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# Enhanced enzymatic synthesis of the enantiopure intermediate for the blockbuster drug intermediate abacavir through a two-step enzymatic cascade reaction

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ABSTRACT

A very efficient enzymatic two-step cascade reaction was devised ($E > 200$) for the resolution of activated γ-lactams (±)-**1** and (±)-**2**. The *N*-hydroxymethyl group worked as a traceless activating group, when the reactions were performed with $H_2O$ (0.5 equiv) in the presence of benzylamine (1 equiv) in *i*-$Pr_2O$ at 60 °C. The ring-opened enantiomerically pure γ-amino acids (1*S*,4*R*)-**6** (*ee* = 99%, intermediate of abacavir) and (1*S*,3*R*)-**8** (*ee* = 99%) and unreacted lactams (1*S*,4*R*)-**1** and (1*R*,4*S*)-**2** (*ee* ⩾ 96%) were obtained in good yields (⩾43%). Treatment of (1*S*,4*R*)-**1** and (1*R*,4*S*)-**2** with 18% HCl or $NH_4OH$ resulted in (1*R*,4*S*)-**6**·HCl and (1*S*,3*R*)-**8**·HCl or (1*S*,4*R*)-**3** and (1*R*,4*S*)-**4** quantitatively, with *ee* ⩾ 96%.



## 1. Introduction

In recent years, some new enzymatic and asymmetric methods for the preparation of enantiopure γ-amino acids and γ-lactams have been published.[1–4] The enantiomers of 2-azabicyclo[2.2.1]hept-5-en-3-one are building blocks of pharmaceutically important molecules, which have antiviral or antibacterial activity.[5,6] (1*S*,4*R*)-4-Aminocyclopent-2-ene-1-carbocyclic acid (1*S*,4*R*)-**6** is a key intermediate for the blockbuster abacavir, which is a nucleoside analogue reverse transcriptase inhibitor.[7] The importance of abacavir is also accentuated in the *WHO's List of Essential Medicines*, a list of the most important medication needed in a basic health system. A number of enzymatic methods have been developed for its synthesis. For example, Evans et al.[8] worked out a method for the resolution of 2-azabicyclo[2.2.1]hept-5-en-3-one by using lactamase ENZA-1 [*Rhodococcus equi* NCIMB 41213] or ENZA-20 [*Pseudomonas solanacearum* NCIMB 40249]). Taylor et al.[6] described a more useful procedure for the enantioselective ring opening of the above racemic lactam by using ENZA-25 or ENZA-22 strains. Later, a very efficient lipase-catalysed route[9] was developed for the ring cleavage of both β-[10–12] and γ-lactams.[13] Very recently, a new enzymatic two-step cascade procedure was devised for rapid access to diverse β-amino acids from *N*-hydroxymethyl-β-lactams.[9] Herein our aim was to devise an enantioselective cascade reaction for the synthesis of racemic *N*-hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (±)-**1** and *N*-hydroxymethyl-2-azabicyclo[2.2.1]heptan-3-one (±)-**2** (Scheme 1). Transformations of the enantiomeric *N*-activated γ-lactams into the desired inactivated γ-lactam and γ-amino acid hydrochlorides (Scheme 3) were also planned.

## 2. Results and discussion

Racemic γ-lactam **1** was synthesized from the commercially available 2-azabicyclo[2.2.1]hept-5-en-3-one (±)-**3** with paraformaldehyde under sonication.[14] Catalytic transfer hydrogenation of (±)-**1** and (±)-**3** in the presence of cyclohexene as a hydrogen donor gave racemic **2** or **4** (Scheme 1).[13]

Based on the results achieved on the ring cleavage of *N*-hydroxymethyl-β-lactams, ring-cleavage reactions of (±)-**1** and (±)-**2** catalysed by CAL-B (lipase B from *Candida antarctica*, produced by the submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) were performed with $H_2O$ (0.5 equiv) in the presence of benzylamine (1 equiv) in *i*-$Pr_2O$ at 60 °C (Scheme 2, Table 1, entries 1 and 3).[15] The role of benzylamine, as demonstrated earlier,[9] is to restrict any adverse side-reactions capturing formaldehyde.

