Synthesis and yeast - mediated bioreduction of α-keto-β-lactams bearing a functionalized and rigid side chain

Marko D. Mihovilovic,* Markus Spina, and Peter Stanetty

Institute of Applied Synthetic Chemistry, Vienna University of Technology
Getreidemarkt 9/163-OC, Vienna, Austria
E-mail: mmihovil@pop.tuwien.ac.at

Abstract

Bakers' yeast (*Saccharomyces cerevisiae*) mediated reductions were investigated for the potential of a fermentation approach towards novel paclitaxel C-13 side-chain analogs bearing a polar methoxy-functionality in combination with a rigid structural core unit. Synthesis of correspondingly substituted α-keto-β-lactam compounds as biotransformation precursors was carried out following a straightforward strategy involving Staudinger [2+2] cyclization. Bioreductions were carried out using growing whole-cells of *S. cerevisiae* and the dia- and enantioselectivity of the process was studied.

Keywords: Biocatalysis, biotransformation, reduction, yeast, paclitaxel

Introduction

The introduction of enzymes as catalysts into synthetic organic chemistry opened up a variety of new transformations. The employment of microorganisms provides access to enzymes that are difficult to use in an isolated form. In addition, such organisms are self-propagating sources of the desired biocatalyst and all cofactors necessary for the reaction. Therefore, the use of such "biocatalytic reagents" has spread among synthetic organic chemists over the past decade. Since fermentations are generally carried out in aqueous buffers the use of organic solvents and heavy metals in the course of a synthesis is minimized, hence this methodology representing a "green chemistry" approach is of increasing interest for industrial applications.

Bakers' yeast (*Saccharomyces cerevisiae*) has been the first and most popular whole-cell biocatalyst, particularly for asymmetric reductions of carbonyl compounds. Reductions catalyzed by this organism tolerate a large variety of ketones and side-reactions are rarely observed. This broad substrate acceptance is due to the presence of a number of reductase enzymes, some of which have been isolated and characterized.

While α-keto acids and derivatives thereof have received relatively less attention than β-keto esters as substrates for yeast reductions, the resulting α-hydroxy carbonyl compounds are equally valuable chiral building blocks and several routes to these compounds have been devised. Among a number of biologically interesting targets microbial reduction of α-keto-β-lactams allows access to the paclitaxel side chain. In this contribution we are disclosing initial studies of the bioreduction of functionalized precursors of this type, aimed at the subsequent synthesis of modified taxoids.

Taxol (paclitaxel; R' = phenyl, R'' = benzoyl, R''' = acetyl) and Taxotere (docetaxel; R' = phenyl, R'' = *tert.*-butoxycarbonyl, R''' = H) are block-buster chemotherapeutic agents (Scheme 1). The compounds are used in clinical therapy against ovarian, breast, and non-small cell lung cancer and preclinical screens have revealed significant activity against resistant murine melanoma. Paclitaxel was originally extracted in small quantities from the bark of the Pacific yew tree, *Taxus brevifolia*, and was quickly regarded as a most promising anti cancer agent. The compound class promotes microtubule assembly *in vitro* and, consequently, upsets the delicate dynamic equilibrium between soluble dimeric tubules and the microtubule polymers. The stabilization of the latter inhibits the late part of phase G_2 and M of cell mitosis in cancer cells. Due to this particular mode of action paclitaxel represents an important addition to the cancer armamentarium.

Presently, the drug is prepared *via* a semisynthetic process utilizing baccatin III, which is isolated from needles of the Atlantic yew tree. The precursor represents the polycyclic core structure of taxol and the biologically highly relevant C-13 side chain incorporated by the "β-lactam approach" (Scheme 1).
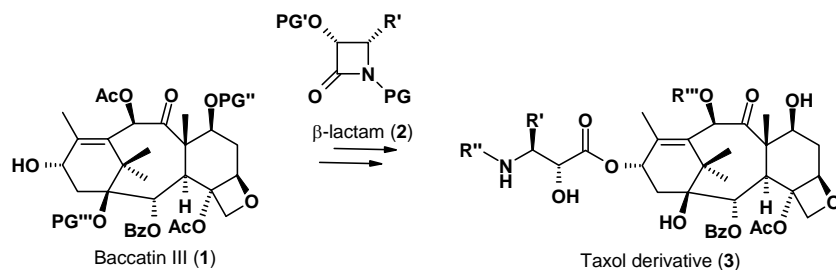
**Scheme 1.** β-Lactam approach to taxoids.

A number of structure-activity studies suggests that significant changes in the pharmacological activity of the compound can be achieved by modifications of the C-13 side chain. This led to the development of a number of "second generation" taxoids with improved administration profiles and/or cytotoxic potential.

In this context we are presenting our synthetic efforts to incorporate rigid cyclic systems as key structural elements of novel C-13 side chain analogs bearing additional functional groups. The key step in our sequence is the stereoselective bioreduction of appropriate α-keto-β-lactams using whole yeast cells in a fermentation process. Previously, we could demonstrate, that yeast cells carry the required biocatalytic arsenal to generate the natural side chain precursor for

natural paclitaxel from a suitable keto precursor. In addition, yeast reduction is also suitable to access second generation taxoids in a resolution process of corresponding α-keto-β-lactams.

