An Efficient and Practical Chemoenzymatic Method for Preparing Optically Active Secondary Amines

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Supporting Information

Experimental Section

General Procedure for the preparation of oxalamic esters (1b-12b): To 5.0 mmol of racemic amine (**1a-12a**) in CH₂Cl₂ (20 mL) was added triethylamine (1.0eq, 5.0 mmol) at 0 °C. Then ethyl chlorooxoacetate (1.0eq, 5.0 mmol) in CH₂Cl₂ (2 mL) was added dropwise over a 30 minutes period under argon. The solution was allowed to stir for 2-4 h at room temperature. Afterwards, the reaction solution was washed with 1N HCl, 1N NaHCO₃, and brine. The organic phase was dried over MgSO₄. After solvent evaporation, the crude product was submitted to enzymatic reaction directly with no further purification. In most cases, the purity of the products was in the range of 95 % to 99%.

1b: (±)-2-Methylpyrrolidine-oxo-ethyl acetate: 92% isolated yield. ESIMS [M+Na]⁺ *m/z* 208.1. ¹H NMR (CDCl₃, 300MHz) δ 4.12-4.36 (m, 3H), 3.50-3.65 (m, 2H), 1.82-2.10 (m, 2H), 1.56-1.70 (m, 2H), 1.35 (m, 3H), 1.24 (m, 3H). ¹³C NMR (CDCl₃, 75 MHz)(*cis/trans*): δ 163.00, 162.91, 159.98, 159.16, 62.12, 60.65, 54.32, 53.96, 47.72, 45.96, 33.52, 32.26, 24.40, 21.97, 21.82, 19.36, 14.50, 14.33. IR (KBr) 2977, 1733, 1656, 1438, 1228, 1178 cm⁻¹

2b: (±)-2-Phenyl-pyrrolidine-oxo-ethyl acetate: 99% isolated yield. ESIMS [M+Na]⁺ *m/z* 270.1, [M+H]⁺ *m/z* 248.1. ¹H NMR (CDCl₃, 300MHz) δ 7.04-7.29 (m, 5H), 4.29 (m, 2H), 3.65-3.88 (m, 3H), 1.89 (m, 1H), 1.62 (br s, 1H), 1.31 (m, 1H), 1.13 (m, 1H), 0.83 (t, *J* = 6.0 Hz, 3H). ¹³C NMR (CDCl₃, 75 MHz) δ 164.20, 162.17, 144.46, 130.51(2), 129.33(2), 127.98, 64.07, 62.80, 62.23, 50.10, 44.34, 38.64, 35.79, 25.82, 24.09, 22.88, 16.07, 14.41. IR (KBr) 2980, 1732, 1656, 1441, 1230 cm⁻¹.

4b: (±)-2-Methylpiperidine-oxo-ethyl acetate: 84% isolated yield. ESIMS [M+Na]⁺ *m/z* 222.1. ¹H NMR (CDCl₃, 300MHz) δ 4.27 (m, 2H), 3.29 (m, 1H), 3.16 (dt, *J* = 3.0 and 12.9 Hz, 1H), 2.72 (dt, *J* = 3.0 and 12.9 Hz, 1H), 1.49-1.74 (m, 6H), 1.30 (t, *J* = 7.2 Hz, 3H), 1.18 (dd, *J* = 6.9 and 18.6 Hz, 3H). ¹³C NMR (CDCl₃, 75 MHz) δ 163.92, 160.97, 62.14, 50.34, 44.33, 42.82, 30.97, 26.32, 19.19, 12.88. IR (KBr) 2940, 1734, 1656, 1433, 1210 cm⁻¹.

