Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 17 (2006) 3193–3196

Do lipases also catalyse the ring cleavage of inactivated cyclic *trans*-β-lactams?

Enikő Forró and Ferenc Fülöp*

Institute of Pharmaceutical Chemistry, University of Szeged, H-6701 Szeged, PO Box 427, Hungary

Received 8 November 2006; accepted 20 November 2006
Available online 12 December 2006

Abstract—Twelve-membered cyclic *cis*- and *trans*-β-lactams **1b** and **2b** and the corresponding cyclic *cis*- and *trans*-β-amino acid enantiomers, **1a**, **1c** and **2a**, **2c** were prepared through the CAL-B-catalysed enantioselective ring cleavage of racemic *cis*-13-azabicyclo-[10.2.0]tetradecan-14-one, (±)-**1**, and *trans*-13-azabicyclo[10.2.0]tetradecan-14-one, (±)-**2**. High enantioselectivities (E >200) were observed for the ring opening of both the *cis*- and *trans*-β-lactams when the Lipolase-catalysed reactions were performed with 0.5 equiv of H_2O in *i*-Pr_2O at 70 °C. The resolved β-lactams **1b** and **2b** (yield ⩾47%) and β-amino acids **1a** and **2a** (yield ⩾32%) could be easily separated.

1. Introduction

Small to medium-sized cyclic β-amino acids have been intensively investigated, as a consequence of their potential biological activity (e.g., cispentacin)[1] and their utility in synthetic chemistry.[2] They are particularly attractive building blocks for the synthesis of modified peptides with well-defined three-dimensional structures (e.g., β-peptides with possible antibiotic activity) similar to those of natural peptides.[3] Cyclic β-amino acids are likewise used in heterocyclic[4] and combinatorial[5] chemistry. Thus, the relatively large number of synthetic approaches for both *cis*- and *trans*-β-amino acids are not surprising. While a range of stereoselective syntheses for enantiopure small (three- or four-membered)[6] and medium (five- to eight-membered)[7] -sized cyclic β-amino acids or their derivatives are reported, including indirect[8] and direct[9] enzymatic methods, only one example has been reported for the synthesis of enantiopure 12-membered β-amino acid derivatives.[8d] Those authors describe the enzymatic kinetic resolutions of model compounds (±)-**1** and (±)-**2** through an indirect enzymatic method: *N*-hydroxymethyl-(±)-**1** was acylated with 2,2,2-trifluoroethyl butanoate in the presence of lipase PS (lipase from *Pseudomonas cepacia*) in acetone ($E \sim 98$), while *N*-hydroxymethyl-(±)-**2** was acylated with vinyl butanoate in the presence of CAL-B (lipase B from *Candida antarctica*) ($E \sim 57$). We have developed a very simple and efficient new enzymatic hydrolysis method for the enantioselective (E >200) ring opening of medium-sized inactivated cyclic *cis*-β-lactams (the synthesis of cispentacin, for instance).[9a] An advantage of this method is that the lactam ring does not necessarily need to be activated and the product β-amino acid and β-lactam can be obtained in good chemical yields (⩾36%). These results on the lipase-catalysed enantioselective hydrolysis of cyclic five- to eight-membered *cis*-β-lactams suggested the possibility of the enantioselective ring cleavage of racemic *cis*-13-azabicyclo[10.2.0]tetradecan-14-one and *trans*-13-azabicyclo[10.2.0]tetradecan-14-one. Herein, we report a lipase-catalysed direct method for the enantioselective ring cleavage of the larger ring-fused cyclic *cis*-β-lactam (±)-**1**, and also give, an enzymatic method for the enantioselective ring cleavage of cyclic *trans*-β-lactam (±)-**2**.

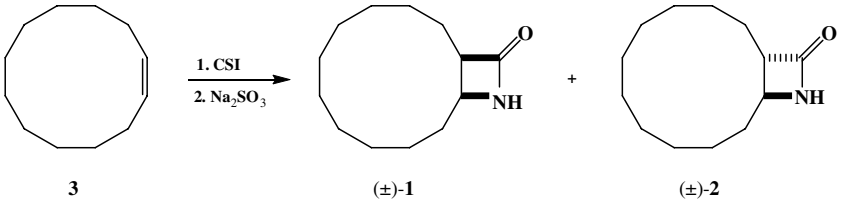
2. Results and discussion

Racemic β-lactams **1** and **2** were prepared from cyclododecene **3** (a mixture of *cis* and *trans*) by 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (CSI) (Scheme 1), as described earlier.[8d] The products **1** and **2** (∼1:1), obtained as a mixture, were separated by column chromatography.

