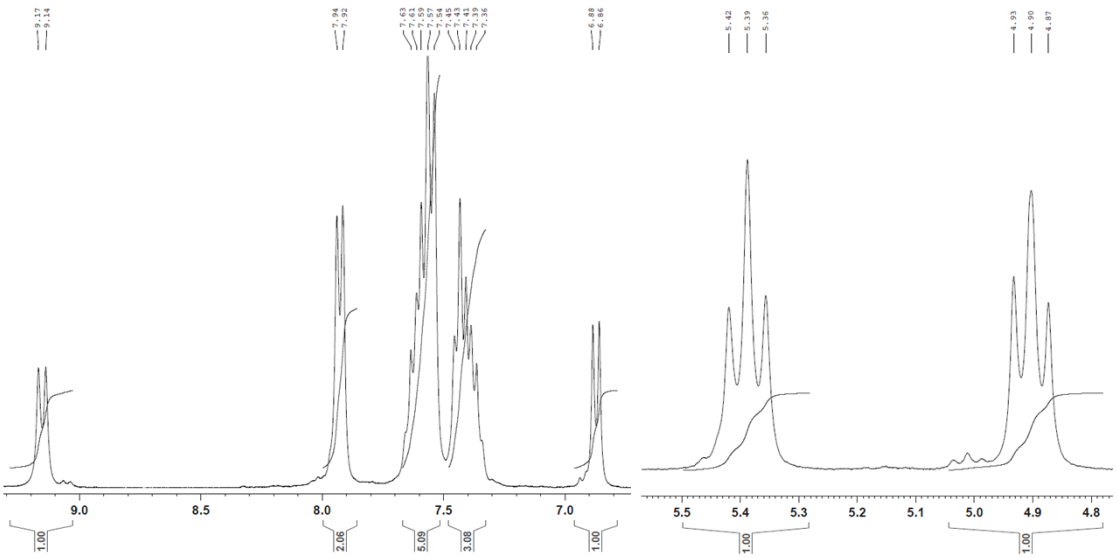


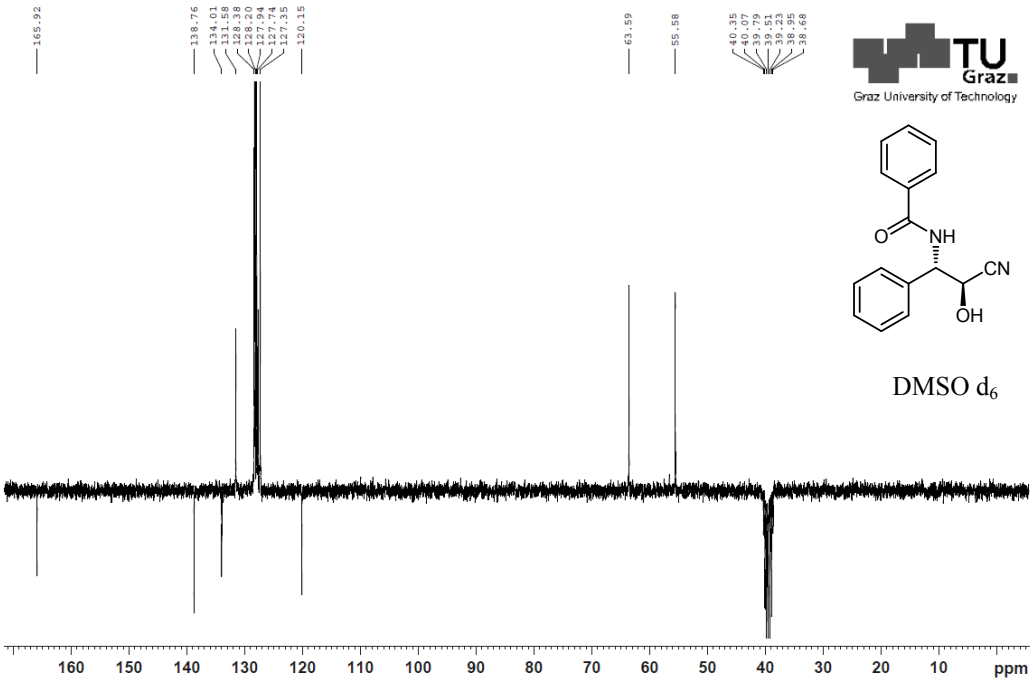




***(±)-anti-N*-(2-cyano-2-hydroxy-1-phenylethyl)benzamide**





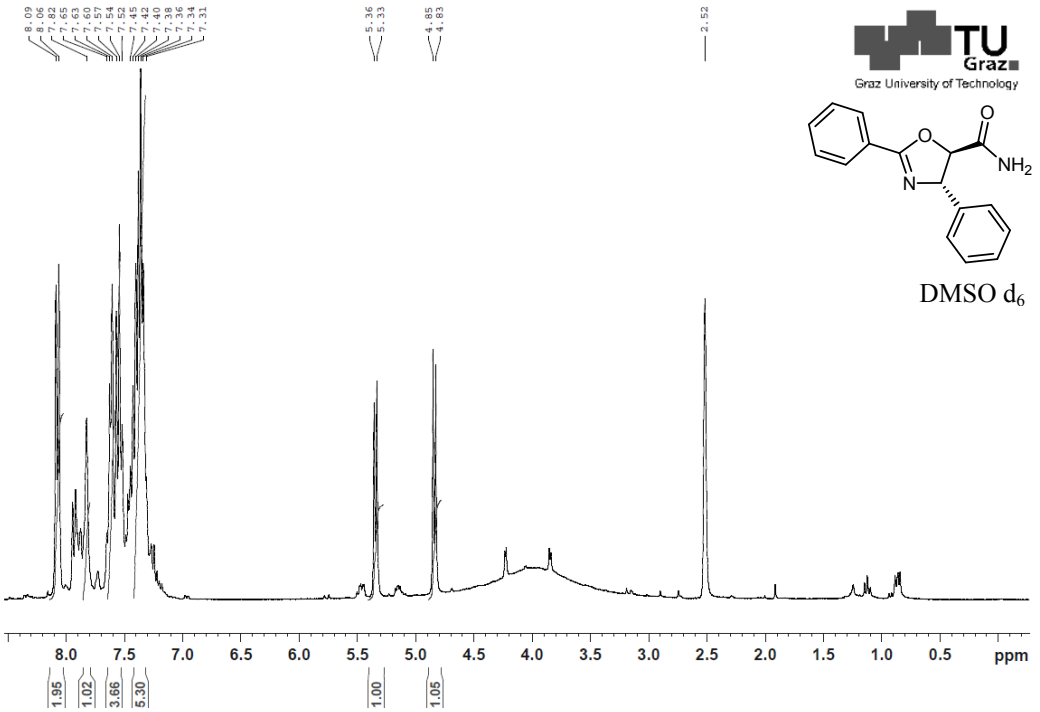


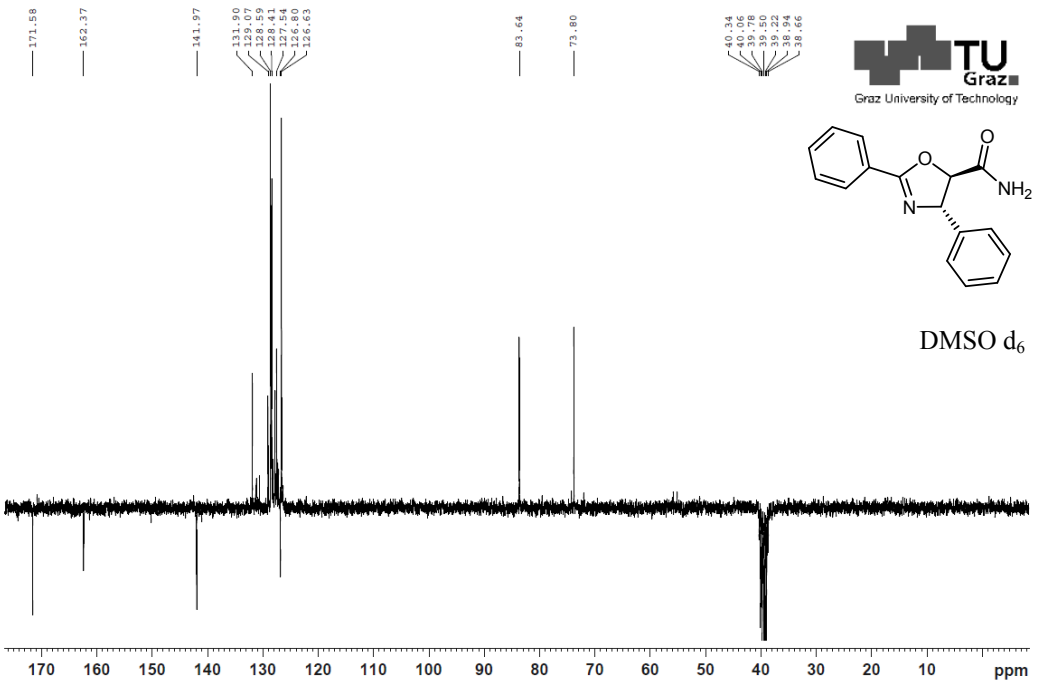
TU Graz
Graz University of Technology

DMSO d_6

(±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxamide

Prepared as reference material only, contains impurities.





