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Tetrahedron: *Asymmetry* 16 (2005) 2521–2524

Microbial Baeyer–Villiger oxidation applied to the synthesis of the *N*-protected (1*R*,5*R*)-Geisman–Waiss lactone

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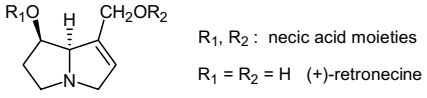
Received 17 May 2005; accepted 30 June 2005

Abstract—Using whole cell Baeyer–Villiger biooxidation as a key step and depending on the choice of the biocatalyst {*E. coli* TOP10[pQR239] or *Acinetobacter* TD63}, the synthesis of nearly enantiopure *N*-protected (1*R*,5*R*)-(−)-Geisman–Waiss lactone (92% ee) or its (1*R*,5*S*)-(−) regioisomer (98% ee) be performed.

1. Introduction

Whole-cell or enzyme mediated Baeyer–Villiger (BV) biooxidations allow the efficient transformation of ketones into lactones with high regio-, stereo- and enantioselectivity. They favourably compare with BV oxidations using transition metal-based catalysts.[1,2] The enzymes responsible for this type of reaction, that is, Baeyer–Villiger monooxygenases (BVMOs), belong to the flavin monooxygenase family and, because they are NAD(P)H dependent, need cofactor recycling.[1,3] However, due to several practical bottlenecks such as substrate and/or product inhibition, such biotransformation processes have not been amenable to efficient upscaling until now.[4] As an interesting breakthrough, we have recently shown that the combination of a whole cell process (using a recombinant strain overexpressing a BVMO) with a resin-based in situ substrate feeding product removal methodology[5] allowed us to overcome these drawbacks and potentially pave the way to industrial development, as exemplified by a very recent kilogram scale application.[6] This encouraged us to broaden the scope of substrates transformable in high regio- and/or enantioselectivity.

To date, only a few examples of BV biooxidation of ketones bearing a nitrogen atom have been reported.[1] We therefore focused our attention on the biooxidation of *N*-protected 2-aza-bicyclo[3.2.0]heptan-6-one **1**, a precursor of the so-called Geisman–Waiss lactone (2-oxa-6-azabicyclo[3.3.0]octan-3-one). This lactone and its *N*-protected derivatives **2** are versatile intermediates for the synthesis of necine base pyrrolizidine alkaloids,[7] a large class of natural products widely found in plants (Scheme 1).[8]



Scheme 1. General scheme of necine base pyrrolizidine alkaloids.

Pyrrolizidine alkaloids are responsible for livestock poisoning through contaminated crops and are involved in the chemical defence of insects or amphibians. However, in spite of their various biological activities (ranging from anti-feedant to insecticidal, fungicidal, bactericidal and virucidal effects[8]), studies focusing on the biological effect of synthetic pyrrolizidine alkaloids analogues or of unnatural stereoisomers of these compounds are scarce, a feature that may be partly due to the lack of appropriate synthetic methods.[9]

Herein, we report the preparation of nearly enantiopure *N*-protected (1*R*,5*R*)-Geisman–Waiss lactone **2** and of its (1*R*,5*S*) regioisomer **3** via the whole cell mediated Baeyer–Villiger oxidation of *rac*-**1** (Scheme 2).

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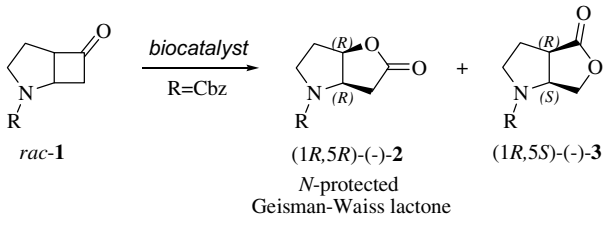
doi:10.1016/j.tetasy.2005.06.038