On the basis of our earlier results on the lipase-catalysed ring cleavage of cyclic *cis*-β-lactams,[9] preliminary experiments[10] on (±)-**1** and (±)-**2** were started with water

* Corresponding author. Tel.: +36 62 545564; fax: +36 62 545705; e-mail: fulop@pharm.u-szeged.hu

0957-4166/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.tetasy.2006.11.030



Scheme 1.

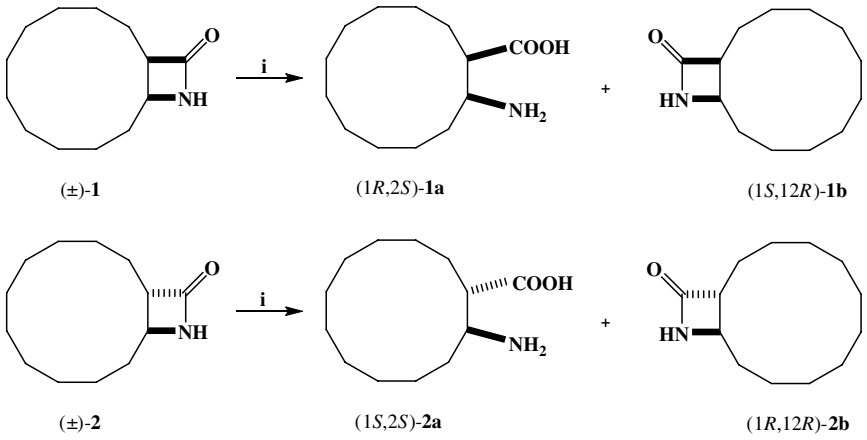
(0 or 0.5 or 1 equiv.), added in the presence of Lipolase (50 mg mL^{-1}) in *i*-Pr_2O, initially at 60 °C (Scheme 2).

Since no significant changes in enantioselectivity (E >200) or reaction rate [conversion 45–46% after 8 h for (±)-**1** and conversion 45–50% after 137 h for (±)-**2**] were observed, we concluded that the water present in the reaction medium (<0.1%) or in the enzyme preparation (<5%) was responsible for the lactam ring opening. Although the E-values were high, the long reaction times, in particular for (±)-**2** (49% conversion after 137 h with 0.5 equiv of water), suggested to us that we should increase the reaction temperature. When the reactions were performed at 70 °C, higher reaction rates were observed [50% conversion after 16 h for (±)-**1** and 50% conversion after 91 h for (±)-**2**], without any loss in enantioselectivity.

The preparative-scale resolutions of (±)-**1** and (±)-**2** were performed with 0.5 equiv of water in the presence of Lipolase in *i*-Pr_2O at 70 °C. The products were characterised by an excellent enantiomeric excess at 50% conversion. The results are shown in Table 1.

The transformations involving the ring opening of β-lactams **1b** and **2b** with 18% aqueous HCl[13] resulted in the enantiomers of β-amino acid hydrochlorides **1c** and **2c** (Scheme 3). Treatment of amino acids **1a** and **2a** with 22% HCl/EtOH[14] resulted in enantiopure hydrochlorides **1a**·HCl and **2a**·HCl. The physical data of the enantiomers prepared, are reported in Table 2.

In order to determine the stereoselectivity of the enzyme, methyl *cis*-2-aminocyclododecanecarboxylate **1d**[15] and the hydroxymethylated **2d**[8c] were also prepared. The absolute configurations were determined by comparing the [α] values with the literature data (Table 2, row 5 with row 6, and row 7 with row 8). Accordingly, the absolute configuration for **1b** is (1*S*,12*R*) and that for **2b** is (1*R*,12*R*).