Results and Discussion

Synthesis of the required substrate α-keto-β-lactams **10a-c** followed a straight forward strategy, and this concept was previously developed by us for the preparation of similar compounds (Scheme 2, Table 1). We started the sequence from aldehydes **4a-c**, which are readily available from simple cyclic ketone precursors, as recently reported by us. Formation of imines **5a-c** was accomplished by reaction with *p*-anisidine in the presence of Na_2SO_4 and addition of molecular sieves led to a significant acceleration of the conversion.
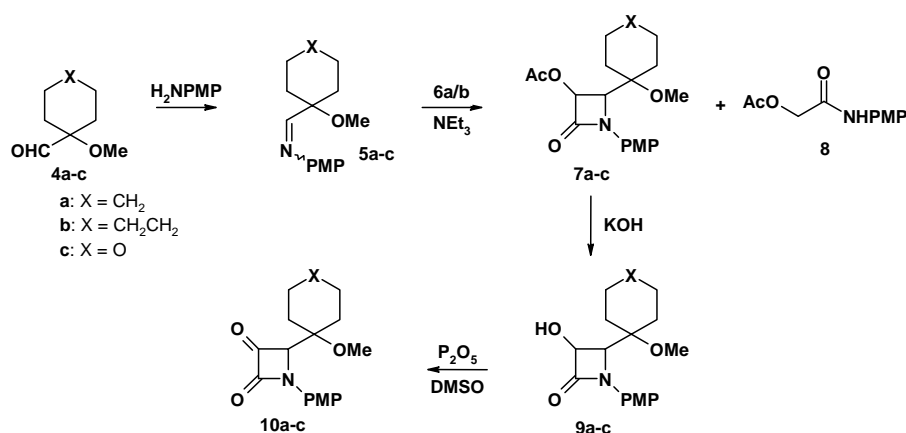
Scheme 2. Synthetic sequence towards α-keto-β-lactams **10a-c** (PMP = *p*-methoxyphenyl).

Table 1. Synthetic sequence towards α-keto-β-lactams **10a-c**

| precursor	X	5	7	9	10
4a	CH_2	69%	25%[a]	87%	64%
4b	CH_2CH_2	81%	30%[a]	91%	76%
4c	O	55%	24%[b]	92%	72%

[a] using **6a**; [b] using **6b**

Subsequent cyclization to the β-lactam core **7a-c** was carried out *via* a Staudinger-type methodology: The intermediate ketene was either generated in a one pot procedure from acetoxy acetic acid **6a** using $POCl_3$ or from acid chloride **6b** in the presence of excess NEt_3. Due to sterical hindrance by the quaternary carbon adjacent to the cyclization site, the cycloaddition reaction was rather sluggish and required several days to reach complete conversion. For such prolonged reaction times, stability of the imine under the conditions applied became a limiting factor, as in all cases aldehyde **4a-c** was recovered. This decomposition of the imines **5a-c** gave rise to significant amounts of *p*-anisidine, which led to the formation of amid **8** as by-product of

the cyclization reaction. The observed problems with sterically demanding reactions partners in the Staudinger reaction are consistent with a significantly decreased yield for the conversion to the corresponding 4-*tert.*-butyl-azetidinone as found in a previous study.

While lactams **7a** and **7b** were formed as *cis-* and *trans*-isomers in approximately equal amounts, tetrahydropyran derivative **7c** was isolated in *cis*-form, exclusively. Assignment of diastereomers is based on previous studies of coupling constants for azetidin protons H-3 and H-4. Hydrolysis of the acetoxy protecting group under basic conditions yielded alcohols **9a-c** in a similar *cis/trans* ratio. Subsequent oxidation using P_2O_5/DMSO finally gave α-keto-β-lactams **10a-c** in good overall yields after chromatographic purification.

Bioreductions with commercial bakers' yeast were carried out in 10% glucose solution supplemented by 1 equivalent of β-cyclodextrin to improve solubility and membrane penetration of the substrates and to decrease toxicity *vis-à-vis* the living cells. Biotransformations at 30°C in an orbital shaker required 2-5 days to reach completion (Scheme 3, Table 2). Alcohols **9a-c** were obtained in good chemical yields as *cis*-isomers, exclusively, when using ordinary bakers' yeast obtained at a local supermarket. However, enantioselectivity of the microbial transformation was low for all ketone precursors. This result was rather unexpected, as previous yeast mediated reductions of α-keto-β-lactams displayed a distinguished diastereo- and enantioselectivity. In the case of substrates **10a-c** the responsible enzyme seems unable to differentiate the existing chiral center of the racemic substrate. Consequently, the reduction is mainly governed by thermodynamic effects, leading to the energetically most stable product. In the case of the azetidine core this is the *cis*-substitution pattern of groups in position 3 and 4, consistent with the chemical reduction of ketones **10a-c** using sodium borohydride.
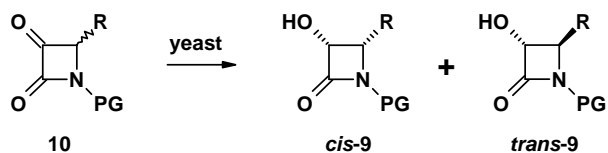
Scheme 3. Yeast mediated reductions of α-keto-β-lactams to *cis-* and *trans*-alcohols **9**.

Table 2. Yeast mediated reductions of α-keto-β-lactams to *cis-* and *trans*-alcohols **9**

ketone	R	*cis*-**9**		*trans*-**9**	
		Yield	e.e.	Yield	e.e.
10a	1-methoxy-1-cyclohexyl[a]	62%	~5%	0%	n.a.[d]
10b	1-methoxy-1-cycloheptyl[a]	62%	8%	0%	n.a.[d]
10c	4-methoxy-tetrahydropyran-4-yl[a]	74%	~5%	0%	n.a.[d]
10d	phenyl[b]	62%	55%	38%	>97%
10e	*tert.*-butyl[c]	48%	>95%	44%	>98%

[a] data obtained in this study; [b] data from ref. **Error! Bookmark not defined.**; [c] data from ref. **Error! Bookmark not defined.**; [d] not applicable

Previous bioreduction studies with phenyl (**10d**) and *tert.*-butyl (**10e**) ketolactams utilizing whole yeast cells showed a distinguished trend (Scheme 3): Using commercial bakers' yeast, racemic substrate **10d** gave complete conversion to two diastereomeric alcohols *cis*- and *trans*-**9d**. Stereochemistry at the secondary alcohol was *R* in both cases. While *trans*-**9d** was obtained almost exclusively as (3*R*,4*R*) enantiomer, *cis*-**9d** was only obtained in moderate optical purity, however predominantly in (3*R*,4*S*) configuration as suitable precursor for paclitaxel. A control experiment with the mutant yeast strain ATCC 26403 gave a classical kinetic resolution, yielding only *cis*-**9d**, however, again in moderate enantiomeric excess, as in the previous transformation. This strain is deficient in fatty acid synthase (FAS), which suggests that FAS is responsible for the formation of *trans*-**9d** and this enzyme is stereoselective.

