Tetrahedron: *Asymmetry* 19 (2008) 1333–1338

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Enzymatic desymmetrization of pyrrolidine and pyrroline derivatives

Robert Chênevert [a,*], Frédéric Jacques [a], Pascall Giguère [a], Mohammed Dasser [b]

[a] *Département de Chimie, CREFSIP, Faculté des Sciences et de Génie, Université Laval, Québec (Qc), Canada G1K 7P4*
[b] *OmegaChem, 8800 boul. de la Rive-Sud, Lévis (Qc), Canada G6V 9H1*

ARTICLE INFO

Article history:
Received 13 March 2008
Accepted 19 May 2008

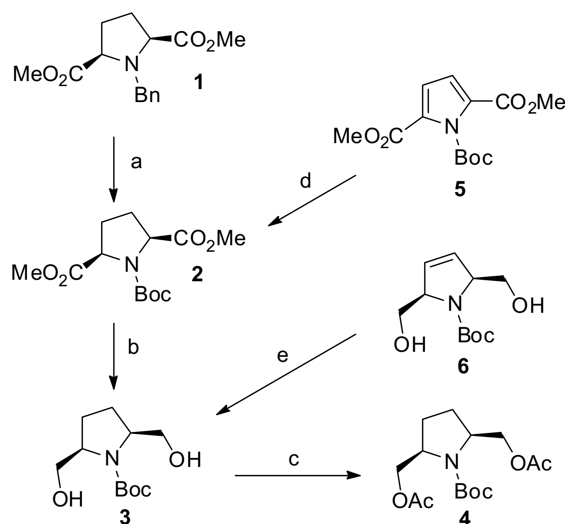
ABSTRACT

The enzymatic desymmetrization of various *meso*-*N*-Boc-2,5-*cis*-disubstituted pyrrolidines and pyrrolines compounds by ester hydrolysis or transesterification provided the corresponding monoesters in high enantiomeric excess.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Pyrrolidine and pyrroline rings are found in numerous natural products, such as pyrrolizidine and indolizidine alkaloids.[1–3] These five-membered heterocycles are common structural features of synthetic pharmaceutical and agrochemical compounds.[2,4,5] Many polyhydroxylated pyrrolidines, also known as imino or azasugars, are inhibitors of glycosidase enzymes, showing therapeutic potential in the treatment of diabetes, AIDS, and cancer.[6–9] Chiral pyrrolidines have been used as organocatalysts[10,11] and chiral auxiliaries or ligands in asymmetric synthesis.[12–15] The interest in pyrrolidine and pyrroline derivatives is well displayed by the wealth of published material detailing their synthesis.[2,4,16–21] Enzymatic differentiation of enantiotopic groups in *meso*-substrates (desymmetrization) is an efficient method for the preparation of enantiomerically enriched products with multiple stereogenic centers.[22] Herein, we report the enzymatic desymmetrization of *meso*-pyrrolidines and *meso*-pyrrolines.



Scheme 1. Reagents and conditions: (a) H_2, $Pd(OH)_2/C$, Boc_2O, MeOH, 99%; (b) $LiAlH_4$, THF, 84%; (c) AcCl, Et_3N, CH_2Cl_2, 88%; (d) H_2, Pt/C, 96%; (e) H_2, Pd/C, 95%.

2. Results and discussion

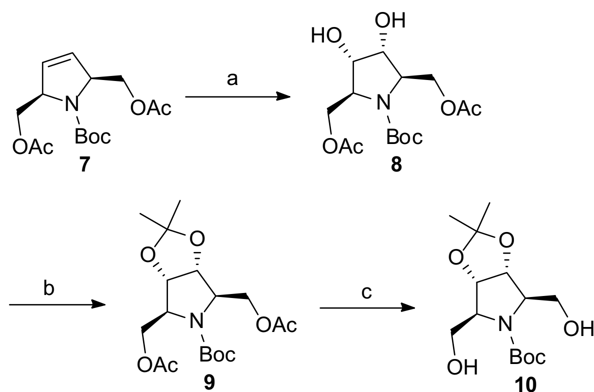
2.1. Substrate preparation

N-Benzyl *cis*-diester **1**, which was prepared in four steps starting from adipic acid,[23–26] was converted into the corresponding *N*-*tert*-butyloxycarbonyl (*N*-Boc) *cis*-diester **2** by catalytic hydrogenolysis in the presence of di-*tert*-butyl dicarbonate (Boc_2O) in a one-pot procedure (Scheme 1). Reduction of **2** with $LiAlH_4$ in THF[27] gave *meso* diol **3**. The corresponding *meso* diacetate **4** was obtained by acetylation of diol **3** with acetyl chloride. The preparation of **1** from adipic acid is tedious and gives low yields; therefore we sought a more suitable synthesis of **2** and **3**. In an alternative route, catalytic hydrogenation of known compound **5**[28] provided a nearly quantitative yield of a *cis*–*trans* mixture rich (93%) in the *cis*-compound **2**. Similarly, hydrogenation of **6**[29] gave **3** in high yield.

The synthesis of substrates **9** and **10** is described in Scheme 2. Diol **8** was prepared by the reaction of the known olefin **7**[29] with catalytic osmium tetroxide in the presence of *N*-methylmorpholine *N*-oxide (NMO) as the co-oxidant. Reaction of diol **8** with dry acetone in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) and sodium sulfate as a dehydrating agent provided isopropylidene acetal **9**. Saponification of the acetate groups with K_2CO_3 in water–methanol gave diol **10**.

