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Chemoenzymatic preparation of fluorine-substituted β-lactam enantiomers exploiting *Burkholderia cepacia* lipase

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Abstract—Both enantiomers of fluorinated and non-fluorinated 4-phenyl-2-azetidinones are prepared in high enantiopurities (ee 99%) by a chemoenzymatic method, using a double resolution technique to N-hydroxymethylated β-lactams in the presence of *Burkholderia cepacia* lipase as the source of enantiopurity. N-Deprotections yield the β-lactam enantiomers from the corresponding hydroxymethylated counterparts using $KMnO_4$ in a mixture of acetone and water.
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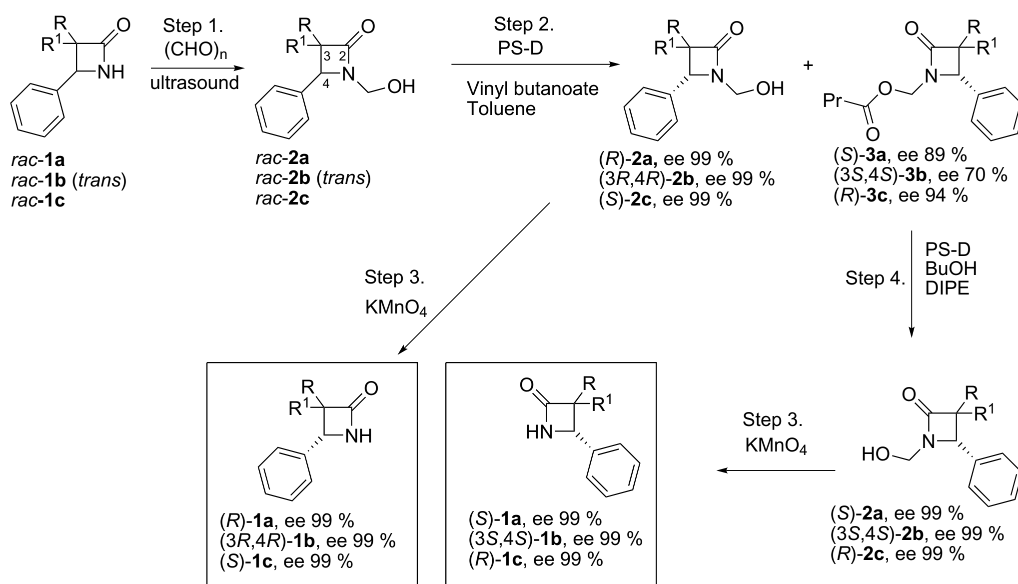
1. Introduction

β-Lactams (2-azetidinones) are important chiral intermediates and building blocks for various types of pharmaceuticals, the preparation of which are thoroughly described in a number of reviews.[1–7] The substitution of one or more of the hydrogen atoms with isosteric fluorine in the β-lactam skeleton can further enhance their importance for medicinal chemistry, often leading to derivatives with desirable effects on physiological activity and metabolism. The (*S*)-enantiomer of 3,3-difluoro-4-phenyl-2-azetidinone (*S*)-**1a** (Scheme 1) is a good example of an activated acyl



Scheme 1. Chemoenzymatic preparation of enantiopure lactams: (a) $R = R^1 = F$, (b) $R = F$, $R^1 = H$ and (c) $R = R^1 = H$.

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donor which, as was shown previously for the reaction of *N*-Boc activated β-lactams with alcohols,[5,6,8] likely gives fluorotaxol analogues when coupled with the free alcohol group of the taxane ring.