5b: (±)-2-Ethylpiperidine-oxo-ethyl acetate: 94% isolated yield. ESIMS $[M+Na]^+$ m/z 236.1. 1H NMR ($CDCl_3$, 300MHz): δ 4.41 (br t, $J = 8.1$ Hz, 1H), 4.20 (m, 2H), 2.95-3.44 (m, 2H), 1.28-1.78 (m, 4H), 1.22 (t, $J = 8.1$ Hz, 3H), 1.06 (m, 2H), 0.75 (q, $J = 5.8$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75MHz) (*cis/trans*): δ 163.89, 163.60, 161.44, 161.31, 62.14, 56.50, 50.09, 42.80, 42.48, 38.00, 36.78, 28.92, 27.85, 26.35, 25.38, 23.19, 22.63, 19.42, 14.51, 12.50, 10.94, 10.77. IR (KCl) 2939, 1735, 1650, 1454, 1208 cm^{-1} .

6b: (±)-2-Propylpiperidine-oxo-ethyl acetate: >95% isolated yield. ESIMS $[M+Na]^+$ m/z 250.1. 1H NMR ($CDCl_3$, 300MHz) (*cis/trans*, 1:1) *cis*: δ 4.55 (br t, $J = 8.0$ Hz, 1H), 4.18 (m, 2H), 3.52 (brs, 1H), 3.25 (d, $J = 13.0$ Hz, 1H), 1.10-1.85 (m, 11H), 0.85 (m, 3H). *trans*: 4.20 (m, 1H), 3.05 (t, $J = 13.0$ Hz, 1H), 2.60 (t, $J = 13.0$ Hz, 1H), 1.10-1.85 (m, 11H), 0.85 (m, 3H). IR (KCl) 2935, 1735, 1650, 1448, 1206 cm^{-1} .

7b: (±)-2-(4-Fluorophenyl)piperidine-oxo-ethyl acetate. >95% isolated yield. 1H NMR (300 MHz, $CDCl_3$) (*cis/trans*). *cis*: δ 7.7.19-7.32 (m, 2H), 7.02-7.12 (m, 2H), 5.81 (brd, $J = 4.8$ Hz, 1H), 4.38 (dq, $J = 1.2, 7.2$ Hz, 2H), 3.46 (m, 1H), 3.06 (m, 1H), 2.00-2.50 (m, 3H), 1.50-1.80 (m, 2H), 1.40 (t, $J = 7.2$ Hz, 3H). *trans*: δ 7.7.19-7.32 (m, 2H), 7.02-7.12 (m, 2H), 4.88 (brd, $J = 3.6, 1H$), 4.27 (q, $J = 7.2$ Hz, 2H), 2.63 (m, 1H), 2.00-2.50 (m, 3H), 1.50-1.80 (m, 2H), 1.26 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (75 ppm, $CDCl_3$) (*cis/trans*): δ 163.71, 163.52, 163.28, 163.23, 161.59, 160.44, 133.51, 128.53, 115.91, 115.59, 62.11, 55.99, 50.13, 43.09, 37.65, 28.42, 26.97, 26.04, 25.05, 19.54, 19.47, 14.07, 13.91.

8b: (±)-2-(1,3-Thiazol-2-yl)piperidine-oxo-ethyl acetate. Quantitative yield. ESIMS $[M+Na]^+$ m/z 269.1. 1H NMR (300 MHz, $CDCl_3$) (*cis/trans*, 1: 2) *cis*: δ 7.77 (d, $J = 3.0$ Hz, 1H), 7.38 (d, $J = 3.0$ Hz, 1H), 4.50 (d, $J = 15.0$ Hz, 1H), 4.32 (q, $J = 6.0$ Hz, 2H), 2.85 (dt, $J = 3.0, 12.0$ Hz, 1H), 2.66 (d, $J = 12.0$ Hz, 1H), 1.36-2.10 (m, 6H), 1.30 (t, $J = 12.0$ Hz, 3H). *trans*: 7.75 (d, $J = 3.0$ Hz, 1H), 7.34 (d, $J = 3.0$ Hz, 1H), 4.38 (q, $J = 6.0$ Hz, 2H), 3.55 (d, $J = 15.0$ Hz, 1H), 3.32 (dt, $J = 3.0, 12.0$ Hz, 1H), 2.60 (d, $J = 15.0$ Hz, 1H), 1.36-2.10 (m, 6H), 1.39 (t, $J = 12.0$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) (*cis/trans*): δ 169.70, 169.26, 163.09, 161.65, 161.49, 143.09, 142.95, 120.67, 120.15, 62.59, 56.68, 50.83, 44.00, 38.82, 29.44, 28.29, 25.86, 25.00, 20.2, 14.38, 14.27.