* Corresponding author.
E-mail address: robert.chenevert@chm.ulaval.ca (R. Chênevert).

0957-4166/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2008.05.022



Scheme 2. Reagents and conditions: (a) OsO_4, NMO, acetone, H_2O, 91%; (b) acetone, *p*-TsOH, 91%; (c) K_2CO_3, MeOH–H_2O, 95%.

2.2. Enzymatic desymmetrization

We first completed some screening experiments in order to find hydrolases with the ability to distinguish the enantiotopic groups of *meso*-substrates **2**, **3**–**4**, **6**–**7**, **9**–**10**. The pig liver esterase (PLE)-catalyzed hydrolysis of ester **1** has been reported and found to be very dependent on the reaction conditions.[25] High enantioselectivity is obtained only in the presence of a co-solvent (25% DMSO in Tris buffer at pH 7.5). Reactions run in this buffer were faster and gave higher enantioselectivity but Tris acts as a competitive nucleophile to water and lowers the yield of monoester. We needed an easily reproducible reaction on a preparative scale and a more convenient protecting group such as *tert*-butyloxycarbonyl (Boc).

Diester **2** was hydrolyzed by PLE in water at pH 7.5 to give optically active monoester **11** in good yield and high enantiomeric excess (ee) (Table 1). *Candida antarctica* lipase fraction B (CAL-B) provided monoester **11** in high ee (⩾99%) but low yield (40%). The reaction stopped after 24 h and the low yield was attributed to product inhibition. The hydrolysis of the corresponding diethyl ester gave identical results.

The results of the enzymatic desymmetrization of *meso*-substrates **3**–**4**, **6**–**7** and **9**–**10** via acylation or hydrolysis are summarized in Tables 2–4. The enantiomeric excess of the monoesters were determined by HPLC or GC on a chiral phase. The reactions were monitored by this chromatographic analysis and terminated when all of the starting material (diol or diacetate) was consumed (conversion = 100%).

Acylation of diol **3** with vinyl acetate as the acylation reagent and solvent in the presence of CAL-B provided monoester (2*S*,5*R*)-**12** in fair yield (70%) and high enantiomeric excess (97%) (Table 2, entry 1). The opposite enantiomer (2*R*,5*S*)-**12** was obtained in better yield (83%) but in lower ee (85%) in the presence of *Candida rugosa* lipase (CRL) (entry 2). Interestingly, acylation and hydrolysis with CRL afforded the same enantiomer (entries 2 and 3). When both the *meso*-alcohol and the corresponding *meso*-ester are substrates for a lipase, acylation and hydrolysis are usually complementary and give the opposite enantiomers. While acylation and hydrolysis represent reactions in opposite directions, the enzyme displays the same selectivity (the same enantiomer or the same prochiral group) in both cases. This empirical rule applies to kinetic resolutions and desymmetrizations but exceptions have been reported.[30,31] However, with both CRL and PLE, extensive overhydrolysis to achiral diol **3** occured and monoester **12** was obtained in poor yield (entries 3 and 4).

Table 3 displays several biotransformations leading to pyrroline monoester **13**. CRL, *Pseudomonas cepacia* lipase (PCL), *Pseudomonas* sp. lipase (PSL), and CAL-B were found to catalyze the enantioselective esterification of diol **6** (entries 1–4). Acylation of diol **6** by treatment with PSL in vinyl acetate gave the best results (yield = 85%, ee = 96%, entry 3). Hydrolysis of diester **7** in the presence of PSL and CRL gave monoester (2*S*,5*R*)-**13** with high enantioselectivity, but yields were lowered by overhydrolysis. As the desymmetrization of **6**–**7** was being developed, we became aware of a report by Donohoe et al.[29] describing similar and complementary results.

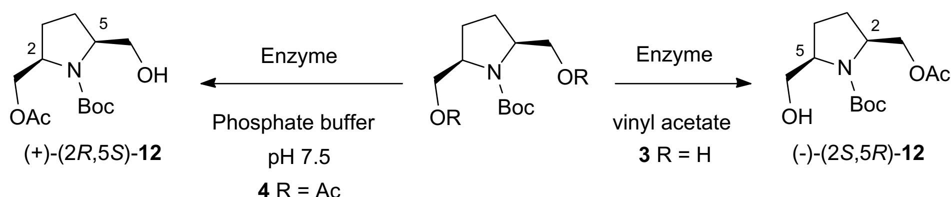
Table 1
Enzymatic desymmetrization of diester **2**

MeO_2C / CO_2Me / N / Boc / **2** → hydrolase, H_2O, pH 7.5 → MeO_2C / 5 / 2 / CO_2H / N / Boc / **11**

Enzyme	Time (h)	Yield[c] (%)	ee[d] (%)	Absolute configuration
PLE[a]	36	75	⩾99	(+)-(2*S*,5*R*)
CAL-B[b]	24	40	⩾99	(+)-(2*S*,5*R*)

[a] PLE: pig liver esterase.
[b] CAL-B: *Candida antarctica* lipase B.
[c] Isolated yield.
[d] Determined by HPLC analysis using a Chiralcel OD-H column.