Lipases are very versatile biocatalysts for the production of enantiopure compounds, which in the best cases can afford both enantiomers at the same time through kinetic resolution. Enantioselective hydrolysis and alcoholysis of the β-lactam ring with lipase catalysis has proven to be an applicable kinetic resolution method, producing the less reactive enantiomer as a β-lactam.[9–12] We had previously shown that the kinetic resolution of fluorine-activated β-lactams (4-phenyl-2-azetidinones *rac*-**1a** and *rac*-**1b**, Scheme 1) as acyl donors with alcohols and *Burkholderia cepacia* lipase (commercial lipase PS-D or lipase PS as adsorbed on Celite in the presence of sucrose[13]) affords the corresponding enantiopure (*S*)-β-amino esters, leaving the (*R*)-β-lactams (*R*)-**1a** and (3*R*,4*R*)-**1b** unreacted.[12] Another widely used lipase-catalyzed kinetic resolution method exploits N-hydroxymethylated β-lactams as racemic alcohols for O-acylation, including the preparation of the enantiomers of *rac*-**1c**.[9,10,14,15] The benefit of the latter method is that the kinetic resolution leaves the β-lactam ring of both enantiomers untouched.

Herein we report the chemoenzymatic preparation of both enantiomers of β-lactams **1a** and **1b** (Scheme 1). The key racemates for kinetic resolution are N-hydroxymethylated β-lactams, *rac*-**2a** and *rac*-**2b**. Special emphasis is needed for the chemical N-deprotection of the prepared **2a** and **2b** enantiomers through step 3 in such a way that the ring opening of the fluorine activated rings can be prevented. The non-fluorinated **1c** is included in the present work in order to obtain comparable data based on the use of lipase PS-D catalysis. In addition, the double kinetic resolution technique now exploited has not been used before for the preparation of (*R*)- and (*S*)-**2c**. The asymmetric acylation of *rac*-**2c**, which gives the unreacted (*S*)-**2c**, and the asymmetric alcoholysis of the corresponding racemic ester which gives the produced (*R*)-**2c**, have earlier produced the enantiomers.[14]

2. Results and discussion

A simple chemoenzymatic protocol to provide the enantiomers of *rac*-**1a–c** in enantiopure forms (ee 99%) is shown in Scheme 1. Fluorine-substituted *rac*-**1a** and **-1b** used as starting materials were available from our previous work whereby a Reformatsky type reaction allowed the formation of the β-lactam structure.[12] Non-fluorinated *rac*-**1c** was prepared as reported using the 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (CSI) to styrene.[14] N-Hydroxymethylation of the β-lactams with paraformaldehyde under ultrasound easily afforded racemic **2a–c** as substrates for lipase-catalyzed O-acylation (Scheme 1).[14,15]

There are two key steps in the chemoenzymatic synthesis of the β-lactam enantiomers **1a–c**: lipase-catalyzed kinetic resolution of *rac*-**2a–c** through step 2 and *N*-deprotection of enantiomers **2a–c** through step 3 (Scheme 1). When enzymatic enantioselectivity does not provide excellent results, a double resolution technique with the minimal number of reaction steps proved to be the best choice for the preparation of both enantiomers of a racemic alcohol. This can be conveniently carried out by performing the first enzymatic acylation followed by enzymatic alcoholysis of the ester produced.[15] The acylation conditions were previously optimized for the kinetic resolution of *rac*-**2c**, except that our catalyst now is commercial lipase PS-D. Accordingly, *rac*-**2a–c** was subjected to acylation with vinyl butanoate and lipase PS-D in dry toluene at room temperature. Vinyl butanoate was used in 4 equiv rather than in more usual 2 equiv excess in order to minimize the risk of the enzymatic hydrolysis of the formed **3a–c** back to the corresponding **2a–c** by the water in the seemingly dry enzyme preparation and to obtain the unreacted substrate smoothly in an enantiopure form.[15] Two enzyme contents (15 and 20 mg mL^{-1}) were tested. Although the enzyme contents had an effect on the reactivity, they both conveniently gave enantioselective reactions, leading to unreacted enantiomers in enantiopure forms at reasonable times (Table 1). The same high enantioselectivity (enantiomeric ratio, $E > 200$) is clear for the acylation of *rac*-**2c** with lipase PS-D (rows 4 and 5) and with the lipase PS preparation (row 6). The presence of fluorine in *rac*-**2a** and *rac*-**2b** lowers the enantioselectivity for the acylation. For effective sequential gram-scale resolution, the acylations with 15 mg mL^{-1} for *rac*-**2b** and *rac*-**2c** and 20 mg mL^{-1} for *rac*-**2a** were stopped when the unreacted enantiomers were enantiopure at 58% and 51% and 53% conversions (rows 1, 2 and 4), respectively. Separation of the resolution products on a silica gel column yielded the unreacted (*R*)-**2a**, (3*R*,4*R*)-**2b** and (*S*)-**2c** with the desired ee = 99%.