(±)-*cis*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxamide

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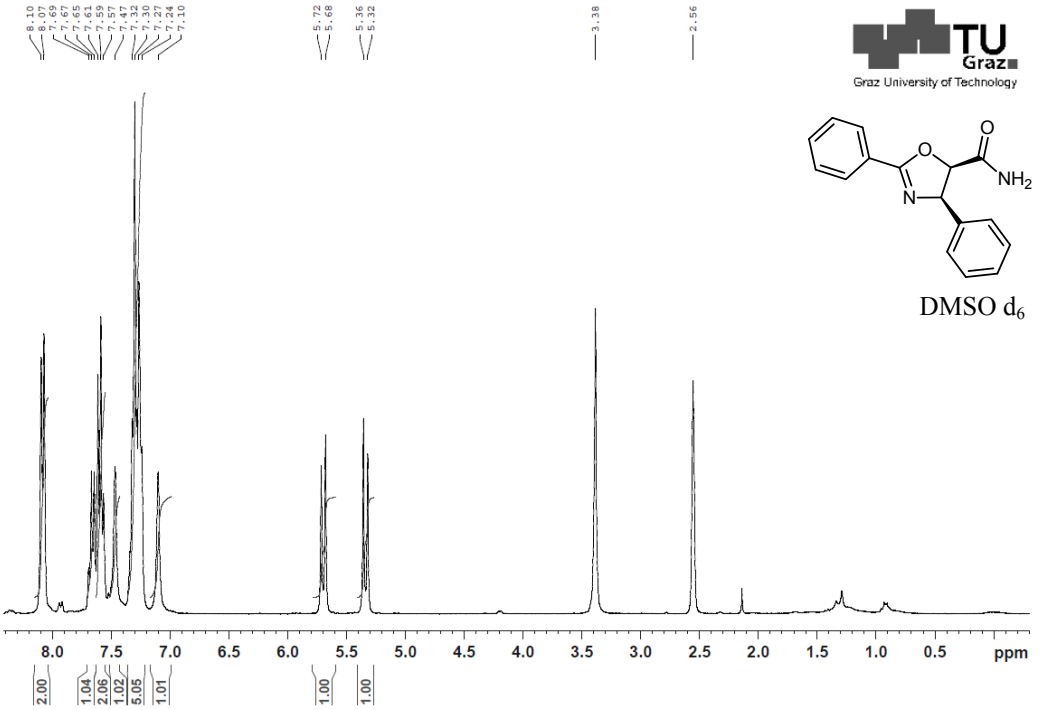
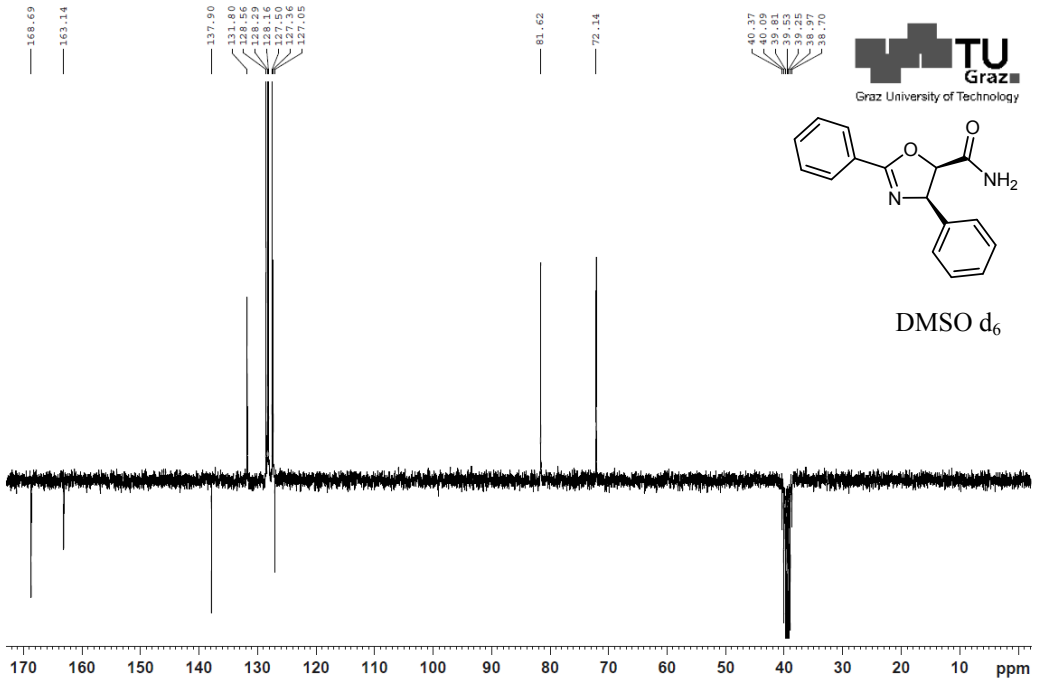
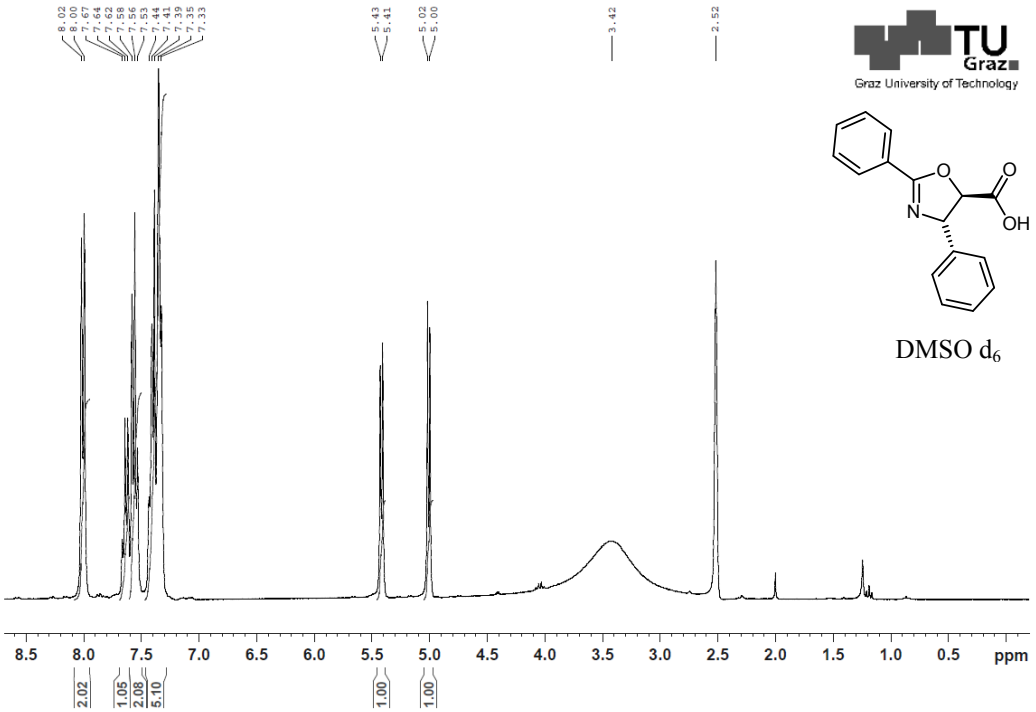
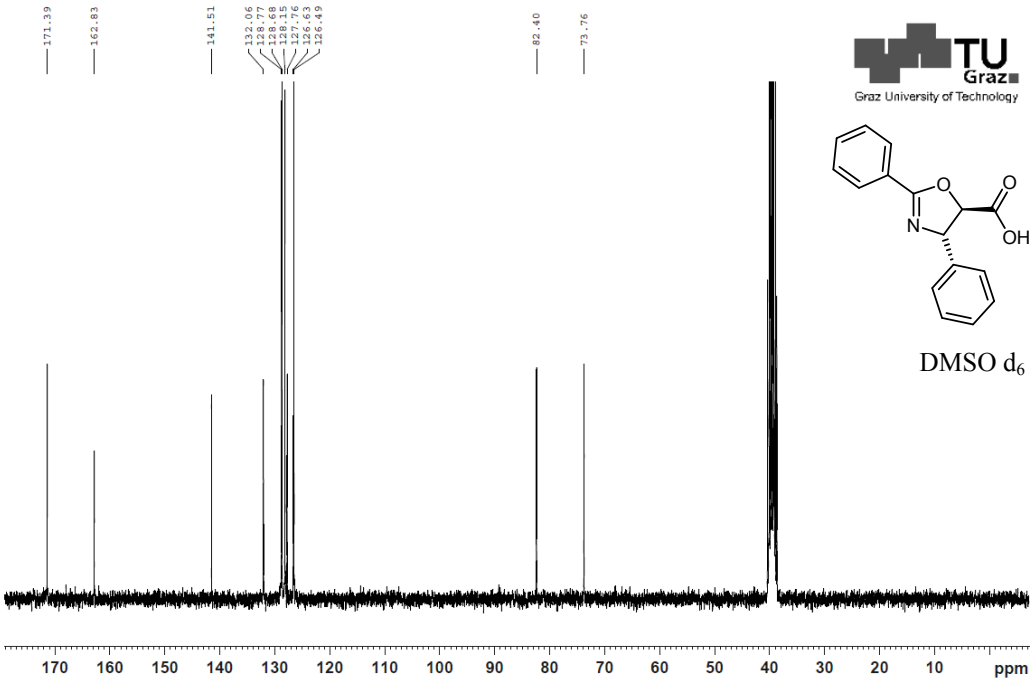




(±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxylic acid

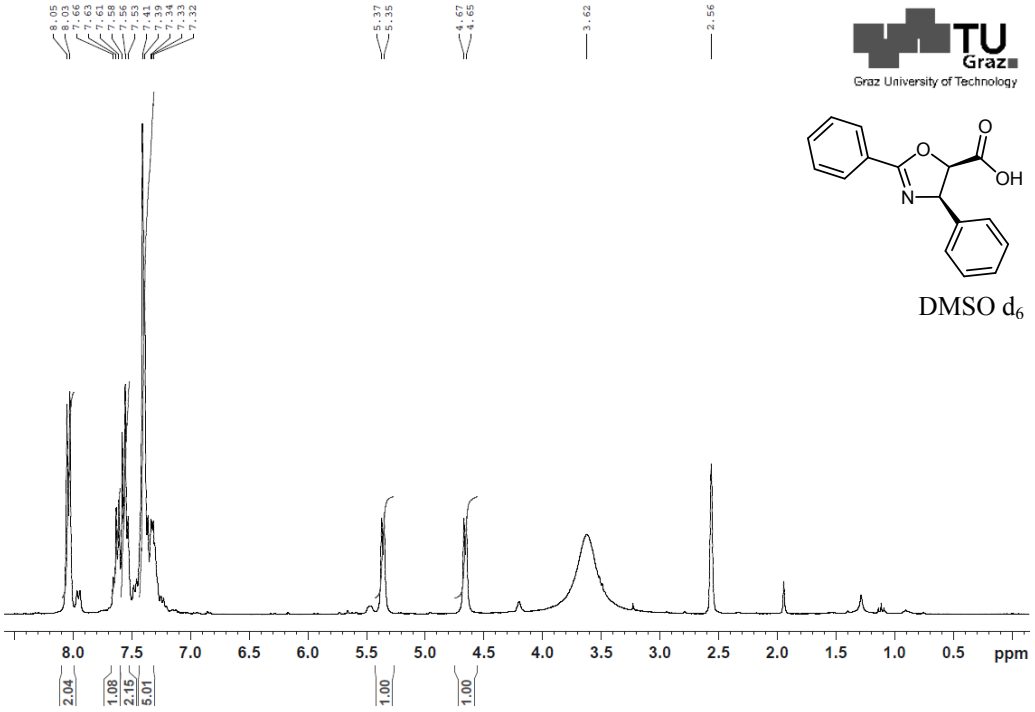
Prepared as reference material only, contains minor impurities.

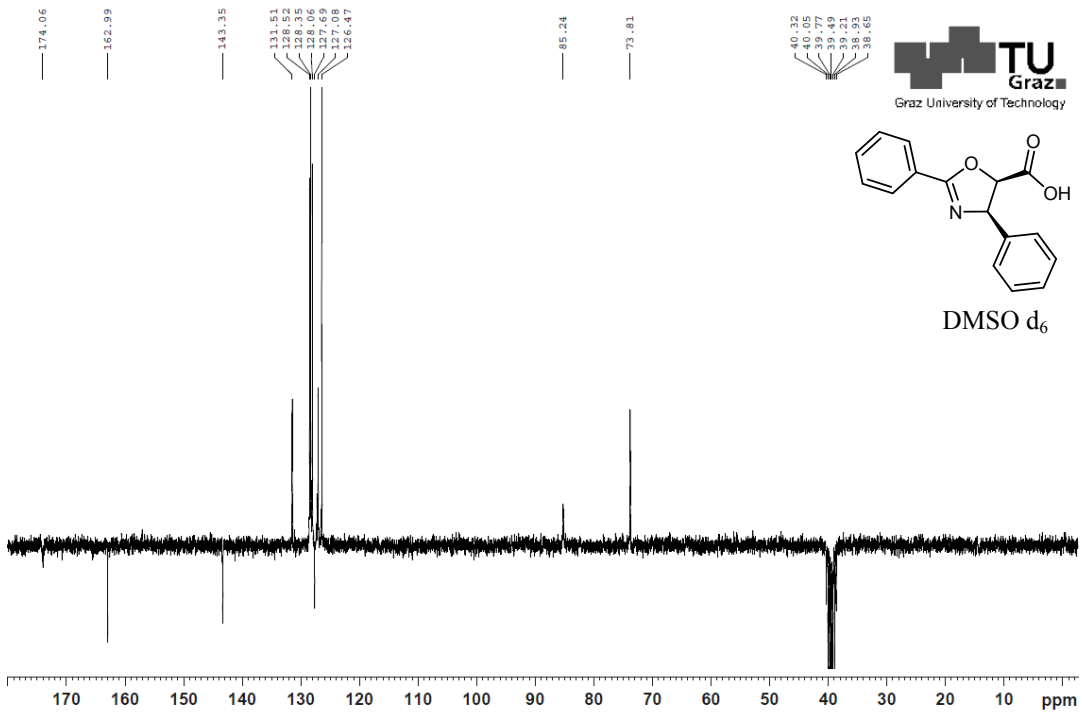




(±)-*cis*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxylic acid

Prepared as reference material only, contains minor impurities.





