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Chemoenzymatic preparation of non-racemic *N*-Boc-pyrrolidine-3,4-dicarboxylic acid 3-ethyl esters and their 4-hydroxymethyl derivatives

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Abstract—For the synthesis of metalloproteinase inhibitors the (*R*,*R*)- and (*S*,*S*)-monoethyl esters of *N*-Boc-pyrrolidine-3,4-dicarboxylic acid were prepared as key intermediates from the *trans*-diester racemate by two consecutive, highly selective enzymatic reactions. Reduction of the formed acids to the corresponding enantiopure hydroxymethyl derivatives ((*R*,*R*)- and (*S*,*S*)-ethyl *N*-Boc-4-hydroxymethyl-3-carboxylate) gives access to a new series of chiral building blocks.

1. Introduction

Stereoisomerically pure 3,4-substituted pyrrolidines are valuable basic units useful in the synthesis of biologically important azabicyclic systems,[1] metalloproteinase inhibitors[2] and important scaffolds for the synthesis of many natural products and pharmaceuticals.[3]

As an alternative to an asymmetric approach[4] for the synthesis of the chiral key intermediates **2a** and **2b**, we considered the *enzymatic* resolution of *trans* diester racemate **1**. Various different *cis*- and *trans*-configurated diesters of five-membered ring systems have been resolved enzymatically as described in literature,[5] among them, however, are only few pyrrolidines, predominantly 2,5-diesters. Achiwa et al.[6] described the asymmetric monohydrolysis of both the *N*-benzylated and unprotected pyrrolidine *trans*-3,4-diesters with pig liver esterase which, however, proceeded only with moderate stereoselectivity. Herein we describe the preparation of both half ester enantiomers of Boc-protected pyrrolidine *trans*-3,4-dicarboxylate **2a** and **2b** by two consecutive, highly selective enzymatic reactions (Scheme 1).

2. Results and discussion

The two-step synthesis of *N*-Boc-protected diethyl pyrrolidine *trans*-3,4-dicarboxylate **1** was carried out on the multi-gram scale (25 g) with high overall yield (86%).

The first step was a 1,3-dipolar cycloaddition[7] reaction between diethyl fumarate and the azomethine ylide generated from the decarboxylative condensation of *N*-benzylglycine and paraformaldehyde to give the *trans*-diethyl ester pyrrolidine, and the second step was a hydrogenation in the presence of BOC anhydride for the replacement of the benzyl group (Scheme 2).

In a pH-indicator assay with racemic *trans*-diethyl ester **1** as the substrate 21 out of 145 esterases or lipases showed activity. The active enzymes were re-evaluated in small scale experiments (15–20 mg) on an automated pH-stat system resulting in three reasonably active enzyme preparations (ESP-ESL-1199 and the *Candida rugosa* lipases OF and Chirazyme L-3). The *Candida* lipases showed no enantioselectivity in contrast to the nearly complete selectivity displayed by hydrolase ESP-ESL-1199. But all three enzymes were specific with respect to monohydrolysis. Therefore, the synthesis of the racemic monoester **2** as well as its enantiomerically pure forms **2a** (98% ee) and **2b** (>99% ee) were possible

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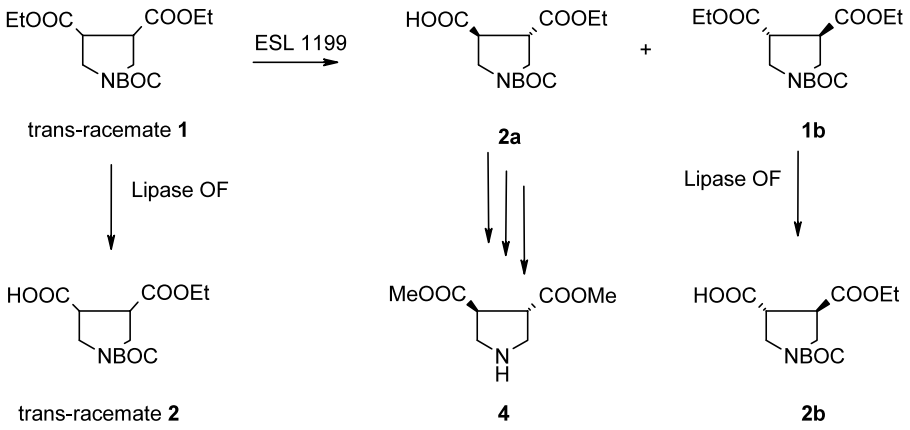
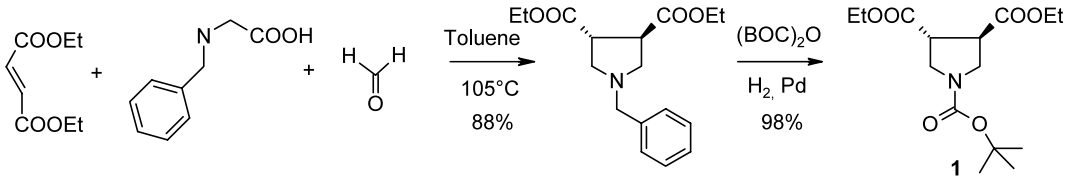