In addition to enantiopure **2a–c**, enantiomerically enriched esters **3a–c** were produced by lipase PS-D-catalyzed acylation (Table 1). In the next step, the isolated **3a–c** were transformed into the corresponding enantiopure alcohols under mild and enantiomeric excess increasing conditions,

Table 1. Kinetic resolution of *rac*-**2a–c** with vinyl butanoate (4 equiv) in toluene at room temperature

Row	Substrate	Lipase (mg mL^{-1})	Time (h)	Conversion (%)	ee**2** (%) (abs. config.)	ee**3** (%) (abs. config.)	*E*
1	**2a**	20	1	53	99 (*R*)	89/(*S*)	90
2	**2b**	15	1/2.3	30/58	37/99 (3*R*,4*R*)	86/70 (3*S*,4*S*)	19
3	**2b**	20	1/1.5	41/50	56/76 (3*R*,4*R*)	82/76 (3*S*,4*S*)	18
4	**2c**	15	1/1.5	49/51	95/99 (*S*)	99/94 (*R*)	>200
5	**2c**	20	1/1.5	50/51	97/99 (*S*)	98/95 (*R*)	>200
6	**2c**[a]	30	1.5	49	94/(*S*)	97/(*R*)	>200

[a] Vinyl butanoate (2 equiv), lipase PS on Celite in the presence of sucrose;[13] see Ref. 14.

Table 2. Alcoholysis of enantiomerically enriched **3a–c** with butanol (2 equiv) in DIPE in the presence of lipase PS-D (30 mg mL^{-1}) at room temperature

Row	Substrate [ee (%)]	Time (h)	Conversion (%)	ee^2 (%) (abs. config.)
1	(*S*)-**3a** [89]	1.7	92	99 (*S*)
2	(3*S*,4*S*)-**3b** [70]	4.2	83	99 (3*S*,4*S*)
3	(*R*)-**3c** [94]	1.0	94	99 (*R*)

by subjecting the esters to alcoholysis with 1-butanol in diisopropyl ether (DIPE) in the presence of lipase PS-D (Scheme 1, step 4; Table 2). The reaction conditions corresponded to those previously used when the enantiomerically enriched 4-benzyl-substituted analogue of **3c** was transformed into the corresponding enantiopure alcohol by lipase PS catalysis.[15] A low butanol concentration (2 equiv) allowed high reactivity. The reactions were carried out to the stage (82–94% conversion depending on the initial ee of the ester) where the **2** produced was still enantiopure. As a total outcome of the double resolution, 45% of *rac*-**2a** was transformed into (*R*)-**2a** and 42% into (*S*)-**2a**; 41% of *rac*-**2b** was transformed into both (3*R*,4*R*)-**2b** and (3*S*,4*S*)-**2b**; and finally, 49% of *rac*-**2c** was transformed into (*R*)-**2c** and 47% into (*S*)-**2c**. These values closely correspond to the theoretical 50% proportions of the enantiomers in the racemates.