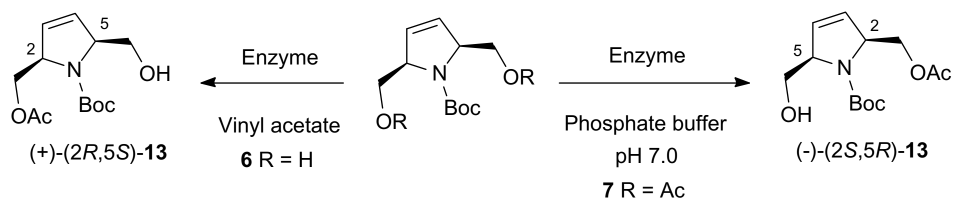
Table 2
Enzymatic desymmetrization of diol **3** and diacetate **4**



Entry	Substrate	Reaction	Enzyme	Time (h)	Yield[d] (%)	ee[e] (%)	Absolute configuration
1	Diol **3**	Acylation	CAL-B[a]	36	70	97	(−)-(2*S*,5*R*)
2	Diol **3**	Acylation	CRL[b]	22	83	85	(+)-(2*R*,5*S*)
3	Diester **4**	Hydrolysis	CRL[b]	26	30	94	(+)-(2*R*,5*S*)
4	Diester **4**	Hydrolysis	PLE[c]	26	15	75	(+)-(2*R*,5*S*)

[a] CAL-B: *Candida antarctica* lipase B.
[b] CRL: *Candida rugosa* lipase.
[c] PLE: pig liver esterase.
[d] Isolated yield.
[e] Determined by GC analysis using a Chiraldex B-DM column.

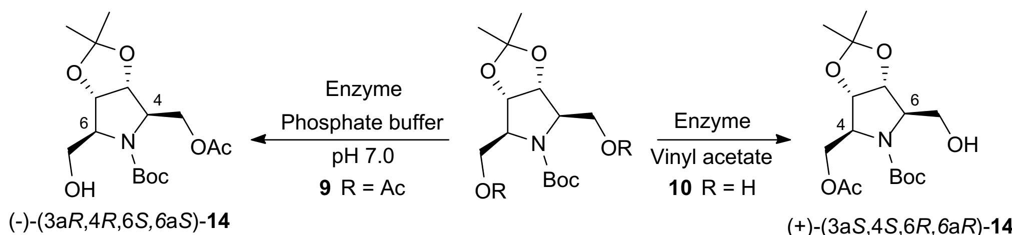
Table 3
Enzymatic desymmetrization of diol **6** and diacetate **7**



Entry	Substrate	Reaction	Enzyme	Time (h)	Yield[e] (%)	ee[f] (%)	Absolute configuration
1	Diol **6**	Acylation	CRL[a]	39	71	80	(+)-(2*R*,5*S*)
2	Diol **6**	Acylation	PCL[b]	39	60	81	(+)-(2*R*,5*S*)
3	Diol **6**	Acylation	PSL[c]	26	85	96	(+)-(2*R*,5*S*)
4	Diol **6**	Acylation	CAL-B[d]	84	65	88	(−)-(2*S*,5*R*)
5	Diester **7**	Hydrolysis	PSL[c]	24	75	94	(−)-(2*S*,5*R*)
6	Diester **7**	Hydrolysis	CRL[a]	3.5	65	96	(−)-(2*S*,5*R*)

[a] CRL: *Candida rugosa* lipase.
[b] PCL: *Pseudomonas cepacia* lipase.
[c] PSL: *Pseudomonas* sp. lipase.
[d] CAL-B: *Candida antarctica* lipase B.
[e] Isolated yield.
[f] Determined by HPLC analysis using a Chiralcel OD-H column.

Table 4
Enzymatic desymmetrization of diacetate **9** and diol **10**



Entry	Substrate	Reaction	Enzyme	Time (h)	Yield[c] (%)	ee[d] (%)	Absolute configuration
1	Diol **10**	Acylation	CAL-B[a]	96	90	97	(−)-(3a*R*,4*R*,6*S*,6a*S*)
2	Diol **10**	Acylation	PSL[b]	24	85	96	(+)-(3a*S*,4*S*,6*R*,6a*R*)
3	Diester **9**	Hydrolysis	CAL-B[a]	120	30	60	(+)-(3a*S*,4*S*,6*R*,6a*R*)
4	Diester **9**	Hydrolysis	PSL[b]	72	60	90	(−)-(3a*R*,4*R*,6*S*,6a*S*)

[a] CAL-B: *Candida antarctica* lipase B.
[b] PSL: *Pseudomonas* sp. lipase.
[c] Isolated yield.
[d] Determined by HPLC analysis using a Chiralcel OJ-H column.

The results obtained for the desymmetrization compounds **9**–**10** are shown in Table 4. The enantioselective acylation of **10** was performed successfully with both PSL and CAL-B (entries 1 and 2). These enzymes have a complementary enantiopreference and both enantiomers of monoester **14** were obtained in good yield (85–90%) and high ee (96–97%). The hydrolysis of diester **9** by PSL or CAL-B provided product **14** with opposite absolute stereochemistry with lower yield and ee (entries 3 and 4).

