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Enantioselective hydrolysis of 3,4-disubstituted β-lactams. An efficient enzymatic method for the preparation of a key Taxol side-chain intermediate

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ABSTRACT

3,4-Disubstituted β-lactams 3-benzyloxy-4-(4-chlorophenyl)azetidin-2-one [(3*S**,4*R**)-(±)-**1**], 3-benzyloxy-4-phenylazetidin-2-one [(3*S**,4*R**)-(±)-**2**] and 4-(4-chlorophenyl)-3-phenoxyazetidin-2-one [(3*S**,4*R**)-(±)-**3**] were resolved through immobilized CAL-B-catalysed ring-cleavage reactions. Excellent enantioselectivities ($E > 200$) were obtained for (3*S**,4*R**)-(±)-**1** and (3*S**,4*R**)-(±)-**2** when the reactions were performed with added H_2O as nucleophile in *tert*-butyl methyl ether at 70 °C, whereas only moderate E (12) was achieved for (3*S**,4*R**)-(±)-**3** under the same conditions but in diisopropyl ether. The resulting ring-opened β-amino acids [(2*R*,3*S*)-**4** (*ee* > 98%), (2*R*,3*S*)-**5** (*ee* > 98%) and (2*R*,3*S*)-**6** (*ee* = 50%)] and the unreacted β-lactams [(3*S*,4*R*)-**1**–**3**] (*ee* > 98%) could be easily separated.

1. Introduction

A large number of recent published articles and reviews have stressed the biological and chemical importance of β-lactams and β-amino acids [1]. Molecules containing a 2-azetidinone ring may possess antibacterial activity, e.g., carumonam is a β-lactamase-resistant monobactam antibiotic [2], while others containing a *cis* 3,4-disubstituted β-lactam ring may display PPAR α/γ agonist [3], vasopressin VIa agonist [4] or anticancer [5,6] activity. β-Amino acids and some of their derivatives are widely used in combinatorial, peptide, organic and medicinal chemistry [7–9]. Numerous non-proteinogenic amino acids are available can serve as relevant components of fibrinogen receptor antagonists [10]. Taxol®, one of the most efficient anticancer agents of the past decade [11,12], contains (2*R*,3*S*)-3-amino-3-phenyl-2-hydroxypropanoic acid [(2*R*,3*S*)-**7**] in its side-chain. Since the total synthesis of Taxol is a very lengthy and expensive process [13,14], chemists are continuously working on the development of semi-synthetic methods which involve coupling of the C(13)-O of baccatin III derivatives [15] to the corresponding side-chain.

Earlier enzymatic studies on the ring opening of a set of cyclic and acyclic β-lactams [16–19] were continued with successful enzymatic syntheses of a Taxol side-chain key intermediate through the enantioselective ring opening of racemic *cis*-3-hydroxy-4-phenylazetidin-2-one (0.5 equiv. of H_2O in *t*-BuOMe at 60 °C, with immobilized CAL-B) and sequential kinetic resolution of racemic *cis*-3-acetoxy-4-phenylazetidin-2-one (1 equiv. of H_2O in iPr_2O at 60 °C, with immobilized CAL-B) [20]. To extend the substrate scope, and also to analyse how different-sized substituents on C3 or C4 influence the ring cleavage of β-lactams, in the present work we set out to develop immobilized CAL-B-catalysed methods for the enzymatic ring opening of racemic 3,4-disubstituted β-lactams, such as 3-benzyloxy-4-(4-chlorophenyl) azetidin-2-one, 3-benzyloxy-4-phenylazetidin-2-one and 4-(4-chlorophenyl)-3-phenoxyazetidin-2-one [(3*S**,4*R**)-(±)-**1**–**3**] (Scheme 1), and then to synthetize (2*R*,3*S*)-3-phenylisoserine (2*R*,3*S*)-**7**, the key intermediate of the Taxol side-chain, from the corresponding enantiomeric compound.

2. Results and discussion

2.1. Synthesis of (3S*,4R*)-(±)-1–3

Racemic β-lactams (3*S**,4*R**)-(±)-**1**–**3** were synthesized according to a literature method [21]. A mixture of *p*-ethoxyaniline

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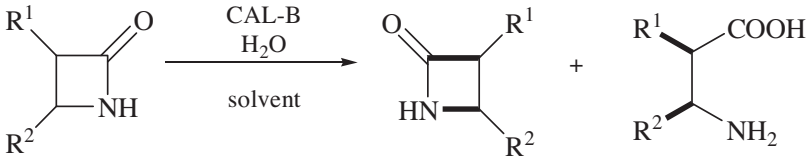


1, 4: R^1 = BnO, R^2 = *p*-ClPh
2, 5: R^1 = BnO, R^2 = Ph
3, 6: R^1 = PhO, R^2 = *p*-ClPh

Scheme 1. Immobilized CAL-B-catalysed hydrolysis of (±)-**1**–**3**.

and the appropriate aldehyde furnished the Schiff bases (Z)-*N*-(4-chlorobenzylidene)-4-ethoxybenzenamine (**10**) and (Z)-*N*-benzylidene-4-ethoxybenzenamine (**11**), which, through cycloadditions in the presence of the appropriate acyl chlorides, 2-phenoxyacetyl chloride (**8**) or 2-benzyloxyacetyl chloride (**9**), resulted in the *N*-protected *β*-lactams **12**–**14**. CAN-mediated oxidative removal of the 4-ethoxyphenyl groups gave the desired β-lactams **1**–**3** (Scheme 2).