The transformation of N-hydroxymethylated β-lactams **2** into the corresponding β-lactams **1** is another key step of the present work. This is usually carried out using NH_4OH/MeOH treatment.[10,14,15] When the transformation of the fluorine activated **2a** with NH_4OH/MeOH did not give product **1a**, it was clear that another method had to be found. Oxidative cleavage with $KMnO_4$ was previously reported to lead to the formation of free β-lactams from the *N*-formyl protected compounds.[16] On the other hand, oxidation of primary alcohols with $KMnO_4$ is known to give aldehydes.[17] Inspired by these two precedents, we expected that oxidative N-deprotection with $KMnO_4$ would be a suitable procedure in the present work as shown in Scheme 2. In practice, enantiomers **2a–c** were successfully transformed into the corresponding **1a–c** using $KMnO_4$ (2 equiv) as an oxidant in the mixture of acetone and water without a sign of racemization. However, the transformation became progressively slower when decreasing the number of fluorines in the ring. Thus, the formation of (*R*)- and (*S*)-**1c** was only about 20% in seven days, although for the last two days, the reaction proceeded at room temperature rather than at normal 2 °C. On the other hand, (*R*)- and (*S*)-**1c** were obtained at 86–89% isolated yield in 20 h under the NH_4OH/MeOH treatment.

3. Conclusion

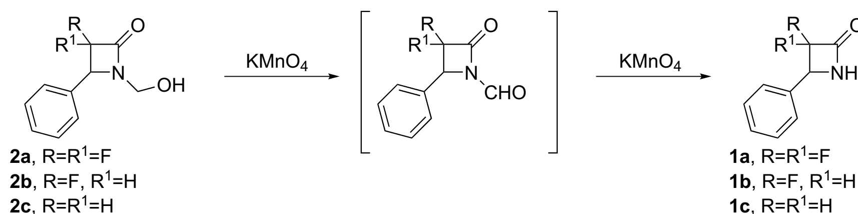
In conclusion, the chemoenzymatic route shown in Scheme 1 describes a highly effective method to prepare both enantiomers of the β-lactams **1a–c**. Although the enantioselectivities in the case of kinetic resolution through acylation only range from moderate to good, a double resolution technique allows the transformation of racemates to enantiopure (ee 99%) counterparts with close to theoretical yields.

4. Experimental

4.1. Materials and methods

All solvents were of the highest analytical grade and were dried by standard methods. Compounds *rac*-**1a** and *rac*-**1b** (with the *trans*-configuration) were available from our previous work.[12] Compound *rac*-**1c** and *rac*-**2c** were prepared as previously reported with identical spectroscopic properties to those in the literature.[14] Lipase PS-D (immobilized on Celite) from *B. cepacia* (previously lipase PS from *Pseudomonas cepacia*) was purchased from Amano Europe, England. Preparative chromatographic separations were performed by column chromatography on Merck Kieselgel 60 (0.063–0.200 μm). TLC was carried out with Merck Kieselgel $60F_{254}$ sheets. All enzymatic reactions were performed at room temperature (23 °C). Melting points were measured on a Sanyo instrument.

The progress of the reactions (4-fluorophenyl acetonitrile as an internal standard) was followed by taking samples from the reaction mixture. Theoretical yields for each of the resolution products are based on the conversion where the reaction was stopped. The products were analyzed in the case of **1a**, **1b**, **2a**, **2b** and **3a** by HPLC on a CHIRACEL-OD column (0.46 × 25 cm) and in the case of **1c**, **2c**, **3b** and **3c** by GC on a Chrompack CP-Chirasil-DEX CB column. The ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 spectrometer with tetramethylsilane (TMS) as an internal standard. ^1H–^1H COSY, ^1H–^{13}C HQSC and ^1H–^{13}C HMBC spectra were used for the assignment



Scheme 2. N-Deprotection of **2** with $KMnO_4$.

of the chemical shifts when necessary. Mass spectra were taken on a VG 7070E mass spectrometer. Optical rotations were determined with a Perkin–Elmer polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