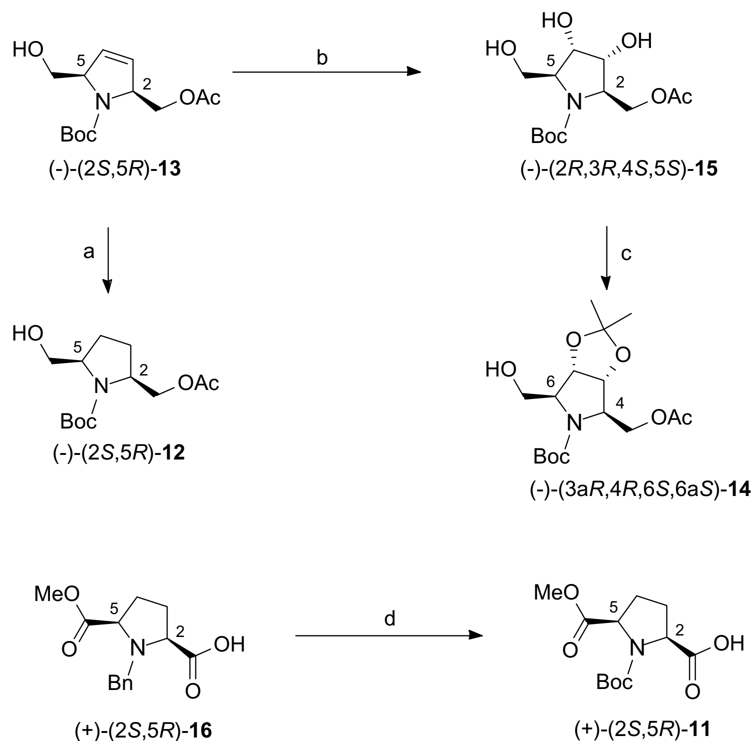
2.3. Determination of the absolute configurations

The absolute configurations of compounds **12** and **14** (Scheme 3) were determined by chemical correlation with compound (−)-**13** of a known absolute configuration being (2*S*,5*R*).[29] Hydrogenation of **13** in methanol in the presence of 10% Pd/C gave monoester (−)-(2*S*,5*R*)-**12**. Compound (−)-(3a*R*,4*R*,6*S*,6a*S*)-**14** was prepared by dihydroxylation of **13** followed by acetalization of intermediate **15** with acetone. Hydrogenolysis of the known (+)-(2*S*,5*R*)-**16**[25] with $H_2/Pd(OH)_2/C$ in the presence of Boc_2O provided (+)-(2*S*,5*R*)-**11**.

3. Experimental

3.1. General

NMR spectra were recorded on a Varian Inova AS400 spectrometer (400 MHz). The majority of the ^{1}H and ^{13}C NMR spectra exhibit doubling of some signals because of the presence of *N*-Boc rotamers. Infrared spectra were recorded on a Bomem MB-100 spectrometer. Optical rotations were measured using a JASCO DIP-360 polarimeter (*c* as gram of compound per 100 mL). Flash column chromatography was carried out using 40–63 μm (230–400 mesh) silica gel. The enantiomeric excesses (ee) were determined by chiral HPLC analysis on Chiralcel OD-H or Chiralcel OJ-H columns and by chiral GC analysis on a Chiraldex B-DM column using racemic compounds as references. *C. antarctica* lipase B (Chirazyme L-2) was obtained from Boehringer Mannheim. Porcine liver esterase (PLE), *Pseudomonas* sp. lipase (PSL), *P. cepacia* lipase (PCL), and *C. rugosa* lipase (CRL) were purchased from Aldrich.



Scheme 3. Reagents and conditions: (a) Pd/C, MeOH, 95%; (b) OsO_4, NMO, acetone–H_2O, 90%; (c) acetone, *p*-TsOH, 90%; (d) H_2, $Pd(OH)_2/C$, Boc_2O, MeOH, quantitative.

3.2. *cis*-1-*tert*-Butyl 2,5-dimethyl pyrrolidine-1,2,5-tricarboxylate 2

Method 1: To a solution of diester **1** (3.16 g, 11.4 mmol) and di-*tert*-butyl dicarbonate (2.73 g, 12.5 mmol) in freshly degassed methanol (30 mL) was added 20% $Pd(OH)_2/C$ (54 mg). Hydrogenolysis was performed at 54 psi for 24 h at room temperature. EtOAc (75 mL) was added and the mixture was filtered through a pad of Celite. The organic layer was washed with 1 N HCl (3 × 75 mL), saturated aqueous $NaHCO_3$ (3 × 75 mL) and brine (75 mL). The organic layer was dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (hexanes–EtOAc, 7:3) to give **2**[24,27] (3.25 g, 99%). IR (NaCl) 2983, 1748, 1687, 1365, 1158 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 9H), 2.02–2.15 (m, 4H), 3.66 (s, 6H), 4.19–4.34 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3, 28.9, 29.7, 52.2, 52.3, 59.7, 60.2, 80.9, 153.6, 172.3, 172.6.