***(±)-syn*-3-benzamido-2-hydroxy-3-phenylpropanamide**

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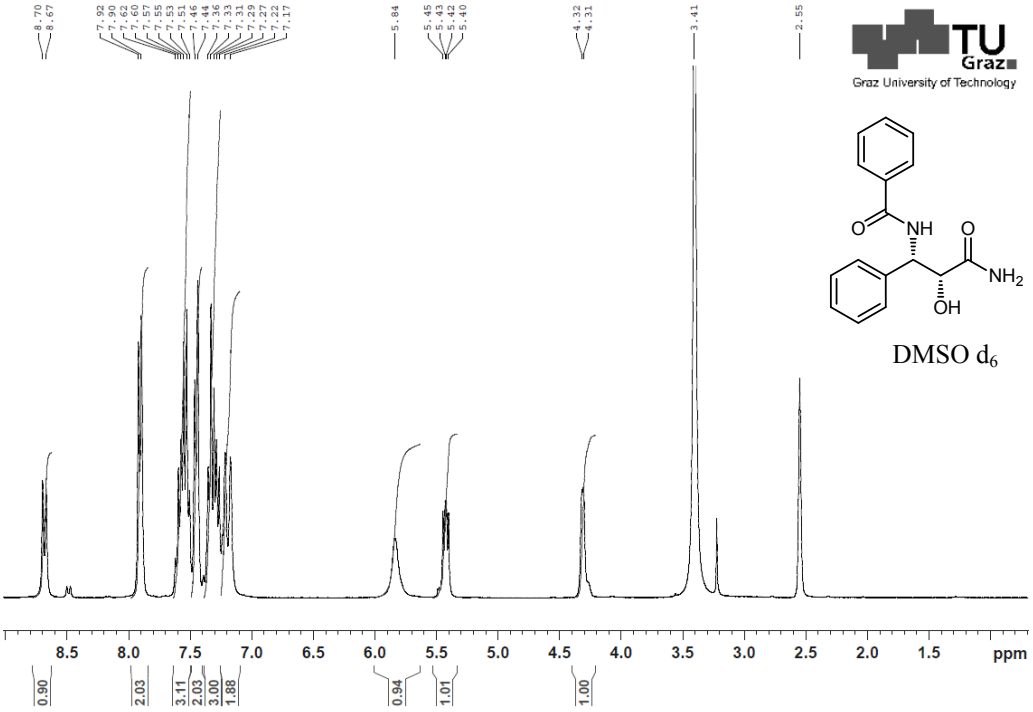

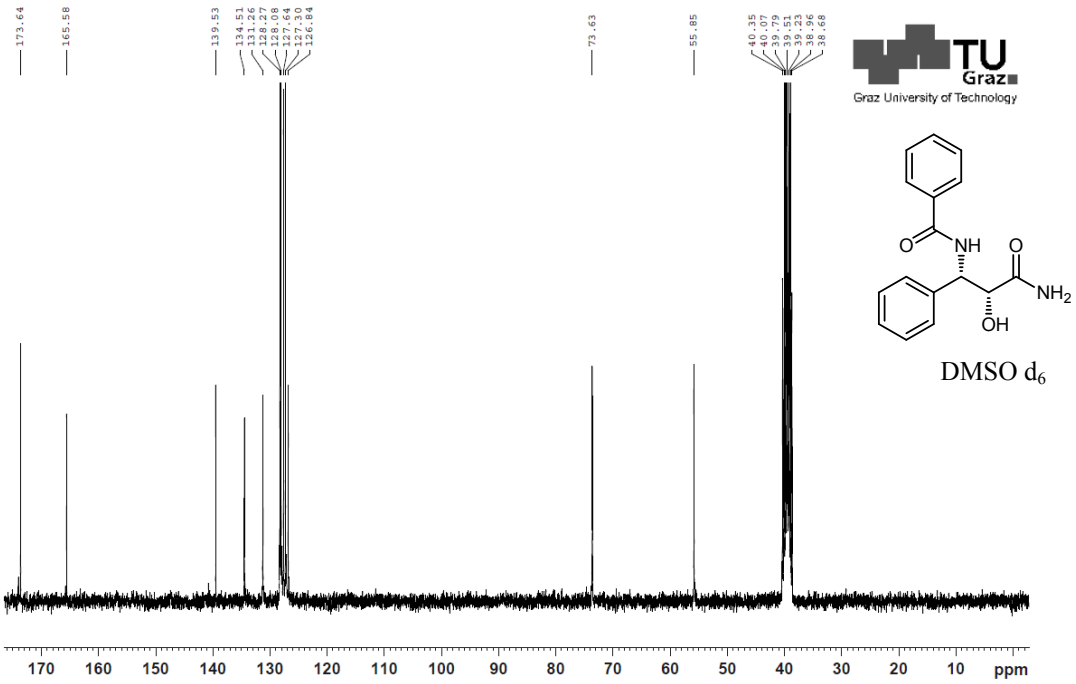

***(±)-anti*-3-benzamido-2-hydroxy-3-phenylpropanamide**

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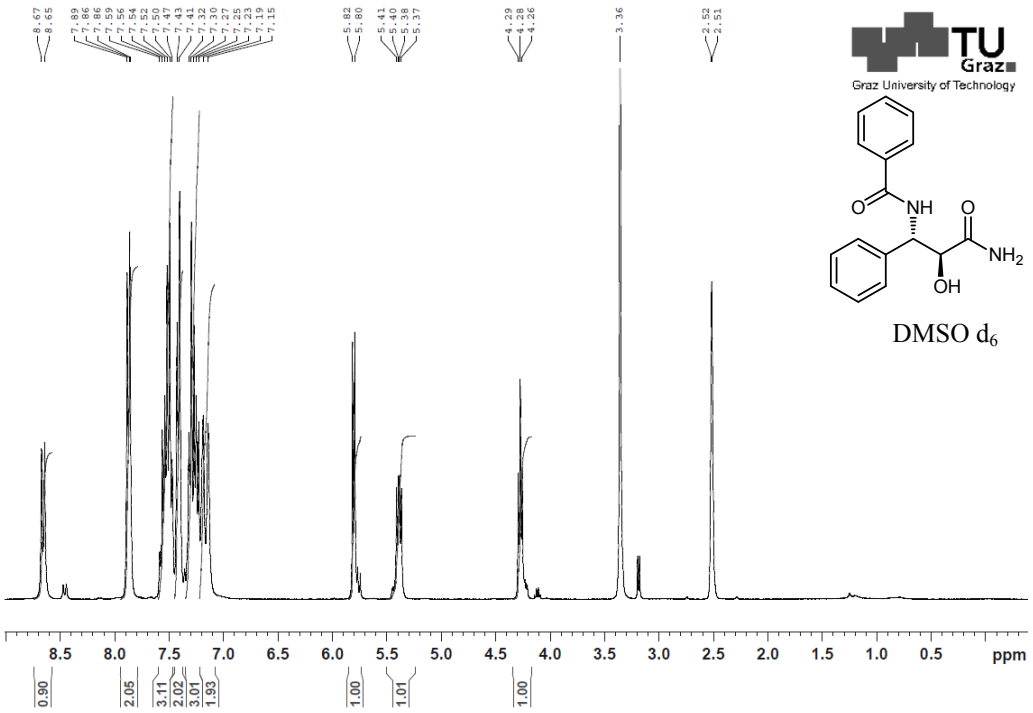

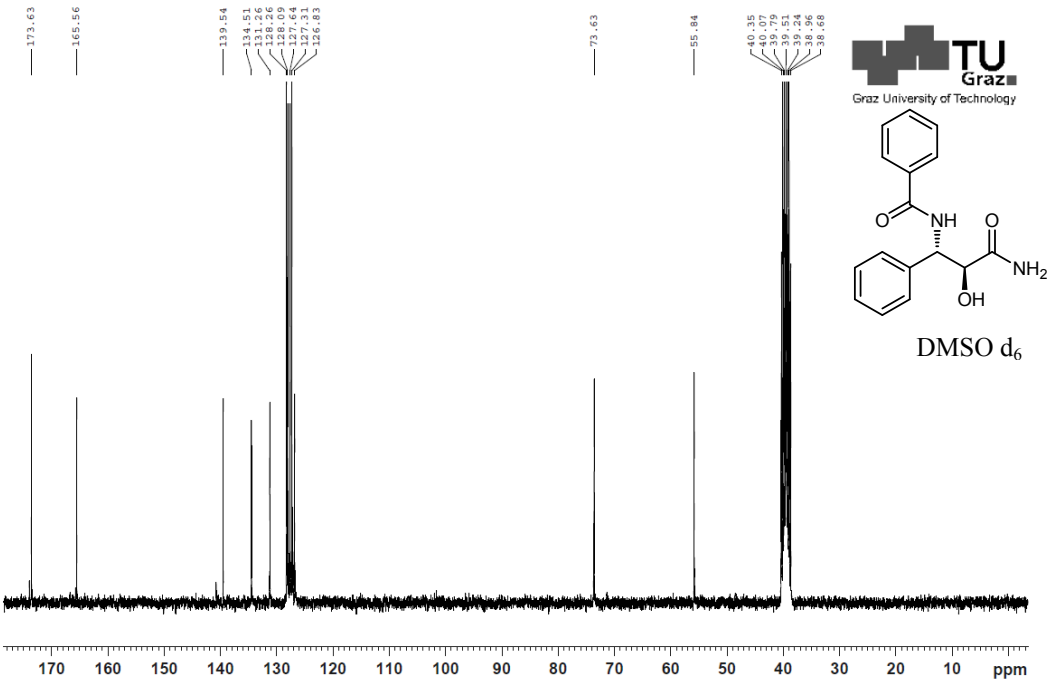

***(±)-syn*-3-benzamido-2-hydroxy-3-phenylpropanoic acid**

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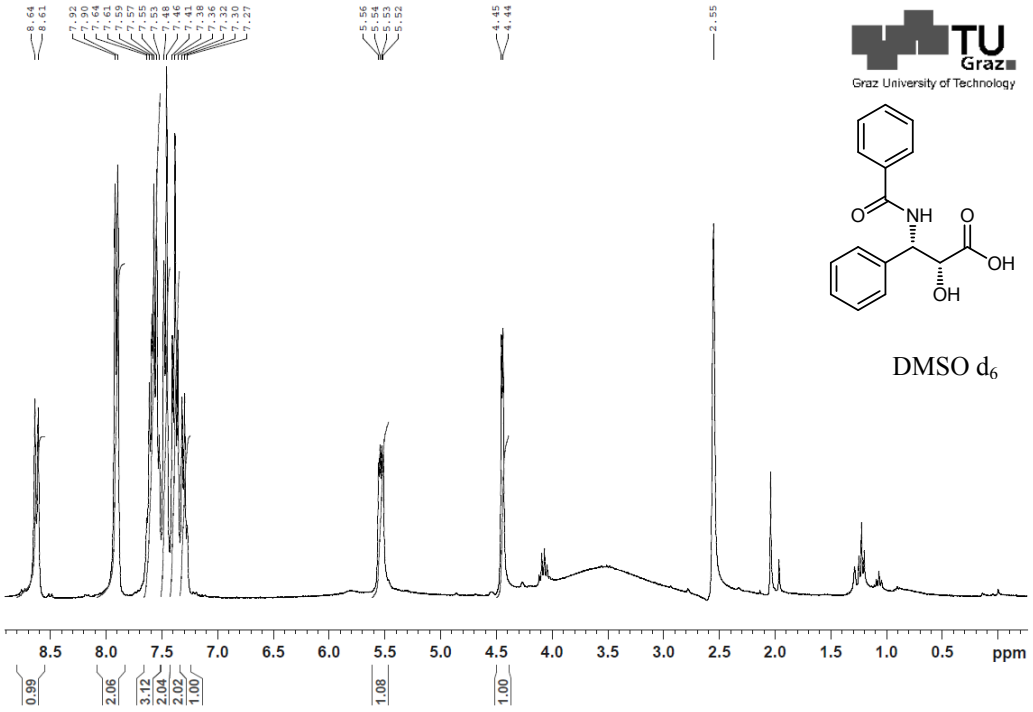