2. Results and discussion

2.1. Synthesis of *rac*-1

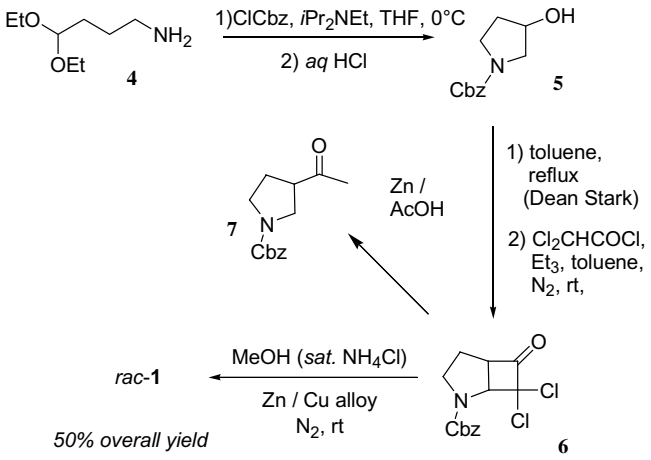
Over the course of studies aimed at the [2+2] cycloaddition of endocyclic enecarbamates,[10] Correia et al. described a synthesis of ketone **1** via the unstable 1-pyrroline intermediate.[10c] However, because of the low yield we obtained following this method (<10%), we decided to follow a slightly different route involving cyclization of an aminobutyraldehyde[11] (Scheme 2). The commercially available aminobutyraldehyde diethyl acetal **4** was protected by a Cbz ($PhCH_2OC(O)$–) moiety at the nitrogen atom and was cyclized under acid catalysis, to afford the corresponding hydroxy-carbamate **5**. Subsequent dehydration of **5** in toluene (using a Dean–Stark device) led to the corresponding endocyclic enecarbamate. Because of its easy rehydration, the [2+2] cycloaddition was carried out directly in the same pot and solvent. The dichloroketene was formed in situ by reaction of dichloroacetyl chloride with triethylamine.[10] The classical treatment of dichloroketone **6** with AcOEt/Zn led to the undesired compound **7** (82% yield). However, a reductive dechlorination could be successfully carried out using a Zn/Cu mixture in methanol (prior saturated with ammonium chloride).[10b] The desired *N*-Cbz-2-azabicycloheptanone **1** was finally obtained in 50% overall yield (Scheme 3).



Scheme 2. Whole-cell biotransformation of *rac*-**1**.

2.2. Biocatalysts

In order to explore the BV oxidation of *rac*-**1**, we selected two Baeyer–Villiger monooxygenases among the most studied. These enzymes are cyclohexanone monooxygenases, named here CHMO A and B. The employed biocatalysts were the two wild type bacterial strains [respectively, *Acinetobacter calcoaceticus* NCIMB 9871 (CHMO A)[12] and *Acinetobacter* TD63[13] (CHMO B)] and one recombinant strain overexpressing CHMO A, *E. coli* TOP10[pQR239], which was constructed by Ward et al.[14] Wild type cells were grown in a mineral media supplemented with 1,2-cyclohexanediol as previously described.[15] Recombinant cells were grown in a glycerol-based medium while the enzyme expression was induced by L-arabinose as previously described.[5]



Scheme 3. Synthesis of racemic azabicycloheptanone **1**.

2.3. Biotransformations

Analytical scale biotransformations were carried out in 250 mL shake flasks at 30 °C using 2 mM of *rac*-**1** and 50 mL of cell broth. These were monitored by GC analysis of samples taken at regular intervals. Ketone and lactone ees were determined by HPLC (Chiralcel AS column-Daicel) as well as by chiral GC (Chirasil Dex-Chrompak- and CycloSilβ-JW Scientific-columns) analyses. The results reported in Table 1 were obtained at ca. 50–60% conversion ratio.

Whatever be the biocatalyst, the whole cell mediated biotransformation afforded two lactones in high ees: the 'normal' one, the *N*-protected Geisman–Waiss lactone **2** (corresponding to the usual oxygen insertion between the more substituted carbon atom and the carbonyl group) and the 'abnormal' lactone **3** (arising from oxygen insertion on the other side of the carbonyl group).