9b: (±)-6-Fluoro-2-methyl-1,2,3,4-tetrahydroquinoline-oxo-ethyl acetate: 90% isolated yield. ESIMS $[M+Na]^+$ m/z 287.1. 1H NMR ($CDCl_3$, 300 MHz) δ 7.02 (dd, $J = 4.8, 8.4$ Hz, 1H), 6.91 (d, $J = 8.7$ Hz, 1H), 6.83 (dt, $J = 4.5, 8.4$ Hz, 1H), 4.75 (m, 1H), 4.12 (dd, $J = 6.9, 14.1$ Hz), 2.63 (m, 1H), 2.41 (m, 1H), 1.37 (m, 2H), 1.20 (d, $J = 6.3$ Hz, 3H), 1.12 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.19, 161.50(2), 137.67, 132.14, 124.92, 115.44, 113.85, 62.19, 53.75, 49.19, 31.91, 26.4, 20.01, 14.04. IR (KBr) 2983, 1739, 1656, 1495, 1235, 1195 cm^{-1} .

10b: (±)-3-Methylpiperidine-oxo-ethyl acetate. 99% yield. ESIMS $[M+Na]^+$ m/z 222.2. 1H NMR (300 MHz, $CDCl_3$) (*cis/trans*: ~1:1) δ 4.20 (m, 4H), 4.20-4.60 (m, 4H), 2.95 (m, 1H), 2.60 (m, 2H), 2.25 (m, 1H), 1.40-1.90 (m, 14H), 1.30 (t, $J = 7.0$ Hz, 6H), 1.12 (m, 4H), 0.85 (m, 6H). ^{13}C NMR (75 ppm, $CDCl_3$) δ 163.61, 163.57, 160.66, 160.64, 62.19, 62.16, 53.82, 48.73, 47.12, 42.06, 33.21, 33.16, 31.93, 31.07, 26.14, 24.60, 19.19, 18.95, 14.53, 14.34.

11b: (±)-N-Methyl-1,2,3,4-tetrahydronaphthalen-1-amino-oxo-ethyl acetate: 92% yield. ESIMS $[M+Na]^+$ m/z 284.1. 1H NMR (300 MHz, $CDCl_3$) δ 7.04-7.25 (m, 5H), 5.82 (t, $J = 9.9$ Hz, 1H), 4.83 (t, $J = 6.3, 10.2$ Hz, 1H), 4.36 (m, 2H), 2.81 (m, 2H), 2.71 (t, $J = 8.1$ Hz, 3H), 1.70-2.25 (m, 4H), 1.41 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (75 ppm, $CDCl_3$) δ 163.80, 163.25,

139.06, 133.76, 129.80, 128.01, 127.56, 127.54, 127.48, 126.80, 62.38, 58.27, 52.52, 31.01, 29.78, 29.68, 28.82, 28.26, 27.19, 22.25, 14.42.

