Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 1593–1600

TETRAHEDRON: *ASYMMETRY*

Enzymatic resolution of alicyclic β-lactams

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Received 8 February 2000; accepted 18 February 2000

Abstract

Racemates of *N*-hydroxymethylated β-lactams **4–6** were resolved through the lipase-catalyzed asymmetric acylation of the primary hydroxy group at the 6*S* stereogenic centre. High enantioselectivity ($E > 200$) was observed when the enzymatic reactions were performed in acetone with lipase PS as catalyst and vinyl butyrate as acyl donor. The hydrolysis of the enantiomeric azetidinones **4a–6a** and **4b–6b** resulted in the enantiomerically pure alicyclic β-amino acids **4c–6c** and **4d–6d**. When the less reactive enantiomers **4b–6b** were treated with NH_4OH/MeOH, enantiomerically pure β-lactams **4e–6e** were formed.

1. Introduction

The alicyclic β-amino acids, derived from the alicycle-condensed azetidinones, are of biological importance, e.g. β-amino acids can be introduced into peptides in order to increase or modify their biological activities.[1,2] Through such exchanges, the stability of the natural peptides can be increased. Among such amino acids, cispentacin, (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid, is a simple natural product with effective antifungal properties. This compound has been prepared, for example, by enantioselective synthesis[3] and enzymatic resolution.[4] (1*R*,2*S*)-2-Amino-3-cyclohexenecarboxylic acid was recently described as an anti-*Candida* agent.[5] The (1*R*,2*S*) enantiomer of 2-amino-4-cyclohexenecarboxylic acid is a key intermediate in the synthesis of carbapenem antibiotics.[6,7] (1*R*,2*S*)-2-Aminocyclohexanecarboxylic acid as part of a peptide exhibited thrombin-inhibitory activity.[8] Since these compounds are interesting from both chemical and pharmacological aspects, our aim was to study the lipase-catalyzed resolution of **1–3** in order to obtain them in optically active form and to hydrolyze them to the corresponding enantiomerically pure amino acids. The unsaturated amino acids can also be functionalized, e.g. by making use of the reactivity of the double bond.

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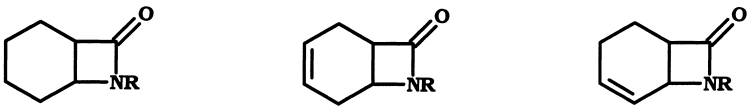
0957-4166/00/$ - see front matter
PII: S0957-4166(00)00070-7