Scheme 1. Preparation of both non-racemic *trans*-configurated monoesters **2a** and **2b** from diethyl ester **1** by two consecutive enzymatic reactions.

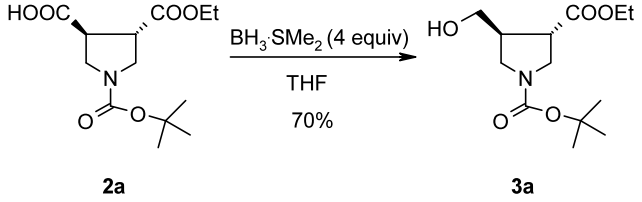


Scheme 2. Preparation of the *trans*-diethyl ester substrate **1**.

by using lipase OF from *C. rugosa* and esterase ESP-ESL-1199, respectively, as indicated in Scheme 1 and demonstrated on a g-scale.

Workup was not straightforward as the mono- and diester products were not properly separated after three extraction steps (observed with several organic solvents). Therefore, both the aqueous and the organic phases had to be subjected to an additional washing step. The absolute configuration of the reaction products was established by conversion of monoester **2a** to the unprotected dimethyl ester (*S*,*S*)-**4**,[6] thereby revealing that ESP-ESL-1199 exhibits the opposite selectivity as compared to pig liver esterase.[6]

The reduction of the chiral monoacids **2a** and **2b** with borane dimethyl sulfide complex to the corresponding hydroxymethyl groups gives access to the interesting non-racemic building blocks **3a** and **3b** (Scheme 3).



Scheme 3. Selective reduction of the monoacids **2a** (equivalent **2b**) to the corresponding hydroxymethyl compound **3a**.

3. Conclusion

The preparation of ethyl (*S*,*S*)- and (*R*,*R*)-*N*-Boc-pyrrolidine-3,4-dicarboxylates **2a** and **2b** by two consecutive, highly selective enzymatic reactions was demonstrated on the gram-scale. Reduction of the non-racemic monoacids to the corresponding non-racemic hydroxymethyl compounds **3a** and **3b** and possible further derivatization[8] gives access to a series of interesting non-racemic building blocks.

4. Experimental

4.1. General

NMR-spectra: Bruker DPX 400 MHz. IR-spectra: Nicolet, FT-IR 20 SXB. MS-spectra: Finnigan MAT SSQ 7000, EI at 70 eV. Optical rotations: Perkin–Elmer Polarimeter 241.

4.2. Materials

Hydrolase ESP-ESL 1199 was purchased from Diversa Corporation (San Diego), Chirazyme L-3 (ex *C. rugosa*) from Roche Diagnostics and Lipase OF (ex *C. rugosa*) from Meito Sangyo Co. (Tokyo). The filter aid Dicalite was from Acros, and all other reagents were from Fluka or Merck.

4.2.1. Diethyl *trans*-*N*-benzyl-pyrrolidine-3,4-dicarboxylate. In 15 min intervals a mixture of 25.4 g (845 mmol) paraformaldehyde and 25.03 g (151 mmol) *N*-benzyl-

glycine was added portionwise during 1 h to a solution of 21.7 g (126 mmol) (*E*)-butenedioic acid diethyl ester in 1000 ml toluene at 105°C. The reaction mixture was refluxed overnight and the water collected in a Deam-Stark. After evaporation of the organic solvent, the remaining crude product was dissolved in 500 ml hexane, filtrated and the organic solvent evaporated. The excess of paraformaldehyde was removed by distillation at 175°C. The remaining product 34 g (89%), a brown oil, was identified by NMR. *Analysis*: >95% pure by ^{1}H NMR. ^{1}H NMR (400 MHz; $CDCl_3$): 1.27 (t, 6H, -CH_3), 2.80–2.77 (m, 2H, -NCH_2-), 2.92–2.88 (m, 2H, -NCH_2-), 3.44–3.42 (m, 2H, -CH-CH-), 3.60 (s, 2H, N-CH_2-Ph), 4.16 (q, 4H, OCH_2-), 7.30–7.29 (m, 5H, Ph). ISP MS: 306.3 (M+H^+). IR (neat): 1727 (ester), 1171 (ester).