The low selectivity in the bioreduction to *cis*-**9d** was attributed to the similarity of the 4-phenyl substituent and the PMP protecting group. Consequently, switching to a 4-*tert.*-butyl precursor, a key structural element of second generation butitaxels, was expected to improve the selectivity of the yeast mediated reductions. This effect was successfully demonstrated for ketone **10e**, which was converted diastereoselectively to *cis*-(3*R*,4*S*)-**9e** and *trans*-(3*R*,4*R*)-**9e** in high optical purity.

Based on the structural similarity of the sterically strained compounds, we had expected a similar trend for the biotransformation of ketones **10a-c**. However, these compounds seem to be unsuitable substrates for FAS, as no *trans*-alcohols were formed in the biocatalytic conversion. A control experiment with **10b**, using the mutant strain ATCC 26403 in a suitable growth media containing fatty acid supplements, did not show any conversion to alcohols **9b**. As a result, ketones **10a-c** were only accepted by a still not identified enzyme, which is responsible for the formation of *cis*-products but with rather limited stereoselectivity for the secondary alcohol functionality generated in the biocatalytic process. The biotransformation of substrates **10a-c** is most likely the result of a single enzyme, while for the bioreduction of **10d/e** a two enzyme process seems most likely.

Nevertheless, such conclusions should be handled with caution, as yeast harbors a large variety of different reductases with partially overlapping substrate specificity. Unambiguous assignment of enzyme activity and stereoselectivity can only be made after biotransformations using isolated enzymes or recombinant expression systems with a suitable genetically clean background. Such experiments are currently under way in our laboratory.

Experimental Section

General Procedures. Unless otherwise noted, chemicals and microbial growth media were purchased from commercial suppliers and used without further purification. All solvents were distilled prior to use. Flash column chromatography was performed on silica gel 60 from Merck (40-63μm). *Kugelrohr* distillation was carried out using a Büchi GKR-51 apparatus. Melting points were determined using a Kofler-type Leica Galen III micro hot stage microscope and are

uncorrected. NMR-spectra were recorded from $CDCl_3$ or DMSO-d_6 solutions on a Bruker AC 200 (200 MHz) or Bruker Avance UltraShield 400 (400 MHz) spectrometer and chemical shifts are reported in ppm using TMS as internal standard. Combustion analysis was carried out in the Microanalytic Laboratory, University of Vienna. Enantiomeric excess was determined by HPLC using a Chiracel OD-H column (4.6 x 150mm) on a Beckmann Gold system (hexane/2-propanol = 9/1, UV-detection). Specific rotation $[\alpha]_D^{20}$ was determined using a Perkin Elmer Polarimeter 241.

General procedure for the preparation of imines 5

p-Anisidine (1 equiv.), Na_2SO_4 (4 equiv.) and molecular sieves (3Å) were added to aldehyde **4** (5% solution in dry CH_2Cl_2). After stirring for 24 hours at RT the mixture was passed through a bed of Celite® and concentrated.

***N*-(1-Methoxy-cyclohexylmethylene)-4-methoxyaniline (5a).** 1-Methoxycyclohexanecarboxaldehyde **4a** (1.00g, 7.0mmol) was converted according the above procedure to give **5a** (1.19g, 69%) as yellow liquid after *Kugelrohr* distillation; bp 140-150°C/0.2mbar (KRD); ^{1}H-NMR (200MHz, δ, $CDCl_3$): 1.20–2.00 (m, 10H), 3.25 (s, 3H), 3.81 (s, 3H), 6.90 (d, J= 6Hz, 2H), 7.04 (d, J= 6Hz, 2H), 7.70 (s, 1H); ^{13}C-NMR (50MHz, δ, $CDCl_3$): 21.6 (t), 25.6 (t), 31.8 (t), 50.6 (q), 55.5 (q), 77.5 (s), 114.2 (d), 121.9 (d), 144.5 (s), 158.1 (s), 167.6 (d).

***N*-(1-Methoxy-cycloheptylmethylene)-4-methoxyaniline (5b).** 1-Methoxycycloheptanecarboxaldehyde **4b** (7.00g, 45mmol) was converted according the above procedure to give **5b** (10.03g, 81%) as brown liquid. ^{1}H-NMR (200MHz, δ, $CDCl_3$): 1.40 – 1.82 (m, 8H), 1.89 – 2.00 (m, 4H), 3.25 (s, 3H), 3.80 (s, 3H), 6.89 (d, J= 6Hz, 2H), 7.07 (d, J= 6Hz, 2H), 7.72 (s, 1H); ^{13}C-NMR (50MHz, δ, $CDCl_3$): 21.4 (t), 29.5 (t), 34.4 (t), 51.0 (q), 55.4 (q), 81.5 (s), 114.2 (d), 121.8 (d), 144.5 (s), 158.0 (s), 167.7 (d).