1 R = H
4 R = CH_2OH

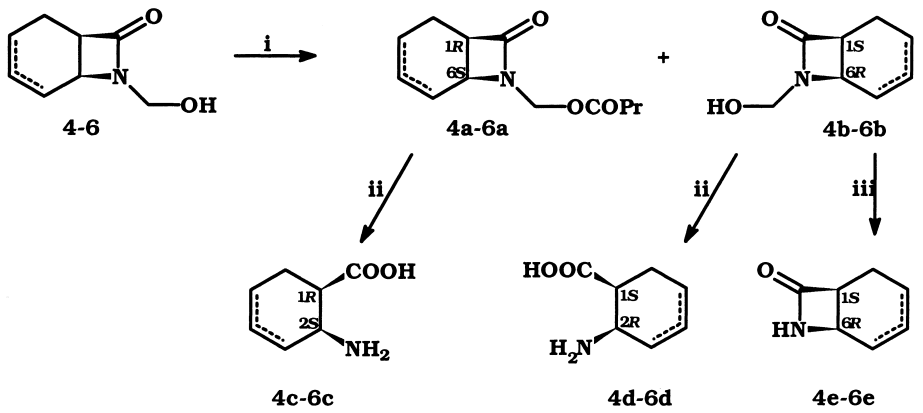
2 R = H
5 R = CH_2OH

3 R = H
6 R = CH_2OH

2. Results and discussion

There are several possibilities to resolve alicyclic-condensed azetidinones. Evans et al. investigated the enantiospecific hydrolysis of (±)-6-azabicyclo[3.2.0]hept-3-en-7-one. Even though a large number of commercially available isolated enzymes proved unsuccessful, lactamases in a special whole-cell preparation, ENZA-1 (*Rhodococcus equi* NCIB 40213), did give rise to the enantioselective ring opening of this compound.[9] If the lactam ring was activated with acyl-protecting groups such as *tert*-butoxycarbonyl or acetyl, the lactam bond could be hydrolyzed enantioselectively by numerous hydrolytic enzymes (esterases, lipases and proteases).[10] One other possibility to resolve such compounds is the enzyme-catalyzed kinetic resolution of the *N*-hydroxymethylated derivatives. 5,5-Disubstituted hydantoins, substituted pyrrolidinones, photopyridones and 2-azabicyclo[2.2.1]hept-5-en-3-ones have been prepared in enantiomerically pure form. In these cases, the resolutions were based on the lipase PS or AK-catalyzed asymmetric acylation of the primary hydroxy group or on the hydrolysis of the corresponding ester derivatives.[11–14]

Since the preparation of *N*-hydroxymethyl derivatives of **4–6** (starting from **1–3**) is simple[4] and the above group can easily be removed under basic reaction conditions, the possibilities of lipase-catalyzed asymmetric acylation of **4–6** were investigated in the present work (Scheme 1).



Scheme 1. (i) Lipase PS, vinyl butyrate in acetone; (ii) 18% HCl, then ion-exchange chromatography; (iii) NH_4OH/MeOH

In order to find the optimal conditions for gram-scale resolutions of **4–6**, some preliminary experiments were needed. The effects of enzyme, acyl donor and solvent on the enantioselectivity and on the reaction rate in the enzymatic reactions were investigated for compound **4**.

Lipase PS and lipase AK are the enzymes most commonly used in the resolution of alcohols with remote stereocentres.[15,16] As concerns the butyrylation of **4** under the same conditions, lipase PS displayed a somewhat higher selectivity (Table 1, rows 2–5) than that with lipase AK. The acylation of **4** with vinyl butyrate was also investigated in the presence of Novozym 435 in tetrahydrofuran (THF). In this case, low enantioselectivity (after 45 min, conv. = 43%, ee_s = 32%, ee_p = 40%, E = 3) was observed.

Table 1
Effects of vinyl ester (0.2 M) and solvent on the acylation of **4** (0.1 M) in the presence of lipase PS (25 mg ml^{-1}), PS[a] or AK[a] (50 mg ml^{-1}) at room temperature

Solvent	Acyl donor	Enzyme	Time (h)	Conv (%)	ee_s (%)	ee_p (%)	E
Acetone	VA	Lipase AK[a]	2	48	82	92	48
Acetone	VB	Lipase AK[a]	2	47	86	96	136
Acetone	VB	Lipase PS[a]	2	48	89	98	>200
THF	VB	Lipase PS[a]	3	46	81	94	81
THF	VB	Lipase AK[a]	3	46	79	93	66
THF	VB	Lipase PS	4	44	70	90	39
Acetonitrile	VB	Lipase PS[a]	4	39	61	97	122
Et_2O	VB	Lipase PS[a]	0.5	40	63	95	74
iPr_2O	VB	Lipase PS[a]	1	47	75	83	24

[a]Contains 20% (w/w) of lipase adsorbed on Celite in the presence of sucrose.

One of the fundamental criteria of enzymatic kinetic resolution is the irreversibility of acyl transfer. For this reason and on the basis of our previous studies,[17–19] vinyl esters were used as acyl donors. A better E value was observed when the lipase AK-catalyzed acylation of **4** was performed with vinyl butyrate (VB) instead of vinyl acetate (VA) in acetone (Table 1, rows 1 and 2).

Organic solvents can also have marked effects on the reaction rate and the enantioselectivity of enzymatic reactions. High enantioselectivities were observed in acetone, acetonitrile and THF (Table 1). When the lipase PS-catalyzed butyrylations of **4** were performed in diethyl ether or diisopropyl ether, higher reaction rates but lower enantioselectivities were observed (Table 1, rows 8 and 9).