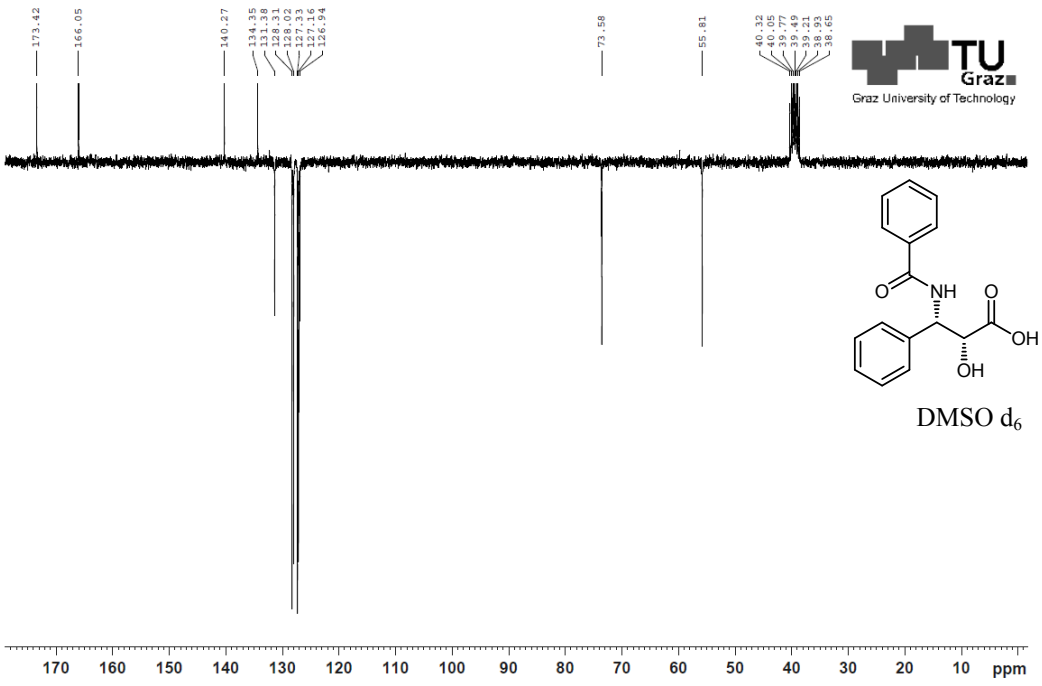

***(±)-anti*-3-benzamido-2-hydroxy-3-phenylpropanoic acid**

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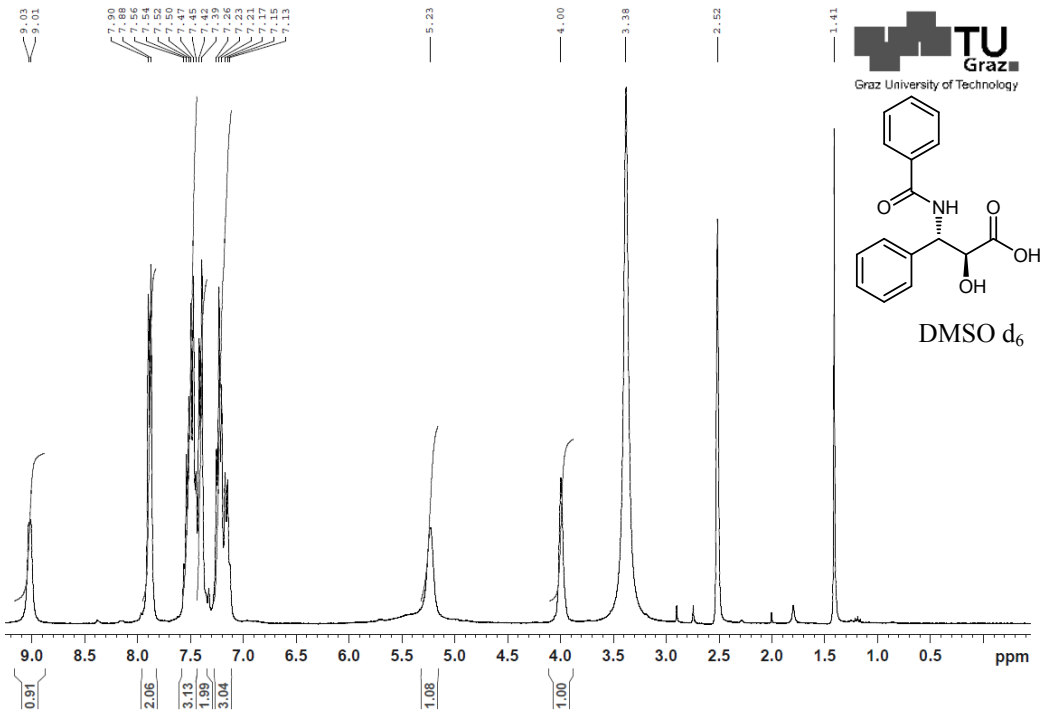
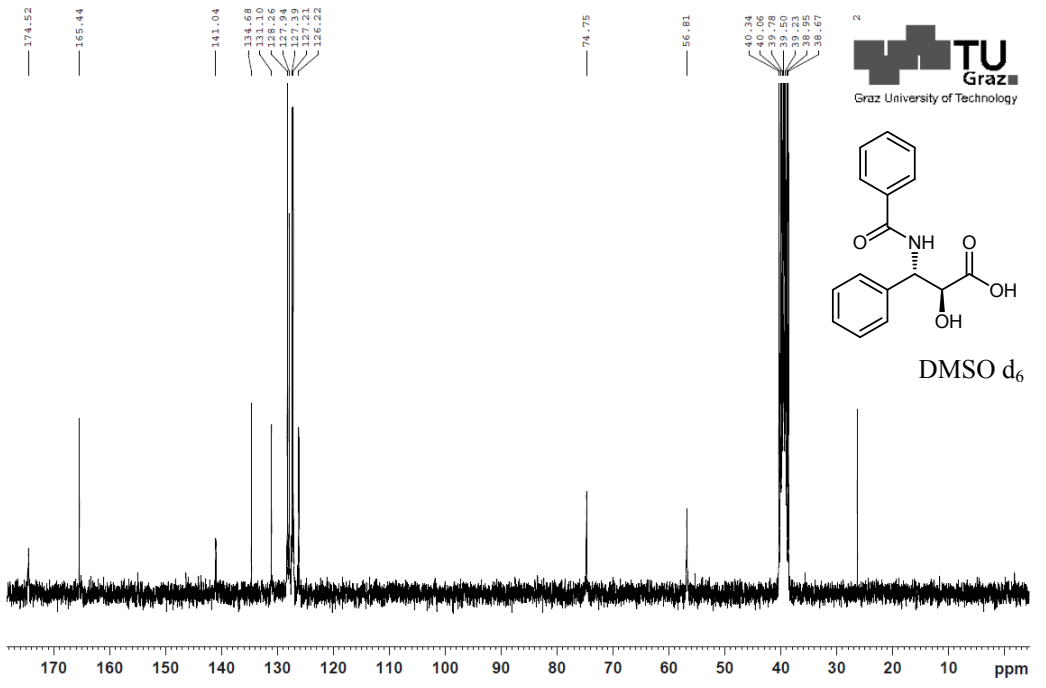
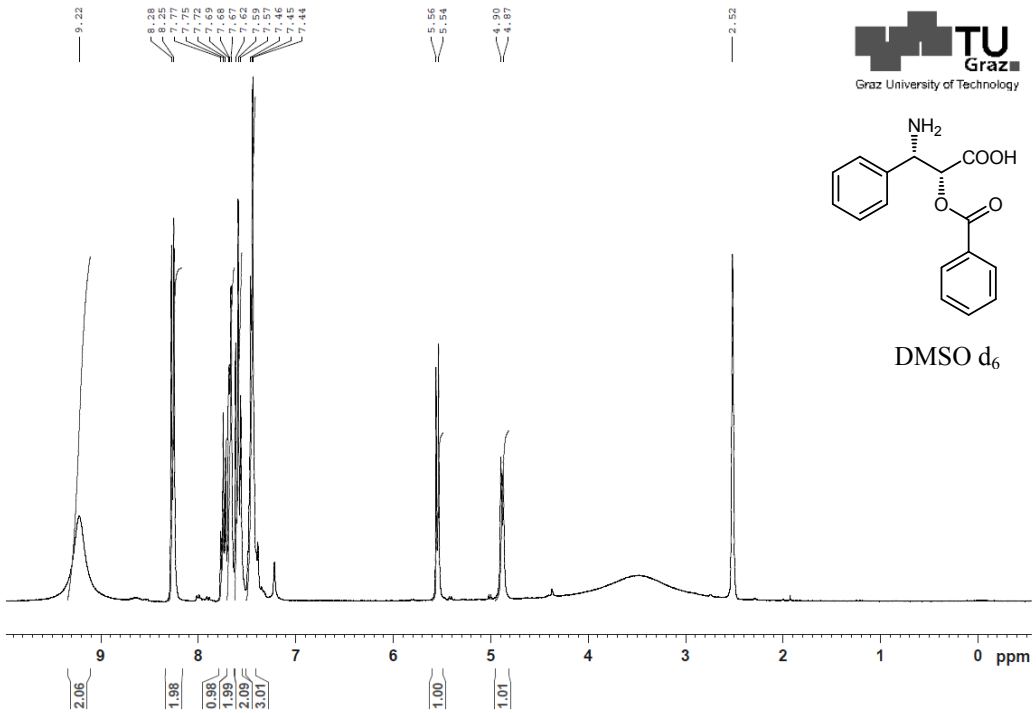
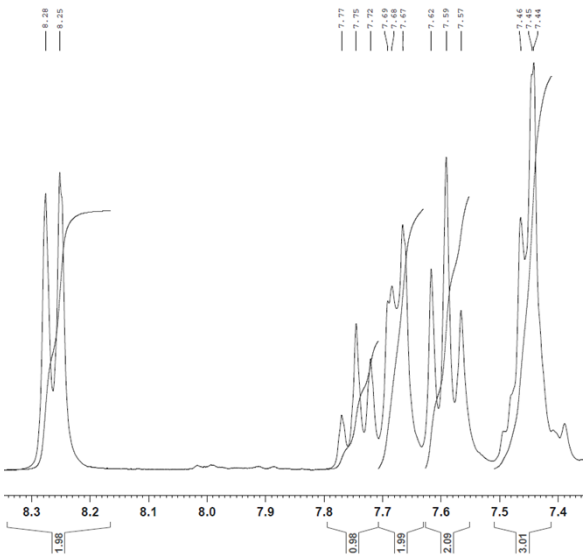
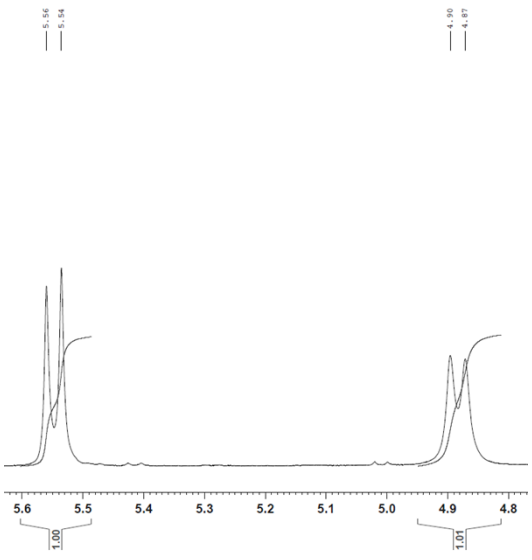


