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Chemoenzymatic synthesis of (3*R*,4*S*)- and (3*S*,4*R*)-3-methoxy-4-methylaminopyrrolidine

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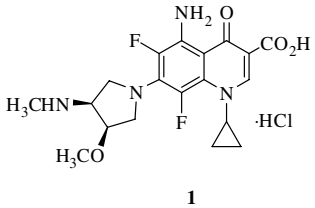
Abstract—An efficient and a convenient enantioselective synthesis of (3*R*,4*S*)-3-methoxy-4-methylaminopyrrolidine has been carried out by a lipase-mediated resolution protocol. This method describes the preparation of (±)-1-Cbz-*cis*-3-azido-4-hydroxypyrrolidine starting from commercially available diallylamine followed by ring-closing metathesis (RCM) via S_N2 displacement reactions. *Pseudomonas cepacia* lipase immobilized on diatomaceous earth (Amano PS-D) provides (3*R*,4*S*)-**11** and (3*S*,4*R*)-**12** in an excellent enantiomeric excess.

1. Introduction

Many synthetic and naturally occurring biologically active compounds contain chiral pyrrolidines as subunits in their structures.[1] It was first demonstrated by Okada et al.[2,3] that (3*R*,4*S*)-3-methoxy-4-methylaminopyrrolidine **3a** is an important subunit linked at the C-7 position of quinolone carboxylic acid derivative **1** and has shown a higher in vitro and in vivo antibacterial activity for both Gram positive and Gram negative bacteria.



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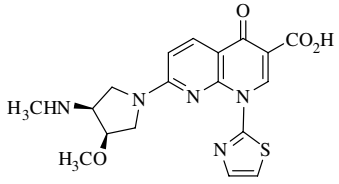

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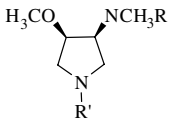

(*R*,*S*)

3a: R = R' = H
3b: R = R' = Boc

Recently, Tomita et al.[4] as well as Tsuzuki et al.[5] have reported a (3*R*,4*S*)-3-methoxy-4-methylaminopyrrolidine **3a** subunit at the C-7 position of naphthyridine ring **2** to obtain a high cytotoxicity against murine P388 leukaemia cells. Furthermore, detailed structure–activity relationship (SAR) studies in this area reveal that a *cis*-configuration and stereochemistry at the C-3,4 positions of the C-7 substituted pyrrolidine ring is responsible for the antibacterial activities in vitro as well as in vivo of these compounds. Literature studies indicate that (3*R*,4*S*)-3-methoxy-4-methylaminopyrrolidine **3a** linked to a quinolone carboxylic acid exhibits a superior in vitro antibacterial activity than did the corresponding racemate. Thus, it is important to develop a practical synthetic strategy for the preparation of this pyrrolidine intermediate in an enantiomerically pure form. There are very few methods that have been reported for the stereospecific synthesis of this biologically active pyrrolidine intermediate. These methods involve either a chiron approach[6,7] or a resolution of the racemates.[3]

Recently, we have reported an highly efficient lipase-mediated resolution protocol for the synthesis of (3*S*,4*S*)- and (3*R*,4*R*)-3-methoxy-4-methylaminopyrrolidines.[8] These results encouraged us to develop a convenient chemoenzymatic method for the synthesis of (3*R*,4*S*)- and (3*S*,4*R*)-3-methoxy-4-methylaminopyrrolidines involving ring-closing metathesis and lipase-mediated kinetic resolution strategies.

2. Results and discussion

In the present synthetic strategy, inexpensive commercially available diallylamine **4** has been utilized as the starting

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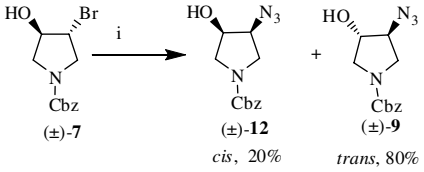
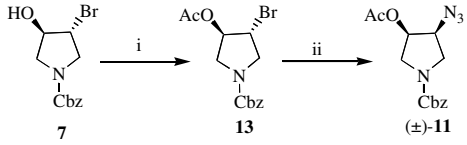
material. The Cbz protection of diallylamine **4** in the presence of a base gives Cbz protected diallylamine **5** followed by ring-closing metathesis employing Grubbs' catalyst to yield 1-Cbz-3-pyrroline **6**. The epoxidation of **6** with *m*-chloroperbenzoic acid gives lower yields[9] (maximum 46%). In order to improve the yields of the epoxidation procedure, a two-step procedure has been employed. In the first step, compound **6** has been converted into its *trans*-bromohydrin **7** employing *N*-bromosuccinimide in DMSO–H_2O. In the second step, alkaline treatment of compound **7** with 1 M NaOH gave pyrroline epoxide **8** in high yields. Epoxide **8**, upon treatment with sodium azide, yielded 1-Cbz-*trans*-3-azido-4-hydroxypyrrolidine **9**. The required *cis* configuration of the azido alcohol has been attained by mesylation of compound **9** to give mesylated *trans*-azido alcohol **10**. The S_N2 displacement reaction of mesylated product **10** with potassium acetate in DMF under reflux conditions gave *cis*-azido acetate **11** with a complete inversion of configuration. The deacetylation was carried out under mild conditions with anhydrous K_2CO_3 to give (±)-1-Cbz-*cis*-3-azido-4-hydroxypyrrolidine **12**, which is required for the lipase-mediated kinetic resolution (Scheme 1).