Method 2: A solution of **5** (295 mg, 1.04 mmol) in MeOH (20 mL) was vigourously stirred at room temperature and degassed with argon for 10 min before Pt/C (90 mg) was added. Hydrogen (1 atm) was added and the mixture stirred for 8 h. The mixture was diluted with CH_2Cl_2 (20 mL) and the catalyst was removed by filtration through a pad of Celite. The pad was washed with CH_2Cl_2 (25 mL) and the solvent evaporated. The crude product was purified by flash chromatography (hexanes–AcOEt, 7:3) to give **2** (288 mg, 96%) as a colorless oil. Spectroscopic data as above.

3.3. *cis*-1-*tert*-Butoxycarbonyl-(2,5)-bis-(hydroxymethyl)-pyrrolidine 3

Method 1: To a suspension of $LiAlH_4$ (0.718 g, 18.9 mmol) in anhydrous THF (100 mL) at 0 °C under a dry nitrogen atmosphere was added a solution of diester **2** (2.71 g, 9.43 mmol) in anhydrous THF (100 mL). The reaction mixture was stirred at room temperature for 20 h and then quenched by the addition of saturated aqueous NH_4Cl (75 mL) and EtOAc (100 mL). The organic layer was washed with 1 M HCl (3 × 100 mL), brine (100 mL), dried over $MgSO_4$, and evaporated. The crude product was purified by flash chromatography (hexanes–EtOAc, 3:7) to give *cis*-diol **3**[27] (1.83 g, 84%) as a colorless oil: IR (NaCl) 3397, 2970, 1657, 1396, 1043 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.72–2.00 (m, 4H), 3.48–3.52 (m, 2H), 3.86 (s, 2H), 3.70–4.02 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.1, 28.7, 60.8, 63.6, 66.4, 80.9, 156.6.

Method 2: A solution of **6** (438 mg, 1.91 mmol) in MeOH (25 mL) was stirred at room temperature with 10% Pd/C (100 mg) under hydrogen (50 psi) for 4 h. The catalyst was then removed by filtration and the solvent evaporated. The crude product was purified by flash chromatography (hexanes–EtOAc, 3:7) to give *cis*-diol **3** (421 mg, 95%) as a colorless oil. Spectral data as above.

3.4. *cis*-1-*tert*-Butoxycarbonyl-(2,5)-bis(acetoxymethyl) pyrrolidine 4

To a solution of diol **3** (400 mg, 1.73 mmol) in anhydrous CH_2Cl_2 (15 mL) at 0 °C under dry atmosphere were added anhydrous Et_3N (532 μL, 3.82 mmol) and acetyl chloride (270 μL, 3.82 mmol). The reaction mixture was stirred at room temperature for 2 h, washed with water, dried over $MgSO_4$, and evaporated. Flash chromatography (hexanes–EtOAc, 1:1) provided diacetate **4** (480 mg, 88%) as a colorless oil. IR (NaCl) 2916, 1742, 1657, 1359, 1036 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.74–2.03 (m, 4H), 2.06 (s, 6H), 3.96–4.19 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1, 26.8, 27.7, 28.6, 56.9, 65.1, 80.4, 154.7, 171.1; HRMS (CI, NH_3) calcd for $C_{15}H_{26}NO_6$ $(MH)^+$: 316.1760. Found 316.1765.

3.5. (2*S*,3*S*,4*R*,5*R*)-1-*tert*-Butoxycarbonyl-3,4-dihydroxy-2,5-bis(acetoxymethyl) pyrrolidine *meso*-8

To a solution of olefin **7** (344 mg, 1.1 mmol) in acetone–water (4:1, 15 mL) was added *N*-methylmorpholine-*N*-oxide monohydrate (396 mg, 3.3 mmol) followed by a solution of osmium tetroxide in water (200 μL, 4% w/w) and a catalytic amount of quinidine. The mixture was stirred at room temperature for 24 h, then ex-

tracted with diethyl ether (2 × 25 mL). The organic phase was washed with brine (1 × 25 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (Et_2O) to yield **8** (348 mg, 91%) as a colorless oil. IR (NaCl) 3419, 1642, 1391, 1249, 1037 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.09 (s, 6H), 3.80–4.40 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1, 28.5, 30.5, 62.2, 63.1, 72.9, 81.3, 155.1, 171.2; HRMS (CI, NH_3) calcd for $C_{15}H_{26}NO_8$ $(MH)^+$: 348.1658. Found: 348.1668.

3.6. [(3a*R*,4*R*,6*S*,6a*S*)-5-(*tert*-Butoxycarbonyl)-2,2-dimethyl-tetrahydro-3a*H*-[1,3]dioxolo[4,5-*c*]pyrrole-4,6-diyl]-bis(methylene) diacetate *meso*-9

To a solution of diol **8** (298 mg, 0.86 mmol) in acetone (25 mL) was added sodium sulfate (500 mg) followed by *p*-toluenesulfonic acid (200 mg). The mixture was stirred at room temperature overnight, then filtered and evaporated. Flash chromatography (CH_2Cl_2–acetone, 3:1) provided **9** (310 mg, 91%) as a colorless oil. IR (NaCl) 2981, 1748, 1391, 1245, 1037 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 6H), 1.44 (s, 9H), 2.11 (m, 6H), 3.35–4.61 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 21.0, 25.4, 27.3, 28.4, 50.6, 63.3, 63.6, 63.8, 64.0, 66.0, 81.1, 81.9, 82.7, 112.4, 154.0, 170.9; HRMS (CI, NH_3) calcd for $C_{18}H_{30}NO_8$ $(MH)^+$: 388.1971. Found: 388.1974.