Table 1. Biotranformations of *rac*-**1**

Enzyme	Microorganism	Reaction time (min)	Ketone **1** yield[a] % (ee %)	Normal lactone **2** yield[a] % (ee %)	Abnormal lactone **3** yield[a] % (ee %)	Ratio **2**:**3**
CHMO A	*A. calcoaceticus* NCIMB 9871	120	41 (86)	36 (96)	10 (93)	3.6:1
	E. coli TOP10[pQR239]	60	nd[b] (>95)	37 (94)	11 (87)	3.4:1
CHMO B	*Acinetobacter* TD63	300	45 (74)	11 (87)	29 (98)	1:2.6

[a] Ketone and lactone yields were measured by GC using internal standard method.
[b] An impurity from the growth medium prevented us from a correct calculation of the ketone yield.

2.4. Regioselectivity

In the case of both CHMO A expressing strains (wild type or recombinant strain), the desired Geisman–Waiss lactone **2** was the predominant product in a regioisomeric **2/3** ratio of about 3.5:1. This ratio was similar to that obtained upon mCPBA-mediated BV oxidation (3:1). However, an inversion of this ratio was observed with *Acinetobacter* TD63 (CHMO B), which led to the preferential formation of the 'abnormal' lactone **3** (in a regioisomeric ratio **2/3** of about 1:2.6). This was rather unexpected since, until now, we had never observed any noticeable qualitative differences between both CHMOs, A and B, the latter exhibiting only a slightly lower enantioselectivity.[15,16] As observed previously with other substrates, **1** also underwent a reduction to afford alcohols in <5% yield.

2.5. Enantioselectivity

In all the biotransformations, ketone ee regularly increased whereas the lactone ee was quite high during the first part of the reaction ($87\% < ee < 98\%$) and decreased later on. Apparent *E* value (enantiomeric ratio calculated using substrate and product ees), could be estimated to superior to 80 for both CHMOs.

In order to determine the absolute configuration of the lactones and to validate this biotransformation as a potential synthetic method, a semi-preparative scale experiment was carried out in a 2 L fermenter (750 mL broth, 30 °C, pH 7.2) using *E. coli* [pQR239] cells. As the recombinant strain mediated oxidation was very fast, grown cells were diluted twofold before use (with a fresh culture medium) to provide a cell suspension of 1.4 g L^{-1} dry weight. Thus, the observed drop of lactone ee was slowed down enough to allow, thanks to a timely monitoring, the quenching of the reaction at high lactone ee. A solution of ketone *rac*-**1** (in ethanol) was added to reach a 2.7 mM initial concentration (0.67 g L^{-1}). The biotransformation was stopped once the unreacted ketone **1** reached about 80% ee (ca. 40 min). The time course of the biotransformation is reported in Figure 1.

After continuous extraction of the broth with dichloromethane and purification by flash chromatography, lactone **2**[†] was isolated in 32% yield and 92% ee $\{[\alpha]_D^{32} = -107$ (*c* 0.7, $CHCl_3)\}$. The minor lactone **3**[‡] was obtained in 10% yield and 94% ee ($[\alpha]_D^{32} = -170$ (*c* 0.3, $CHCl_3)$), whereas the unreacted ketone **1** was recovered in 40% yield and 79% ee. A small amount (4%) of *endo*-alcohol was also isolated. The deprotection of **2** to give the (−)-Geisman–Waiss lactone could be easily carried out by hydrogenation on Pd as previously reported.[7c]

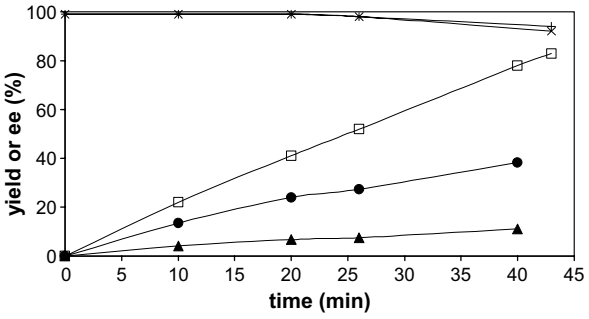


Figure 1. Time course of the semi-preparative biotransformation with *E. coli* [pQR239] cells. Ketone: □ ee; normal lactone: ● yield, × ee; abnormal lactone: ▲ yield, + ee.