Attempts have been made to reduce the number of synthetic steps. With this in mind, an S_N2 displacement reaction of the bromo functionality of compound **7** was carried out by an azide nucleophile and it was observed that a mixtures of *cis*- and *trans*-azido alcohols (20:80%) was obtained during this reaction. A plausible mechanism for the formation of *trans*-azido alcohol (±)-**9** from compound **7** may be by the formation of an epoxide under refluxing conditions, followed by the ring opening by azide nucleophile. The products are confirmed by ^{1}H NMR spectroscopy and their percentage ratio is calculated by HPLC analysis (Scheme 2).

To eliminate the formation of *trans*-azido alcohol and to obtain *cis*-azido alcohol exclusively, a two-step procedure was employed. In the first step, the hydroxyl functionality



Scheme 2. Reagents and conditions: (i) NaN_3, DMF, reflux, 10 h.

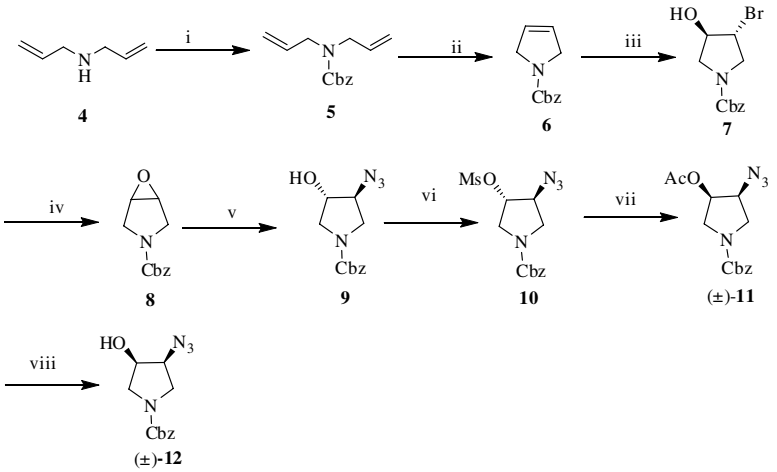


Scheme 3. Reagents and conditions: (i) CH_3COCl, Et_3N, 0 °C, 3 h; (ii) NaN_3, DMSO, 100 °C, 6 h.

of compound **7** is protected as its acyl derivative **13** under mild conditions. The next step is the S_N2 displacement reaction with sodium azide of compound **13** in dry DMSO at 100 °C, which gives racemic *cis*-azido acetate **11** as shown in Scheme 3. The resulting *cis*-azido acetate, which upon treatment with anhydrous K_2CO_3 gave racemic *cis*-azido alcohol **12**. In view of our interest in enzyme-mediated kinetic resolution of chiral building blocks,[10] we performed the resolution of (±)-**11** and (±)-**12** by employing different lipases.

2.1. Lipase screening and enzyme concentration

The selection of suitable lipases is an important aspect for developing an efficient resolution protocol.[11] Herein we carried out the transesterification as well as alcoholysis. Ten lipases from different sources were screened for this lipase-mediated transesterification of racemic vicinal azido alcohol (±)-**12** with isopropenyl acetate in diisopropyl ether. The results obtained are summarized in Table 1. It was observed that lipases from *Pseudomonas cepacia*



Scheme 1. Reagents and conditions: (i) CbzCl, Et_3N, CH_2Cl_2, rt, 10 h; (ii) $(Pcy_2)_2Cl_2Ru{=}CHPh$, CH_2Cl_2, rt, 6 h; (iii) NBS, DMSO–H_2O, rt, 2 h; (iv) 1 M NaOH, rt, 1 h; (v) NaN_3, 1,4-dioxane–H_2O, reflux, 12 h; (vi) MsCl, Et_3N, CH_2Cl_2, rt, 4 h; (vii) AcOK, DMF, reflux, 3 h; (viii) anhydrous K_2CO_3, MeOH, rt, 1 h.

Table 1. Transesterification of (±)-1-Cbz-*cis*-3-azido-4-hydroxypyrrolidine **12**[a]

Lipase	Equivalents (w/w)	Time[b] (h)	ee[c] (%)		Conversion (%)
			Alcohol (3*S*,4*R*)-**12**	Acetate (3*R*,4*S*)-**11**	
PS-C	1	8	97	59	62.1
PS-D	1	9	94	86	52.2
PS-C	0.5	12	94	72	56.6
PS-D	0.5	14	92	97	48.6
PS	1	68	64	88	42.1
CAL	1	72	47	65	41.9
CRL	1	60	52	89	36.8
CCL	1	120	38	74	33.9
Lipozyme	1	120	14	86	14
PFL	1	168	—	—	—
AK-20	1	168	—	—	—
PPL	1	168	—	—	—

[a] Conditions: **12** (1 mmol), diisopropyl ether (10 mL), lipase, isopropenyl acetate (6 mmol).
[b] Time taken for transesterification.
[c] Determined by chiral HPLC analysis of the reaction mixture employing a Daicel Chiralcel OD column (0.46 × 25 cm); eluent: hexane/isopropanol = 80:20; flow rate: 0.5 mL/min; detector: 254 nm.