Comparing the ring-cleavage reaction rates of the *N*-hydroxymethyl lactams (±)-**1** and (±)-**2** to those of inactivated lactams (±)-**3** and (±)-**4** (Table 1, entries 1 and 3 vs 2 and 4) performed under the same conditions demonstrated the beneficial accelerator

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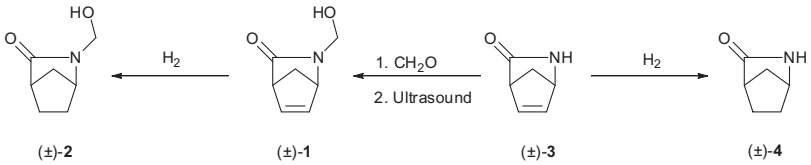
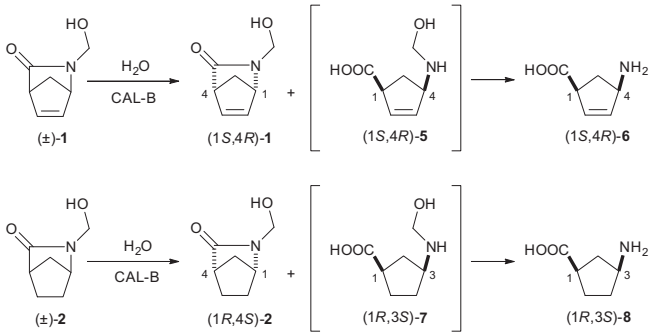
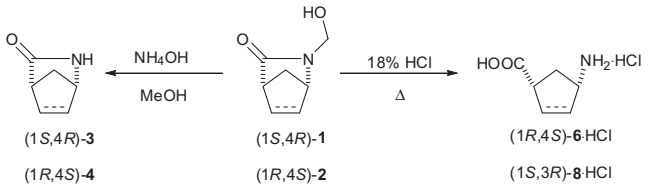
0957-4166/




**Scheme 1.**




**Scheme 2.**




**Scheme 3.**

**Table 1**
CAL-B-catalysed ring opening of (±)-**1**[a], **2**[a], **3**[b] and **4**[b]

| Entry | Racemate | Reaction time (h) | Conv.[c] (%) | $ee_S$[d] (%) | $ee_P$[e] (%) | *E* |
|---|---|---|---|---|---|---|
| 1 | (±)-**1** | 0.5 | 45 | 80 | 99 | >200 |
| 2 | (±)-**3** | 0.5 | 33 | 50 | 99 | >200 |
| 3 | (±)-**2** | 48 | 41 | 70 | 99 | >200 |
| 4 | (±)-**4** | 48 | 33 | 50 | 99 | >200 |

[a] 0.05 M substrate, 0.5 equiv of $H_2O$, 1 equiv of benzylamine, *i*-$Pr_2O$, 60 °C.
[b] 0.05 M substrate, 0.5 equiv of $H_2O$, *i*-$Pr_2O$, 60 °C.
[c] Calculated from $ee_S$ and $ee_P$.
[d] According to GC analysis.
[e] According to GC analysis after double derivatisation.[16]

effect of the activating group (Table 1, entries 1 vs 2 and 3 vs 4), as stated earlier for β-lactams.[9]

On the basis of the preliminary results, the preparative-scale reactions of (±)-**1**[17] and (±)-**2**[18] were performed with $H_2O$ (0.5 equiv) in the presence of benzylamine (1 equiv) in *i*-$Pr_2O$ at 60 °C. The results are reported in Table 2.

Hydrolysis of unreacted *N*-hydroxymethyl-γ-lactams (1*S*,4*R*)-**1** and (1*R*,4*S*)-**2** with 18% aqueous HCl gave γ-amino acid hydrochlorides (1*R*,4*S*)-**6**·HCl and (1*S*,3*R*)-**8**·HCl (Scheme 3).[20] The deprotection of *N*-hydroxymethyl-γ-lactam enantiomers was performed with $NH_4OH$ and MeOH[21] affording γ-lactam enantiomers (1*S*,4*R*)-**3** and (1*R*,4*S*)-**4** (Scheme 3).[22]

The absolute configurations were determined by comparing the specific rotation values with the literature data (footnote of Table 2).