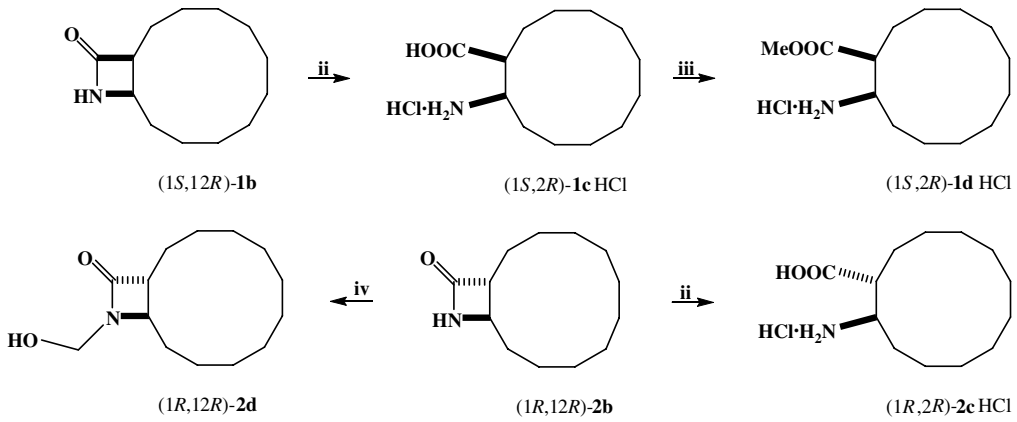


Scheme 2. Reagents: (i) Lipolase, H_2O, *i*-Pr_2O.

Table 1. Lipolase-catalysed ring cleavage of (±)-**1**[11] and (±)-**2**[12]

	Time (h)	Conv. (%)	E	β-Lactam **1b** and **2b**				β-Amino acid **1a** and **2a**			
				Yield[a] (%)	Isomer	ee[b] (%)	$[\alpha]_D^{25}$ [c]	Yield[a] (%)	Isomer	ee[d] (%)	$[\alpha]_D^{25}$ [c]
(±)-**1**	18	50	>200	47	(1*S*,12*R*)	>99	−6.9[e]	32	1*R*,2*S*	>98	+5[f]
(±)-**2**	99	50	>200	47	(1*R*,12*R*)	98	−140[e]	46	1*S*,2*S*	>98	+8.3[g]

[a] Maximum yield 50%.
[b] According to GC.[10]
[c] Optical rotations were measured with a Perkin–Elmer 341 polarimeter.
[d] Determined by GC, using *n*-hexadecane as an internal standard.[10]
[e] *c* 0.5, EtOH.
[f] *c* 0.2, H_2O.
[g] *c* 0.4, EtOH.



Scheme 3. Reagents and conditions: (ii) 18% HCl, reflux for 2 h; (iii) CH_2N_2; (iv) CH_2O, ultrasound.

Table 2. Physical data of enantiomers prepared

Row	Enantiomers	ee (%)	$[\alpha]_D^{25}$
1	(1*R*,2*S*)-**1a**·HCl	>98	+15 (*c* 0.2, H_2O)
2	(1*S*,2*R*)-**1c**·HCl	>98	−15.8 (*c* 0.4, H_2O)
3	(1*S*,2*S*)-**2a**·HCl	>98	+6.2 (*c* 0.4, H_2O)
4	(1*R*,2*R*)-**2c**·HCl	>98	−6 (*c* 0.2, H_2O)
5	(1*S*,2*R*)-**1d**·HCl	>98	−36.1 (*c* 0.4, MeOH)
6	(1*R*,2*S*)-**1d**·HCl[8d]	99	+33.4 (*c* 1, MeOH)
7	(1*R*,12*R*)-**2d**	>98	−150.6 (*c* 0.4, $CHCl_3$)
8	(1*R*,12*R*)-**2d**[8d]	91	−146.6 (*c* 1, $CHCl_3$)

3. Conclusion

In conclusion, β-lactam and β-amino acid enantiomers (ee ⩾98%) were prepared through the Lipolase (*C. antarctica* lipase B)-catalysed enantioselective ring cleavage of racemic *cis*-13-azabicyclo[10.2.0]tetradecan-14-one, (±)-**1** and *trans*-13-azabicyclo[10.2.0]tetradecan-14-one, (±)-**2**. The ring cleavage reaction of the *trans*-β-lactam proved to be a very efficient and facile new route to enantiopure *trans*-β-amino acids. The products could be easily separated. Transformations by ring opening of the β-lactams **1b** and **2b** with 18% aqueous HCl resulted in enantiomers of the β-amino acid hydrochlorides **1c**·HCl and **2c**·HCl (ee >98%).