4-Methoxy-*N*-(4-methoxy-tetrahydropyran-4-ylmethylene)-aniline (5c). 4-Methoxy-tetrahydropyran-4-carboxaldehyde **4c** (1.00g, 6.9mmol) was converted according the above procedure to give **5c** (0.94g, 55%) as dark liquid. ^{1}H-NMR (200MHz, δ, $CDCl_3$): 1.79 – 2.31 (m, 4H), 3.25 (s, 3H), 3.62 – 3.92 (m, 4H), 3.80 (s, 3H), 6.88 (d, J= 6Hz, 2H), 7.09 (d, J= 6Hz, 2H), 7.71 (s, 1H); ^{13}C-NMR (50MHz, δ, $CDCl_3$): 32.1 (t), 50.6 (q), 55.4 (q), 63.5 (t), 75.1 (s), 114.2 (d), 121.8 (d), 143.9 (s), 158.3 (s), 164.7 (d).

Azetidine cyclization – Method A

Acetoxyacetic acid **6a** (1.3 equiv.) and NEt_3 were added to imine **5** (5% solution in dry CH_2Cl_2). After cooling to 5°C, $POCl_3$ was added dropwise (N_2 atmosphere). This mixture was stirred for 7-9 days at RT. After hydroysis with 2N HCl the solution was extracted with CH_2Cl_2. The combined organic layers were washed with satd. $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated.

Azetidine cyclization – Method B

NEt_3 (3 equiv.) was added to acetoxyacetyl chloride **6b** (1.3 equiv., 5% solution in dry CH_2Cl_2). Imine **5** was added slowly at -20°C. After stirring for 5-6 days at RT the solution was hydrolyzed

with 2N HCl and extracted with CH_2Cl_2. The combined organic layers were washed with satd. $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated.

3-Acetoxy-1-(4-methoxyphenyl)-4-(1-methoxycyclohexyl)-azetidin-2-one (7a). Imine **5a** (1.19g, 4.8mmol) was converted according to method A to give **7a** (406mg, 25%) as yellow crystals (*cis/trans* isomers approx. 3/2) after flash column chromatography (silica gel, LP/EtOAc 4/1); ^{1}H-NMR (200MHz, δ, CDCl$_3$, *cis*): 1.20–2.00 (m, 10H), 2.18 (s, 3H), 3.28 (s, 3H), 3.81 (s, 3H), 4.50 (d, J = 6Hz, 1H), 6.10 (d, J = 6Hz, 1H), 6.89 (d, J = 6Hz, 2H), 7.40 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$, *cis*): 20.9 (q), 20.9 (t), 21.2 (t), 25.5 (t), 29.5 (t), 31.9 (t), 49.4 (q), 55.4 (q), 61.2 (d), 75.1 (d), 77.2 (s), 114.2 (d), 121.8 (d), 130.2 (s), 157.2 (s), 163.7 (s), 169.5 (s); ^{1}H-NMR (200MHz, δ, CDCl$_3$, *trans*): 1.20–2.00 (m, 10H), 2.16 (s, 3H), 3.25 (s, 3H), 3.81 (s, 3H), 4.24 (d, J ~ 1Hz, 1H), 5.79 (d, J ~ 1Hz, 1H), 6.89 (d, J = 6Hz, 2H), 7.35 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$, *trans*): 20.8 (q), 20.8 (t), 21.0 (t), 25.2 (t), 29.1 (t), 31.7 (t), 48.9 (q), 55.4 (q), 60.1 (d), 72.8 (d), 75.5 (s), 114.2 (d), 121.4 (d), 129.9 (s), 157.1 (s), 162.8 (s), 169.3 (s).

3-Acetoxy-1-(4-methoxyphenyl)-4-(1-methoxycycloheptyl)-azetidin-2-one (7b). Imine **5b** (1.40g, 5.4mmol) was converted according to method A to give **7b** (591mg, 30%) as beige crystals (*cis/trans* isomers approx. 1/1) after flash column chromatography (silica gel, LP/EtOAc 4/1); ^{1}H-NMR (200MHz, δ, CDCl$_3$, *cis*): 1.20–2.00 (m, 10H), 2.19 (s, 3H), 3.27 (s, 3H), 3.79 (s, 3H), 4.53 (d, J = 6Hz, 1H), 6.10 (d, J = 6Hz, 1H), 6.89 (d, J = 6Hz, 2H), 7.38 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$, *cis*): 20.8 (q), 21.5 (t), 22.3 (t), 28.5 (t), 29.4 (t), 32.3 (t), 35.8 (t), 49.7 (q), 55.4 (q), 66.2 (d), 75.4 (d), 79.7 (s), 114.2 (d), 122.3 (d), 130.1 (s), 157.3 (s), 163.9 (s), 169.4 (s); 1H-NMR(200MHz, δ, CDCl$_3$, *trans*): 1.20–2.00 (m, 10H), 2.17 (s, 3H), 3.22 (s, 3H), 3.79 (s, 3H), 4.26 (d, J ~ 1Hz, 1H), 5.81 (d, J ~ 1Hz, 1H), 6.89 (d, J = 6Hz, 2H), 7.38 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$, *trans*): 20.6 (q), 20.9 (t), 21.8 (t), 28.2 (t), 29.3 (t), 31.9 (t), 35.6 (t), 49.3 (q), 55.4 (q), 62.5 (d), 72.7 (d), 78.6 (s), 114.2 (d), 121.4 (d), 129.9 (s), 157.1 (s), 163.0 (s), 169.2 (s).