On the basis of the small-scale experiments (Table 1), the gram-scale resolutions of **4–6** were performed in acetone with vinyl butyrate in the presence of lipase PS at room temperature. The results are reported in Table 2 and in the Experimental. Although the stereogenic centre is two atoms removed from the reaction centre, excellent selectivities (E > 200) were observed in every lipase PS-catalyzed gram-scale resolution (Table 2).

2.1. Absolute configurations

The stereochemistry was confirmed in each set of compounds. Thus, treatment of **4a** with 18% HCl resulted in **4c**·HCl with specific rotation $[\alpha]_D^{25} = -0.9$ (c = 0.51, MeOH). This is close to the value of –1 (c = 0.59, MeOH) reported for (1*R*,2*S*)-2-aminocyclohexanecarboxylic acid hydrochloride.[20]

Table 2
Lipase PS[a] (50 mg ml^{-1})-catalyzed resolution of **4–6** (0.1 M) in the presence of vinyl butyrate (0.2 M) in acetone, at room temperature

	Time (h)	Conv (%)	$ee_{4a\text{-}6a}$ (%)	Isomer 4a-6a	Yield[b] (%)	$[\alpha]_D^{25}$	$ee_{4b\text{-}6b}$ (%)	Isomer 4b-6b	Yield[b] (%)	$[\alpha]_D^{25}$	E
4	5	50	98[c]	1*R*,6*S*	80	-15.5[d]	97[c]	1*S*,6*R*	72	-31.7[d]	>200
5	4	49	99[c]	1*R*,6*S*	67	-6.3[d]	97[c]	1*S*,6*R*	50	-46.1[d]	>200
6	6	49	99[c]	1*R*,6*S*	68	-43.3[e]	94[c]	1*S*,6*R*	64	-9.1[f]	>200

[a]Contains 20% (w/w) of lipase adsorbed on Celite in the presence of sucrose. [b]Yield 100% at 50% conversion. [c]According to chiral GC. [d]c = 1, MeOH. [e]c = 0.5, $CHCl_3$. [f]c = 1.88, MeOH.

Compound **4c**·HCl was purified by ion-exchange chromatography and converted to amino acid **4c** with $[\alpha]_D^{25} = -19.6$ (c = 0.25, H_2O).

When **5b** was hydrolyzed with 18% HCl, amino acid **5d** was formed with $[\alpha]_D^{25} = +34$ (c = 0.27, H_2O). This value is close to the +36.4 (c = 0.45, H_2O) reported for (1*S*,2*R*) 2-amino-4-cyclohexenecarboxylic acid.[21]

The hydrolysis of **6a** resulted in amino acid **6c**, which was converted into **4c** by hydrogenation in the presence of Pd/C in methanol.[22] The resulting amino acid **4c** was derivatized with TAGIT reagent and its HPLC peak was found to be identical with that of **4c** prepared by hydrolysis of **4a**.[23] The configuration is therefore proved to be 1*R*,2*S*.

In conclusion, the lipase PS-catalyzed butyrylation of **4–6** displayed 6*S* selectivity.

3. Experimental

3.1. Materials and methods

The racemic alicyclic-condensed azetidinones **1–3** were obtained by cycloaddition of chlorosulfonyl isocyanate to the corresponding cycloalkenes.[24–26] Vinyl acetate was purchased from Aldrich Co. and vinyl butyrate from Fluka. Lipase PS and lipase AK were obtained from Amano Pharmaceuticals, and Novozym 435 as an immobilized preparation from NOVO Nordisk. Before use, lipase PS (5 g) and lipase AK (5 g) were dissolved in Tris–HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (7 g; Sigma). The lipase preparations thus obtained contained 20% (w/w) of lipase. All the solvents were of the highest analytical grade. For gram-scale resolutions, acetone was dried over Na_2SO_4 for some days before use.