Acknowledgements

The authors acknowledge receipt of OTKA Grants T 046440 and T 049407, GVOP-3.1.1.-2004-05-0255/3.0 and a Bolyai Fellowship for E.F.

References

1. (a) Fülöp, F. *Chem. Rev.* **2001**, *101*, 2181–2204; (b) Kuhl, A.; Hahn, M. G.; Dumic, M.; Mittendorf, J. *Amino Acids* **2005**, *29*, 89–100.
2. (a) Kiss, L.; Forró, E.; Bernáth, G.; Fülöp, F. *Synthesis* **2005**, 1265–1268; (b) Fülöp, F.; Palkó, M.; Forró, E.; Dervarics, M.; Martinek, T. A. *Eur. J. Org. Chem.* **2005**, 3214–3220; Kiss, L.; Forró, E.; Fülöp, F. *Tetrahedron Lett.* **2006**, 2855–2858.
3. (a) Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, 2015–2022; (b) Porter, E. A.; Wang, X. F.; Lee, H. S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565; (c) Martinek, T. A.; Tóth, G. K.; Vass, E.; Hollósi, M.; Fülöp, F. *Angew. Chem.* **2002**, *114*, 1794–1797; *Angew. Chem., Int. Ed.* **2002**, *41*, 1718–1721; (d) Hetényi, A.; Mándity, I. M.; Martinek, T. A.; Tóth, G. K.; Fülöp, F. *J. Am. Chem. Soc.* **2005**, *127*, 547–553; (e) Fülöp, F.; Martinek, T. A.; Tóth, G. K. *Chem. Soc. Rev.* **2006**, *35*, 323–334; (f) Martinek, T. A.; Mándity, I. M.; Fülöp, L.; Tóth, G. K.; Vass, E.; Hollósi, M.; Forró, E.; Fülöp, F. *J. Am. Chem. Soc.* **2006**, *128*, 13539–13544.
4. (a) Davies, S. G.; Bhalay, G. *Tetrahedron: Asymmetry* **1996**, *7*, 1595–1596; (b) Szakonyi, Z.; Martinek, T.; Hetényi, A.; Fülöp, F. *Tetrahedron: Asymmetry* **2000**, *11*, 4571–4579.
5. Gedey, S.; Van der Eycken, J.; Fülöp, F. *Org. Lett.* **2002**, *4*, 1967–1969.
6. (a) Izquierdo, S.; Rua, F.; Sbai, A.; Parella, T.; Alvarez-Larena, A.; Branchadell, V.; Ortuno, R. M. *J. Org. Chem.* **2005**, *70*, 7963–7971; (b) Ortuno, R. M.; Moglioni, A. G.; Moltrasio, G. Y. *Curr. Org. Chem.* **2005**, *9*, 237–259; (c) Zhong, J. C.; Liu, S. Z.; Bian, Q. H.; Yin, M. M.; Wang, M. *Chinese Chem. Lett.* **2006**, *17*, 584–586; (d) D'hooghe, M.; Mangelinckx, S.; Persyn, E.; Van Brabandt, W.; DeKimpe, N. *J. Org. Chem.* **2006**, *71*, 4232–4236; (e) Gauzy, C.; Saby, B.; Pereira, E.; Faure, S.; Aitken, D. J. *Synlett* **2006**, 1394–1398.
7. (a) Priego, J.; Flores, P.; Ortiz-Nava, C.; Escalante, J. *Tetrahedron: Asymmetry* **2004**, *15*, 3545–3549; (b) Davies, S. G.; Garner, A. C.; Long, M. J. C.; Morrison, R. M.; Roberts, P. M.; Savory, E. D.; Smith, A. D.; Sweet, M. J.; Withey, J. M. *Org. Biomol. Chem.* **2005**, 2762–2775; (c) Fuller, A. A.; Chen, B.; Minter, A. R.; Mapp, A. K. *J. Am. Chem. Soc.* **2005**, *127*, 5376–5383; (d) Winkler, M.; Martinkova, L.; Knall, A. C.; Krahulec, S.; Klempier, N. *Tetrahedron* **2005**, *61*, 4249–4260.
8. (a) Csomós, P.; Kanerva, L. T.; Bernáth, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1789–1796; (b) Kámán, J.; Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2000**, *11*, 1593–1600; (c) Forró, E.; Árva, J.; Fülöp, F. *Tetrahedron: Asymmetry* **2001**, *12*, 643–649; (d) Gyarmati, Z. C.; Liljeblad, A.; Rintola, M.; Bernáth, G.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2003**, *14*, 3805–3814.
9. (a) Forró, E.; Fülöp, F. *Org. Lett.* **2003**, *5*, 1209–1212; (b) Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2004**, *15*, 573–575; (c) Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2004**, *15*, 2875–2880; (d) Forró, E.; Fülöp, F. *Chem. Eur. J.* **2006**, *12*, 2587–2592.
10. In a typical small-scale experiment, racemic β-lactam (0.05 M solution) in *i*-Pr_2O (2 mL) was added to Lipolase