4.2.2. Diethyl *trans*-*N*-Boc-pyrrolidine-3,4-dicarboxylate *rac*-1. 24.5 g (80 mmol) of 1-benzyl-pyrrolidine-3,4-dicarboxylic acid diethyl ester and 19.2 g (88 mmol) di-*tert*-butyldicarbonate were dissolved in 500 ml ethanol, and 2.55 g (2.4 mmol) Pd–C 10% was added. The reaction was stirred under hydrogen for 2 h and then the balloon with hydrogen was replaced because of CO_2 formation. After 3.5 h the reaction was finished. The Pd/C was removed by filtration and rinsed with ethanol. The filtrate was concentrated and the residue purified by column chromatography (silica gel, EtOAc/hexane 2/3) to give 24.6 g (98%) of a colorless oil. *Analysis*: >95% pure by ^{1}H NMR. ^{1}H NMR (400 MHz; $CDCl_3$): 1.27 (t, 6H, -CH_3), 1.46 (s, 9H, Boc), 3.30–3.44 (m, 2H, -NCH_2-), 3.46–3.58 (m, 2H, -NCH_2-), 3.68–3.83 (m, 2H, -CH-CH-), 4.18 (q, 4H, OCH_2-). ISP MS: 338.2 (M+Na^+), 333.3 (M+NH_4^+), 316.2 (M+H^+). IR (neat): 1732 (ester), 1695 (carbamate), 1161 (ester).

4.2.3. pH-indicator assay. Into pre-loaded 96 well plates containing 0.5 mg enzyme/well a buffered indicator solution (190 μl, 7.5 mM Tris–HCl, pH 8.0, 0.02% NaN_3, 50 mg/l cresol red) and the substrate solution (0.5 mg **1** in 10 μl EtOH) were added with a liquid handler (Lissy; Zinsser Analytics). For up to 2 days the color change of the indicator from red to yellow was monitored at 410 nm with a well plate reader (Tecan Sunrise) and a well plate autosampler (Twister).

4.2.4. Automated pH-stat (Metrohm) screening procedure[9]. 15–20 mg (47–63 μmol) chiral diester **1** (99.4%) was added as an ethanol solution (0.3 ml) into a freshly prepared reaction solution (19.5 ml 0.1 M NaCl, 3 mM potassium phosphate buffer pH 7.0, 0.02% NaN_3) containing an enzyme aliquot (~3–10 mg lyophilisate). Under vigorous stirring the pH was kept constant by the controlled addition (pH-stat) of 0.05N NaOH solution. After termination of the reaction (~10–50% conversion; maximal 3–5 h) the reaction mixture was acidified to pH 2.5 with 0.5N HCl and extracted with 10–20 ml CH_2Cl_2. The organic phase was subjected to GLC analysis for ee-determination of the reaction products.

4.2.5. Ethyl *trans*-*N*-Boc-pyrrolidine-3,4-dicarboxylate *rac*-2. 5.0 g (15.85 mmol) chiral diester **1** was emulsified under vigorous stirring in 300 ml 0.1 M NaCl, 3 mM potassium phosphate buffer pH 7.5. 200 mg of Lipase OF was added and the pH kept constant by the controlled addition (pH-stat) of 1.0N NaOH solution. After termination of the reaction the mixture was acidified to pH 2.0 with 25% hydrochloric acid and extracted twice with 300 ml ethyl acetate. The combined organic phases were dried over Na_2SO_4, evaporated and dried on a HV to give 4.60 g crude product. After chromatography on silica gel (hexane/ethyl acetate 1/1) 3.45 g (75.7%) of the racemic monoacid **2** was obtained. *Analysis:* purity: 99.6% GLC (methylated).

