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

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## Biotransformations with colsolvents

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Synthesis of substrates and reference materials

3-Phenyloxirane-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 BnBu₃NCl (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 Na₂SO₄ and purified by column chromatography. (±)-trans-phenyloxirane-2-carbonitrile was isolated as white solid (5.57g, 19.3%).

(±)-trans-phenyloxirane-2-carbonitrile was isolated as white solid (5.57g, 19.3%).

1H NMR (CDCl₃) 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'), 7.29-7.35 (3H, m, H-3', H-4', H-5'); [13C NMR (CDCl₃) 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%.

1H NMR (DMSO-d₆) 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'); [13C NMR (CDCl₃) 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

(±)-trans-2,4-Diphenyl-4,5-dihydrooxazole-5-carboxamide (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 Na₂SO₄ 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]+, C₁₆H₁₄N₂O₂– C₁H₃NO, calc. 221.0841);

1H NMR (DMSO-d₆) 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, CONH₂), 8.07 (2H, d, J=7.1Hz, H-2', H-6'); [13C NMR (DMSO-d₆) δ 73.80 (C-4), 83.64 (C-5), 126.63 (C-4''), 126.80 (C-1'), 127.54, 128.16, 128.29 (C-2'', C-3'', C-5'', C-6''), 129.07 (C-2', C-6'), 131.90 (C-4'), 141.97 (C-1''), 162.37 (C-2), 171.58 (CONH₂).

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

(±)-cis-2,4-Diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carboxamide was prepared analogously from (±)-cis-2,4-diphenyl-4,5-dihydrooxazole-5-carboxamide (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]+, C₁₆H₁₃N₂O₂-C₁H₂NO, calc. 221.0841); 1H NMR (DMSO-d₆) δ 5.34 (1H, d, J=10.7Hz, H-5), 5.70 (1H, d, J=17.0Hz, H-4), 7.10 (1H, s, NH₂), 7.21-7.36 (5H, m, H-2''', H-3''', H-4''', H-5''', H-6'''), 7.67 (1H, t, J=7.2Hz, H-3', H-5'), 8.09 (2H, d, J=7.2Hz, H-2', H-6'); [13C NMR (DMSO-d₆) 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-carboxonitrile, 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.
Figure S3: Synthesis of (±)-trans-2,4-diphenyl-4,5-di hydroxazole-5-carboxylic acid

(±)-trans-2,4-diphenyl-4,5-di hydroxazole-5-carboxylic acid 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-di hydroxazole-5-carboxylic acid was isolated as white solid (292.9mg, 54.1%). m.p. 205°C; 1H NMR (DMSO-d6) 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'); 13C NMR (DMSO-d6) 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-di hydroxazole-5-carboxylic acid

(±)-cis-2,4-Diphenyl-4,5-di hydroxazole-5-carboxylic acid was prepared analogously from (±)-cis-2,4-diphenyl-4,5-di hydroxazole-5-carbonitrile (500.1mg, 2.01mmol). (±)-cis-2,4-Diphenyl-4,5-di hydroxazole-5-carboxylic acid was isolated as white solid (59.0mg, 11.0%). m.p. 152°C; 1H NMR (DMSO-d6) 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'); 13C NMR (DMSO-d6) 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).

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

(±)-syn-3-benzamido-2-hydroxy-3-phenylpropanamide was isolated as white solid (26.4mg, 11.8%). 1H NMR (DMSO-d6) 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, NH2), 7.28-7.39 (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); 13C NMR (DMSO-d6) δ 55.85 (C-3), 73.63 (C-2), 126.84,

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


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

(±)-Anti-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%).

\[ \text{H NMR (DMSO-d}_6\text{)} \delta 4.28 (1H, t, J=5.5Hz, H-2), 5.39 (1H, dd, H-3), 5.80 (1H, d, J=5.8Hz, OH), 7.17 (d, J=12.9Hz, NH\text{2}), 7.21-7.35 (3H, m, H-3'', H-4'', H-5''), 7.41 (2H, d, J=7.0Hz, H-2'', H-6''), 7.45-7.61 (3H, m, H-3', H-4', H-5'), 7.88 (2H, d, J=7.6Hz, H-2', H-6'). 8.66 (1H, s, J=8.4Hz, NH); \]

\[ \text{13C NMR (DMSO-d}_6\text{)} \delta 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

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

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

(+)-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%).