(PS), *Candida antarctica* (CAL), *Candida rugosa* (CRL), *Candida cylindracea* (CCL) and *Mucor miehei* (Lipozyme) showed comparatively low enantioselectivities, whereas *Pseudomonas fluorescens* (PFL), AK-20 and *porcine pancreatic* lipase (PPL) did not show any conversions, even after prolonged reactions. The lipases from *P. cepacia* immobilized on ceramic particles (PS-C) and lipase *P. cepacia* immobilized on diatomaceous earth (PS-D) provided encouraging results with respect to conversion and enantioselectivity. Immobilized *P. cepacia* on ceramic particles (PS-C) gives a lower enantioselectivity of the azido acetate (3*R*,4*S*)-**11** 59% ee, when 1 equiv (w/w) of lipase is employed and gives a 72% ee, with 0.5 equiv (w/w) of lipase. However, the corresponding azido alcohol (3*S*,4*R*)-**12** was obtained in a 97% ee and a 94% ee. Immobilized *P. cepacia* on diatomaceous earth (PS-D) has also been examined under similar reaction conditions. When 1 equiv (w/w) of lipase was used, azido alcohol (3*S*,4*R*)-**12** was obtained in a 94% ee, whereas azido acetate (3*R*,4*S*)-**11** in a 86% ee. However, when 0.5 equiv (w/w) lipase was used, azido alcohol (3*S*,4*R*)-**12** was obtained in a 92% ee and the corresponding azido acetate (3*R*,4*S*)-**11** in a 97% ee. Therefore, based on these results, lipase PS-D was chosen for further studies. However, in earlier studies the transesterification of *trans*-azido alcohol (±)-**9** using lipase PS-C gave better results compared to lipase PS-D.[8]

2.2. Effect of solvent

Solvent variation in lipase-catalyzed kinetic resolution is known to influence the enantiomeric and enantiotopic selectivity as well as the reaction rate.[12] In the present study, five different solvents were examined using PS-D lipase and isopropenyl acetate. It has been observed that THF, *tert*-butyl methyl ether (TBME) and hexane are not suitable solvents for obtaining good enantioselectivities and conversions. Toluene takes a longer reaction time to reach 50% conversion and shows comparatively lower enantiomeric purities as shown in Table 2. Diisopropyl ether gives good results with respect to conversions and enantioselectivities. Moreover, it takes a shorter reaction time. Therefore, diisopropyl ether has been selected as a suitable solvent for these resolution processes.

2.3. Effect of acyl donor

Acyl donors also play a crucial role in achieving better enantioselectivities.[13] In the present protocol two different acyl donors, that is, isopropenyl acetate and vinyl acetate, have been employed using lipase PS-D in diisopropyl ether at 40 °C. It was observed that vinyl acetate takes 9 h to reach about 50% conversion and the enantiomeric excess of azido alcohol (3*S*,4*R*)-**12** and azido acetate (3*R*,4*S*)-**11**

Table 2. Effect of different solvents on transesterification of (±)-1-Cbz-*cis*-3-azido-4-hydroxypyrrolidine **12**[a]

Solvent	Lipase equivalents (w/w)	Time[b] (h)	ee[c] (%)		Conversion (%)
			Alcohol (3*S*,4*R*)-**12**	Acetate (3*R*,4*S*)-**11**	
DIPE	0.5	14	92	97	48.6
Toluene	0.5	66	81	67	54.7
TBME	0.5	47	61	88	40.9
Hexane	0.5	60	38	72	34.5
THF	0.5	48	41	89	31.5

[a] Conditions: **12** (1 mmol), solvent (10 mL), lipase PS-D, isopropenyl acetate (6 mmol).
[b] Time taken for transesterification.
[c] Determined by chiral HPLC analysis of the reaction mixture employing Daicel Chiralcel OD column (0.46 × 25 cm); eluent: hexane/isopropanol = 80:20; flow rate: 0.5 mL/min; detector: 254 nm.

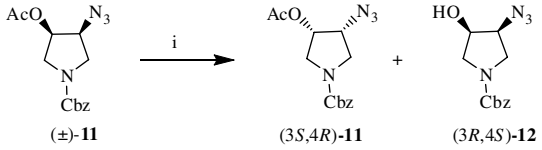
are 82% and 68% ee, respectively. However, isopropenyl acetate takes 14 h to reach about 50% conversion and azido alcohol (3*S*,4*R*)-**12** is obtained in a 92% ee and azido acetate (3*R*,4*S*)-**11** in a 97% ee.

2.4. Enzymatic alcoholysis

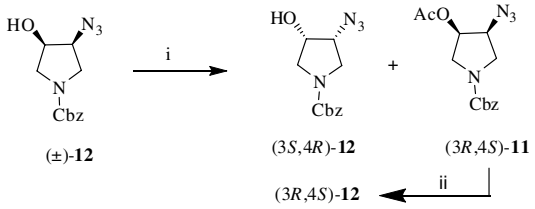
Lipase-mediated alcoholysis of (±)-1-Cbz-*cis*-3-acetyloxy-4-azidopyrrolidine **11** has been attempted by employing two different alcohols, namely, *n*-butanol and 2-propanol. The reaction rates of lipase-mediated alcoholysis are quite slow when 1 equiv of lipase (w/w) was used, as shown in Scheme 4. The lipase PS-D in *n*-butanol gives moderately better results. This reaction required nearly 54 h to reach about 50% conversion and azido alcohol (3*R*,4*S*)-**12** was obtained in a 76% ee and azido acetate (3*S*,4*R*)-**11** in a 84% ee. The enantiopurities of the corresponding products are lower in comparison to the transesterification process. Therefore, lipase-mediated alcoholysis was not considered practical for achieving good enantiopurities although some good results obtained in this investigation have been summarized in Table 3.