12b: ($\pm$) **N-Ethyl-3a,4,5,6,7,7a-hexahydrobenzo[b]thiophene-1-amino-2-sulphonamide-oxo-ethyl acetate:** 70% yield. ESIMS $[M+Na]^+$ m/z 383.2. 1H NMR (300 MHz, $CDCl_3$) (*cis/trans*, 1:4). *trans*: δ 8.03 (s, 1H), 7.42 (s, 1H), 5.45 (brs, 1H), 4.35 (m, 2H), 3.42 (m, 1H), 2.84 (m, 2H), 1.80-2.30 (m, 4H), 1.35 (m, 3H), 1.18 (m, 3H). ^{13}C NMR (75 ppm, $CDCl_3$) *cis*: δ 163.52, 162.62, 145.99, 140.67, 135.32, 130.50, 62.66, 56.34, 52.05, 40.80, 38.42, 29.04, 27.60, 16.75. *trans*: δ 163.22, 163.11, 146.72, 141.23, 134.75, 130.68, 62.66, 53.79, 51.90, 36.91, 31.88, 23.06, 20.89, 14.31.

General procedure for preparing a racemic oxalamic acid: 1 N NaOH (10.0 eq) was added dropwise to oxalamic esters (**1b-12b**) (0.1 mmol). The reaction was allowed to stir at room temperature for 2 hours. The reaction was monitored by HPLC and LC-MS. After acidifying with 1N HCl, the crude product was extracted with ethyl acetate, and dried over Na_2SO_4 . After the solvent was evaporated, the crude acid was afforded and used as a chemical standard.

Enzyme screening. A screening kit⁷ was thawed for 5 minutes and 80 μ L of potassium phosphate buffer (0.1 M, pH 7.0) was then dispensed into each well via a multi-channel pipette. 10 μ L of ACN solution of the substrate (10-20 mg of **1b-12b** /mL) was then added to each well, and the plate was incubated at 23°C on a thermomixer (900 rpm). The reactions were quenched with 100 μ L of acetonitrile after 5 h. The 96-well plate was then centrifuged, and the supernatant was transferred from each well into another 96-well plate and analyzed with automated HPLC using a short C_{18} column (30 x 4.6 mm, 3 μ m) and ACN/ H_2O containing 0.1% TFA as the solvent system.

General Procedure for enzymatic enantioselective hydrolysis of 1b-12b: Oxalamic esters (**1b-12b**) were hydrolyzed with a protease, using *Aspergillus species* (Altus), *Streptomyces griseus* (Sigma), β -chymotrypsin (Sigma), or *Bacillus lentus* (Altus) as the sources. Standard conditions for the hydrolysis include a phosphate buffer (27 mL, 0.1 M, pH 7.0), **1b-12b** (3.0 mmol) dissolved in ACN (3 mL) and protease (enzyme load 1-3%, w/w). The reaction was monitored by HPLC and quenched with CH_2Cl_2 extraction (30 mL) at about 50% conversion (20-48 h). After separation of the organic layer from the aqueous phase, the organic phase gave chiral oxalamic ester. The enantiomeric excess (ee) of the oxalamic ester was analyzed by a chiral HPLC or GC method (see Table 1). For the aqueous phase, the pH was adjusted to 4.0 and extracted by CH_2Cl_2 to afford nearly pure oxalamic acid. The purity of the oxalamic esters and the oxalamic acids were determined by HPLC (90-95% of chemical purity in most cases).

General Procedure for the deprotection of chiral esters and acids: The oxalamic ester or oxalamic acid was separately cleaved with 4 N HCl under reflux for 5-16 hours. The reaction mixture was washed with CH_2Cl_2 and the aqueous layer was then brought to pH 10-11 and extracted with CH_2Cl_2 . After careful evaporation of the solvent, the optically pure amines were obtained with good yields and chemical purity. It was demonstrated using **2a**, **3a**, **5a**, **9a** and **13a** as examples: 92% ee, 93% yld from ester **2b**; 91% ee, 95% yld from ester **3b**; 96% ee, 90% yld from oxalamic acid **3c**; **5a**: 99% ee, 92% yld from ester **5b**; 96% ee, 90% yld from oxalamic acid **5c**; 97% ee, >96% yld from ester **12b**; 95% ee, 90% yld from oxalamic acid **12c**.