The ee values of the unreacted alcohols **4b–6b** and the produced enantiomers of esters **4a–6a** and the enantiomerically pure azetidinones **4e–6e** were determined by gas chromatography on a Chrompack CP-Chirasil-DEX CB column (25 m).

For ion-exchange chromatography, Varion KS resin was used. The ee values of the amino acid enantiomers **4c–6c** and **4d–6d** were determined by HPLC on an APEX ODS column (0.46 cm×25 cm, Jones Chromatography Ltd.), with 0.1% aqueous trifluoroacetic acid:methanol (1:1) as eluent. For chiral derivatization, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (TAGIT) was used, according to the literature.[27]

Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^{1}H NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus.

3.2. Preparation of racemic 7-hydroxymethyl-7-azabicyclo[4.2.0]octan-8-one **4**

7-Azabicyclo[4.2.0]octan-8-one **1** (2 g, 16 mmol) was dissolved in THF (40 ml). Paraformaldehyde (0.58 g, 19.33 mmol), K_2CO_3 (0.22 g, 1.59 mmol) and H_2O (1.6 ml) were added. The solution was sonicated for 5 h. The solvent was evaporated off and the residue was dissolved in diethyl ether (80 ml). The solution was dried over Na_2SO_4 and evaporated down. The residue was recrystallized from diisopropyl ether at –20°C, but the resulting white crystalline product **4** (1.75 g, 11.29 mmol) melted at room temperature. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.46–1.90 (8H, m, 4×CH_2), 3.20–3.24 (1H, m, H-1), 3.94–3.98 (1H, m, H-6), 4.54 (1H, d, CH_2OH, J=11.6), 4.80 (1H, d, CH_2OH, J=11.6). Anal. calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.02; found: C, 62.03; H, 8.31; N, 9.09.

3.3. Preparation of racemic 7-hydroxymethyl-7-azabicyclo[4.2.0]oct-3-en-8-one **5**

With the procedure described above, 7-azabicyclo[4.2.0]oct-3-en-8-one **2** (2 g, 16.26 mmol) afforded white crystals of **5** (1.63 g, 10.65 mmol, mp 59–61°C). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.12–2.18 (2H, m, H-2a and H-5a), 2.42–2.56 (2H, m, H-2b and H-5b), 3.35–3.38 (1H, m, H-1), 4.11–4.13 (1H, m, H-6), 4.50 (1H, d, CH_2OH, J=11.7), 4.74 (1H, d, CH_2OH, J=11.7), 5.69–5.86 (2H, m, C*HCH*). Analysis: calculated for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14; found: C, 62.79; H, 7.13; N, 9.02.

3.4. Preparation of racemic 7-hydroxymethyl-7-azabicyclo[4.2.0]oct-4-en-8-one **6**

With the procedure described above, 7-azabicyclo[4.2.0]oct-2-en-8-one **3** (2 g, 16.26 mmol) afforded white crystals of **6** (1.69 g, 11.04 mmol, mp 56–58°C). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.58–1.61 (1H, m), 2.07–2.12 (3H, m), 3.46–3.53 (1H, m, H-1), 4.14 (1H, t, H-6, J=4.9), 4.43 (1H, d, CH_2OH, J=11.6), 4.66 (1H, d, CH_2OH, J=11.6), 6.01–6.20 (2H, m, C*HCH*). Anal. calcd for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14; found: C, 62.51; H, 7.14; N, 9.21.

3.5. General procedure for a typical small-scale experiment

The appropriate *N*-hydroxymethyl-β-lactams **4**–**6** (0.1 M solution) in an organic solvent (1 ml) were added to lipase PS (50 mg ml^{-1}) or lipase AK (50 mg ml^{-1}) or Novozym 435 (30 mg ml^{-1}). A vinyl ester (0.2 M in the reaction mixture) was added and the mixture was shaken at room temperature. The progress of the reaction was followed by taking samples (0.1 ml) from the reaction mixture at intervals and analyzing them by gas chromatography.