1H 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'); 13C 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-phenylpropanoic acid (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 overnight, 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-hydroxy-3-phenylpropanoic acid was obtained as white solid (342mg, 98.9%). 1H NMR (D$_2$O) 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'); 13C NMR (D$_2$O) 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-benzamido-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$_2$SO$_4$. 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-hydroxy-3-phenylpropanoic acid was obtained as white solid (105.0mg, 31.2%).

1H 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', C-4', C-5'), 7.89 (2H, d, J=7.1Hz, H-2', H-6'), 9.02 (1H, d, J=5.5Hz, NH); 13C 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-4', 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).

NMR spectra

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

DMSO $d_6$
(±)-cis-2,4-diphenyl-4,5-dihydro-1,3-dihydro-1,3-oxazole-5-carbonitrile

DMSO $d_6$
(±)-syn-N-(2-cyano-2-hydroxy-1-phenylethyl)benzamide

DMSO d$_6$
(±)-anti-N-(2-cyano-2-hydroxy-1-phenylethyl)benzamide
(±)-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
Prepared as reference material only, contains impurities.
(±)-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
Prepared as reference material only, contains minor impurities.
(±)-anti-3-benzamido-2-hydroxy-3-phenylpropanamide
Prepared as reference material only, contains minor impurities.
(±)-syn-3-benzamido-2-hydroxy-3-phenylpropanoic acid
Prepared as reference material only, contains impurities.
(±)-anti-3-benzamido-2-hydroxy-3-phenylpropanoic acid
Prepared as reference material only, contains impurities.
(±)-syn-3-amino-2-(benzoyloxy)-3-phenylpropanoic acid
Non-chiral HPLC

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

![Chemical structure of the compound](image)

**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.
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 15 min, line 2: reaction after 1 h, line 3: reaction after 5 h, line 4: reaction after 15 h.
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 TMSCH₂N₂. 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 TMSCH₂N₂ (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 TMSCH₂N₂ 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, erδ 2.8/1, ee 47%.

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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 Na₂HPO₄ (53.65g Na₂HPO₄·7H₂O in 1L deionised water) by combining 55.6mL citric acid stock solution, 44.4mL Na₂HPO₄ 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, (4S,5R)-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 (4S,5R)/enantiomer, *ee* 22.5%.

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.

<table>
<thead>
<tr>
<th>enzyme</th>
<th>conversion to amide [%]; ee-value [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h, 50µL&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PRO-E0256</td>
<td>&lt;5</td>
</tr>
<tr>
<td>PRO-E0257</td>
<td>&lt;5</td>
</tr>
<tr>
<td>PRO-E0258</td>
<td>11.0</td>
</tr>
<tr>
<td>PRO-E0259</td>
<td>25.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.

<table>
<thead>
<tr>
<th>enzyme</th>
<th>conversion to amide [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h, 50µL&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PRO-E0256</td>
<td>91.7</td>
</tr>
<tr>
<td>PRO-E0257</td>
<td>93.7</td>
</tr>
<tr>
<td>PRO-E0258</td>
<td>96.9</td>
</tr>
<tr>
<td>PRO-E0259</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> amount of commercial enzyme preparation used, total volume 500µL.
Biotransformations with cosolvents

In recent examples, the presence of organic solvents has been shown to enhance activity and stereoselectivity in nitrilase catalysed biotransformations. 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 tBuOH 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 diisopropylether (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.

Procedure: For biotransformations with cosolvents, nitrilase NIT-108 was resuspended in buffer (50mM K$_2$HPO$_4$, 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$_2$HPO$_4$, pH 8). The screening reactions were incubated at 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, tBuOH or acetone no conversion was observed.