4.2. Preparation of racemic *rac*-2a and *rac*-2b

Compound *rac*-**1a** (240 mg, 1.31 mmol) was dissolved in tetrahydrofuran (3 mL) and paraformaldehyde (44 mg, 1.48 mmol), K_2CO_3 (18 mg, 0.13 mmol) and H_2O (124 mg, 6.89 mmol) were added. The suspension was sonicated for 2 h. The solvent was evaporated off and the residue dissolved in diethyl ether (6 mL), dried over anhydrous Na_2SO_4 and filtered. After evaporation, the residue was purified on a silica gel column eluting with ethyl acetate/petroleum ether (4:1, v/v) to afford *rac*-**2a** as a solid (258 mg, 1.21 mmol, yield 92%, mp 72–73 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.58 (br s, 1H, O*H*), 4.43–4.45 (d, $J = 11.5$ Hz, 1H), 5.18–5.21 (m, 2H), 7.32–7.48 (m, 5H, arom.); ^{13}C NMR (126 MHz, $CDCl_3$) δ 63.60, 67.48–67.89 (t, $J = 25.2$ Hz), 118.19–122.83 (t, $J = 293.0$ Hz), 127.13, 127.99, 129.11, 129.96, 161.24–161.73 (t, $J = 31.4$ Hz). HRMS: M$^+$ found (M$^+$ calcd for $C_{10}H_9F_2NO_2$) 213.060200 (213.060135); MS: *m*/*z* (relative intensity) 213 (0.15), 183 (2), 140 (100), 104 (6).

Using the above procedure, *rac*-**2b** was obtained as a semi-solid product in 90% isolated yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.12 (br s, 1H, O*H*), 4.23–4.25 (d, $J = 11.5$ Hz, 1H), 4.91–4.94 (dd, $J = 11.0$ Hz, 1.0 Hz, 1H), 5.12–5.14 (d, $J = 11.5$ Hz, 1H), 5.17–5.29 (dd, $J = 54.0$ Hz, 1.5 Hz, 1H), 7.30–7.48 (m, 5H, arom.); ^{13}C NMR (126 MHz, $CDCl_3$) δ 61.75–61.94 (d, $J = 23.9$ Hz), 63.71–63.73 (d, $J = 2.5$ Hz), 96.73–98.53 (d, $J = 226.4$ Hz), 126.76, 129.27, 129.35, 134.31, 164.44, 164.62 (d, $J = 22.6$ Hz). HRMS: M$^+$ found (M$^+$ calcd for $C_{10}H_{10}FNO_2$) 195.069700 (195.069557); MS: *m*/*z* (relative intensity) 195 (0.03), 178 (3), 135 (3), 122 (100), 91 (4), 77 (5).

4.3. Preparation of (*R*)- and (*S*)-2a by lipase-catalyzed acylation/deacylation

Compound *rac*-**2a** (1.0 g, 4.70 mmol) was dissolved in toluene (94 mL), and lipase PS-D (20 mg mL^{-1}) and vinyl butanoate (2.1 g, 18.80 mmol) were added. The reaction was stopped after 1 h by filtering off the enzyme at 53% of the conversion. The solvent was evaporated off and the residue purified on a silica gel column with petroleum ether/ethyl acetate (12:1) to afford (*S*)-**3a** {676 mg, 2.39 mmol, isolated yield, 96%, mp 54–55 °C, ee$^{(S)\text{-}\mathbf{3a}}$ = 89%, $[\alpha]_D^{22} = +49.1$ (*c* 0.65, $CHCl_3$)}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90–0.93 (t, $J = 7.5$ Hz, 3H), 1.56–1.63 (m, 2H), 2.20–2.31 (m, 2H), 5.00–5.02 (d, $J = 11.5$ Hz, 1H), 5.05–5.07 (dd, $J = 8.0$, 2.5 Hz, 1H), 5.43–5.46 (d, $J = 11.5$ Hz, 1H), 7.31–7.33 (m, 2H), 7.44–7.46 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 13.53, 18.06, 35.53, 62.44 (t, $J = 2.5$ Hz), 69.74 (t, $J = 23.9$ Hz), 121.04, 128.09, 129.08, 130.02, 130.11, 161.08, 173.05. HRMS: M$^+$ found (M$^+$ calcd for $C_{14}H_{15}F_2NO_3$) 283.102700 (283.102000); MS: *m*/*z* (relative intensity) 283 (0.01), 206 (0.05), 153 (5), 140 (100), 91 (4). (*R*)-**2a** (447 mg, 2.10 mmol, isolated yield 95%, ee = 99%) was obtained by changing the eluent to petroleum ether/ethyl acetate (4:1).