## 3. Conclusion

In conclusion, a very efficient two-step enzymatic procedure has been devised for the preparation of *N*-hydroxymethyl γ-lactam and γ-aminoacid enantiomers, the abacavir intermediate amino acid [(1*S*,4*R*)-**6**] being one of them. The CAL-B-catalysed ring opening reactions were highly enantioselective (*E* > 200) when the reactions were performed with $H_2O$ (0.5 equiv) in the presence of benzylamine (1 equiv) in *i*-$Pr_2O$ at 60 °C. As the ring-opened amino acids formed, the *N*-hydroxymethyl groups underwent spontaneous degradation, and the desired enantiomeric γ-amino acid and unreacted *N*-hydroxymethyl-γ-lactam enantiomers (*ee* ⩾ 96%) were obtained. The desired γ-amino acid (*ee* = 99%, yield ⩾43%) and γ-lactam enantiomers (*ee* ⩾ 96%, yield ⩾44%) could be easily separated. Transformations of the unreacted *N*-hydroxymethyl-γ-lactam enantiomers (1*S*,4*R*)-**1** and (1*R*,4*S*)-**2** through acidic hydrolysis or deprotection via $NH_4OH$ resulted in the desired (1*S*,4*R*)-**3** and (1*R*,4*S*)-**4** lactams or (1*R*,4*S*)-**6**·HCl and (1*S*,3*R*)-**8**·HCl amino acid hydrochlorides without a loss in *ee* (*ee* ⩾ 96%).

**Table 2**
CAL-B-catalysed ring opening of (±)-**1**[a] and (±)-**2**[b]

| | Reaction time (h) | Product enantiomer | | | | Unreacted enantiomer | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Yield (%) | Isomer | *ee*[c](%) | $[\alpha]_D^{25}$ $H_2O$ | Yield (%) | Isomer | *ee*[d](%) | $[\alpha]_D^{25}$ $CHCl_3$ |
| (±)-**1** | 2 | 49 | (1*S*,4*R*)-**6** | 99 | −240[e,f] | 49 | (1*S*,4*R*)-**1** | 99 | +342[g,h] |
| (±)-**2** | 55 | 43 | (1*R*,3*S*)-**8** | 99 | −11[i,j] | 44 | (1*R*,4*S*)-**2** | 96 | +49.8[k] |

[a] 0.72 mmol substrate, 0.072 M, 0.5 equiv of $H_2O$, 1 equiv of benzylamine, *i*-$Pr_2O$, 300 mg CAL-B (30 mg $mL^{-1}$), 60 °C.
[b] 0.71 mmol substrate, 0.071 M, 0.5 equiv of $H_2O$, 1 equiv of benzylamine, *i*-$Pr_2O$, 300 mg CAL-B (30 mg $mL^{-1}$), 60 °C.
[c] According to GC analysis after double derivatisation.[16]
[d] According to GC analysis.
[e] *c* 0.30.
[f] Lit.[13] $[\alpha]_D^{25}$ = −243 (*c* 0.34, $H_2O$) for (1*S*,4*R*)-**6**.
[g] *c* 0.50.
[h] Lit.[19] $[\alpha]_D^{25}$ = +344 (*c* 0.21, $CHCl_3$) for (1*S*,4*R*)-**1**.
[i] 0.30.
[j] Lit.[13] $[\alpha]_D^{25}$ = −10.6 (*c* 0.35, $H_2O$) for (1*R*,3*S*)-**8**.
[k] *c* 0.72.