***cis*-3-Acetoxy-1-(4-methoxyphenyl)-4-(4-methoxy-tetrahydropyran-4-yl)-azetidin-2-one (7c).** Imine **5c** (1.54g, 6.2mmol) was converted according to method B to give **7c** (507mg, 24%) as colorless crystals after flash column chromatography (silica gel, LP/EtOAc 4/1); mp 165-170°C (decomp.); ^{1}H-NMR(200MHz, δ, CDCl$_3$): 1.61–1.96 (m, 4H), 2.19 (s, 3H), 3.29 (s, 3H), 3.54 – 3.79 (m, 4H), 3.80 (s, 3H), 4.53 (d, J = 6Hz, 1H), 6.12 (d, J = 6Hz, 1H), 6.90 (d, J = 6Hz, 2H), 7.35 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$): 20.8 (q), 30.4 (t), 32.1 (t), 49.4 (q), 55.5 (q), 60.4 (d), 62.7 (t), 63.1 (t), 72.9 (d), 74.5 (s), 114.4 (d), 121.4 (d), 129.5 (s), 157.3 (s), 163.4 (s), 169.5 (s).

Acetic acid (4-methoxy-phenylcarbamoylmethyl)ester (8). Colorless crystals of compound **8** was obtained as by-product in varying amounts upon cyclization to lactams **7a-c** after flash column chromatography (silica gel, LP/EtOAc 4/1); mp 72-75°C; 1H-NMR(200MHz, δ, CDCl$_3$): 2.22 (s, 3H), 3.80 (s, 3H), 4.70 (s, 2H), 6.89 (d, J = 6Hz, 2H), 7.45 (d, J = 6Hz, 2H), 7.70 (bs, 1H); ^{13}C-NMR (50MHz, δ, CDCl$_3$): 20.8 (q), 55.5 (q), 63.3 (t), 114.2 (d), 122.2 (d), 129.6 (s), 156.9 (s), 164.8 (s), 169.3 (s).

General procedure for hydrolysis to alcohols 9

A solution (5%) of compound **7** was treated with the half volume of a 1:1 mixture of 2N KOH and THF at 0°C and stirred at this temperature until conversion was completed. The reaction mixture was poured into water and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated.

3-Hydroxy-1-(4-methoxyphenyl)-4-(1-methoxycyclohexyl)- azetidin-2-one (9a). Product **7a** (380mg, 1.1mmol) was converted according to the above procedure to give **9a** (290mg, 87%) as colorless crystals (*cis/trans* mixture); ^{1}H-NMR (200MHz, δ, CDCl$_3$, *cis*): 1.16–1.78 (m, 10H), 3.42 (s, 3H), 3.80 (s, 3H), 4.25 (d, J = 6Hz, 1H), 4.82 (d, J = 12Hz, 1H), 4.99 (dd, J = 12Hz, J = 6Hz, 1H), 6.89 (d, J = 6Hz, 2H), 7.33 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$, *cis*): 21.1 (t), 21.7 (t), 25.3 (t), 30.8 (t), 32.8 (t), 51.1 (q), 55.2 (q), 63.2 (d), 77.2 (d), 78.0 (s), 114.3 (d), 121.7 (d), 130.6 (s), 157.0 (s), 168.3 (s); ^{1}H-NMR(200MHz, δ, CDCl$_3$, *trans*): 1.05–2.10 (m, 10H), 3.30 (s, 3H), 3.80 (s, 3H), 4.15 (d, J ~ 1Hz, 1H), 4.79 (bs, 1H), 4.82 (d, J = 12Hz, 1H), 6.87 (d, J = 6Hz, 2H), 7.31 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$, *trans*): 20.9 (t), 21.4 (t), 25.2 (t), 28.9 (t), 31.7 (t), 49.2 (q), 55.0 (q), 63.2 (d), 76.5 (d), 77.4 (s), 114.3 (d), 120.5 (d), 130.3 (s), 156.7 (s), 167.6 (s). Anal. calcd. for $C_{17}H_{23}NO_4$ (305.37): C 6.86, H 7.59, N 4.59; found: C 66.81, H 7.79, N 4.46.

3-Hydroxy-1-(4-methoxyphenyl)-4-(1-methoxycycloheptyl)- azetidin-2-one (9b). Product **7b** (685mg, 1.9mmol) was converted according to the above procedure to give **9b** (550mg, 91%) as colorless crystals (*cis/trans* mixture); ^{1}H-NMR (200MHz, δ, CDCl$_3$, *cis*): 1.30–2.09 (m, 12H), 3.31 (s, 3H), 3.80 (s, 3H), 4.32 (d, J = 6Hz, 1H), 4.65 (d, J = 12Hz, 1H), 4.89 (dd, J$_1$ = 12Hz, J$_2$ = 6Hz, 1H), 6.88 (d, J = 6Hz, 2H), 7.30 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$, *cis*): 21.2 (t), 22.8 (t), 30.3 (t), 30.7 (t), 33.3 (t), 35.9 (t), 51.1 (q), 55.4 (q), 64.1 (d), 77.2 (d), 82.3 (s), 114.2 (d), 122.1 (d), 130.6 (s), 157.1 (s), 168.5 (s); ^{1}H-NMR (200MHz, δ, CDCl$_3$, *trans*): 1.30–2.09 (m, 12H), 3.38 (s, 3H), 3.73 (s, 3H), 4.19 (d, J ~ 1Hz), 4.65 (d, J = 13Hz, 1H), 4.79 (m, 1H), 6.86 (d, J = 6Hz, 2H), 7.34 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$, *trans*): 22.2 (t), 22.7 (t), 29.1 (t), 30.2 (t), 31.9 (t), 35.7 (t), 49.5 (q), 55.3 (q), 64.1 (d), 76.0 (d), 78.7 (s), 114.1 (d), 120.8 (d), 130.2 (s), 156.7 (s), 168.2 (s). Anal. calcd. for $C_{18}H_{25}NO_4$ (319.40): C 67.69, H 7.89, N 4.39; found: C 67.56, H 8.05, N 4.66.