3.6. Gram-scale resolution of **4**

Racemic **4** (1 g, 6.45 mmol) was dissolved in dry acetone (65 ml), lipase PS (3.25 g) and vinyl butyrate (1.47 g, 12.90 mmol) were added and the mixture was stirred at room temperature. After 5 h, a few drops of triethylamine were added in order to enhance the stability of the unreacted

acid-labile **4b**, and the enzyme was filtered off at 50% conversion. The acetone was evaporated off. The residue was chromatographed on silica, with elution with dichloromethane for separation of the ester (1*R*,6*S*)-**4a** (0.58 g, 2.58 mmol; $[\alpha]_D^{25} = -15.5$ (c = 1, MeOH); ee = 98%) as a pale-yellow oil. Elution with dichloromethane:ethyl acetate (1:1) afforded the unreacted (1*S*,6*R*)-**4b** (0.36 g, 2.32 mmol; $[\alpha]_D^{25} = -31.7$ (c = 1, MeOH); ee = 97%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **4a**: 0.95 (3H, t, CH_3, $J = 7.4$), 1.45–1.93 (10H, m, 4×CH_2 and $CH_2CH_2CH_3$), 2.31 (2H, t, $CH_2CH_2CH_3$, $J = 7.4$), 3.24–3.25 (1H, m, H-1), 3.87–3.88 (1H, m, H-6), 5.11 (1H, d, CH_2OCOPr, $J = 11.2$), 5.18 (1H, d, CH_2OCOPr, $J = 11.2$). Anal. calcd for $C_{12}H_{19}NO_3$: C, 63.98; H, 8.50; N, 6.22; found: C, 64.03; H, 8.57; N, 6.28. The ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) data for **4b** are similar to those for (±)-**4**. Anal. calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.02; found: C, 61.80; H, 8.36; N, 9.05.

3.7. Gram-scale resolution of *5*

With the procedure described above, the reaction of racemic **5** (1 g, 6.53 mmol) and vinyl butyrate (1.48 g, 13.06 mmol) in dry acetone (65 ml) in the presence of lipase PS (3.25 g) for 4 h afforded the ester (1*R*,6*S*)-**5a** (0.5 g, 2.24 mmol; $[\alpha]_D^{25} = -6.3$ (c = 1, MeOH); ee = 99%) as a pale-yellow oil, together with the unreacted alcohol (1*S*,6*R*)-**5b** (0.25 g, 1.63 mmol; $[\alpha]_D^{25} = -46.1$ (c = 1, MeOH); mp 72–75°C; ee = 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **5a**: 0.95 (3H, t, CH_3, $J = 7.4$), 1.63–1.68 (2H, m, $CH_2CH_2CH_3$), 2.16–2.20 (2H, m, H-2a and H-5a), 2.31 (2H, t, $CH_2CH_2CH_3$ $J = 7.4$), 2.44–2.58 (2H, m, H-2b and H-5b), 3.38–3.41 (1H, m, H-1), 4.02–4.04 (1H, m, H-6), 5.04 (1H, d, CH_2OCOPr, $J = 11.3$), 5.12 (1H, d, CH_2OCOPr, $J = 11.4$), 5.29–5.85 (2H, m, *CHCH*). Anal. calcd for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.67; N, 6.27; found: C, 64.63; H, 7.61; N, 6.35.

The ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) data for **5b** are similar to those for (±)-**5**. Anal. calcd for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14; found: C, 62.65; H, 7.16; N, 9.21.