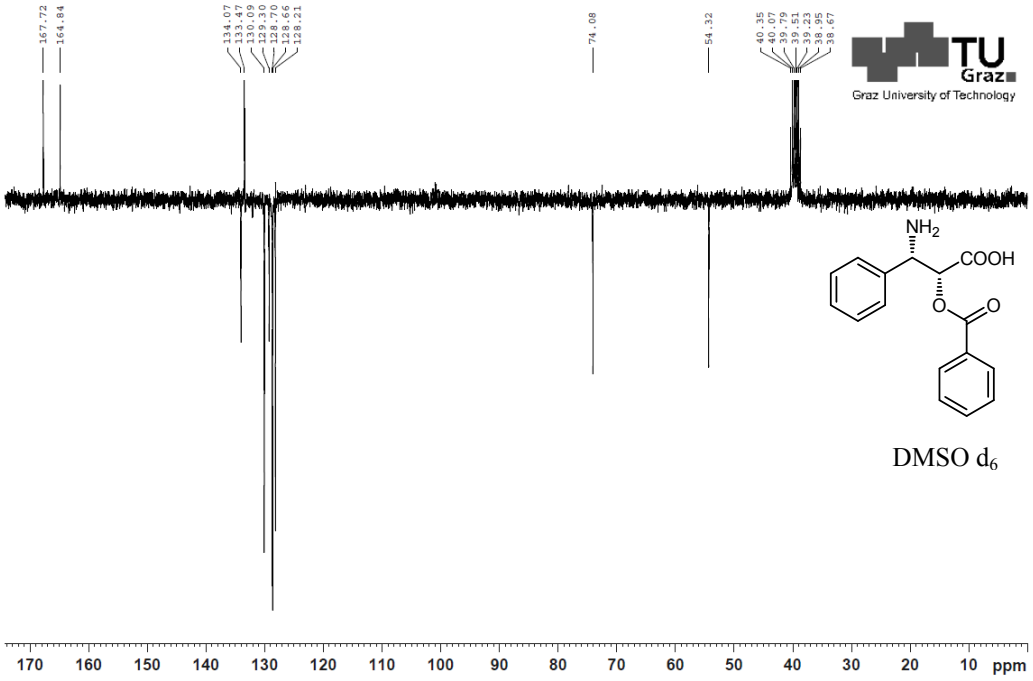


***(±)-syn*-3-amino-2-(benzoyloxy)-3-phenylpropanoic acid**





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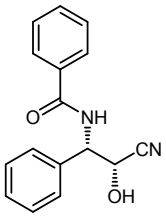
38.95
38.67
TU
Graz
Graz University of Technology
NH2
COOH
O
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

DMSO d_6

Non-chiral HPLC

Biotransformation of *(±)-syn-N*-(2-cyano-2-hydroxy-1-phenylethyl)benzamide



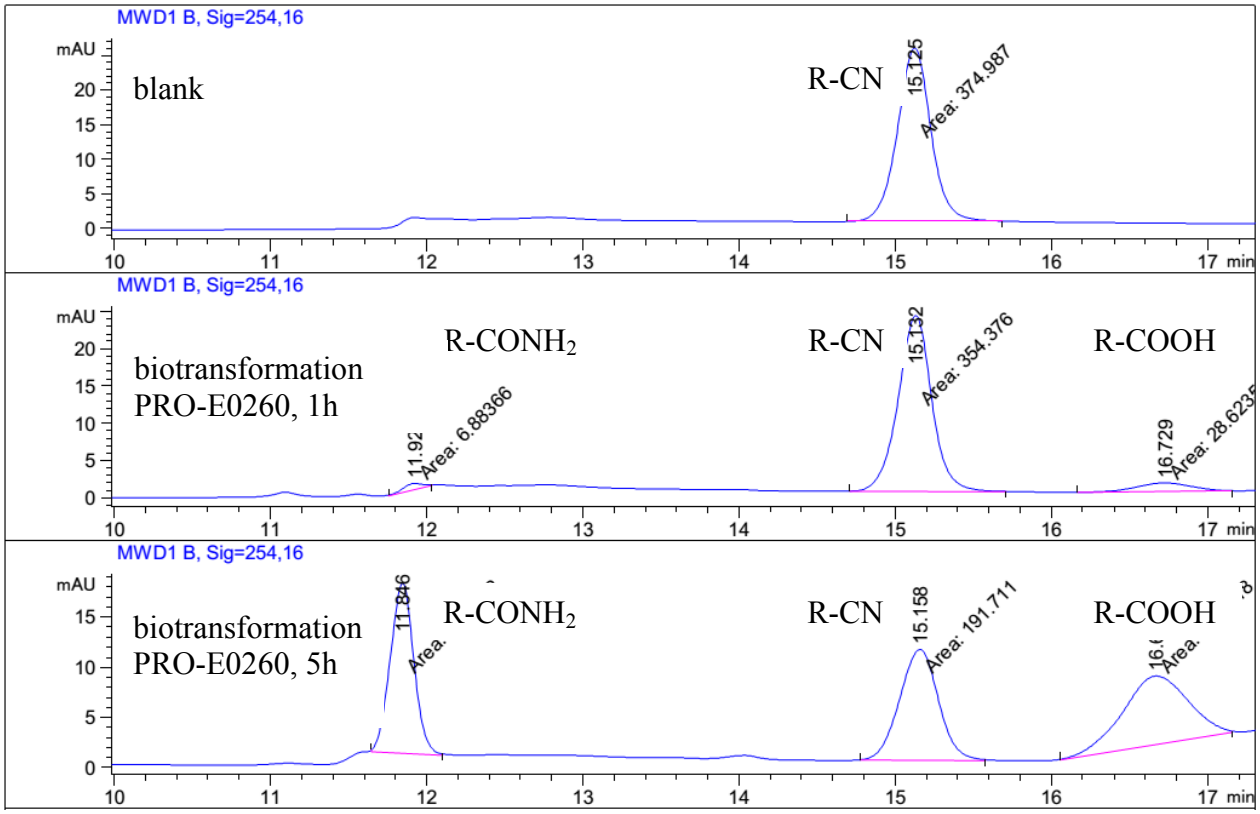
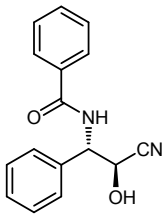


Figure S7: HPLC chromatograms of the biotransformation reactions of *(±)-syn-N*-(2-cyano-2-hydroxy-1-phenylethyl)benzamide with nitrilase PRO-E0260, line 1: blank reaction, line 2: reaction after 1h, line 3: reaction after 5h.

Biotransformation of *(±)-anti-N*-(2-cyano-2-hydroxy-1-phenylethyl)benzamide



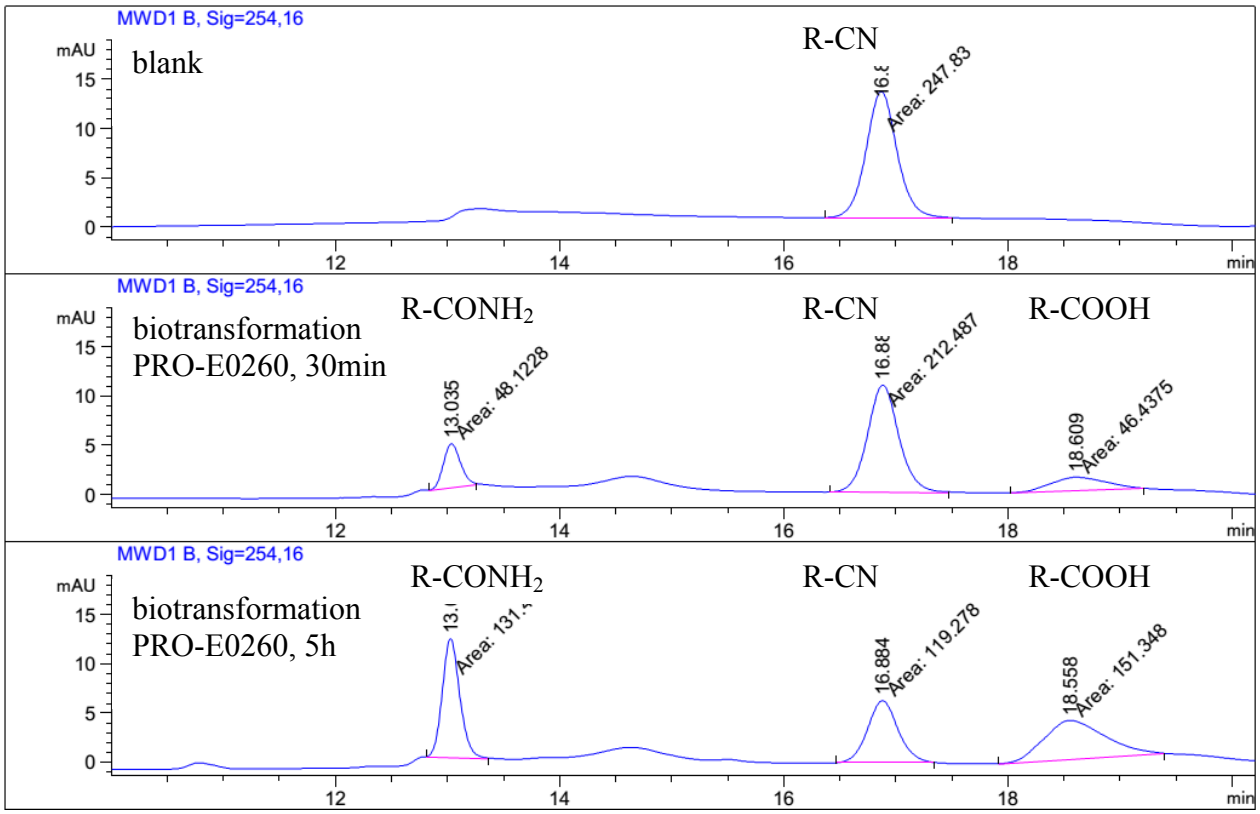
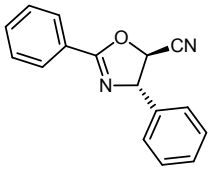


Figure S8: HPLC chromatograms of the biotransformation reactions of *(±)-anti-N*-(2-cyano-2-hydroxy-1-phenylethyl)benzamide with nitrilase PRO-E0260, line 1: blank reaction, line 2: reaction after 1h, line 3: reaction after 5h.