Thus, the lipase-catalyzed transesterification of (±)-1-Cbz-*cis*-3-azido-4-hydroxypyrrolidine **12** has been carried out by employing 0.5 equiv of PS-D lipase (w/w) and isopropenyl acetate in diisopropyl ether at 40 °C. The reaction progress was monitored by chiral HPLC employing a 'Chiralcel OD' column. In order to obtain good enantioselectivities and yields, the reaction was stopped when it reached about 50% conversion. The pure products were isolated by silica gel column chromatography to obtain azido alcohol (3*S*,4*R*)-**12** in a 92% ee and a 48% yield, while the corresponding azido acetate (3*R*,4*S*)-**11** in a 97% ee and a 52% yield. The deacetylation of (3*R*,4*S*)-**11** using anhydrous K_2CO_3 in methanol gave (3*R*,4*S*)-**12** in a 97% ee (Scheme 5).

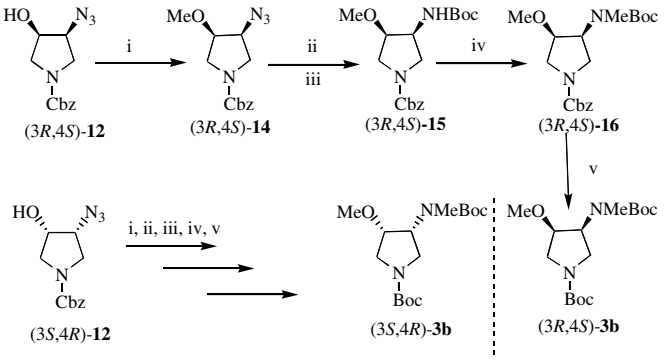
The enantiomerically enriched (3*R*,4*S*)-1-Cbz-3-azido-4-hydroxypyrrolidine **12** obtained by the lipase-mediated kinetic resolution of (±)-**12** was converted to its corresponding methyl ether (3*R*,4*S*)-**14** using CH_3I with NaH as a base. The azide functionality of (3*R*,4*S*)-**14** was selectively reduced under mild reaction conditions using TPP in THF and H_2O at room temperature to give the amine, which has been protected in the same pot with $(Boc)_2O$ to give Boc protected amine (3*R*,4*S*)-**15**. N-Methylation of compound **15** was performed with CH_3I and NaH in dry DMF under an N_2 atmosphere to give (3*R*,4*S*)-**16**. Finally, Cbz deprotection of (3*R*,4*S*)-**16** was carried out under extremely mild conditions employing poly(methylhydrosiloxane) (PMHS) over 10% Pd–C in ethanol followed by treatment with $(Boc)_2O$ to give the desired product (3*R*,4*S*)-**3b** with a good enantiomeric excess (97%). The overall yields of these reactions are also good. The other enantiomer (3*S*,4*R*)-**12** was obtained by employing the same reaction sequence and was converted to (3*S*,4*R*)-**3b** in a 92% ee (Scheme 6). The absolute configuration of these enantiomers (3*R*,4*S*)- and (3*S*,4*R*)-**3b** were



Scheme 4. Reagents and conditions: (i) lipase PS-D, *n*-butanol, DIPE.



Scheme 5. Reagents and conditions: (i) lipase PS-D, IPA, DIPE, 40 °C, 14 h; (ii) anhydrous K_2CO_3, CH_3OH, rt, 1 h.



Scheme 6. Reagents and conditions: (i) NaH, MeI, dry THF, 14 h; (ii) PPh_3, THF:H_2O, rt, 2 h; (iii) $(Boc)_2O$, Et_3N, rt, 6 h; (iv) NaH, MeI, DMF, rt, 6 h; (v) 10% Pd–C, PMHS, $(Boc)_2O$, Et_3N, EtOH, rt, 6 h.

Table 3. Enzymatic alcoholysis of (±)-1-Cbz-*cis*-3-acetyloxy-4-azidopyrrolidine **11**[a]

Lipase	Time[b] (h)	Alcohol	Solvent	ee[c] (%)		Conversion (%)
				Alcohol (3*R*,4*S*)-**12**	Acetate (3*S*,4*R*)-**11**	
PS-D	54	*n*-Butanol	DIPE	76	84	52.5
PS-D	68	2-Propanol	DIPE	72	86	54.4
PS-D	84	*n*-Butanol	Toluene	63	80	55.9
PS-C	72	*n*-Butanol	DIPE	72	75	51.0

[a] Conditions: **11** (0.5 mmol), diisopropyl ether (5 mL), lipase (1 equiv w/w), alcohol (3 mmol).
[b] Time taken for alcoholysis.
[c] Determined by chiral HPLC analysis of the reaction mixture employing a Daicel Chiralcel OD column (0.46 × 25 cm); eluent: hexane/isopropanol = 80:20; flow rate: 0.5 mL/min; detector: 254 nm.

confirmed by comparison of their specific rotations with those previously reported in the literature.[3]

3. Conclusion

In conclusion we have developed a simple and highly efficient method for the preparation of optically active (3*R*,4*S*)-**3b** and (3*S*,4*R*)-**3b**. The lipase-mediated kinetic resolution has been carried out by using *P. cepacia* lipase immobilized on diatomaceous earth PS-D to give excellent results for the *cis*-azido alcohol. We have demonstrated an alternative chemoenzymatic approach for the synthesis of (3*R*,4*S*)- and (3*S*,4*R*)-3-methoxy-4-methylaminopyrrolidine. Moreover, this protocol provides good conversions with a high enantiomeric excess.