3-Hydroxy-1-(4-methoxyphenyl)-4-(4-methoxy-tetrahydropyran-4-yl)-azetidin-2-one (9c). Compound **7c** (450mg, 1.3mmol) was converted according to the above procedure to give **9c** (346mg, 92%) as colorless crystals; mp 152-158°C; ^{1}H-NMR (200MHz, δ, CDCl$_3$): 1.69–2.10 (m, 4H), 3.47 (s, 3H), 3.50 – 3.80 (m, 4H), 3.80 (s, 3H), 4.28 (d, J = 6Hz, 1H), 4.61 (d, J = 12Hz, 1H), 5.01 (dd, J$_1$ = 12, J$_2$ = 6Hz, 1H), 6.88 (d, J = 6Hz, 2H), 7.33 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, CDCl$_3$): 31.0 (t), 33.4 (t), 49.6 (q), 55.4 (q), 63.0 (d), 63.3 (t), 75.8 (s), 77.2 (d), 114.4 (d), 120.2 (d), 130.2 (s), 156.8 (s), 167.8 (s). Anal. calcd. for $C_{16}H_{21}NO_5$ (307.35): C, 62.53; H, 6.89; N 4.56; anal. calcd. for $C_{16}H_{21}NO_5$ x $1H_2O$ (325.36): C 59.07, H 7.13, N 4.30; found: C 59.05, H 7.57, N 3.85.

General procedure for the oxidation to ketones 10

P_4O_{10} (1.5 equiv.) was stirred with dry DMSO (5% solution) for 10min, then alcohol **9** (5% solution in DMSO) was added at RT. This mixture was stirred for 5-10h until TLC showed full conversion. Hydrolysis with satd. $NaHCO_3$ solution was followed by extraction with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated.

1-(4-Methoxyphenyl)-4-(1-methoxycyclohexyl)-azetidin-2,3-dione (10a). Alcohol **9a** (260mg, 0.85mmol) was converted according to the above procedure to give **10a** (164mg, 64%) as yellow crystals after flash column chromatography (silica gel, LP/EtOAc 7/1); mp 94-96°C; ^{1}H-NMR (200MHz, δ, $CDCl_3$): 1.06–2.02 (m, 10H), 3.30 (s, 3H), 3.82 (s, 3H), 4.82 (s, 1H), 6.95 (d, J = 6Hz, 2H), 7.61 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, $CDCl_3$): 20.8 (t), 20.9 (t), 24.9 (t), 28.9 (t), 32.3 (t), 49.2 (q), 55.2 (q), 76.0 (d), 76.1 (s), 114.3 (d), 120.6 (d), 130.1 (s), 157.9 (s), 160.8 (s), 193.5 (s). Anal. calcd. for $C_{17}H_{21}NO_4$ (304.37): C 67.31, H 6.98, N 4.62; found: C 67.09, H 7.10, N 4.64.

1-(4-Methoxyphenyl)-4-(1-methoxycycloheptyl)-azetidin-2,3-dione (10b). Alcohol **9b** (1.5g, 4.7mmol) was converted according to the above procedure to give **10b** (1.14g, 76%) as yellow crystals after flash column chromatography (silica gel, LP/EtOAc 4/1); mp 90-95°C; ^{1}H-NMR (200MHz, δ, $CDCl_3$): 1.30–2.05 (m, 12H), 3.29 (s, 3H), 3.84 (s, 3H), 4.86 (s, 1H), 6.95 (d, J = 6Hz, 2H), 7.61 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, $CDCl_3$): 21.8 (t), 22.0 (t), 29.3 (t), 29.5 (t), 32.5 (t), 35.7 (t), 49.9 (q), 55.5 (q), 77.2 (d), 80.1 (s), 114.4 (d), 120.9 (d), 130.2 (s), 158.0 (s), 161.3 (s), 193.5 (s). Anal. calcd. for $C_{18}H_{23}NO_4$ (318.40): C 68.12, H 7.30, N 4.41; found: C 68.20, H 7.52, N 4.54.

1-(4-Methoxyphenyl)-4-(4-methoxy-tetrahydropyran-4-yl)-azetidin-2,3-dione (10c). Alcohol **9c** (290mg, 1.0 mmol) was converted according to the above procedure to give **10c** (207mg, 72%) as yellow crystals after flash column chromatography (silica gel, LP/EtOAc 7/1); mp 118-120°C; ^{1}H-NMR (200MHz, δ, $CDCl_3$): 1.54–1.96 (m, 4H), 3.38 (s, 3H), 3.45 – 3.81 (m, 4H), 3.85 (s, 3H), 4.85 (s, 1H), 6.95 (d, J = 6Hz, 2H), 7.60 (d, J = 6Hz, 2H); ^{13}C-NMR (50MHz, δ, $CDCl_3$): 29.6 (t), 32.8 (t), 50.1 (q), 55.5 (q), 62.7 (t), 63.1 (t), 74.2 (s), 75.7 (d), 114.6 (d), 120.8 (d), 130.0 (s), 158.3 (s), 160.1 (s), 192.7 (s). Anal. calcd. for $C_{16}H_{19}NO_5$ (305.33): C 62.94, H 6.24, N 4.67; found: C 62.66, H 6.27, N 4.59.

General procedure for the yeast reduction

Commercial bakers' yeast (4g, obtained from a local supermarket) was added to a 10% solution of glucose in water (100mL) and incubated for 10 minutes at 30°C. Ketone **10** (100mg in 1mL dioxane) and β-cyclodextrin (1 equiv.) were added to this fermentation culture. The mixture was incubated for 2-5 days at 30°C. The biomass was separated by centrifugation (5min at 3000 rpm). After extraction with EtOAc the combined organic layers were dried over Na_2SO_4, filtered, and concentrated.