Biotransformation of (±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile



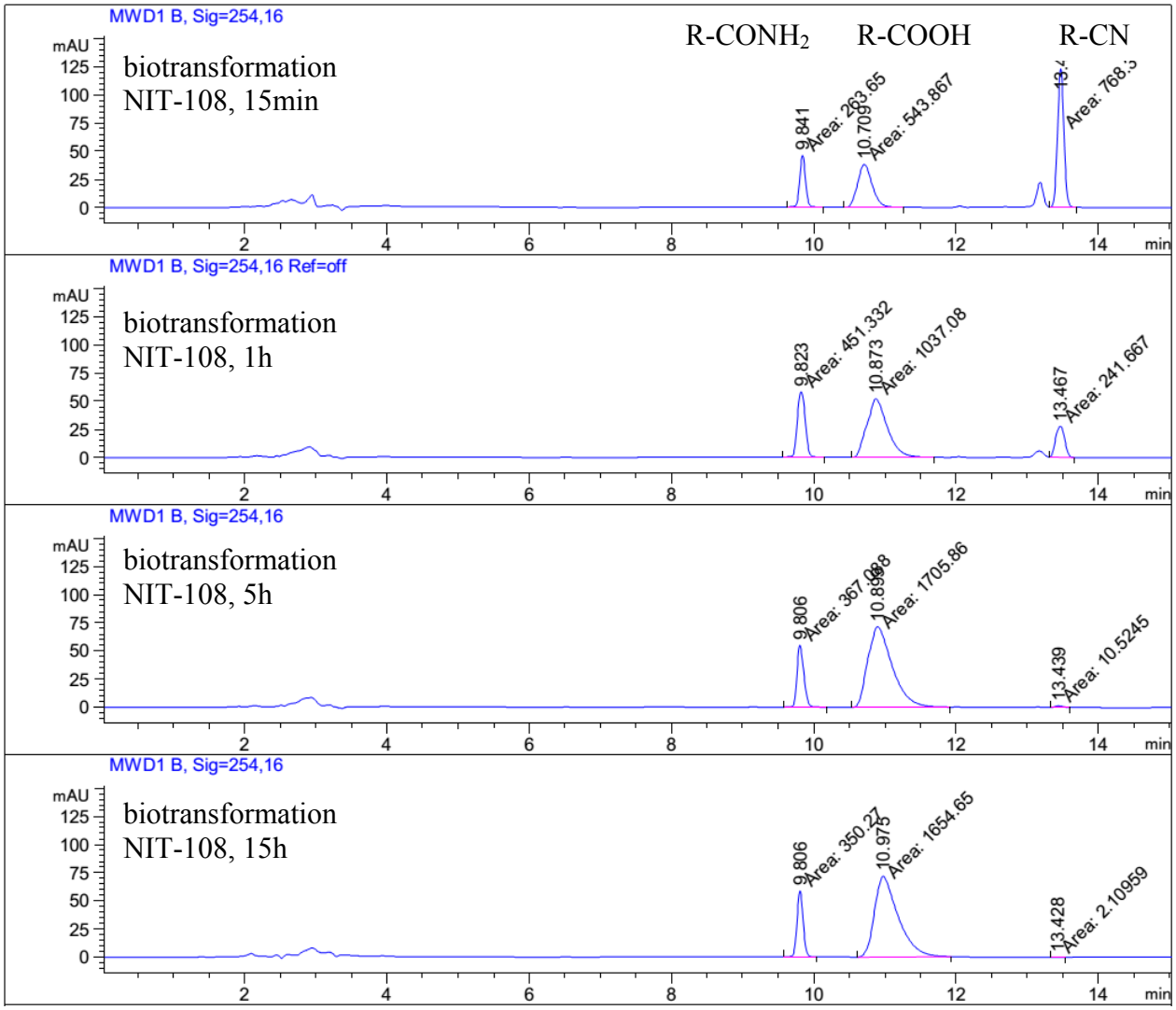
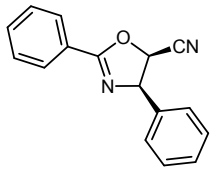


Figure S9: HPLC chromatograms of the biotransformation reactions of *(±)-trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile with nitrilase NIT-108, line 1: reaction after 15min, line 2: reaction after 1h, line 3: reaction after 5h, line 4: reaction after 15h.

Biotransformation of *(±)-cis*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile



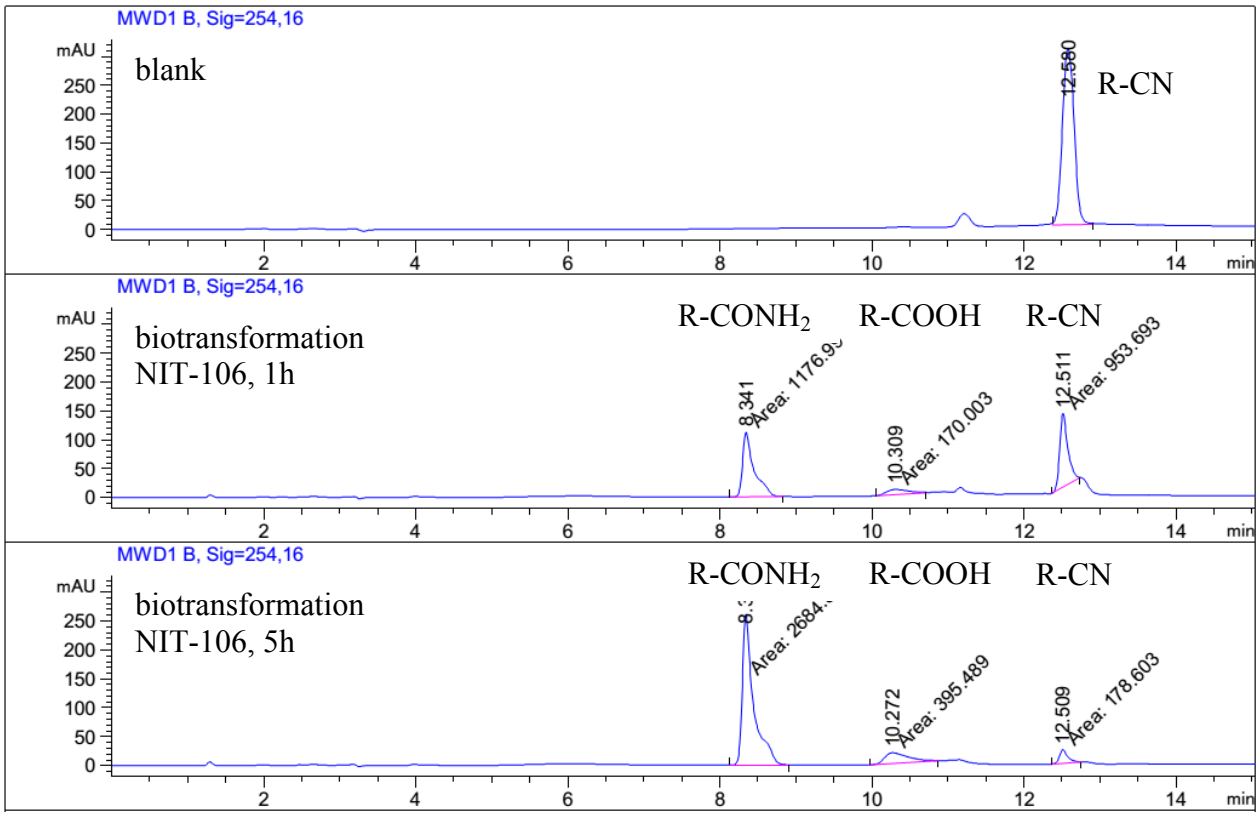


Figure S10: HPLC chromatograms of the biotransformation reactions of *(±)-cis*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile with nitrilase NIT-106, line 1: blank reaction, line 2: reaction after 1h, line 3: reaction after 5h.

Chiral HPLC

Chiralpak AD-H column

Chiralpak® AD-H column (Daicel Chemical Industries, Ltd., 0.46x25cm): isocratic method with 100% ethanol as eluent, flow 0.55mL/min, column oven temperature 40°C.

(±)-trans-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxylic acid was separated on a Chiralpak® AD-H column after derivatisation with $TMSCH_2N_2$. The mixture of substrate and product was extracted from the aqueous biotransformation reaction by adding 10µL 0.1M aqueous HCl and subsequently extracting two times with ethyl acetate (500µL each). The combined organic layers were dried over magnesium sulphate and 200µL THF, 100µL methanol and 100µL $TMSCH_2N_2$ (2.0M solution in hexane) was added. The reaction was shaken on a thermomixer at 500rpm and 22°C for 30 minutes. The resulting sample was analysed by chiral HPLC.

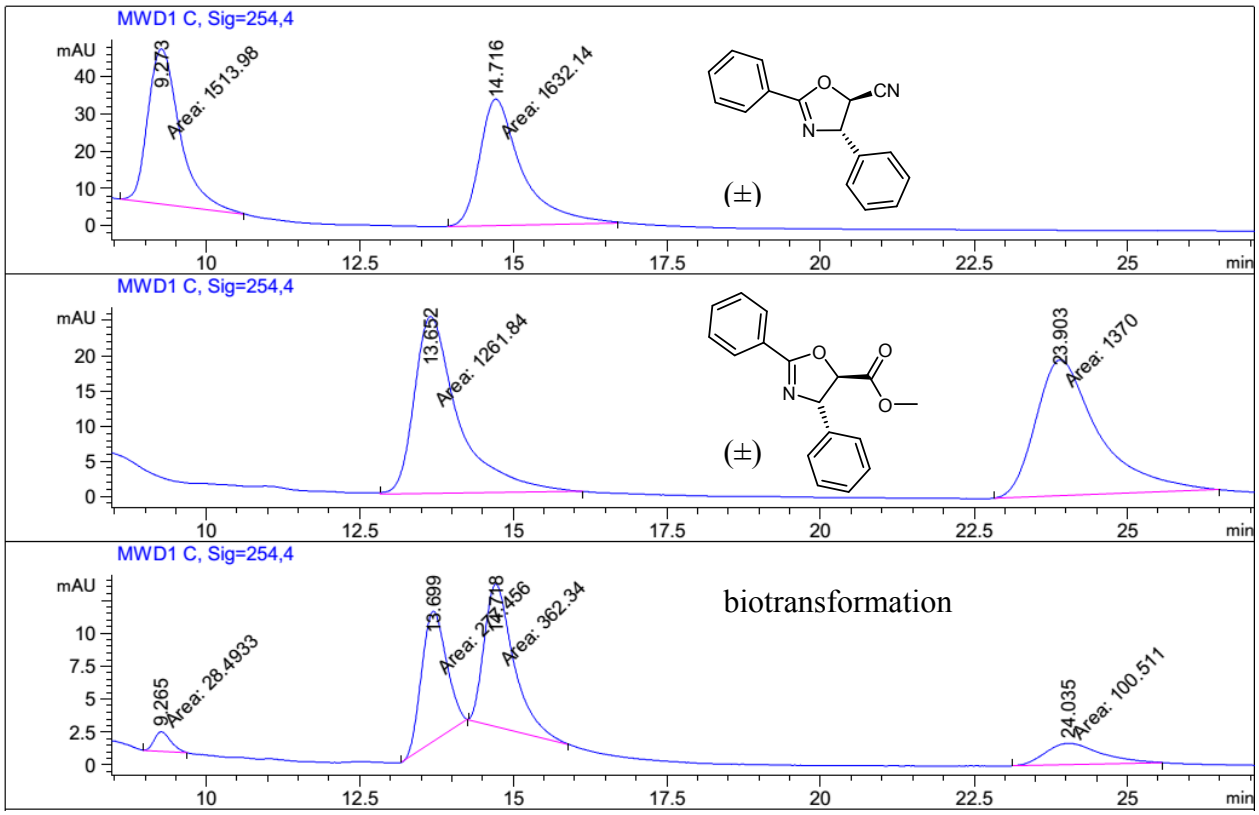


Figure S11: HPLC chromatograms obtained after derivatisation of the samples with $TMSCH_2N_2$ on a Chiralpak® AD-H column. Line 1: *(±)-trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile, line 2: *(±)-trans*- methyl 2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxylate, line 3: biotransformation reaction of (±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile with NIT-108 in the presence of 10%v/v cyclohexane, *er*[6] 2.8/1, *ee* 47%.

[6] R. E. Gawley, *J. Org. Chem.* 2006, **71**, 2411-2416.

Chiralpak AGP column

Chiralpak® AGP column (Daicel Chemical Industries, Ltd., 150x4mm, 5µm): isocratic method with 10% acetonitrile and 90% acetate buffer (100mM, pH 4.4), flow 0.9mL/min, column oven 25°C.

Acetate buffer (100mM, pH 4.4) was prepared from the following stock solutions: 0.2M solution of acetic acid (11.55g in 1L deionised water), 0.2M solution of sodium acetate (16.4g anhydrous sodium acetate in 1L deionised water) by combining 61mL acetic acid stock solution, 39mL sodium acetate stock solution and 300mL deionised water.