Compound (*S*)-**3a** (676 mg, 2.39 mmol, ee = 89%) was dissolved in DIPE (48 mL), and BuOH (356 mg, 4.80 mmol) and PS-D (30 mg mL^{-1}) were added. The reaction was stopped after 1.7 h by filtering off the enzyme at 92% conversion. The solvent was evaporated off and the residue separated on a silica gel column as above to afford (*S*)-**2a** (421 mg, 1.98 mmol, isolated yield 90%, ee$^{(S)\text{-}\mathbf{2a}}$ = 99%) and (*R*)-**3a** (50 mg, 0.18 mmol, ee$^{(R)\text{-}\mathbf{3a}}$ = 80%).

4.4. Preparation of (3*R*,4*R*)- and (3*S*,4*S*)-2b by lipase-catalyzed acylation/deacylation

Compound *rac*-**2b** (490 mg, 2.51 mmol) was dissolved in toluene (50 mL), and lipase PS-D (15 mg mL^{-1}) and vinyl butanoate (1.1 g, 10.00 mmol) were added. The reaction was stopped after 2.3 h by filtering off the enzyme at 58% conversion. The solvent was evaporated off and the residue purified on a silica gel column with petroleum ether/ethyl acetate (8:1) to afford (3*S*,4*S*)-**3b** (373 mg, 1.41 mmol, isolated yield 96%, ee$^{(3S,4S)\text{-}\mathbf{3b}}$ = 70%, $[\alpha]_D^{22} = -6.0$ (*c* 0.80, $CHCl_3$)) as an oily product. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90–0.93 (t, $J = 7.5$ Hz, 3H), 1.56–1.62 (m, 2H), 2.22–2.30 (m, 2H), 4.79–4.81 (dd, $J = 11.5$ Hz, 2.0 Hz, 1H), 4.87–4.89 (d, $J = 11.0$ Hz, 1H), 5.22–5.33 (dd, $J = 54.0$ Hz, 2.0 Hz, 1H), 5.35–5.37 (d, $J = 11.0$ Hz, 1H), 7.28–7.33 (m, 2H), 7.39–7.46 (m, 3H). HRMS: M$^+$ found (M$^+$ calcd for $C_{14}H_{16}FNO_3$) 265.113700 (265.111422); MS: *m*/*z* (relative intensity) 265 (0.05), 178 (6), 135 (23), 122 (100), 91 (10), 77 (3). (3*R*,4*R*)-**2b** (202 mg, 1.03 mmol, isolated yield 98%, ee$^{(3R,4R)\text{-}\mathbf{2b}}$ = 99%) was obtained by changing the eluent to petroleum ether/ethyl acetate (1:1).

Compound (3*S*,4*S*)-**3b** (360 mg, 1.36 mmol, ee = 70%) was dissolved in DIPE (27 mL), and BuOH (200 mg, 2.70 mmol) and PS-D (30 mg mL^{-1}) were added. The reaction was stopped after 4.2 h by filtering off the enzyme at 83% conversion. The solvent was evaporated off and the residue was separated on a silica gel column as above to afford (3*S*,4*S*)-**2b** (199 mg, 1.02 mmol, isolated yield 90%, ee$^{(3S,4S)\text{-}\mathbf{2b}}$ = 99%) and unreacted (3*R*,4*R*)-**3b** (60 mg, 0.23 mmol, ee$^{(3R,4R)\text{-}\mathbf{3b}}$ = 71%).