***cis*-3-Hydroxy-1-(4-methoxyphenyl)-4-(1-methoxycyclohexyl)- azetidin-2-on (*cis*-9a).** Substrate **10a** (100mg, 0.33mmol) was converted according to the above procedure to give *cis*-**9a** (64mg,

64%, ee: 5%) as colorless crystals after flash column chromatography (silica gel, LP/EtOAc 4/1); mp 146-150°C; e.e. ~ 5%; $[\alpha]_D^{20} = +11.8$ (c 0.34, CHCl$_3$).

cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(1-methoxycycloheptyl)- azetidin-2-on (cis-9b). Substrate **10b** (100mg, 0.33mmol) was converted according to the above procedure to give cis-**9b** (64mg, 64.0%) as colorless crystals after flash column chromatography (silica gel, LP/EtOAc 4/1); mp 140-145°C; e.e. = 8%; $[\alpha]_D^{20} = -15.0$ (c 1.3, CHCl$_3$).

cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(4-methoxy-tetrahydropyran-4-yl)-azetidin-2-on (cis-9c). Substrate **10c** (100mg, 0.34mmol) was converted according to the above procedure to give cis-**9c** (74mg, 74%, ee: 5%) as colorless crystals after column chromatography (silica gel, LP/EtOAc 5/1); mp 152-158°C; e.e. ~ 5%; $[\alpha]_D^{20} = -15.4$ (c 1.3, CH$_2$Cl$_2$).

Acknowledgements

References

1. (a) Faber, K. *Biotransformations in Organic Chemistry*, Springer: Berlin, 1997. Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994. (b) Davis, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in Preparative Organic Chemistry*; Academic Press: London, 1989.
2. Stewart, J. D. *Biotechnol. Genet. Eng. Rev.* **1997**, *14*, 67.
3. (a) Stewart, J. D. *Curr. Opinion in Drug Discovery and Development* **1998**, *1*, 278. (b) D'Arrigo, P.; Pedrocchi-Fantoni, G.; Servi, S. *Adv. Appl. Microbiol.* **1997**, *44*, 81. (c) Csuk, R.; Glanzer, B. I. *Chem. Rev.* **1991**, *91*, 49. (d) Servi, S. *Synthesis* **1990**, 1. (e) Neuberg, C. *Adv. Carb. Chem.* **1949**, *4*, 75.
4. Wada, M.; Kataoka, M.; Kawabata, H.; Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Shimizu, S. *Biosci. Biotech. Biochem.* **1998**, *62*, 280. (b) Kuhn, A.; van Zyl, C.; van Donder, A.; Prior, B. A. *Appl. Environ. Microbiol.* **1995**, *61*, 1580. (c) Nakamura, K.; Kondo, S.; Kawai, Y.; Nakajima, N.; Ohno, A. *Biosci. Biotech. Biochem.* **1994**, *58*, 2236. (d) Nakajima, N.; Ishihara, K.; Kondo, S.; Tsuboi, S.; Utaka, M.; Nakamura, K. *Biosci. Biotech. Biochem.* **1994**, *58*, 2080. (e) Ishihara, K.; Nakajima, N.; Tsuboi, S.; Utaka, M. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 3314. (f) Nakamura, K.; Kawai, Y.; Nakajima, N.; Ohno, A. *J. Org. Chem.* **1991**, *56*, 4778. (g) Heidlas, J.; Engel, K.-H.; Tressl, R. *Eur. J. Biochem.* **1988**, *172*, 633. (h) Shieh, W.-R.; Gopalin, A. S.; Sih, C. J. *J. Am. Chem. Soc.* **1985**, *107*, 2993. (i) Murata, K.; Fukuda, Y.; Simosaka, M.; Watanabe, K.; Saikusa, T.;

Kimura, A. *Eur. J. Biochem.* **1985**, *151*, 631. (j) Furuichi, A.; Akita, H.; Matsukura, H.; Oishi, T.; Horikoshi, K. *Agric. Biol. Chem.* **1985**, *49*, 2563. (k) King, H. L., Jr.; Dyar, R. E.; Wilken, D. R. *J. Biol. Chem.* **1974**, *249*, 4689. (l) Francke, A. *Biochem. J.* **1965**, *95*, 633.

5. Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071.

6. *Chemical syntheses:* Corey, E. J.; Link, J. O.; Shao, Y. *Tetrahedron Lett.* **1992**, 3435. (b) Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346. (c) *Biocatalytic routes:* Adam, W.; Boland, W.; Hartmann-Schreier, J.; Humpf, H.-U.; Lazarus, M.; Saffert, A.; Saha-Möller, C. R.; Schreier, P. *J. Am. Chem. Soc.* **1998**, *120*, 11044. (d) Adam, W.; Lazarus, M.; Boxx, B.; Saha-Möller, C. R.; Humpf, H.-U.; Schreier, P. *J. Org. Chem.* **1997**, *62*, 7841. (e) Adam, W.; Fell, R. T.; Hoch, U.; Saha-Möller, C. R.; Schreier, P. *Tetrahedron: Asymmetry* **1995**, *6*, 1047. (f) Effenberger, F. *Ang. Chem. Int. Ed. Engl.* **1994**, *33*, 1555; (g) Sugai, T.; Ohta, H. *Tetrahedron Lett.* **1991**, 7063. (h) Ushio, K.; Yamauchi, S.; Masuda, K. *Biotechnol. Lett.* **1991**, *13*, 495; (i) Wong, C.-H.; Matos, J. R. *J. Org. Chem.* **1985**, *50*, 1992.