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## Commercial enzyme preparations

### Prozomix

<table>
<thead>
<tr>
<th>Nitrile hydratase</th>
<th>Source organism</th>
<th>specific activity</th>
<th>protein</th>
<th>substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRO-E0256</td>
<td><em>Rhodococcus erythropolis AJ270</em> (Q7AZY7 (α-subunit); Q7AZY6 (β-subunit))</td>
<td>964.7 U/mg</td>
<td>2.00 mg/mL</td>
<td>methacrylnitrile (10mM)</td>
</tr>
<tr>
<td>PRO-E0257</td>
<td><em>Rhodopseudomonas palustris HaA2</em> (Q21WK4 (α-subunit), Q21WK3 (β-subunit))</td>
<td>13.7 U/mg</td>
<td>3.35 mg/mL</td>
<td>methacrylnitrile (10mM)</td>
</tr>
<tr>
<td>PRO-E0258</td>
<td><em>Rhodopseudomonas palustris CGA009</em> (Q6N613 (α-subunit), Q6N612 (β-subunit))</td>
<td>71.28 U/mg</td>
<td>5.10 mg/mL</td>
<td>methacrylnitrile (10mM)</td>
</tr>
<tr>
<td>PRO-E0259</td>
<td><em>Sinorhizobium meliloti 1021</em> (Q92NS3 (α-subunit), Q92NS2 (β-subunit))</td>
<td>33.82 U/mg</td>
<td>4.9 mg/mL</td>
<td>methacrylnitrile (10mM)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Nitilas</th>
<th>Source organism</th>
<th>Activity</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRO-E0260</td>
<td><em>Bradyrhizobium japonicum USDA 110</em> (Q89GE3)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>PRO-E0261</td>
<td><em>Rhodopseudomonas palustris CGA009</em> (Q6N284)</td>
<td>1.041 U/mg solid</td>
<td>acetonitrile (50mM)</td>
</tr>
<tr>
<td>PRO-E0262</td>
<td><em>Bacillus cereus ATCC 14579</em> (Q819F0)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>PRO-E0263</td>
<td><em>Silicibacter promerovii DSS-3</em> (Q5LLB2)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>PRO-E0264</td>
<td><em>Bradyrhizobium japonicum USDA 110</em> (Q89PT3)</td>
<td>1.166 U/mg solid</td>
<td>acetonitrile (50mM)</td>
</tr>
</tbody>
</table>

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

### Codexis

<table>
<thead>
<tr>
<th>Nitilas</th>
<th>Specific activity</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIT-101</td>
<td>3.8 U/mg solid</td>
<td>3-phenylpropionitrile (10mM)</td>
</tr>
<tr>
<td>NIT-102</td>
<td>11 U/mg solid</td>
<td>3-phenylpropionitrile (10mM)</td>
</tr>
<tr>
<td>NIT-103</td>
<td>2.1 U/mg solid</td>
<td>3-phenylpropionitrile (10mM)</td>
</tr>
<tr>
<td>NIT-104</td>
<td>2.4 U/mg solid</td>
<td>n/a</td>
</tr>
<tr>
<td>NIT-105</td>
<td>10 U/mg solid</td>
<td>benzonitrile (20mM)</td>
</tr>
<tr>
<td>NIT-106</td>
<td>43 U/mg solid</td>
<td>p-tolylacetonitrile (5mM)</td>
</tr>
<tr>
<td>NIT-108</td>
<td>5.9 U/mg solid</td>
<td>cinnamionitrile (10mM)</td>
</tr>
<tr>
<td>NIT-109</td>
<td>19 U/mg solid</td>
<td>3-phenylpropionitrile (10mM)</td>
</tr>
<tr>
<td>NIT-110</td>
<td>17 U/mg solid</td>
<td>3-phenylpropionitrile (10mM)</td>
</tr>
<tr>
<td>NIT-111</td>
<td>2.2 U/mg solid</td>
<td>p-tolylacetonitrile (5mM)</td>
</tr>
<tr>
<td>NIT-112</td>
<td>12 U/mg solid</td>
<td>3-phenylpropionitrile (10mM)</td>
</tr>
<tr>
<td>NIT-113</td>
<td>10 U/mg solid</td>
<td>3-phenylpropionitrile (10mM)</td>
</tr>
<tr>
<td>NIT-114</td>
<td>72 U/mg solid</td>
<td>p-tolylacetonitrile (5mM)</td>
</tr>
</tbody>
</table>

Purity of nitilas from Codexis: n/a

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