4.5. Preparation of (*R*)- and (*S*)-2c by lipase-catalyzed acylation/deacylation

Compound *rac*-**2c** (1.2 g, 6.72 mmol) was dissolved in toluene (134 mL), and lipase PS-D (15 mg mL^{-1}) and vinyl butanoate (3.1 g, 26.89 mmol) were added. The reaction was stopped after 1.5 h by filtering off the enzyme at 51% of the conversion. The solvent was evaporated off and the residue purified on a silica gel column with petroleum ether/ethyl acetate (6:1) to afford an oily product (*R*)-**3c** {820 mg, 3.32 mmol, isolated yield 97%, ee$^{(R)\text{-}\mathbf{3c}}$ = 94%, $[\alpha]_D^{22} = +61.3$ (*c* 1.00, EtOH)}. (*S*)-**2c** (554 mg, 3.13 mmol, isolated yield 95%, ee$^{(S)\text{-}\mathbf{2c}}$ = 99%, mp 85–87 °C) was obtained by changing the eluent to petroleum ether/ethyl acetate (1:3). The spectroscopic data are as reported.[14]

Compound (*R*)-**3c** (789 mg, 3.19 mmol, ee = 94%) was dissolved in DIPE (64 mL), and BuOH (474 mg, 6.40 mmol) and PS-D (30 mg mL^{-1}) were added. The reaction was stopped after 1 h by filtering off the enzyme at 94% conversion. The solvent was evaporated off and the residue was separated on a silica gel column as above to afford (*R*)-**2c** (509 mg, 2.88 mmol, isolated yield 96%, $ee^{(R)\text{-}\mathbf{2c}} = 99\%$) and *rac*-**3c** (33 mg, 0.13 mmol).

4.6. Preparation of (*R*)- and (*S*)-1a, (3*R*,4*R*)- and (3*S*,4*S*)-1b and (*R*)-1c and (*S*)-1c

Compound (*S*)-**2a** (85 mg, 0.40 mmol, ee = 99%) was added to a solution of $KMnO_4$ in acetone (32 mL) and H_2O (32 mL) at room temperature. Immediately after the addition, the reaction mixture was put into a fridge at 2 °C. After 87 h, the starting material was totally consumed according to TLC. 2-Propanol (4 mL) was added to quench the reaction and the resulting suspension was filtered. The filtrate was concentrated to half of its original volume and extracted with CH_2Cl_2 (2 × 32 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. Silica gel column purification resulted in the product (*S*)-**1a** (57 mg, 0.31 mmol, isolated yield 77%, mp 65–66 °C, $ee^{(S)\text{-}\mathbf{1a}} = 99\%$).

Using a similar procedure the enantiomers of other N-hydroxymethylated β-lactams **2** were transformed in 87 h into (*R*)-**1a** (isolated yield 75%, mp 66–69 °C, $ee^{(R)\text{-}\mathbf{1a}} = 99\%$), in 125 h into (3*R*,4*R*)-**1b** (isolated yield 84%, $ee^{(3S,4S)\text{-}\mathbf{1b}} = 99\%$), in 120 h into (3*S*,4*S*)-**1b** (isolated yield 80%, $ee^{(3S,4S)\text{-}\mathbf{1b}} = 99\%$) and in 120 h (2 °C) followed by an additional 48 h (room temperature) into both (*R*)-**1 c** (isolated yield 20%, mp 106–107 °C, $ee^{(R)\text{-}\mathbf{1c}} = 99\%$) and (*S*)-**1c** (isolated yield 23%, mp 108–109 °C, $ee^{(S)\text{-}\mathbf{1c}} = 99\%$) with the specific rotations shown in Table 3.

Compounds (*R*)- and (*S*)-**1c** were also prepared by adding NH_4OH (1 mL, 25% in water) to a solution of the corresponding **2c** enantiomer (100 mg, 0.56 mmol, ee = 99%) in methanol (10 mL). The reaction proceeded for 20 h at room temperature before the solvents were evaporated. The residue was purified on silica gel column using ethyl acetate in petroleum ether [33% (v/v)], affording (*R*)-**1c** (71 mg, 0.48 mmol, isolated yield 86%, $ee^{(R)\text{-}\mathbf{1c}} = 99\%$) and (*S*)-**1c** (89% isolated yield, $ee^{(S)\text{-}\mathbf{1c}} = 99\%$), respectively.