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15. In a typical small-scale experiment, to the racemic substrate (0.05 M solution) in *i*-$Pr_2O$ (1 mL) CAL-B (30 mg), $H_2O$ (0.5 equiv) and then benzylamine (1 equiv) were added. The mixture was shaken (167 rpm) at 60 °C. The progress of the reaction was followed by taking samples from the reaction mixtures and analyzing them by a GC method on a Chrompack Chirasil-Dex CB column [140 °C for 25 min → 190 °C (temperature rise 20 °C $min^{-1}$; 140 kPa; retention times (min), (1*S*,4*R*)-**1**: 5.84 (antipode: 5.66)], (1*R*,4*S*)-**2**: 7.47 (antipode: 7.11)]. The *ee* values for the product γ-amino acids [after pre-column derivatization[16] with $CH_2N_2$ (*Caution! derivatization with $CH_2N_2$ should be performed under a well-ventillating hood*)] were determined by a GC method [120 °C for 25 min → 160 °C (temperature rise 10 °C $min^{-1}$; 140 kPa; retention times (min), (1*S*,4*R*)-**6**: 27.38 (antipode: 27.84)], (1*R*,3*S*)-**8**: 28.74 (antipode: 28.98)].
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17. (±)-**1** (100 mg, 0.72 mmol) was dissolved in *i*-$Pr_2O$ (10 mL). Next, CAL-B (300 mg, 30 mg $mL^{-1}$), benzylamine (79 μL, 0.72 mmol) and $H_2O$ (6.5 μL, 0.36 mmol) were added and the mixture was shaken in an incubator shaker at 60 °C for 120 min. The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated and the residue was subjected to column chromatography (EtOAc:*n*-hexane 1:1). The resulting γ-lactam (1*S*,4*R*)-**1** was [49 mg, 49%; viscous oil {$[\alpha]_D^{25}$ = +342 (*c* 0.50, $CHCl_3$); *ee* = 99%, lit.[19] = +344 (*c* 2.1, $CHCl_3$); *ee* > 99%}. The filtered enzyme was washed with distilled $H_2O$ (3 × 15 mL) and after evaporation of $H_2O$ yielded the crystalline γ-amino acid (1*S*,4*R*)-**6** [46 mg, 49%; $[\alpha]_D^{25}$ = −240 (*c* 0.30, $H_2O$), *ee* = 99%, lit.[13] = −243 (*c* 0.34, $H_2O$), *ee* > 99%; mp >260 °C with decomposition (recrystallized from $H_2O$/$Me_2CO$), lit.[13] mp >260 °C with decomposition (recrystallized from $H_2O$/$Me_2CO$)]. The $^1$H NMR (400 MHz, $CDCl_3$, 25 °C, TMS) data for (1*S*,4*R*)-**1**: δ = 2.10–2.34 (m, 2H, $CH_2$); 3.31–3.37 (m, 1H, *CH*CO); 4.30–4.37 (m, 1H, *CH*N); 4.45–4.52 (d, 1H, *J* = 10.96 Hz, $CH_2$OH); 4.80–4.88 (d, 1H, *J* = 10.96 Hz, $CH_2$OH); 6.52–6.96 (m, 2H, *CHCH*). Analysis: calculated for $C_7H_9NO_2$: C, 60.42; H, 6.52; N, 10.07; found: C, 61.10; H, 6.48; N, 10.02. The $^1$H NMR (400 MHz, $D_2O$) data for (1*S*,4*R*)-**6**: δ = 2.05–2.64 (m, 2H, $CH_2$); 3.56–3.63 (m, 1H, *CH*COOH); 4.39–4.45 (m, 1H, *CH*$NH_2$); 5.99–6.39 (m, 2H, *CHCH*). Analysis: calculated for $C_6H_9NO_2$: C, 56.68; H, 7.13; N, 11.02; found: C, 56.74; H, 7.14; N, 10.97.
18. Via the procedure described above,[13] the reaction of racemic (±)-**2** (100 mg, 0.71 mmol), benzylamine (79 μL, 0.71 mmol.) and $H_2O$ (6.4 μL, 0.35 mmol) in *i*-$Pr_2O$ (10 mL) in the presence of CAL-B (300 mg, 30 mg $mL^{-1}$) at 60 °C after 55 h afforded the unreacted (1*R*,4*S*)-**2** [44 mg, 44%; viscous oil, $[\alpha]_D^{25}$ = +49.8 (*c* 0.72, $CHCl_3$), *ee* = 96%] and amino acid (1*R*,3*S*)-**8** [39 mg, 43%; $[\alpha]_D^{25}$ = −11.0 (*c* 0.30, $H_2O$), *ee* = 99%; lit.[13] = −10.6 (*c* 0.35, $H_2O$), *ee* = 98%; mp >260 °C with decomposition (recrystallized from $H_2O$/$Me_2CO$), lit.