(50 mg mL^{-1}). Water (0, 0.5 or 1 equiv) was added. The mixture was shaken at either 60 or 70 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analysing them by gas chromatography. The ee values for the unreacted β-lactam enantiomers were determined by gas chromatography on a Chromopak Chiralsil-Dex CB column (25 m) at 190 °C and 140 kPa [retention times (min): compound **1b**: 30.98 (antipode: 30.53); 100 °C for 5 min → 150 °C for 45 min → 180 °C (temperature rise 5 °C min^{-1}), 140 kPa, retention times (min): compound **2b**: 95.45 (antipode: 96.13)]. The ee values for the ring-opened amino acids produced (during preliminary experiments) were calculated by using *n*-hexadecane [CP-Chirasil-Dex CB column, 120 °C for 4 min *arrow* 190 °C (temperature rise 10 °C min^{-1}; 140 kPa) retention time 7.22 min] as an internal standard.[9a]

11. Racemic **1**[8d] (500 mg, 2.38 mmol) was dissolved in *i*-Pr_2O (20 mL). Lipolase (1 g, 50 mg mL^{-1}) and water (36 μL, 1.19 mmol) were added and the mixture was shaken in an incubator shaker at 70 °C for 18 h. The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated off and the residue of (1*S*,12*R*)-**1b** crystallised [235 mg, 47%; $[\alpha]_D^{25} = -6.9$ (*c* 0.5, EtOH); mp 167–171 °C (recrystallised from *i*-Pr_2O); ee >99%]. The filtered enzyme was washed with H_2O (3 × 15 mL), and the water was evaporated off, yielding the crystalline β-amino acid (1*R*,2*S*)-**1a** {211 mg, 39%; $[\alpha]_D^{25} = +5$ (*c* 0.2, H_2O); mp 240–243 °C (recrystallised from H_2O and Me_2CO); ee >98% [after derivatisation with Marfey's reagent (Nova-Pak C18 column, eluent TFA–MeCN = 60:40, retention times (min): compound **2a**: 28.21 (antipode: 32.89)]}.
^{1}H NMR (400 MHz, D_2O): δ (ppm) for **1a**: 1.35–1.91 (20H, m, 10 × CH_2), 2.84–2.86 (1H, m, CHCOOH), 3.58–3.59 (1H, m, C*H*NH_2). Anal. Calcd. for $C_{13}H_{25}NO_2$: C, 68.68; H, 11.08; N, 6.16. Found: C, 68.46; H, 11.25; N, 6.16.
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) for **1b**: 1.21–1.78 (20H, m, 10 × CH_2), 3.11–3.14 (1H, m, CHCO), 3.64–3.69 (1H, m, C*H*NH), 5.90 (1H, br s, NH). Anal. Calcd. for $C_{13}H_{23}NO$: C, 74.59; H, 11.07; N, 6.69. Found: C, 74.50; H, 10.99; N, 6.52.