7. *Other synthetic routes:* Kearns, J.; Kayser, M. M. *Tetrahedron Lett.* **1994**, 2845. (b) Hattori, K.; Miyata, M.; Yamamoto, H. *J. Am. Chem. Soc.* **1993**, *115*, 1151. (c) Holton, R. A.; Liu, J. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2475. (d) Georg, G. I.; Gou, D. M.; Liu, Y. C.; Chen, C. S. *J. Org. Chem.* **1993**, *58*, 1287. (e) Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R.; Burke, C. T. *J. Med. Chem.* **1992**, *35*, 4230. (f) Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985. (g) Commerçon, A.; Bézard, D.; Bernard, F.; Bourzat, J. D. *Tetrahedron Lett.* **1992**, 5185. (h) Ojima, I.; Habus, I.; Zhao, M.; Georg, G. I.; Jayasinghe, L. R. *J. Org. Chem.* **1991**, *56*, 1681. (i) Denis, J. N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1991**, *56*, 6939. Denis, J.-N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1990**, *55*, 1957. (k) Denis, J.-N.; Greene, A. E.; Serra, A. A.; Luche, M.-J. *J. Org. Chem.* **1986**, *51*, 46.

8. Munson, P.L. *Principles of Pharmacology*, Chapman & Hall: New York 1994; 1503.

9. Wani, M.C.; Taylor, H.L.; Wall, M.E.; Coggon, P.; McPhail, A.T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.

10. Borman, S. *Chem. Eng. News* **1991**, *69*, 11. (b) Junad, T. *Life* **1992**, *5*, 71. (c) Nicolaou, K. C.; Guy, R. K. *Angew. Chem.* **1995**, *107*, 2247. (d) Nicolaou, K. C.; Sorensen, E.J. *Classics in Total Synthesis*, VCH: Weinheim, 1996.

11. Gringauz, A. *Introduction to Medicinal Chemistry*; Wiley-VCH: New York, 1997, 132.

12. Georg, G. I.; Boge, T.C.; Cheruvallath, Z.S.; Clowers, J.S.; Harriman, G.C.B.; Hepperle, M.; Park, H. The Medicinal Chemistry of Taxol. In *Taxol: Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, FL, USA.

13. Denis, J.-N.; Greene, A.E.; Guenard, D.; Gueritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917.

14. Ojima, I., Sun, C. M.; Zucco, M.; Park, Y. H.; Duclos, O.; Kuduk, S. *Tetrahedron Lett.* **1993**, *34*, 4149. (b) Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985. (c) Ojima, I.; Habus, I.; Zhao, M.; Georg, G. I.; Jayasinghe, L. R. *J. Org. Chem.* **1991**, *56*, 1681. (d) Holton, R. A. *Eur. Pat. Appl. EP400, 971*, 1990 *Chem. Abstr.* 1990, 114, 164568q.

15. Hepperle, M.; Georg, G. I. *Drugs of the Future* **1994**, *19*, 573.

16. Ojima, I.; Wang, T.; Miller, M. L.; Lin, S.; Borella, C. P.; Geng, X.; Pera, P.; Bernacki, R. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3423. (b) Yamaguchi, T.; Harada, N.; Ozaki, K.; Arakawa, H.; Oda, K.; Nakanishi, N.; Tsujihara, K.; Hashiyama, T. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1639. (c) Ojima, I.; Lin, S. *J. Org. Chem.* **1998**, *63*, 224. (d) Syed, M. A.; Hoemann, M. Z.; Aube, J.; Georg, G. I.; Mitscher, L. A.; Jayasinghe, L. R. *J. Med. Chem.* **1997**, *40*, 236. (e) Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P.-Y.; Vrignaud, P.; Bissery, M.-C.; Veith, J. M.; Pera, P.; Bernacki, R. J. *J. Med. Chem.* **1996**, *39*, 3889. (f) Syed, M. A.; Hoemann, M. Z.; Aube, J.; Mitscher, L. A.; Georg, G. I.; McCall, R.; Jayasinghe, L. R. *J. Med. Chem.* **1995**, *38*, 3821. (g) Boge, T. C.; Himes, R. H.; Vander Velde, D. G.; Georg, G. I. *J. Med. Chem.* **1994**, *37*, 3337.

17. Kayser, M. M.; Mihovilovic, M. D.; Kearns, J.; Feicht, A.; Stewart, J. D. *J. Org. Chem.* **1999**, *64*, 6603.

18. Kayser, M. M.; Yang, Y.; Mihovilovic, M. D.; Feicht, A.; Rochon, F. D. *Can. J. Chem.* **2002**, *80*, 796.

19. Mihovilovic, M. D.; Feicht, A.; Kayser, M. M. *J. Prakt. Chem.* **2000**, *342*, 585.

20. Mihovilovic, M. D.; Spina, M.; Müller, B.; Stanetty, P. *Monatsh. Chem.* **2004**, *135*, 899.

21. N. DeKimpe, M. Nagy, M. Boeykens, D. Van der Schneren, *J. Org. Chem.* **1992**, *57*, 5761. (b) N. DeKimpe, D. DeSmaele, *Tetrahedron Lett.* **1994**, *35*, 8023.

22. Staudinger, H. *Liebigs Ann. Chem.* **1907**, *51*, 356.

23. Rubottom, G.; Marrero, R.; Gruber J. *Tetrahedron* **1983**, *39*, 861.

24. Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Carrequx, F.; Cuevas, C.; Maneiro, E.; Ontoria, J. M. *J. Org. Chem.* **1994**, *59*, 3132. (b) Palomo, C.; Aizpurua, J. M.; Cossio, F. P.; Garcia, J. M.; Lopez, M. C.; Oiarbide, M. *J. Org. Chem.* **1990**, *55*, 2070.

25. Bar, R. *Trends Biotechnol.* **1989**, *7*, 2.

26. Schweizer, E.; Bolling, H. *Proc. Natl. Acad. Sci. USA* **1970**, *67*, 660.