Enzymatic Preparation of (R) and (S)-5: To a potassium phosphate buffer (43 mL, pH 7.0, 0.1 M) was added *Aspergillus species* protease (2.0 g). **5b** (1.06g, 5.0 mmol) in acetonitrile (5.0 mL) was then added to the solution, and the reaction

mixture was stirred vigorously at 23°C and pH 7.0 controlled by a continuous addition of 1N NaOH. After about 50% conversion as shown in HPLC, the reaction was quenched and extracted with CH₂Cl₂. After removal of the solvent, the remaining ester **5b** was recovered (0.52 g, 49%). The aqueous layer was adjusted to pH 4.0 and extracted with CH₂Cl₂ to afford acid **5c** (415 mg). **5b** and **5c** were then refluxed separately with 4 N HCl for 5 hours. The reaction mixture was washed with CH₂Cl₂ and the aqueous layer was then brought to pH 10 and extracted with CH₂Cl₂. After careful solvent evaporation, the amines (*R*)-**5a** and (*S*)-**5a** were produced in good yields and optical purity (*R*-**5a**: 45%, 99% ee; *S*-**5a**: 43%, 96% ee). The absolute configurations of the amines were determined by comparing with commercially available standards.

Chiral method development for free amines and oxalamic esters/acids:

1 mg of oxalamic esters, oxalamic acids or free amines in 1-5 mL of ACN were analyzed on an Agilent HPLC, using a set of chiral HPLC columns (Chiracel AS-RH, AD-RH, OD-RH, and OJ-RH, the reversed phase; Chiralpak AS-H, AD-H, OD-H, and OJ-H, the normal phase). In several cases, chiral methods were developed on an Agilent GC, using a set of chiral GC columns, such as G-TA, B-TA, B-DM, P-DM. In most cases, the chiral methods were successfully developed for the oxalamic ester and the free amines. However, the attempts to develop chiral methods for the oxalamic acids were failed in most cases. For chiral method details please see Table 1.

Table 1. Chiral chromatographic methods for the amines and the oxalamic esters

No.	compound	oxalamic ester	free amine
1a		chiraldex B-TA (GC) 170°C (iso)	chiraldex B-TA (GC) 110°C (iso)
2a			chiralcel OD-RH (HPLC) 50% H ₂ O: 50% ACN ^a
3a		chiralcel AS-RH (HPLC) 70% H ₂ O: 30% ACN ^b	chiralcel OD-R (HPLC) 75% H ₂ O: 25% ACN ^a
4a		chiraldex G-TA (GC) 150°C (iso)	chiraldex G-TA (GC) 110°C (iso)
5a		chiralcel AS-RH (HPLC) 40% H ₂ O: 60% ACN ^a	chiral G-TA (GC) 110°C (iso)
6a		chiraldex BTA (GC) 60-150 °C (15°C/min), 150 °C, hold 6 min	chiralcel AD-RH (HPLC) 50% H ₂ O: 50% ACN ^a
7a			chiralcel OJ-RH (HPLC) 50% H ₂ O: 50% ACN ^a
8a			chiralcel OJ-RH (HPLC) 65% H ₂ O: 35% ACN ^a
9a		chiralcel AS-RH (HPLC) 70% H ₂ O: 30% ACN ^a	chiralcel AS-RH (HPLC) 70% H ₂ O: 30% ACN ^a
		chiralcel OJ-RH (HPLC): ACN/MeOH/H ₂ O, 20:20:60.	
10a
11a			chiralpak AS-H (HPLC) 95% heptane: 5% IPA ^c
12a		ChiraDex (HPLC) 10% MeOH: 90% buffer, (0.1% TEA, pH 4.0)	

*containing 0.1% TFA for HPLC mobile phase; ^bfor the oxalamic acid and no chiral separation methods available for the oxalamic ester. ^c+0.2% DEA. HPLC column 4.5 mm x 150 mm; detection at 205 nm.