3.8. Gram-scale resolution of *6*

With the procedure described above, the reaction of racemic **6** (1 g, 6.53 mmol) and vinyl butyrate (1.48 g, 13.06 mmol) in dry acetone (65 ml) in the presence of lipase PS (3.25 g) for 6 h afforded the ester (1*R*,6*S*)-**6a** (0.49 g, 2.21 mmol; $[\alpha]_D^{25} = -43.3$ (c = 0.5, $CHCl_3$); ee = 99%) as a pale-yellow oil, together with the unreacted alcohol (1*S*,6*R*)-**6b** (0.32 g, 2,09 mmol; $[\alpha]_D^{25} = -9.5$ (c = 1.88, MeOH); ee = 94%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **6a**: 0.95 (3H, t, CH_3, $J = 7.4$), 1.56–2.15 (6H, m, 2×CH_2 and $CH_2CH_2CH_3$), 2.30 (2H, t, $CH_2CH_2CH_3$, $J = 7.5$), 3.48–3.51 (1H, m, H-1), 4.11 (1H, t, H-6, $J = 4.9$), 5.00 (1H, d, CH_2OCOPr, $J = 11.2$), 5.16 (1H, d, CH_2OCOPr, $J = 11.2$), 6.02–6.21 (2H, m, *CHCH*). Anal. calcd for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.67; N, 6.27; found: C, 64.72; H, 7.81; N, 6.33. The ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) data for **6b** are similar to those for (±)-**6**. Anal. calcd for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14; found: C, 62.61; H, 7.32; N, 9.23.

3.9. Acid hydrolysis of *4a* and *4b*

(1*R*,6*S*)-**4a** (0.3 g, 1.33 mmol) was dissolved in 18% HCl (6 ml) and refluxed for 2 h at 70°C. The solvent was evaporated off, the residue was purified by ion-exchange chromatography and the product was recrystallized from ethanol, which afforded white crystals of (1*R*,2*S*)-**4c** (0.07 g,

0.39 mmol; $[\alpha]_D^{25} = -19.6$ (c = 0.25, H_2O); mp 229–230°C; ee = 98%). ^1H NMR (400 MHz, D_2O) δ (ppm) for **4c**: 1.39–1.95 (8H, m, 4×CH_2), 2.62–2.66 (1H, m, H-1), 3.45–3.49 (1H, m, H-2). Anal. calcd for $C_7H_{13}NO_2$: C, 64.55; H, 7.67; N, 6.27; found: C, 64.31; H, 7.51; N, 6.39.

Similarly, (1*S*,6*R*)-**4b** (0.2 g, 1.29 mmol) afforded white crystals of (1*S*,2*R*)-**4d** (0.1 g, 0.56 mmol; $[\alpha]_D^{25} = +20$ (c = 0.25, H_2O); mp 230–231°C; ee = 97%).

The ^1H NMR (400 MHz, D_2O) δ (ppm) data for **4d** are similar to those for **4c**. Anal. found: C, 64.25; H, 7.43; N, 6.15.

3.10. Acid hydrolysis of *5a* and *5b*

Similarly, (1*R*,6*S*)-**5a** (0.2 g, 0.89 mmol) afforded white crystals of (1*R*,2*S*)-**5c** (69 mg, 0.39 mmol; $[\alpha]_D^{25} = -36.2$ (c = 0.5, H_2O); mp 224–225°C; ee = 99%). ^1H NMR (400 MHz, D_2O) δ (ppm) for **5c**: 2.16–2.44 (4H, m, 2×CH_2), 2.67–2.71 (1H, m, H-1), 3.69–3.73 (1H, m, H-2), 5.57–5.76 (2H, m, C*HCH*). Anal. calcd for $C_7H_{11}NO_2$: C, 59.56; H, 7.85; N, 9.92; found: C, 59.28; H, 7.78; N, 9.81.

Similarly, (1*S*,6*R*)-**5b** (0.15 g, 0.98 mmol) afforded white crystals of (1*S*,2*R*)-**5d** (45 mg, 0.32 mmol; $[\alpha]_D^{25} = +34$ (c = 0.27, H_2O); mp 223–226°C; ee = 97%); lit.[21] $[\alpha]_D^{25} = +36.4$ (c = 0.45, H_2O).

The ^1H NMR (400 MHz, D_2O) δ (ppm) data for **5d** are similar to those for **5c**. Anal. found: C, 59.73; H, 7.83; N, 9.75.