4. Experimental

4.1. Material and methods

Enzymatic reactions were carried out on a 'Lab-line environ-shaker' at 150 rpm. Infrared spectra of neat samples are reported in wave numbers (cm^{-1}). ^1H NMR was recorded as solutions in $CDCl_3$ and chemical shifts are reported in parts per million (ppm, δ) on a 200 MHz instrument. Coupling constants are reported in hertz (Hz). LSIMS mass spectra were recorded on Autospec M. with a 7 kV acceleration voltage and 25 kV gun voltage. HPLC analysis was performed on an instrument that consisted of a Shimadzu LC-10AT system controller, SPD-10A fixed wavelength UV monitor as the detector. Specific rotations were recorded on SEPA-300 Horiba high sensitive polarimeter, fixed with a sodium lamp of wavelength 589 nm.

4.2. Chemicals and enzymes

Diallylamine, sodium azide, NBS, Grubb's catalyst, methane sulfonylchloride, PMHS, sodium hydroxide and solvents were obtained commercially and used without purification. *P. cepacia* lipase immobilized on ceramic particles (PS-C) and *P. cepacia* lipase immobilized on diatomaceous earth (PS-D) were purchased from Amano (Nagoya, Japan).

4.2.1. 1-Cbz-*N*,*N*-Diallylamine 5. To a solution of diallylamine **4** (4.85 g, 50 mmol) and Et_3N (9 mL, 65 mmol) in dry CH_2Cl_2 (75 mL) was added benzyl chloroformate (18.4 mL, 55 mmol of 50% solution in toluene) dropwise over a period of 30 min at 0 °C. The reaction mixture was stirred at room temperature for 10 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel column chromatography to give pure 10.62 g of **5**. Yield: 92%; IR (neat): 3072, 1703, 1629 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.86 (4H, s), 5.11 (6H, m), 5.74 (2H, s), 7.30 (5H, m); EIMS (*m*/*z*): 231 (M^+). Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.61; H, 7.32; N, 5.99.

4.2.2. 1-Cbz-3-Pyrroline 6. The 1-Cbz-*N*,*N*-diallylamine **5** (6.5 g, 28.1 mmol) was dissolved in dry CH_2Cl_2 (950 mL) under nitrogen. Grubbs' catalyst (0.464 g, 0.588 mmol, 2 mol %) was added and the solution stirred at room temperature under nitrogen. After 6 h, the solvent was evaporated and the residue purified on silica gel column chromatography to afford 5.14 g pure 1-Cbz-3-pyrroline **6**. Yield: 90%; IR (neat): 3061, 1705, 1624 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 4.19 (4H, s), 5.12 (2H, s), 5.67–5.85 (2H, q, $J = 13.91$, 6.59 Hz), 7.32 (5H, m); EIMS (*m*/*z*): 203 (M^+). Anal. Calcd for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 70.86; H, 6.35; N, 6.82.

4.2.3. 1-Cbz-*trans*-3-Bromo-4-hydroxypyrrolidine 7. To a stirred mixture of **6** (5.1 g, 24.8 mmol), DMSO (70.7 mL) and H_2O (3.4 mL), NBS (5.36 g, 29.8 mmol) was gradually added over 15 min at 0 °C. After stirring at room temperature for 2 h, water was added and the reaction mixture extracted with ethyl acetate. The organic layer was washed with a saturated solution of NaCl, dried over anhydrous Na_2SO_4 and then concentrated. The residue obtained was chromatographed on silica gel to give 6.48 g of pure bromohydrin **7**. Yield: 86%; IR (neat): 3380, 3010, 1710 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 3.44 (1H, d, $J = 12.02$ Hz), 3.68–4.16 (5H, m), 4.36 (1H, s), 5.09 (2H, s), 7.30 (5H, m); FABMS (*m*/*z*): 302 (M^++2). Anal. Calcd for $C_{12}H_{14}BrNO_3$: C, 48.02; H, 4.70; N, 4.67. Found: C, 47.96; H, 4.64; N, 4.62.

4.2.4. 1-Cbz-*trans*-3-Acetyloxy-4-bromopyrrolidine 13. To a stirred solution of **7** (0.3 g, 1 mmol) in dry CH_2Cl_2 (10 mL) was added Et_3N (0.16 mL, 1.5 mmol) and then acetyl chloride (0.078 mL, 1.1 mmol) gradually at 0 °C. After stirring at room temperature for 3 h, water was added and the reaction mixture extracted with CH_2Cl_2. The organic layer was washed with the saturated solution of NaCl, dried over anhydrous Na_2SO_4 and then concentrated. The residue obtained was chromatographed on silica gel to give 0.29 g of pure **13**. Yield: 86%; IR (neat): 3021, 1705, 1739 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.07 (3H, s), 3.82–4.07 (4H, m), 4.23 (1H, d, $J = 3.3$ Hz), 5.13 (2H, s), 5.27 (1H, d, $J = 4.2$ Hz), 7.34 (5H, m); FABMS (*m*/*z*): 344 (M^++2). Anal. Calcd for $C_{14}H_{16}NBrO_4$: C, 49.14; H, 4.71; N, 4.09. Found: C, 49.08; H, 4.64; N, 4.01.

4.2.5. 1-Cbz-3,4-Epoxypyrrolidine 8. Bromohydrin **7** (6.2 g, 20.6 mmol) was dissolved in MeOH (72.9 mL) and a solution of aqueous NaOH (30.9 mL solution of 1 M, 30.9 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 1 h. Methanol was evaporated under reduced pressure, and water added and extracted with ethyl acetate. The organic layer was washed with a saturated solution of NaCl, dried over anhydrous Na_2SO_4 and then concentrated, the residue obtained was chromatographed on silica gel to give of 3.68 g pure epoxide **8**. Yield: 82%; IR (neat): 3023, 1698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.35 (2H, d, $J = 12.08$ Hz), 3.63 (2H, d, $J = 3.3$ Hz), 3.78–3.90 (2H, t, $J = 12.84$ Hz), 5.08 (2H, m), 7.30 (5H, m); EIMS (*m*/*z*): 219 (M^+). Anal. Calcd for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.69; H, 5.91; N, 6.35.