4.2.6. Diethyl (*R*,*R*)-*N*-Boc-pyrrolidine-3,4-dicarboxylate 1b. 13.00 g (40.98 mmol) chiral diester **1** (99.4%) was emulsified under vigorous stirring in 650 ml 0.1 M NaCl, 3 mM potassium phosphate buffer pH 7.0. 130 mg of Hydrolase ESP-ESL 1199 was added and the pH kept constant by the controlled addition (pH-stat) of 1.0N NaOH solution. After termination of the reaction (~50% conversion; 20 h) the reaction mixture was extracted thrice with 500 ml CH_2Cl_2. The combined organic phases were washed with 400 ml 2% $NaHCO_3$ and 500 ml water (phase separation after filtration on 75 g dicalite prewashed with CH_2Cl_2 and water). The organic phase was dried over Na_2SO_4, evaporated and dried on a HV to give 6.00 g (46.4%) of the chiral diester **1b** as a yellowish oil. *Analysis*: purity: 100% GLC (methylated); >99% ee. Specific rotation $[\alpha]_D$ +27.8 [*c* 0.92; $CHCl_3$]. ^{1}H NMR (400 MHz; $CDCl_3$): 1.27 (t, 6H, -CH_3), 1.46 (s, 9H, Boc), 3.30–3.44 (m, 2H, -NCH_2-), 3.46–3.58 (m, 2H, -NCH_2-), 3.68–3.83 (m, 2H, -CH-CH-), 4.18 (q, 4H, OCH_2-). EI MS: 316.2 (M+H^+), 300.2 (M–Me), 258.0 (M–^{t}Bu), 242.1 (M–COOEt). IR (neat): 1732 (ester), 1695 (carbamate), 1161 (ester).

4.2.7. Ethyl (*S*,*S*)-*N*-Boc-pyrrolidine-3,4-dicarboxylate 2a. The aqueous phase of the above experiment was washed twice with 500 ml EtOAc, acidified to pH 2.5 with 25% HCl and extracted thrice with 500 ml CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, evaporated and dried on a HV to give 4.50 g (38.1%) of the chiral monoacid **2a** as a yellowish viscous oil. *Analysis:* purity: 99.7% GLC (methylated; 0.3% diacid); 98% ee. Specific rotation $[\alpha]_D$ −23.4 [*c* 1.09; $CHCl_3$]. ^{1}H NMR (400 MHz; $CDCl_3$): 1.28 (t, 3H, -CH_3), 1.46 (s, 9H, Boc), 3.32–3.64 (2×m, 4H, NCH_2-), 3.68–3.86 (m, 2H, -CH-CH-), 4.20 (m, 2H, OCH_2), ~9.6 (bs, ~1H, COOH). EI MS: 288.2 (M+H^+), 230.1 (M–^{t}Bu), 214.1 (M–COOEt). IR (neat): 2400–3200 (acid), 1733 (ester), 1698 (carbamate), 1673 (acid).

4.2.8. Ethyl (*R*,*R*)-*N*-Boc-pyrrolidine-3,4-dicarboxylate 2b. 5.70 g (18.07 mmol) chiral diester **1b** was emulsified under vigorous stirring in 430 ml 0.1 M NaCl, 3 mM potassium phosphate buffer pH 7.5. 40 mg of Lipase OF was added and the pH kept constant by the controlled addition (pH-stat) of 1.0N NaOH solution. After termination of the reaction (40 h) the reaction mixture was extracted twice with 400 ml CH_2Cl_2 and once with 400 ml EtOAc. The aqueous phase was acidified to pH 2.5 with 25% hydrochloric acid and extracted thrice with 400 ml CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, evaporated

and dried on a HV to give 5.18 g (100%) of the chiral monoacid **2b**. *Analysis:* purity: 100% GLC (methylated); >99% ee. Specific rotation $[\alpha]_D$ +25.1 [*c* 1.08; $CHCl_3$].

4.2.9. Absolute configuration of monoacid 2a. 154 mg (0.528 mmol) chiral monoacid **2a** (98.6%; 99% ee) was dissolved in 1.0 ml methanol. A total of 1.11 ml 1.0N NaOH solution (2.1 equiv.) was added in portions under stirring. After 2 h the reaction mixture was adjusted to pH 3.5 with 4% citrate buffer pH 1.9 and subsequently to pH 2.0 with 1.0N hydrochloric acid. The solution was washed thrice with 10 ml CH_2Cl_2 and then extracted twice with 10 ml EtOAc. The combined EtOAc phases were dried over Na_2SO_4, evaporated and dried on a HV to give the diacid (104 mg; 99% ee) which was methylated in 40 ml diazomethane/ether solution. The solution was washed with 25 ml 0.1 M sodium phosphate buffer pH 7.0, dried over Na_2SO_4 and evaporated to give the dimethyl ester (94 mg; 99% ee) which was dissolved in 4 ml ether/TFA 3:1 for deprotection. After 45 min incubation the solvent was evaporated (30°C/5 mbar) and the brown residue dissolved in 3 ml 0.1 M sodium phosphate buffer pH 7.0. The solution (pH 2.7) was washed with 5 ml EtOAc, adjusted to pH 8.0 with 1.0N NaOH solution and extracted twice with 10 ml EtOAc. The combined organic phases were dried over Na_2SO_4 and evaporated to give 19.4 mg (S,S)-**4**. *Analysis:* purity: 97.1% GLC; Specific rotation $[\alpha]_D$ −123.9 [*c* 1.27; $CHCl_3$]. The specific rotation of the reference compound[6] (*R*,*R*)-**4** of 23% ee is: $[\alpha]_D$ +37.0 [*c* 1.20; $CHCl_3$]. MS: 188.3 (M+H^+). A control check of the enantiomeric purity after derivatization with Boc anhydride revealed an enantiomeric excess of 95%.