2.2. Immobilized CAL-B-catalysed ring-opening of (3S*,4R*)-(±)-1–3

In earlier studies, immobilized CAL-B proved to be applicable for the enantioselective ($E > 200$) ring opening of both 4-aryl-substituted [17] and carbocyclic β-lactams [22], and we therefore carried out the ring opening of model compound (3S*,4R*)-(±)-**1** with 1 equiv. of H_2O in *i*Pr_2O at 60 °C, with immobilized CAL-B as catalyst (Table 1, entry 1).

In order to find the optimum conditions for the gram-scale resolution of (3S*,4R*)-(±)-**1**, solvent screening (Table 1, entries 1–6) was first performed in order to determine the effects on *E* and the reaction rate. Practically, no reaction was detected during 65 h when the reactions were performed in THF (entry 4) or 2-Me-THF (entry 5). The reactions proceeded enantioselectively ($E > 200$), but slowly in *t*-BuOMe and *i*Pr_2O (conv. = 5–8% after 65 h) (entries 1 and 6) and with somewhat higher conversions in toluene (conv. = 15% after 65 h, $E = 32$) (entry 2) or *n*-hexane (conv. = 17% after 65 h, $E = 39$) (entry 3). In view of the results, *t*-BuOMe was chosen for further preliminary experiments.

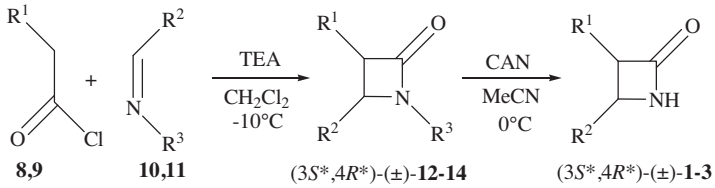
H_2O, as a nucleophile, is essential for the ring-opening reaction, through its quantity in the reaction medium can affect the enzymatic activity [18,22]. Experiments were therefore also performed with different quantities of added H_2O (Table 1, entries 7–10 and 12–15). On increase of the amount of H_2O up to 50 equiv., the reactions became faster without a drop in *E* (entries 8–10), but a further increase of the H_2O content resulted in considerably decreases in both reaction rate and *E* (entries 12–15). It is noteworthy that, in accordance with our earlier observation that a hydrolytic reaction proceeded even without added H_2O in the reaction mixture (due to the H_2O present in the reaction medium) [22], the quantity of H_2O present in the reaction medium (<0.1%) or at the surface of the immobilized CAL-B (2–5%) was sufficient for the ring cleavage of (±)-**1** (entry 7). Finally, 25 equiv. of H_2O was chosen as the optimum quantity.

On increase of the temperature of the ring-opening reaction from 60 °C (Table 1, entry 10) to 70 °C, the reaction rate increased without any decrease in enantioselectivity (Table 1, entry 11). Accordingly, 70 °C was chosen as the reaction temperature.

The above-optimized reaction conditions (25 equiv. of H_2O, *t*-BuOMe, 70 °C) were next applied for the ring cleavage of (±)-**2** and (±)-**3**. Excellent results were observed for (±)-**2** ($E > 200$), but a very poor *E* (5) for (±)-**3** (Table 2, entry 1). We therefore continued the optimizations for (±)-**3** with a new solvent screening, changing the amount of added H_2O and also the temperature of the reaction (Table 2).

The reactions in toluene and *n*-hexane proceeded relatively slowly, with low *E* (entries 2 and 3) while in MeCN and THF the enzyme did not display activity during 65 h (entries 6 and 7). A slightly increased *E* (8) was noted in *i*Pr_2O vs. *t*BuOMe ($E = 2$) (entries 4 and 5). Variation of the quantity of water (from 2 to 100 equiv., entries 8–11) and temperature (50 and 70 °C, entries 12 and 13) led to the same results as observed earlier for (±)-**1**. In summary, *E* was increased slightly ($E = 14$, entry 13) when the reaction was carried out with 25 equiv. of water in *i*Pr_2O at 70 °C (Scheme 3).

On the basis of the preliminary results, the immobilized CAL-B-catalysed preparative-scale ring-opening reactions of (±)-**1** and (±)-**2** were performed with 25 equiv. of H_2O in *t*-BuOMe at 70 °C, while the preparative-scale resolution of (±)-**3** was performed with 25 equiv. of H_2O in *i*Pr_2O at 70 °C. In order to obtain (2*R*,3*S*)-**6** with a good *ee* value, the reaction was overrun to 66% conversion. The results are reported in Table 3 and in Section 3 (Experimental part).



8: R^1 = BnO
9: R^1 = PhO
10: R^2 = Ph, R^3 = *p*-EtOPh
11 R^2 = *p*-ClPh, R^3 = *p*-EtOPh
12: R^1 = BnO, R^2 = *p*-ClPh, R^3 = *p*-EtOPh
13: R^1 = BnO, R^2 = Ph, R^3 = *p*-EtOPh
14: R^1 = PhO, R^2 = *p*-ClPh, R^3 = *p*-EtOPh
1: R^1 = BnO, R^2 = *p*-ClPh
2: R^1 = BnO, R^2 = Ph
3: R^1 = PhO, R^2 = *p*-ClPh

Scheme 2. Synthesis of (±)-**1**–**3**.