4.2.6. 1-Cbz-*trans*-3-Azido-4-hydroxypyrrolidine 9. Epoxide **8** (3.65 g, 16.5 mmol) was dissolved in 1,4-dioxane (50 mL) and water (10 mL), after which sodium azide (1.40 g, 20 mmol) was added at room temperature. The reaction mixture was stirred at 100 °C for 12 h. After completion of the reaction, the reaction mixture was brought to room temperature and water was added and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue subjected for silica gel column chromatography to give 3.78 g of pure racemic *trans*-azido alcohol **9**. Yield: 86%; IR (neat): 3401, 3033, 2107, 1689 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 3.31–3.73 (5H, m), 3.89 (1H, s), 4.13 (1H, s), 5.07 (2H, s), 7.31 (5H, m); FABMS (*m*/*z*): 263 (M^++1). Anal. Calcd for $C_{12}H_{14}N_4O_3$: C, 54.96; H, 5.38; N, 21.36. Found: C, 54.91; H, 5.31; N, 21.29.

4.2.7. 1-Cbz-*trans*-3-Azido-4-[(methylsulfonyl)oxy]pyrrolidine 10. A solution of methanesulfonyl chloride (1.2 mL, 15 mmol) in dry CH_2Cl_2 (10 mL) was added to a mixture of **9** (3.75 g, 14 mmol) and Et_3N (2.49 mL, 17 mmol) in dry CH_2Cl_2 over a period of 30 min at 0 °C. The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and solvent removed under reduced pressure. The residue was chromatographed on silica gel to give 4.37 g of pure mesylated product **10**. Yield: 90%; IR (neat): 3026, 2110, 1705, 1357, 1176, 936, 906 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 3.04 (3H, s), 3.52–3.81 (4H, m), 4.29 (1H, m), 4.95 (1H, m), 5.12 (2H, s), 7.33 (5H, m); FABMS (*m*/*z*): 341 (M^++1). Anal. Calcd for $C_{13}H_{16}N_4O_5S$: C, 44.88; H, 4.74; N, 16.46. Found: C, 44.79; H, 4.70; N, 16.39.

4.2.8. 1-Cbz-*cis*-3-Acetyloxy-4-azidopyrrolidine 11. *Method A*: Bromo acetate **13** (0.25 g, 0.728 mmol) was dissolved in dry DMSO (15 mL) and sodium azide (0.7 g, 1.09 mmol) was added at room temperature. The reaction mixture stirred at 100 °C for 6 h. After completion of the reaction, the reaction mixture was brought to room temperature and ice cold water was added and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue subjected for silica gel column chromatography to give 0.170 g of pure racemic *cis*-azido acetate **11**. Yield: 78%.

Method B: A mixture of **10** (4 g, 11 mmol) and potassium acetate (2.3 g, 23.5 mmol) in dry DMF (40 mL) was heated at 100 °C for 3 h under nitrogen atmosphere. The reaction mixture was poured into an cold ice water and extracted with ether. The ether layer was washed with brine, dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residue was chromatographed on silica gel to give 3 g of pure *cis*-azido acetate **11**. Yield: 86%; IR (neat): 3032, 2107, 1740, 1706 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.15 (3H, s), 3.37–3.58 (2H, m), 3.64–3.81 (2H, m), 4.08 (1H, m), 5.10 (2H, m), 5.22–5.36 (1H, m), 7.32 (5H, m); FABMS (*m*/*z*): 305 (M^++1). Anal. Calcd for $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.19; H, 5.24; N, 18.33.

4.2.9. 1-Cbz-*cis*-3-Azido-4-hydroxypyrrolidine 12. *cis*-Azido acetate **11** (3 g, 10.1 mmol) was dissolved in methanol (20 mL) and then anhydrous K_2CO_3 (3.49 g, 25.2 mmol) was added and the reaction stirred at room temperature for 1 h. The reaction mass was filtered on a Celite pad, the methanol evaporated and subjected to silica gel column chromatography to give 2.53 g pure *cis*-azido alcohol **12**. Yield: 98%; IR (neat): 3384, 3030, 2097, 1665 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.31–3.78 (4H, m), 3.90–4.05 (1H, m), 4.32 (1H, br s), 5.10 (2H, m), 7.32 (5H, m); FABMS (*m*/*z*): 263 (M^++1). Anal. Calcd for $C_{12}H_{14}N_4O_3$: C, 54.96; H, 5.38; N, 21.36. Found: C, 54.88; H, 5.34; N, 21.28.

4.2.10. General procedure for lipase-mediated alcoholysis. To a solution of racemic *cis*-azido acetate **11** (100 mg, 0.32 mmol) in diisopropyl ether (5 mL) lipase (100 mg, 1 equiv w/w) and alcohol (1.97 mmol) were added. The suspension was shaken at 220 rpm at 40 °C. The reaction was monitored by chiral HPLC and at 50% conversion the reaction was stopped, filtered and solvent was evaporated. The residue was chromatographed on silica gel to give enantiopure azido alcohol (3*R*,4*S*)-**12** and the corresponding azido acetate (3*S*,4*R*)-**11**. The enantiopurity of these products was analyzed by chiral HPLC and compared with the corresponding racemic products.