2.6. Absolute configuration assignment

Comparison of the specific rotation sign of purified **2** with the literature data[7c] led us to assign the (1*R*,5*R*) absolute configuration to this lactone. Interestingly, it was the desired enantiomer for the synthesis of natural necine bases as (+)-retronecine. Chemical (mCPBA) oxidation of the recovered substrate **1** (79% ee) afforded a mixture of **2** and **3** exhibiting the same ee. Since all enantiomers were separable by chiral GC, the retention time comparison of the thus synthesized lactone **2** with that of (1*R*,5*R*)-enantiomer formed by bioconversion entitled us to assign the (1*S*,5*S*)-configuration to the recovered **1**. Finally, the retention time comparison of chemically and microbiologically obtained **3** allowed us to assign the (1*R*,5*S*)-configuration to the 'microbial' abnormal lactone.

It is noteworthy that, using CHMOs, both regioisomeric lactones were obtained from the same single ketone enantiomer, allowing consequently the recovery of optically active **1**. This feature is quite different from that observed using bicyclo[3.2.0]hept-2-en-6-one (a structurally very close compound) as substrate.[16] In this experiment, an almost perfect regiodivergent parallel kinetic resolution was described:[17] both lactones were formed simultaneously, each of them arising from a different ketone enantiomer. Obviously, the presence of a nitrogen atom and/or of an increased steric hindrance due to the nitrogen protecting group strongly influences on the overall enzyme behaviour.

3. Conclusion

Nearly enantiopure (1*R*,5*R*)-*N*-Cbz-2-oxa-6-azabicyclo-[3.3.0]octan-3-one **2** (*N*-protected Geisman–Waiss lactone) and its regioisomer (1*R*,5*S*)-**3** were obtained in high ee by whole-cell Baeyer–Villiger biooxidations of ketone *rac*-**1**. CHMO A expressing strains favoured the formation of the desired lactone **2** whereas

[†] (1*R*,5*R*)-*N*-Cbz-2-oxa-6-azabicyclo[3.3.0]octan-3-one **2** was identified by comparison of its spectroscopic data with those already described in the literature.[7c]

[‡] For (1*R*,5*S*)-*N*-Cbz-3-oxa-6-azabicyclo[3.3.0]octan-2-one **3**: ^1H NMR ($CHCl_3$-*d*, 250 MHz): δ 2.13 (m, 1H), 2.29 (m, 1H), 3.20 (m, 2H), 3.73 (m, 1H), 4.42 (m, 3H), 5.08 (m, 2H), 7.29 (s, 5H); ^{13}C NMR ($CHCl_3$-*d*, 250 MHz), mixture of rotamers: δ 27.0 (CH_2), 27.6 (CH_2), 43.9 (CH), 44.7 (CH), 45.2 (CH_2), δ 45.6 (CH_2), 57.7 (CH_2), 58.4 (CH), 67.3 (CH_2), 72.5 (CH_2), 73.4 (CH_2), 128.0 (CH), 128.1 (CH), 128.6 (CH), 136.2 (C), 154.0 (C=O), 177.8 (C=O).

CHMO B expressing strain predominantly led to the 'abnormal' lactone **3**.

Work is currently in progress in our laboratory to study the influence of the size and nature of the nitrogen atom protecting group towards the enzyme selectivity and to determine the requirements for high enantioselectivity.

Acknowledgements

This work was funded by the European Community 5th Framework Program (QLK3-CT2001-00403). We are very grateful to Prof. J. Ward (University College of London) for providing us with recombinant strain and also Dr. Correia (University of Campinas, Brazil) for helpful discussions.

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