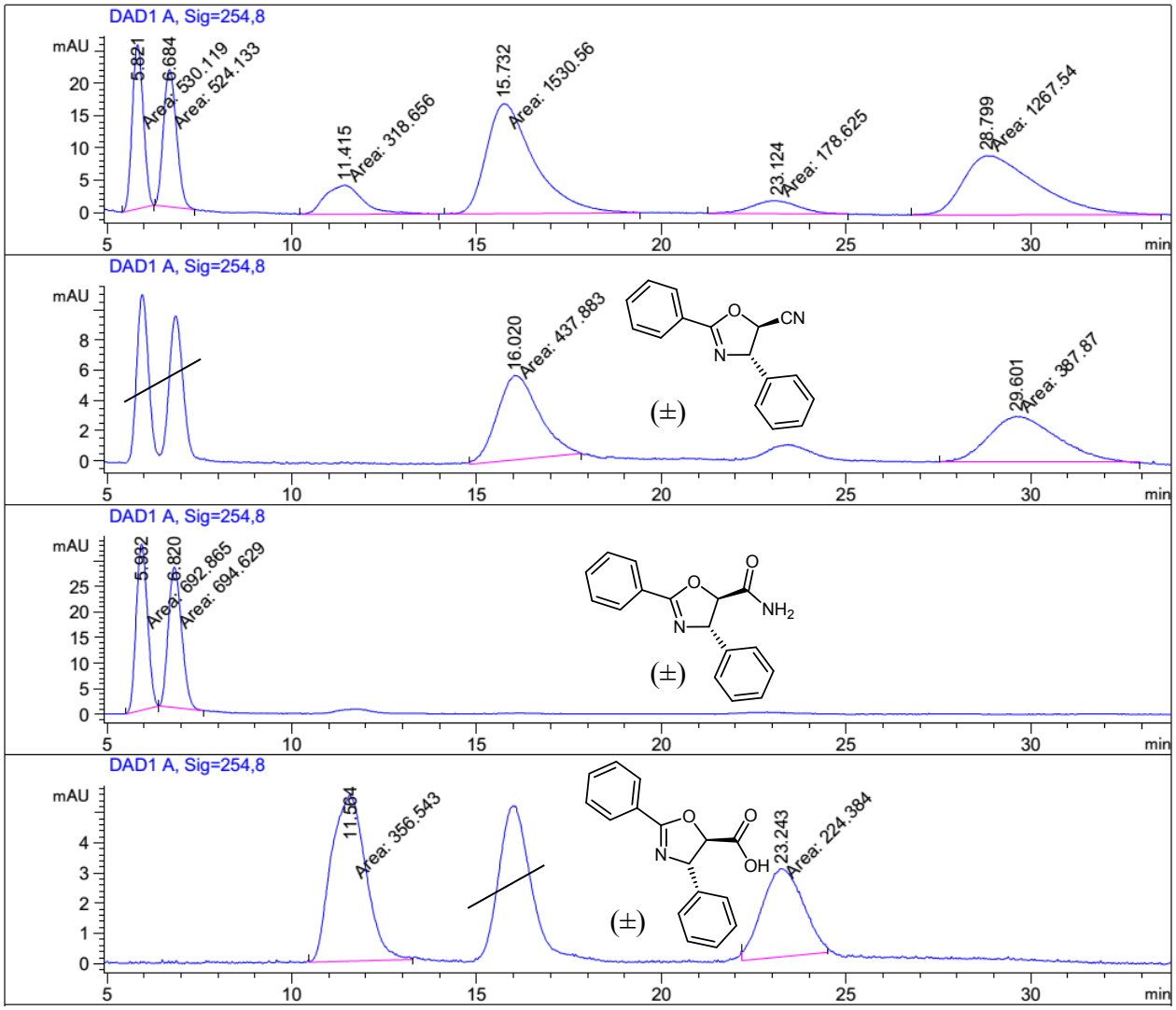


Figure S12: HPLC chromatograms obtained on a Chiralpak® AGP column with acetate buffer and acetonitrile as eluents. Line 1: mixture of references of *(±)-trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile and the corresponding amide and acid, line 2: *(±)-trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile, line 3: (±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxamide, line 4: (±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxylic acid.

Chiralpak® AGP column (Daicel Chemical Industries, Ltd., 150x4mm, 5µm): isocratic method with 10% iso-propanol and 90% citrate buffer (50mM, pH 4.4), flow 0.9mL/min, column oven 22°C.
Citrate buffer (50mM, pH 4.4) was prepared from the following stock solutions: 0.1M solution of citric acid (19.21g in 1L deionised water), 0.2M solution of Na_2HPO_4 (53.65g Na_2HPO_4*$7H_2O$ in 1L deionised water) by combining 55.6mL citric acid stock solution, 44.4mL Na_2HPO_4 stock solution and 188.8mL deionised water.

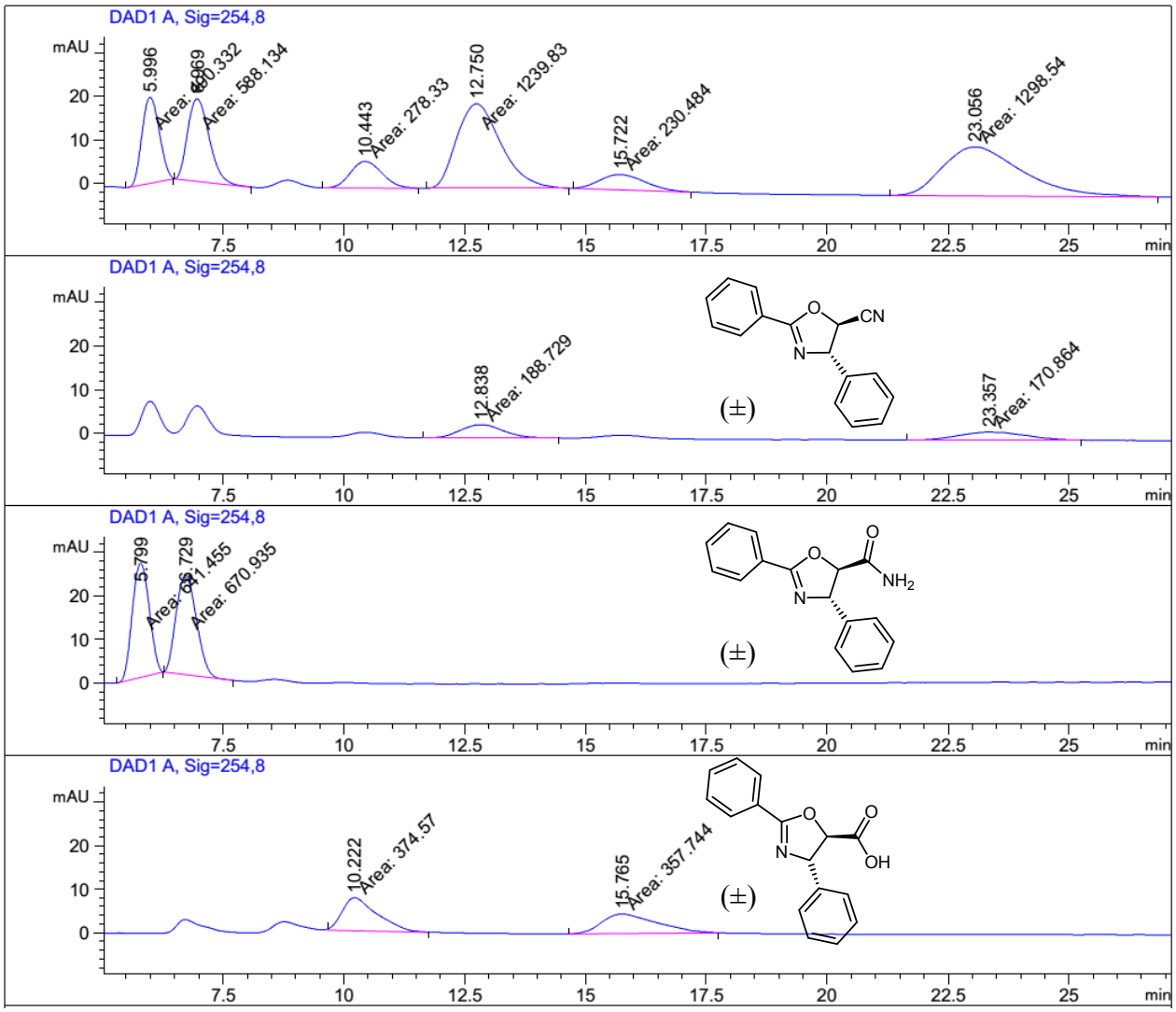


Figure S13: HPLC chromatograms obtained on a Chiralpak® AGP column with citrate buffer and *iso*-propanol as eluents. Line 1: mixture of references of *(±)-trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile and the corresponding amide and acid, line 2: *(±)-trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile, line 3: (±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxamide, line 4: (±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxylic acid.

Preparative scale biotransformation of (±)-*trans*-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile

Preparative scale biotransformations were carried out with whole cells of *E. coli* expressing the nitrilase from *Neurospora crassa* OR74A (see experimental procedure in the article).
Chiral HPLC: Chiralpak® AGP column (Daicel Chemical Industries, Ltd., 150x4mm, 5µm): isocratic method with 6% iso-propanol and 94% citrate buffer (50mM, pH 4.6), flow 0.9mL/min.

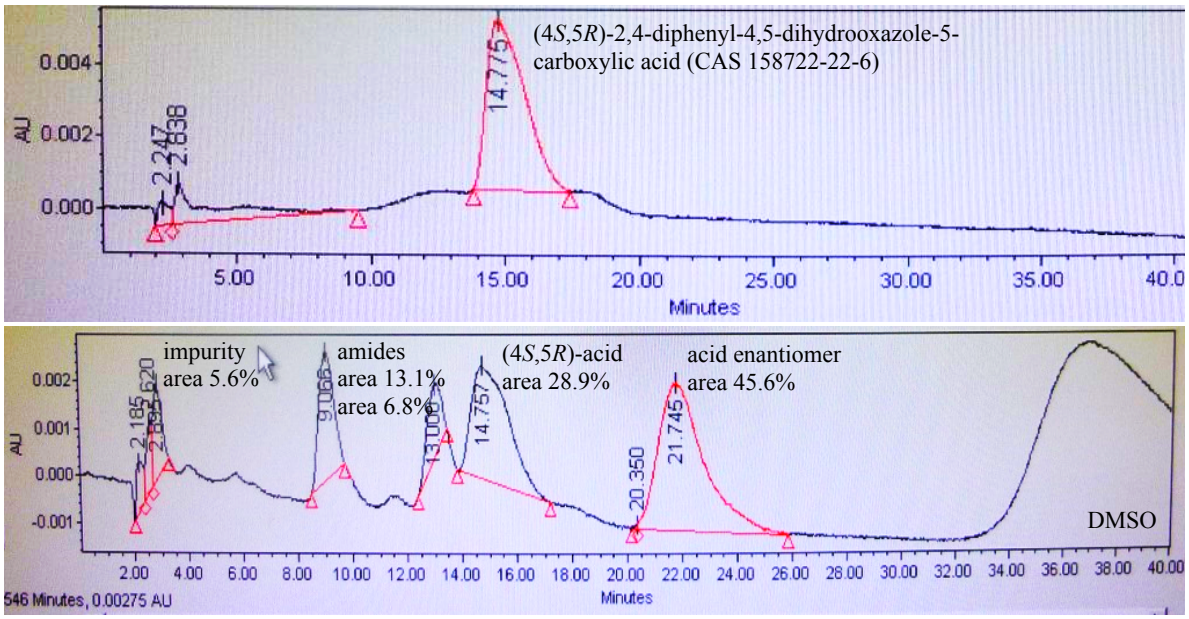


Figure S14: HPLC chromatograms obtained on a Chiralpak® AGP column with citrate buffer and *iso*-propanol as eluents. Line 1: enantiopure acid reference, (4*S*,5*R*)-2,4-diphenyl-4,5-dihydrooxazole-5-carboxylic acid (CAS 158722-22-6) obtained from A Chemtek Inc., catalog number 068-15362; line 2: isolated acid product from the preparative scale biotransformation (purity 71%, contains 29% amide and other impurities), *er*[7] 1/1.6 (4*S*,5*R*)/enantiomer, *ee* 22.5%.