3.7. (3a*R*,4*R*,6*S*,6a*S*)-*tert*-Butyl 4,6-bis(hydroxymethyl)-2,2-dimethyldihydro-3a*H*-[1,3]dioxolo[4,5-*c*]pyrrole-5(4*H*)-carboxylate *meso*-10

To a solution of **9** (310 mg, 0.80 mmol) in MeOH–H_2O (20 mL, 1:1) was added K_2CO_3 (553 mg, 4.00 mmol). The solution was stirred vigourously at room temperature overnight, then extracted with CH_2Cl_2 (5 × 20 mL), dried over $MgSO_4$, and evaporated to dryness. Flash chromatography (CH_2Cl_2–acetone, 1:1) gave **10** (231 mg, 95%) as a colorless oil. IR (NaCl) 3583, 2934, 2120, 1704, 1479 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 3H), 1.44 (s, 9H), 3.67–4.12 (m, 9H), 4.69 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.5, 27.6, 28.5, 63.1, 63.3, 66.5, 66.8, 81.0, 81.6, 82.2, 111.8, 155.2;HRMS (CI, NH_3) calcd for $C_{14}H_{26}NO_6$ $(MH)^+$: 304.1760. Found: 304.1755.

3.8. (2*S*,5*R*)-1-(*tert*-Butoxycarbonyl)-5-(methoxycarbonyl)pyrrolidine-2-carboxylic acid 11

Diester **2** (292 mg, 1.30 mmol) was emulsified in a phosphate buffer (15 mL, 0.5 M, pH 7.5). Porcine liver esterase was added (50 mg). After 3 d, the aqueous mixture was filtered on a 0.22 μm nylon membrane filter. The aqueous solution was acidified to pH 4–5 with 1 M HCl and extracted with CH_2Cl_2 (5 × 50 mL). The organic layer was dried over $MgSO_4$ and concentrated. Flash chromatography (EtOAc–MeOH, 9:1) gave (+)-**11** (270 mg, 76%) as a colorless oil. $[\alpha]_D^{20} = +12.9$ (*c* 1.40, MeOH); ee ⩾ 99% chiral HPLC; IR (NaCl) 3408, 2983, 1705, 1389, 1140 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.98–2.30 (m, 4H), 3.72 (s, 3H), 4.23–4.37 (m, 2H), 4.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3, 29.3, 30.0, 54.1, 60.0, 61.6, 82.9, 153.2, 172.9, 177.9; HRMS (CI, NH_3) calcd for $C_{12}H_{20}NO_6$ $(MH)^+$: 274.1291. Found: 274.1289.

3.9. 1-*tert*-Butoxycarbonyl-2-(acetoxymethyl)-5-(hydroxy-methyl)pyrrolidine 12

3.9.1. Enzymatic acylation of diol 3

To a solution of diol **3** (1.50 g, 6.49 mmol) in vinyl acetate (15 mL) was added *C. antarctica* lipase B (240 mg). The mixture was stirred at room temperature for 36 h. The enzyme was removed by filtration, and the filtrate was evaporated to dryness. The crude product was purified by flash chromatography (hexanes–EtOAc, 1:1) to provide (−)-(2*S*,5*R*)-**12** (1.24 g, 70%) as a colorless oil. $[\alpha]_D^{20} = -10.9$ (*c* 1.60, $CHCl_3$); ee = 97%, chiral GC; IR (NaCl) 3397, 2922, 1657, 1395, 1042 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.50–2.00 (m, 5H), 2.04 (s, 3H), 3.45–4.20 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1, 27.0, 27.2, 28.6, 57.7, 61.7, 65.3, 67.5, 81.2, 154.6, 171.1; HRMS (CI, NH_3) calcd for $C_{13}H_{24}NO_5$ $(MH)^+$: 274.1654. Found: 274.1649.

3.9.2. Enzymatic hydrolysis of diacetate 6

Diester **4** (100 mg, 0.32 mmol) was emulsified in a phosphate buffer (10 mL, 0.5 M, pH 7.5) and *C. rugosa* lipase was added (25 mg). After 26 h, the aqueous mixture was filtered and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated. Flash chromatography (hexane–EtOAc, 1:1) gave (+)-(2*R*,5*S*)-**12** (26 mg, 30%) as a colorless oil. $[\alpha]_D^{20} = +10.6$ (*c* 1.60, $CHCl_3$); ee = 94%, chiral GC. Spectroscopic data as above.

3.10. *tert*-Butyl 2-(acetoxymethyl)-5-(hydroxymethyl)-2,5-dihydro-1*H*-pyrrole-1-carboxylate 13

3.10.1. Enzymatic acylation of diol 6

To a solution of diol **6** (250 mg, 1.09 mmol) in vinyl acetate (5 mL) was added *Pseudomonas* sp. lipase (90 mg). The mixture was stirred at room temperature for 26 h. The enzyme was removed by filtration, and the filtrate was evaporated to dryness. The crude product was purified by flash chromatography (hexane–EtOAc, 1:1) to provide (+)-(2*R*,5*S*)-**13**[29] (251 mg, 85%) as a colorless oil. $[\alpha]_D^{20} = +79.6$ (*c* 2.00, $CHCl_3$); ee = 96%, chiral HPLC; IR (NaCl) 3442, 2922, 1745, 1395, 1039 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 9H), 2.04 (s, 3H), 3.54–4.79 (m, 7H), 5.72 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1, 28.6, 64.5, 64.6, 66.9, 68.4, 81.6, 128.1, 128.3, 155.3, 171.1.