Table 3. Specific rotations for the prepared enantiomer pairs

Compound	ee (%)	$[\alpha]_D^{22}$ (10^{-1} deg cm^2 g^{-1})
(*R*)-**1a**	99	−76.1 (*c* 1.00, $CHCl_3$)
(*S*)-**1a**	99	+76.3 (*c* 1.00, $CHCl_3$)
(3*R*,4*R*)-**1b**	99	−19.3 (*c* 1.00, $CHCl_3$)
(3*S*,4*S*)-**1b**	99	+19.3 (*c* 1.00, $CHCl_3$)
(*R*)-**1c**[a]	99	+141.0 (*c* 0.50, EtOH)
(*S*)-**1c**[a]	99	−140.5 (*c* 0.50, EtOH)
(*R*)-**2a**	99	−139.4 (*c* 1.05, $CHCl_3$)
(*S*)-**2a**	99	+139.2 (*c* 1.00, $CHCl_3$)
(3*R*,4*R*)-**2b**	99	−64.2 (*c* 1.10, $CHCl_3$)
(3*S*,4*S*)-**2b**	99	+64.3 (*c* 1.10, $CHCl_3$)
(*R*)-**2c**[b]	99	+171.0 (*c* 1.00, EtOH)
(*S*)-**2c**[b]	99	−171.2 (*c* 1.00, EtOH)

[a] $[\alpha]_D^{25} = +132.4$ (*c* 0.5, EtOH) for (*R*)-**1c** (ee = 97%) and $[\alpha]_D^{25} = -136.3$ (*c* 0.5, EtOH) for (*S*)-**1c** (ee = 99%); Ref. 14.

[b] $[\alpha]_D^{25} = +161$ (*c* 0.35, EtOH) for (*R*)-**2c** (ee = 96%) and $[\alpha]_D^{25} = -166.7$ (*c* 1, EtOH) for (*S*)-**2c** (ee = 97%); Ref. 14.

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References

1. Yoakim, C.; Ogilvie, W. W.; Cameron, D. R.; Chabot, C.; Guse, I.; Haché, B.; Naud, J.; O'Meara, J. A.; Plante, R.; Déziel, R. *J. Med. Chem.* **1998**, *41*, 2882–2891.
2. Singh, G. S. *Mini-Rev. Med. Chem.* **2004**, *4*, 69–92.
3. Singh, G. S. *Mini-Rev. Med. Chem.* **2004**, *4*, 93–109.
4. Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Oiarbide, M. *Eur. J. Org. Chem.* **1999**, 3223–3235.
5. Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Oiarbide, M. *Synlett* **2001**, 1813–1826.
6. Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Oiarbide, M. In *Enantioselective Synthesis of β-Amino Acids*, 2nd ed.; Juaristi, E., Soloshonok, V., Eds.; Wiley-VHC: New York, 2005; pp 477–495.
7. Benaglia, M.; Cinquini, M.; Cozzi, F. *Eur. J. Org. Chem.* **2000**, 563–572.
8. Uoto, K.; Ohsuki, S.; Takenoshita, H.; Ishiyama, T.; Iimura, S.; Hirota, Y.; Mitsui, I.; Terasawa, H.; Soga, T. *Chem. Pharm. Bull.* **1997**, *45*, 1793–1804.
9. Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831–5854.
10. Forró, E.; Fülöp, F. *Mini-Rev. Org. Chem.* **2004**, *1*, 93–102.
11. Forró, E.; Paál, T.; Tasnádi, G.; Fülöp, F. *Adv. Synth. Catal.* **2006**, *348*, 917–923.
12. Li, X.-G.; Lähitie, M.; Päiviö, M.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2007**, *18*, 1567–1573.
13. Sundholm, O.; Kanerva, L. T. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2407–2410.
14. Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2001**, *12*, 2351–2358.
15. Li, X.-G.; Kanerva, L. T. *Adv. Synth. Catal.* **2006**, *348*, 197–205.
16. Georg, G. I.; Kant, J.; He, P.; Ly, A. M.; Lampe, L. *Tetrahedron Lett.* **1988**, *29*, 2409–2412.
17. Lou, J.-D.; Zhu, L.-Y.; Wang, L.-Z. *Monatsh. Chem.* **2004**, *135*, 31–34.