4.3. General procedure for lipase-mediated transesterification

To a solution of racemic *cis*-azido alcohol **12** (2.4 g, 9.1 mmol) in diisopropyl ether (50 mL) lipase PS-D (1.2 g, 0.5 equiv w/w) and isopropenyl acetate (6 mL, 54 mmol) were added. The suspension was shaken at 220 rpm at 40 °C. The reaction was monitored by chiral HPLC analysis, and 50% conversion occurred after 14 h. The reaction mixture was filtered and the solvent was evaporated. The residue was chromatographed on silica gel. The enantiopure products were analyzed by chiral HPLC and compared with the corresponding racemic products. Azido alcohol (3*S*,4*R*)-**12** was obtained (1.15 g). Yield: 48%; 92% ee; determined by HPLC analysis using Chiralcel OD column (hexane/isopropanol, 80:20) with 0.5 mL/min flow rate (t_{major} = 19.59, t_{minor} = 22.28 min); $[\alpha]_D^{25} = -9.5$ (*c* 1.01, $CHCl_3$) and the azido acetate (3*R*,4*S*)-**11** in 1.44 g. Yield: 52%, 97% ee; determined by the HPLC analysis using a Chiralcel OD column (hexane/isopropanol, 80:20) with 0.5 mL/min flow rate (t_{major} = 33.65, t_{minor} = 28.32 min); $[\alpha]_D^{25} = -29.3$ (*c* 1.04, $CHCl_3$).

4.3.1. (3*R*,4*S*)-1-Cbz-3-Azido-4-hydroxypyrrolidine 12. Prepared from (3*R*,4*S*)-**11** by a deacetylation procedure using anhydrous K_2CO_3 in methanol at room temperature to give 1.23 g of pure (3*R*,4*S*)-**12** *cis*-azido alcohol. Yield: 98%; 97% ee; determined by the HPLC analysis using a Chiralcel OD column (hexane/isopropanol, 80:20) with 0.5 mL/min flow rate (t_{major} = 22.77, t_{minor} = 21.18 min); $[\alpha]_D^{25} = +11.9$ (*c* 1.03, $CHCl_3$).

4.3.2. (3*R*,4*S*)-1-Cbz-3-Azido-4-methoxypyrrolidine 14. A solution of azido alcohol (3*R*,4*S*)-**12** (1.1 g, 4.1 mmol) in dry THF (10 mL) was added to a suspension of NaH

(60% dispersion in oil, 0.2 g, 5 mmol, washed with dry hexane before use) in dry THF (20 mL). After stirring at room temperature for 1 h, the mixture was gradually warmed to 50 °C over 2 h. The reaction mass was cooled to 0 °C and CH_3I (0.31 mL, 5.03 mmol) added and left at room temperature for 11 h. The reaction mass was quenched with saturated NH_4Cl and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum. The resulting mass was subjected for silica gel column chromatography to give 0.98 g of the methylated product (3*R*,4*S*)-**14**. Yield: 86%; 97% ee; determined by HPLC analysis using Chiralcel OD-H column (hexane/isopropanol, 90:10) with 0.5 mL/min flow rate ($t_{major} = 33.47$, $t_{minor} = 32.00$ min); $[\alpha]_D^{25} = -48.4$ (*c* 1, $CHCl_3$); IR (neat): 2943, 2104, 1693 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.45 (3H, s), 3.46–3.66 (4H, m), 3.97 (2H, m), 5.10 (2H, q, $J = 12.44$ Hz), 7.33 (5H, m); FABMS (*m*/*z*): 277 (M^++1). Anal. Calcd for $C_{13}H_{16}N_4O_3$: C, 56.51; H, 5.84; N, 20.28. Found: C, 56.46; H, 5.78; N, 20.17.

4.3.3. (3*S*,4*R*)-1-Cbz-3-Azido-4-methoxypyrrolidine 14. Prepared from (3*S*,4*R*)-**12** under the above mentioned conditions to give 0.96 g of (3*S*,4*R*)-**14**. Yield: 85%; 92% ee; determined by HPLC analysis using a Chiralcel OD-H column (hexane/isopropanol, 90:10) with 0.5 mL/min flow rate ($t_{major} = 31.12$, $t_{minor} = 34.18$ min); $[\alpha]_D^{25} = +46.0$ (*c* 1.05, $CHCl_3$).

4.3.4. (3*R*,4*S*)-1-Cbz-3-[(*tert*-Butoxycarbonyl)amino]-4-methoxypyrrolidine 15. The methylated azide (3*R*,4*S*)-**14** (0.9 g, 3.3 mmol) was dissolved in THF/H_2O (15:15 mL) and triphenylphosphine (1.73 g, 6.6 mmol) added at room temperature. The reaction was monitored by TLC until the starting material disappeared (approximately 2 h). The reaction mass was cooled to 0 °C after which Et_3N (0.73 mL, 5.2 mmol) and di-*tert*-butyldicarbonate (1.07 g, 4.9 mmol) were added dropwise. The reaction mixture was stirred at room temperature for 6 h and THF then removed under reduced pressure. The reaction mixture was diluted with water and extracted with ether. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on silica gel to give 1.06 g of pure (3*R*,4*S*)-**15**. Yield: 92%; 97% ee; determined by HPLC analysis using a Chiralcel OD-H column (hexane/isopropanol, 80:20) with 0.5 mL/min flow rate ($t_{major} = 12.90$, $t_{minor} = 14.42$ - min); $[\alpha]_{D_1}^{25} = -7.3$ (*c* 1.07, $CHCl_3$); IR (neat): 3336, 2973, 1702 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.44 (9H, s), 3.37 (3H, s), 3.39–3.85 (5H, m), 4.03–4.32 (1H, m), 5.09 (2H, m), 7.32 (5H, m); FABMS (*m*/*z*): 351 (M^++1). Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.67; H, 7.40; N, 7.87.