3.10.2. Enzymatic hydrolysis of diacetate 7

Diester **7** (120 mg, 0.38 mmol) was emulsified in a phosphate buffer (10 mL, 0.25 M, pH 7.6) and *C. rugosa* lipase was added (45 mg). After 3.5 h, the aqueous mixture was filtered and extracted with CH_2Cl_2 (3 × 25 mL). The organic layer was dried over $MgSO_4$ and concentrated. Flash chromatography (hexanes–EtOAc, 1:1) gave (−)-(2*S*,5*R*)-**13** (67 mg, 65%) as a colorless oil. $[\alpha]_D^{20} = -79.6$ (*c* 1.30, $CHCl_3$); ee = 96%, chiral HPLC. Spectral data as above.

3.11. *tert*-Butyl 4-(acetoxymethyl)-6-(hydroxymethyl)-2,2-dimethyldihydro-3a*H*-[1,3]dioxolo[4,5-*c*]pyrrole-5-(4*H*)-carboxylate 14

3.11.1. Enzymatic acylation of diol 10

To a solution of diol **10** (50 mg, 0.16 mmol) in vinyl acetate (5 mL) was added *C. antarctica* lipase B (50 mg). The mixture was stirred at room temperature for 96 h. The enzyme was removed by filtration, and the filtrate was evaporated to dryness. The crude product was purified by flash chromatography (CH_2Cl_2–acetone, 3:1) to provide (−)-**14** (51 mg, 90%) as a colorless oil. $[\alpha]_D^{20} = -38.2$ (*c* 1.20, $CHCl_3$); ee = 97%, chiral HPLC. IR (NaCl) 3391, 2108, 1673, 1398, 869 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 1H), 1.31 (m, 6H), 1.45 (s, 9H), 2.07 (s, 3H), 3.65–4.53 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.2, 25.5, 27.5, 28.5, 63.6, 64.3, 64.5, 67.0, 81.6, 81.8, 82.1, 112.3, 153.9, 164.2; HRMS (CI, NH_3) calcd for $C_{16}H_{28}NO_7$ $(MH)^+$: 346.1866. Found: 346.1871.

3.11.2. Enzymatic hydrolysis of diacetate 9

Diester **9** (120 mg, 0.31 mmol) was emulsified in a phosphate buffer (10 mL, 0.5 M, pH 7.6) and *Pseudomonas* sp. lipase was added (50 mg). After 72 h, the aqueous mixture was

filtered and extracted with CH_2Cl_2 (3×25 mL). The organic layer was dried over $MgSO_4$ and concentrated. Flash chromatography (CH_2Cl_2–acetone, 3:1) provided (−)-**14** (64 mg, 60%) as a colorless oil. $[\alpha]_D^{20} = -35.4$ (*c* 0.80, $CHCl_3$); ee = 90%, chiral HPLC. Spectral data as above.

3.12. Determination of absolute configuration by chemical correlation

3.12.1. Reduction of 13 to 12

Catalytic hydrogenation of (−)-(2*S*,5*R*)-**13** following the procedure used for the transformation of **6** into **3** provided (−)-(2*S*,5*R*)-**12**.

3.12.2. *tert*-Butyl (2*R*,3*R*,4*S*,5*S*)-2-(acetoxymethyl)-3,4-dihydroxy-5-(hydroxymethyl)-pyrrolidine-1-carboxylate 15

Dihydroxylation of (−)-(2*S*,5*R*)-**13** following the procedure used for the transformation of **7** into **8** provided (−)-(2*S*,5*R*)-**15** as a colorless oil. $[\alpha]_D^{20} = -8.1$ (*c* 1.50, $CHCl_3$); from PSL hydrolysis (ee = 94%); IR (NaCl) 3500, 2934, 2101, 1668, 1407, 1254, 1146, 1031 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.06 (s, 3H), 3.70–4.19 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.2, 28.5, 63.1, 63.2, 64.4, 65.1, 72.4, 72.5, 81.9, 156.7, 171.0; HRMS (CI, NH_3) calcd for $C_{13}H_{24}NO_7$ $(MH)^+$: 306.1553. Found: 306.1548.

3.12.3. Preparation of 14 from 15

Acetalization of (−)-(2*R*,3*R*,4*S*,5*S*)-**15** with acetone following the procedure used for the transformation of **8** into **9** provided (−)-(3a*R*,4*R*,6*S*,6a*S*)-**14**. Spectroscopic data as above.