3.11. Acid hydrolysis of *6a* and *6b*

Similarly, (1*R*,6*S*)-**6a** (0.2 g, 0.89 mmol) afforded white crystals of (1*R*,2*S*)-**6c** (48 mg, 0.34 mmol; $[\alpha]_D^{25} = +120$ (c = 0.25, H_2O); mp 220–221°C; ee = 99%). ^1H NMR (400 MHz, D_2O) δ (ppm) for **6c**: 1.80–2.19 (4H, m, 2×CH_2), 2.72–2.77 (1H, m, H-1), 3.95–4.03 (1H, m, H-2), 5.71–6.14 (2H, m, C*HCH*). Anal. calcd for $C_7H_{11}NO_2$: C, 59.56; H, 7.85; N, 9.92; found: C, 59.68; H, 7.96; N, 9.81.

Similarly, (1*S*,6*R*)-**6b** (0.25 g, 1.63 mmol) afforded white crystals of (1*S*,2*R*)-**6d** (80 mg, 0.56 mmol; $[\alpha]_D^{25} = -122$ (c = 0.25, H_2O); mp 217–218°C; ee = 99%).

The ^1H NMR (400 MHz, D_2O) δ (ppm) data for **6d** are similar to those for **6c**. Anal. found: C, 59.40; H, 7.63; N, 9.98.

3.12. Preparation of enantiomerically pure β-lactams *4e–6e*

(1*S*,6*R*)-**4b** (0.1 g, 0.64 mmol) was dissolved in methanol (10 ml), NH_4OH (1 ml) was added and the mixture was stirred at room temperature for 13 h. The solution was evaporated, the residue was chromatographed on silica, and elution with dichloromethane:ethyl acetate (1:1) afforded white crystals of (1*S*,6*R*)-**4e** (65 mg, 0.52 mmol; $[\alpha]_D^{25} = -3.8$ (c = 0.27, $CHCl_3$); mp 69–71°C; ee = 98%).

^1H NMR (400 MHz, $CDCl_3$) δ (ppm) for **4e**: 1.46–1.86 (8H, m, 4×CH_2), 3.22–3.23 (1H, m, H-1), 3.83–3.86 (1H, m, H-6), 6 (1H, br). Anal. calcd for $C_7H_{11}NO$: C, 67.17; H, 8.86; N, 11.19; found: C, 67.35; H, 8.62; N, 11.32.

Similarly, (1*S*,6*R*)-**5b** (50 mg, 0.33 mmol) afforded white crystals of (1*S*,6*R*)-**5e** (32 mg, 0.26 mmol; $[\alpha]_D^{25} = -26.3$ (c = 0.50, $CHCl_3$); mp 152–153°C; ee = 98%); lit.[21] $[\alpha]_D^{25} = -28.6$ (c = 0.585, $CHCl_3$). ^1H NMR (400 MHz, D_2O) δ (ppm) for **5e**: 2.12–2.22 (2H, m, H-2a and H-5a), 2.32–2.51 (2H, m, H–2b and H-5b), 3.42–3.45 (1H, m, H-1), 4.06–4.09 (1H, m, H-6), 5.81–5.89 (2H, m, C*HCH*). Anal. calcd for C_7H_9NO: C, 68.27; H, 7.37; N, 11.37; found: C, 68.45; H, 7.18; N, 11.23.

Similarly, (1*S*,6*R*)-**6b** (50 mg, 0.33 mmol) afforded white crystals of (1*S*,6*R*)-**6e** (27 mg, 0.22 mmol; $[\alpha]_D^{25} = +164$ (c = 0.13, $CHCl_3$); mp 106–108°C; ee = 94%). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) for **6e**: 1.59–2.12 (4H, m, 2×CH_2), 3.48–3.56 (1H, m, H-1), 4.03 (1H, t, H-6, $J = 4.78$), 5.82 (1H, br), 5.94–6.17 (2H, m, C*H*C*H*). Anal. calcd for C_7H_9NO: C, 68.27; H, 7.37; N, 11.37; found: C, 68.03; H, 7.25; N, 11.48.

Acknowledgements

The authors' thanks are due to OTKA (grant No. T 030452) and MKM (grant No. FKFP 0535/1999) for financial support.

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