[7] R. E. Gawley, *J. Org. Chem.* 2006, **71**, 2411-2416.

Biotransformations with commercially available nitrile hydratases

The taxol sidechain precursor ***(±)-trans*-1** and its diasteromer ***(±)-cis*-1** were converted by all commercial nitrile hydratases tested. The corresponding amides of ***(±)-cis*-1** were obtained in yields of up to 75% and ee-values of 65-91% (Table S1). Biotransformations of ***(±)-trans*-1** gave the corresponding amide in almost quantitative yields, even when the enzyme concentration and reaction time were decreased significantly (Table S2). Quantitative yields, however, indicated low enantioselectivity.

Table S1. Screening results of ***(±)-cis*-1**(*(±)-cis*-2,4-diphenyl-4,5-dihydrooxazole-5-carbonitrile, 0.4mM) with commercially available nitrile hydratases. [a]

enzyme	conversion to amide [%]; ee-value [%]		
	3h, 50µL[a]	3h, 100µL[a]	21h, 200µL[a]
PRO-E0256	<5	12.4	31.7; 65.2
PRO-E0257	<5	12.8	51.6; 83.4
PRO-E0258	11.0	20.5	63.2; 87.6
PRO-E0259	25.4	43.7	74.4; 90.9

[a] amount of commercial enzyme preparation used, total volume 500µL.

Table S2 Screening results of ***(±)-trans*-1** *((±)-trans*-2,4-diphenyl-4,5-dihydrooxazole-5-carbonitrile, 0.4mM) with commercially available nitrile hydratases.

enzyme	conversion to amide [%]		
	3h, 50µL[a]	3h, 100µL[a]	21h, 200µL[a]
PRO-E0256	91.7	97.2	99.5
PRO-E0257	93.7	98.9	97.0
PRO-E0258	96.9	100	97.0
PRO-E0259	100	100	100

[a] amount of commercial enzyme preparation used, total volume 500µL.

Biotransformations with colsolvents

In recent examples, the presence of organic solvents has been shown to enhance activity and stereoselectivity in nitrilase catalysed biotransformations.[8] Here, the influence of organic solvents on the biotransformation of ***trans*-1** with nitrilase NIT-108 was investigated. The reactions were compared to the biotransformation in buffer, without the addition of organic solvent. Biotransformation reactions in buffer with different concentrations gave identical conversions. Addition of 10%v/v DMSO still gave similar conversion. Increasing the amount of DMSO to 30%v/v gave conversions of 70% compared to the conversion in buffer. Similar conversions were achieved with 10%v/v *t*BuOH or acetone. When adding 50%v/v of water miscible solvents, no conversion was observed. Cyclohexane (logP 3.2) and *n*-hexane (logP 3.5) gave the most promising results using water-immiscible solvents. With 10%v/v cyclohexane or *n*-hexane, approximately 50% conversion was found. Residual activity was still observed with up to 50% v/v cosolvent. Biotransformations using diisoproylether (logP 1.9) or ethyl acetate (logP 0.68) as cosolvents gave significantly lower conversions. The highest ee-values were achieved using cyclohexane as cosolvent, where an ee-value of 75% was observed. The results obtained for water immiscible solvents comply with the generalization that enzyme activity is usually better retained in cosolvents with a high logP (2-4) than in those with a low logP.[9]

Procedure: For biotransformations with cosolvents, nitrilase NIT-108 was resuspended in buffer (50mM K_2HPO_4, pH 8, 4mg nitrilase in 500µL buffer). Screening reactions were done in test tubes (1.5mL, Eppendorf) using the following conditions and concentrations: nitrilase suspension (240µL), and substrate in DMSO (10µL of a 20mM stock solution, end concentration of substrate 0.4mM, 2%v/v DMSO), co-solvent and buffer to achieve a total volume of 500µL. Blank reactions contained substrate in DMSO (0.4mM, 2%v/v DMSO), and buffer (50mM K_2HPO_4, pH 8). The screening reactions were incubated on a thermomixer at 30°C and 800rpm. The reactions were stopped by adding 1N HCl (10µL) and 500µL ethyl acetate. The protein was precipitated by centrifugation. The layers were separated and the extraction was repeated. The organic layers were analyzed by HPLC-MS.

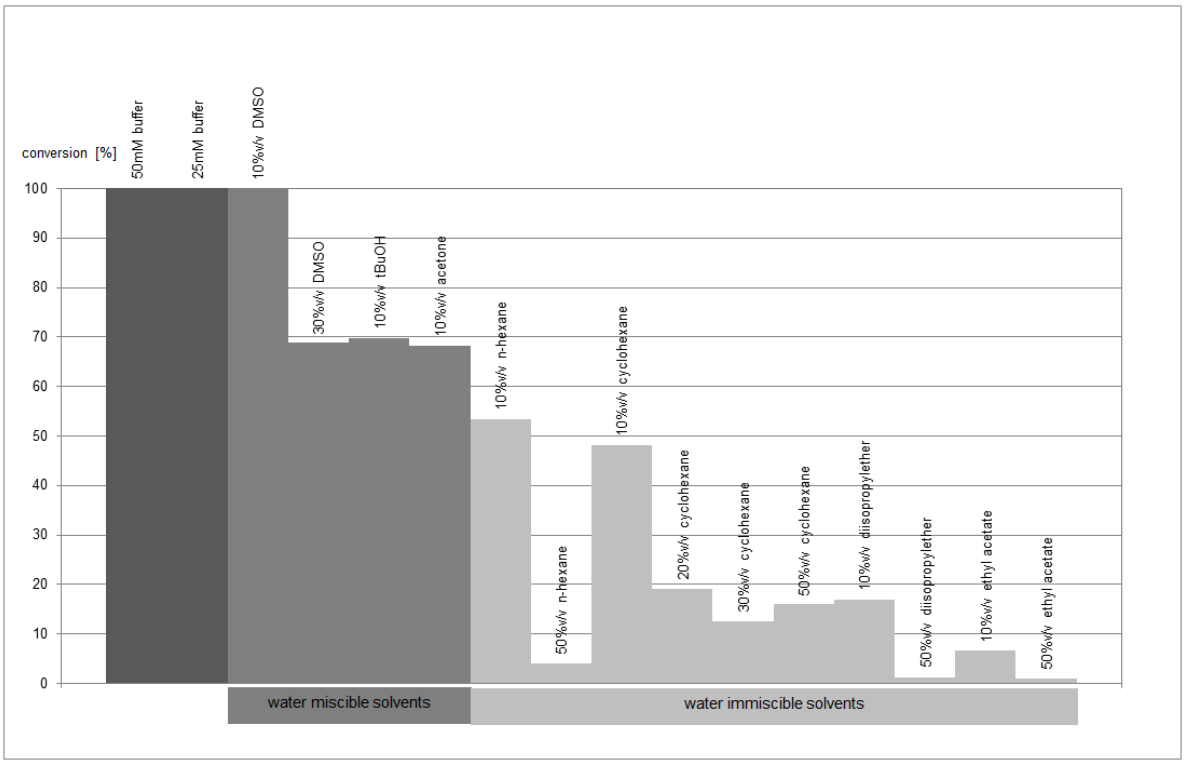


Figure S15: Conversion of *(±)-trans*-2,4-diphenyl-4,5-dihydrooxazole-5-carbonitrile (0.4mM) by NIT-108 (Codexis, Inc.) in the presence of water miscible and water immiscible organic solvents. In the presence of 50%v/v DMSO, *t*BuOH or acetone no conversion was observed.

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[8] Selected references: a) N. Layh, A. Willets, *Biotechnol. Lett.* 1998, **20**, 329-331; b) P. Kaul, U.C. Banerjee, *J. Ind. Microbiol. Biotechnol.* 2008, **35**, 713-720; c) Z.-J. Zhang, J. Pan, J.-F. Liu, J.-H. Xu, Y.-C. He, Y.-Y. Liu, *J. Biotechnol.* 2011, ***152***, 24-29; d) C. Vergne-Vaxelaire, F. Bordier, A. Fossey, M. Besnard-Gonnet, A. Debard, A. Mariage, V. Pellouin, A. Perret, J.-L. Petit, M. Stam, M. Salanoubat, J. Weissenbach, V. De Berardinis, A. Zaparucha, *Adv. Synth. Catal.* 2013, **355**, 1763-1779; e) A. B. Veselá, A. Křenková, L. Martínková, BioTech2014, Prague, Czech Republic, 2014, Poster: Production of (*R*)-mandelic acid by nitrilases from filamentous fungi – comparison of the co-solvent and fed-batch setup.

[9] C. Laane, S. Boeren, K. Vos, C. Veeger, *Biotechnol. Bioeng.* 1986, **30**, 81-87.

Commercial enzyme preparations

Prozomix

Nitrile hydratase	Source organism	specific activity	protein	substrate
PRO-E0256	*Rhodococcus erythropolis AJ270* (Q7AZY7 (α-subunit); Q7AZY6 (β-subunit))	964.7 U/mg	2.00 mg/mL	methocrylnitrile (10mM)
PRO-E0257	*Rhodopseudomonas palustris HaA2* (Q2IWK4 (α-subunit), Q2IWK3 (β-subunit))	13.7 U/mg	3.35 mg/mL	methocrylnitrile (10mM)
PRO-E0258	*Rhodopseudomonas palustris CGA009* (Q6N613 (α-subunit), Q6N612 (β-subunit))	71.28 U/mg	5.10 mg/mL	methocrylnitrile (10mM)
PRO-E0259	*Sinorhizobium meliloti 1021* (Q92NS3 (α-subunit), Q92NS2 (β-subunit))	33.82 U/mg	4.9 mg/mL	methocrylnitrile (10mM)

Purity of all nitrile hydratases from Prozomix: >95% as judged by SDS-PAGE

nitrilase	Source organism	activity	substrate
PRO-E0260	*Bradyrhizobium japonicum USDA 110* (Q89GE3)	n/a	n/a
PRO-E0261	*Rhodopseudomonas palustris CGA009* (Q6N284)	1.041 U/mg solid	acetonitrile (50mM)
PRO-E0262	*Bacillus cereus ATCC 14579* (Q819F0)	n/a	n/a
PRO-E0263	*Silicibactor promeroyi DSS-3* (Q5LLB2)	n/a	n/a
PRO-E0264	*Bradyrhizobium japonicum USDA 110* (Q89PT3)	1.166 U/mg solid	acetonitrile (50mM)

Purity of all nitrilases from Prozomix: approx 40% as judged by SDS-PAGE

Codexis

nitrilase	specific activity	substrate
NIT-101	3.8 U/mg solid	3-phenylpropionitrile (10mM)
NIT-102	11 U/mg solid	3-phenylpropionitrile (10mM)
NIT-103	2.1 U/mg solid	3-phenylpropionitrile (10mM)
NIT-104	2.4 U/mg solid	n/a
NIT-105	10 U/mg solid	benzonitrile (20mM)
NIT-106	43 U/mg solid	*p*-tolylacetonitrile (5mM)
NIT-108	5.9 U/mg solid	cinnamonitrile (10mM)
NIT-109	19 U/mg solid	3-phenylpropionitrile (10mM)
NIT-110	17 U/mg solid	3-phenylpropionitrile (10mM)
NIT-111	2.2 U/mg solid	*p*-tolylacetonitrile (5mM)
NIT-112	12 U/mg solid	3-phenylpropionitrile (10mM)
NIT-113	10 U/mg solid	3-phenylpropionitrile (10mM)
NIT-114	72 U/mg solid	*p*-tolylacetonitrile (5mM)

Purity of nitrilases from Codexis: n/a

Further information can be obtained from the suppliers.
http://www.codexis.com
http://www.prozomix.com/