3.12.4. Preparation of 11 from 16

In a hydrogenation cell, monoester (+)-(2*S*,5*R*)-**16** (130 mg, 0.49 mmol) and *tert*-butoxycarbonyl anhydride (118 mg, 0.54 mmol) were dissolved in MeOH (20 mL). Under a nitrogen atmosphere, $Pd(OH)_2/C$ (20% wt., 20 mg) was added. The cell was purged twice (H_2, then vacuum) and agitated mechanically while hydrogen pressure was adjusted to 54 psi. After 24 h, the mixture was diluted with EtOAc (50 mL), washed with 1 M HCl (3×50 mL), $NaHCO_3$ (3×50 mL), and brine (1×50 mL). The organic phase was dried over $MgSO_4$, concentrated, and the residue was purified by flash chromatography (EtOAc–MeOH, 9:1) to provide (+)-(2*S*,5*R*)-**11** (134 mg, 100%) as a colorless oil. $[\alpha]_D^{22} = +10.7$ (*c* 1.20, MeOH); from PLE hydrolysis of **1** to (+)-**16**: ee = 82%, chiral HPLC. Spectroscopic data as above.

Acknowledgment

We acknowledge the financial support of this work by the Natural Sciences and Engineering Research Council of Canada (NSERC).

References

1. Daly, J. W.; Spande, T. F.; Garraffo, H. M. *J. Nat. Prod.* **2005**, *68*, 1556–1575.
2. O'Hagan, D. *Nat. Prod. Rep.* **2000**, *17*, 435–446.
3. Daly, J. W.; Spande, T. F. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Wiley: New-York, 1986; Vol. 4, chapter 1, pp 1–274.
4. Bellina, F.; Rossi, R. *Tetrahedron* **2006**, *62*, 7213–7256.
5. Devi, N. S.; Perumal, S. *Tetrahedron* **2006**, *62*, 5931–5936.
6. Curtis, K. L.; Fawcett, J.; Handa, S. *Tetrahedron Lett.* **2005**, *46*, 5297–5300.
7. Sun, H.; Abboud, K. A.; Horenstein, N. A. *Tetrahedron* **2005**, *61*, 10462–10469.
8. *Iminosugars as Glycosidase Inhibitors: Nojirimycine and Beyond*; Stutz, A. E., Ed.; Wiley-VCH: Weinheim, 1999.
9. *Iminosugars: From Synthesis to Therapeutic Applications*; Compain, P., Martin, O. R., Eds.; Wiley: Chichester, 2008.
10. Kocovsky, P.; Malkov, A. V. *Tetrahedron* **2006**, *62*.
11. Dalko, P. I.; Moisan, L. *Angew. Chem., Int. Ed.* **2004**, *43*, 5138–5175.
12. Kim, B. H.; Lee, H. B.; Hwang, J. K.; Kim, Y. G. *Tetrahedron: Asymmetry* **2005**, *16*, 1215–1220.
13. Fache, F.; Schulz, E.; Tommasino, M. L.; Lemaire, M. *Chem. Rev.* **2000**, *100*, 2159–2231.
14. Sweet, J. A.; Cavallari, J. M.; Price, W. A.; Ziller, J. W.; McGrath, D. V. *Tetrahedron: Asymmetry* **1997**, *8*, 207–211.
15. Whitesell, J. K. *Chem. Rev.* **1989**, *89*, 1581–1590.
16. Minatti, A.; Muñiz, K. *Chem. Soc. Rev.* **2007**, *36*, 1142–1152.
17. Pandey, G.; Banerjee, P.; Gadre, S. R. *Chem. Rev.* **2006**, *106*, 4484–4517.
18. Davis, F. A.; Xu, H.; Wu, Y.; Zhang, J. *Org. Lett.* **2006**, *8*, 2273–2276.
19. Vasse, J. L.; Joosten, A.; Denhez, C.; Szymoniak, J. *Org. Lett.* **2005**, *7*, 4887–4889.
20. Mitchinson, A.; Nadin, A. *J. Chem. Soc., Perkins Trans. 1* **2000**, 2862–2892.
21. Pichon, M.; Figadère, B. *Tetrahedron: Asymmetry* **1996**, *7*, 927–964.
22. Garcia-Urdiales, E.; Alfonso, I.; Gotor, V. *Chem. Rev.* **2005**, *105*, 313–354.
23. Fraenkel, G.; Duncan, J. H.; Wang, J. *J. Am. Chem. Soc.* **1999**, *121*, 432–443.
24. Kemp, D. S.; Curran, T. P. *J. Org. Chem.* **1988**, *53*, 5729–5731.
25. Boutelje, J.; Hjalmarsson, M.; Hult, K.; Lindbäck, M.; Norin, T. *Bioorg. Chem.* **1988**, *16*, 364–375.
26. Cignarella, G.; Nathansohn, G. *J. Org. Chem.* **1961**, *26*, 1500–1504.
27. Sasaki, N. A.; Sagnard, I. *Tetrahedron* **1994**, *50*, 7093–7108.
28. Donohoe, T. J.; Headley, C. E.; Cousins, R. P. C.; Cowley, A. *Org. Lett.* **2003**, *5*, 999–1002.
29. Donohoe, T. J.; Rigby, C. L.; Thomas, R. E.; Nieuwenhuys, W. F.; Bhatti, F. L.; Cowley, A. R.; Bhalay, G.; Linney, I. D. *J. Org. Chem.* **2006**, *71*, 6298–6301.
30. Izquierdo, I.; Plaza, M. T.; Rodriguez, M.; Tamayo, J. *Tetrahedron: Asymmetry* **1999**, *10*, 449–455.
31. Morgan, B.; Dodds, D. R.; Zaks, A.; Andrews, D. R.; Klesse, R. *J. Org. Chem.* **1997**, *62*, 7736–7743.