[13] mp >260 °C with decomposition (recrystallized from $H_2O$/$Me_2CO$)]. The $^1$H NMR (400 MHz, $CDCl_3$, 25 °C, TMS) data for (1*R*,4*S*)-**2**: δ = 1.35–1.96 (m, 6H, 3×$CH_2$); 2.75–2.86 (m, 1H, *CH*CO); 3.95–4.06 (m, 1H, *CH*N); 4.49–4.87 (m, 2H, $CH_2$OH). Analysis: calculated for $C_7H_{11}NO_2$: C, 59.56; H, 7.85; N, 9.92; found: C, 59.62; H, 7.89; N, 9.86. The $^1$H NMR (400 MHz, $D_2O$) data for (1*R*,3*S*)-**8**: δ = 1.72–2.41 (m, 6H, 3×$CH_2$); 2.84–2.96 (m, 1H, *CH*COOH); 3.79–3.89 (m, 1H, *CH*$NH_2$). Analysis: calculated for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84; found: C, 55.78; H, 8.60; N, 10.84.
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20. The unreacted hydroxymethyl-γ-lactam enantiomers [(1*S*,4*R*)-**1** and (1*R*,4*S*)-**2**] were dissolved in 18% HCl (10 mL) and kept at reflux for 5 h. The solvents were evaporated and the products, obtained almost quantitatively, were recrystallized from EtOH and $Et_2O$. Amino acid hydrochlorides (1*R*,4*S*)-**6**·HCl and (1*S*,3*R*)-**8**·HCl obtained as white crystals were characterized as follows: (1*R*,4*S*)-**6**·HCl: $[\alpha]_D^{25}$ = +110 (*c* 0.20 in $H_2O$), *ee* = 99%; lit.[13] = +111.1 (*c* 0.35, $H_2O$), *ee* > 99%; mp 205–207 °C, lit.[13] 208–209 °C. (1*S*,3*R*)-**8**·HCl: $[\alpha]_D^{25}$ = +10 (*c* 0.20, $H_2O$), *ee* = 96%; lit.[13] = +10.7 (*c* 0.5, $H_2O$), *ee* = 97%; mp 170–173 °C, lit.[13] 175–177 °C. The $^1$H NMR (400 MHz, $D_2O$) data for (1*R*,4*S*)-**6**·HCl: δ = 2.14–2.83 (m, 2H, $CH_2$); 3.79–3.88 (m, 1H, *CH*COOH); 4.45–4.54 (m, 1H, *CH*$NH_2$); 6.03-6.36 (m, 2H, *CHCH*). Analysis: calculated for $C_6H_9NO_2$HCl: C, 44.05; H, 6.16; N, 8.56; found: C, 44.15; H, 6.10; N, 8.55. The $^1$H NMR (400 MHz, $D_2O$) data for (1*S*,3*R*)-**8**·HCl: δ = 1.78–2.55 (m, 6H, 3×$CH_2$); 3.03–3.14 (m, 1H, *CH*COOH); 3.79–3.89 (m, 1H, *CH*$NH_2$). Analysis: calculated for $C_6H_{11}NO_2$·HCl: C, 43.51; H, 7.30; N, 8.46; found: C, 43.61; H, 7.35; N, 8.41.
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22. The unreacted *N*-hydroxymethyl-γ-lactams (1*S*,4*R*)-**1** and (1*R*,4*S*)-**2**, (20 mg, 0.14 mmol) were dissolved in MeOH (2 ml). Next, $NH_4OH$ (2 ml) was added and the mixture was stirred at room temperature for 4 h. The solvent was evaporated, the residue was chromatographed on silica, and elution with ethyl acetate afforded white crystals of (1*S*,4*R*)-**3** [15 mg, 95%; $[\alpha]_D^{25}$ = +545 (*c* 0.3, $CHCl_3$), *ee* = 99%; lit.[13] = +549 (*c* 0.26, $CHCl_3$) *ee* > 99%; mp 95–98 °C (recrystallized from *i*-$Pr_2O$), lit.[13] 97–100 °C] or (1*R*,4*S*)-**9** [15 mg, 93%; $[\alpha]_D^{25}$ = +125.5 (*c* 0.55, $CHCl_3$), *ee* = 96%; lit.[13] = +158 (*c* 0.45, $CHCl_3$) *ee* >99%; mp 78–81 °C (recrystallized from *i*-$Pr_2O$), lit.[13] 78–81 °C]. The $^1$H NMR (400 MHz, $CDCl_3$, 25 °C, TMS) data for (1*S*,4*R*)-**3**: δ = 2.18–2.43 (m, 2H, $CH_2$); 3.18–3.24 (m, 1H, *CH*CO); 4.30–4.37 (m, 1H, *CH*NH); 6.05 (br s, 1H, N*H*); 6.63–6.82 (m, 2H, *CHCH*). Analysis: calculated for $C_6H_7NO$: C, 66.04; H, 6.47; N, 12.84; found: C, 66.12; H, 6.38; N, 12.82. The $^1$H NMR (400 MHz, $CDCl_3$, 25 °C, TMS) data for (1*R*,4*S*)-**4**: δ = 1.33–1.97 (m, 6H, 3×$CH_2$); 2.76–2.85 (m, 1H, *CH*CO); 3.04 (br s, 1H, N*H*); 3.95–4.08 (m, 1H, *CH*NH). Analysis: calculated for $C_6H_9NO$: C, 64.84; H, 8.16; N, 12.60; found: C, 64.81; H, 8.13; N, 12.68.