12. With the procedure described above, the reaction of racemic **2**[8d] (500 mg, 2.38 mmol) in the presence of Lipolase (1 g, 50 mg mL^{-1}) and water (36 μL, 1.19 mmol) for 99 h afforded the β-lactam (1*R*,12*R*)-**2b** [235 mg, 47%; $[\alpha]_D^{25} = -140.1$ (*c* 0.5; EtOH); mp 143–145 °C (recrystallised from *i*-Pr_2O); ee = 98%] and β-amino acid (1*S*,2*S*)-**2a** {221 mg, 41%; $[\alpha]_D^{25} = +8.3$ [*c* 0.4; EtOH (the amino acid is poorly soluble in water)], mp 230–239 °C; ee >98% [after double derivatisation (i) CH_2N_2. (*Caution*! The derivatisation with CH_2N_2 should be performed under a well-ventilated hood); (ii) Ac_2O in the presence of 4-dimethylaminopyridine and pyridine (Chirasil L-Val column, 120 °C for 15 min → 170 °C (temperature rise 10 °C min^{-1}, 100 kPa, retention times (min): **2a**: 22.89 (antipode: 22.34)]}.
^{1}H NMR (400 MHz, D_2O): δ (ppm) for **2a**: 1.37–1.75 (20H, m, 10 × CH_2), 2.61–2.63 (1H, m, C*H*COOH), 3.48–3.50 (1H, m,C*H*NH_2). Anal. Calcd. for $C_{13}H_{25}NO_2$: C, 68.68; H, 11.08; N, 6.16. Found: C, 68.46; H, 11.25; N, 6.16.
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) for **2b**: 1.21–1.93 (20H, m, 10 × CH_2), 2.83–2.87 (1H, m, CHCO), 3.42–3.45 (1H, m, C*H*NH), .5.87 (1H, br s, NH). Anal. Calcd. for $C_{13}H_{23}NO$: C, 74.59; H, 11.07; N, 6.69. Found: C, 70.50; H, 11.18; N, 6.62.

13. Compound (1*S*,12*R*)-**1b** (100 mg, 0.77 mmol) and (1*R*,12*R*)-**2b** (0.1 g, 0.77 mmol) were dissolved separately in 18% HCl (5 mL) and refluxed for 3 h. The solvent was evaporated off, and the product was recrystallised from EtOH–Et_2O, which afforded white crystals of (1*S*,2*R*)-**1c**·HCl [113 mg, 89%, $[\alpha]_D^{25} = -15.8$ (*c* 0.4, H_2O); mp 226–231 °C; ee >99%] and (1*R*,2*R*)-**2c**·HCl [100 mg, 79%; $[\alpha]_D^{25} = -6$ (*c* 0.2, H_2O); mp 229–230 °C; ee >98%]. ^{1}H NMR (400 MHz, D_2O): δ (ppm) for **1c**·HCl: 1.28–1.80 (20H, m, 10 × CH_2), 2.80 (1H, m, C*H*COOH), 3.59 (1H, m, C*H*NH_2). Anal. Calcd. for $C_{13}H_{25}NO_2$·HCl: C, 59.19; H, 9.93; N, 5.31. Found: C, 59.01; H, 9.97; N, 5.20.
^{1}H NMR (400 MHz, D_2O): δ (ppm) for **2c**·HCl: 1.35–1.91 (20H, m, 10 × CH_2), 2.84–2.89 (1H, m, C*H*COOH), 3.56–3.62 (1H, m, C*H*NH_2). Anal. found: C, 59.14; H, 10.02; N, 5.31.

14. When **1a** and **2a** (100 mg) was treated with 22% HCl/EtOH (3 mL), (1*R*,2*S*)-**1a**·HCl [112 mg, 87%; $[\alpha]_D^{25} = +15$ (*c* 0.2, H_2O); mp 228–232 °C (recrystallised from EtOH), ee >98%] and (1*S*,2*S*)–**2a**·HCl [118 mg, 92%; $[\alpha]_D^{25} = +6.2$ (*c* 0.4; H_2O); mp 226–228 °C (recrystallised from EtOH), ee >98%] were formed.
^{1}H NMR (400 MHz, D_2O): δ (ppm) data for (1*R*,2*S*)-**1a**·HCl and (1*S*,2*S*)-**2a**·HCl are similar to those for (1*S*,2*R*)-**1c**·HCl and (1*R*,2*R*)-**2a**·HCl. Anal. found for (1*R*,2*S*)-**1a**·HCl: C, 59.05; H, 9.90; N, 5.30. Anal. found for (1*S*,2*S*)-**2a**·HCl: C, 59.11; H, 9.78; N, 5.22.

15. Compound (1*S*,2*R*)-**1c**·HCl (50 mg, 0.19 mmol) was dissolved in MeOH and a solution of CH_2N_2/Et_2O was added, until the yellowish colour persisted. The solvent was then evaporated off, resulting in (1*S*,2*R*)-**1d**·HCl [50 mg, 96%, $[\alpha]_D^{25} = -36.1$ (*c* 0.4, MeOH); ee >98%].