4.2.10. Ethyl (*S*,*S*)-*N*-Boc-4-hydroxymethyl-pyrrolidine-3-carboxylate 3a. 640 mg (2.23 mmol) ethyl (*S*,*S*)-*N*-Boc-pyrrolidine-3,4-dicarboxylate **2a** was dissolved in 8 ml anhydrous THF. At −20°C 8.9 ml (8.5 mmol) borane dimethyl sulfide as a 1 M CH_2Cl_2 solution was added. The reaction was stirred for 4 h from −20°C to rt 4 ml methanol was added and the mixture stirred for further 10 min. The methanol was removed by evaporation and the mixture was purified by column chromatography (silica gel, EtOAc/hexane 1/1 to 3/1) to give 575 mg (94%) **3a** as a colorless oil. *Analysis*: purity: 100% GLC, >99% ee. Specific rotation $[\alpha]_D$ −20.3 [*c* 1.12; CH_2Cl_2]. ^{1}H NMR (400 MHz; $CDCl_3$): 1.28 (t, 3H, -CH_3), 1.46 (s, 9H, Boc), 2.09 (bs, 1H, OH), 2.57–2.75 (m, 1H, -CH-), 2.84–3.01 (m, 1H, -CH-), 3.09–3.24 (m, 1H), 3.47–3.79 (m, 5H), 4.16–4.21 (q, 2H, OCH_2-). EI MS: 228.0 (M−OEt), 216.1. (M−^{t}Bu), 200.2 (M−O^{t}Bu). IR (neat): 3442 (alcohol), 1732 (ester), 1695 (carbamate).

4.2.11. Ethyl (*R*,*R*)-*N*-Boc-4-hydroxymethyl-pyrrolidine-3-carboxylate 3b. Conversion of 508 mg (1.77 mmol) monoacid **2b** under identical conditions as above gave 490 mg (70%) of **3b** as a colorless oil. *Analysis*: purity 100% GLC, >99% ee. Specific rotation $[\alpha]_D$ +20.4 [*c* 1.12; CH_2Cl_2]. ^{1}H NMR (400 MHz; $CDCl_3$): 1.28 (t, 3H, -CH_3), 1.46 (s, 9H, Boc), 2.10 (t, 1H, OH), 2.57–2.75 (m, 1H, -CH-), 2.84–3.01 (m, 1H, -CH-), 3.09–3.24 (m, 1H), 3.47–3.79 (m, 5H), 4.16–4.21 (q, 2H, OCH_2-). EI MS: 216.1. (M−^{t}Bu), 200.1 (M−O^{t}Bu), 172.2 (M−$CO_2{}^t$Bu). IR (neat): 3442 (alcohol), 1732 (ester), 1695 (carbamate).

4.2.12. Enantiomeric excess. the enantiomeric excess of **1b**, **2a**,**b** and the respective diacid was determined by means of GLC on Chiraldex G-TA (10 m×0.25 mm; H_2; 40 kPa): isothermal at 125°C; Inj.: 200°C. FID: 200°C. Retention times (min): 27.4 (*R*,*R*)-diacid (methylated), 31.4 (*S*,*S*)-diacid (methylated), 35.7 **2b** (methylated), 38.9 **2a** (methylated), 47.4 **1b**, 49.8 (*S*,*S*)-**1**.

The enantiomeric excess of **3a** and **3b** was determined by means of GLC on Chiraldex G-TA (10 m×0.25 mm; H_2; 40 kPa): from 100–180°C with 2°C/min.; Inj.: 200°C. FID: 200°C. Retention times (min): 19.5 **3a** and 18.77 **3b**.

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