4.3.5. (3*S*,4*R*)-1-Cbz-3-[(*tert*-Butoxycarbonyl)amino]-4-methoxypyrrolidine 15. Prepared from (3*S*,4*R*)-**14** under the above mentioned conditions to give 1.06 g of (3*S*,4*R*)-**15**. Yield: 92%; 92% ee; determined by HPLC analysis using a Chiralcel OD-H column (hexane/isopropanol, 80:20) with 0.5 mL/min flow rate ($t_{major} = 14.30$, $t_{minor} = 12.82$ min); $[\alpha]_D^{25} = +5.4$ (*c* 1.07, $CHCl_3$).

4.3.6. (3*R*,4*S*)-1-Cbz-3-[(*tert*-Butoxycarbonyl)methylamino]-4-methoxypyrrolidine 16. Compound (3*R*,4*S*)-**15** (1 g, 2.8 mmol) was dissolved in dry DMF (10 mL) and added into a suspension of NaH (0.13 g, 3.4 mmol, washed with dry hexane before use) in dry DMF at 0 °C over a period of 15 min CH_3I (0.21 mL, 3.6 mmol) was added to the reaction mass at 0 °C and stirred at room temperature for 6 h. The reaction mass was quenched with saturated NH_4Cl and extracted with ether. The ether layer was washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed in vacuo. The residue obtained was subjected to silica gel column chromatography and gave 0.925 g of pure (3*R*,4*S*)-**16**. Yield: 89%; 97% ee; determined by the HPLC analysis using a Chiralcel OD-H column (hexane/isopropanol, 80:20) with 0.5 mL/min flow rate ($t_{major} = 11.12$, $t_{minor} = 15.79$ min); $[\alpha]_D^{25} = -42.6$ (*c* 1.05, $CHCl_3$); IR (neat): 2980, 1726, 1406, 1367, 1166 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.46 (9H, s), 2.89 (3H, s), 3.32 (3H, d, $J = 3.03$ Hz), 3.42–3.68 (4H, m), 3.92 (1H, s), 4.57 (1H, s), 5.12 (2H, m), 7.32 (5H, m); FABMS (*m*/*z*): 365 (M^++1). Anal. Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.57; H, 7.69; N, 7.58.

4.3.7. (3*S*,4*R*)-1-Cbz-3-[(*tert*-Butoxycarbonyl)methylamino]-4-methoxypyrrolidine 16. Prepared from (3*S*,4*R*)-**15** under the above mentioned conditions to give 0.92 g of (3*S*,4*R*)-**16**. Yield: 88%; 92% ee; determined by HPLC analysis using a Chiralcel OD-H column (hexane/isopropanol, 80:20) with 0.5 mL/min flow rate ($t_{major} = 15.22$, $t_{minor} = 11.20$ min); $[\alpha]_D^{25} = +40.6$ (*c* 1.02, $CHCl_3$).

4.3.8. (3*R*,4*S*)-1-*tert*-Butoxycarbonyl-3-methoxy-4{[*tert*-butoxycarbonyl]methylamino}pyrrolidine 3b. To a stirred solution of (3*R*,4*S*)-**16** (0.8 g, 2.1 mmol) in ethanol (10 mL) were added 3 mL of PMHS and 10% Pd–C (80 mg) under an N_2 atmosphere. Di-*tert*-butyldicarbonate (0.71 g, 3.2 mmol) and Et_3N (0.44 mL, 3.2 mmol) were added to the reaction mass and stirred at room temperature for 6 h. The reaction mixture was filtered on a Celite pad and the solvent evaporated in vacuo. The residue was purified on silica gel column chromatography to give 0.6 g of pure (3*R*,4*S*)-**3b**. Yield: 84%; 97% ee; $[\alpha]_D^{25} = -53.2$ (*c* 1.05, MeOH) [lit.[3] $[\alpha]_D^{20} = -54.4$ (*c* 1.07, MeOH), >99% ee]; IR (neat): 2939, 2358, 1689, 1376, 1156 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.46 (9H, s), 1.47 (9H, s), 2.91 (3H, s), 3.34 (3H, s), 3.36–3.60 (4H, m), 3.90 (1H, br s), 4.57 (1H, br s); ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.14, 28.18, 31.22, 44.99, 48.58, 49.33, 57.19, 57.46, 79.33, 79.63, 79.89, 80.51, 154.25, 155.64; FABMS (*m*/*z*): 331 (M^++1). Anal. Calcd for $C_{16}H_{30}N_2O_5$: C, 58.16; H, 9.15; N, 8.48. Found: C, 58.09; H, 9.12; N, 8.41.

4.3.9. (3*S*,4*R*)-1-*tert*-Butoxycarbonyl-3-methoxy-4{[*tert*-butoxycarbonyl]methylamino}pyrrolidine 3b. Prepared from (3*S*,4*R*)-**16** under the above mentioned conditions to give 0.56 g of (3*S*,4*R*)-**3b**. Yield: 80%; 92% ee; $[\alpha]_D^{25} = +50.9$ (*c* 1.01, MeOH), {lit.[3] $[\alpha]_D^{20} = +53.7$ (*c* 1.00, MeOH), >99% ee}.

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