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A new facile chemoenzymatic synthesis of levamisole

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Abstract—An efficient and facile chemoenzymatic synthesis of levamisole by employing lipase-mediated resolution of 3-hydroxy-3-phenylpropanenitrile followed by its conversion to β-amino alcohol as the key intermediate is described.

Levamisole (*lee-VAM-i-sole*) the levo isomer of tetramisole is a broad spectrum anthelmintic.[1] It is a synthetic imidazothiazole derivative belonging to a general class of agents called biologic response modifiers and has been originally designed for anthelmintic properties, has immunomodulating and immuno-stimulating properties and also has been later used in cancer adjuvant therapy and in the treatment of other ailments. It has found wide application in the treatment of worm infestations and eliminating intestinal parasites in both humans[2] and animals.[1b,2a] Levamisole is one of the non-specific immunomodulating agents used in clinical practice. It helps restore the function of certain cells of the body's defense system when they have been impaired. It is currently indicated in combination with 5-flurouracil (5-FU) as adjuvant treatment after surgical resection of stage TNM 3 or Duke's C colon cancer over a duration of one year postoperatively, and is also combined with radiation therapy for Duke's stage B2 and TNM stage 4 cancer.[3]

In spite of the biological importance of this compound there are not many reports of its preparation. The racemic form of this compound has been prepared by Raeymaekers et al.[1b] employing phenacyl bromide. They have also prepared the optically pure form starting from optically pure phenylethylenediamine.[4] Achiwa and co-workers[5] employed rhodium catalyst in the asymmetric synthesis of levamisole.

Based on the retrosynthetic strategy optically pure 3-hydroxy-3-phenylpropanenitrile has been considered as a potential building block for the synthesis of levamisole. History reveals that β-hydroxy nitriles are important both as technical products and as reagents in organic chemistry.[6] They have been extensively investigated and employed in the preparation of various intermediates for naturally occurring bioactive compounds.[7–12] In view of their enormous potential for the construction of chiral organic frameworks they have been exploited in the present investigation. In continuation of our interest[13] in the preparation of optically pure β-hydroxynitriles and their applications towards the preparation of biologically important compounds or their intermediates by employing lipase catalyzed resolution processes we herein wish to report a practical synthesis of levamisole employing (*R*)-3-hydroxy-3-phenylpropanenitrile and (*R*)-3-acetyloxy-3-phenylpropanenitrile. Moreover, this is the first report on the chemoenzymatic synthesis of levamisole.

The transesterification of 3-hydroxy-3-phenylpropanenitrile by vinyl acetate has been carried out as reported earlier by us in good yields with high enantioselectivity (>99% ee).[13b] We herein have also investigated the lipase-mediated hydrolysis of 3-acetoxy-3-phenylpropanenitrile in phosphate buffer to afford required (*R*)-3-hydroxy-3-phenylpropanenitrile (**1*R***) $[\alpha]_D^{30} = +59.9$ (*c* 1.0, $CHCl_3$) in good yields and high enantioselectivity (Scheme 1 and Table 1).

Enantiomerically pure (*R*)-3-hydroxy-3-phenylpropanenitrile (**1*R***) and (*R*)-3-acetoxy-3-phenyl propanenitrile (**2*R***) obtained after lipase-catalyzed resolution have been effectively employed in the preparation of levamisole in a simple reaction sequence as shown in Scheme 2. **1*R*** and **2*R*** on treatment with H_2O_2 in aqueous ammonia have been efficiently transformed into

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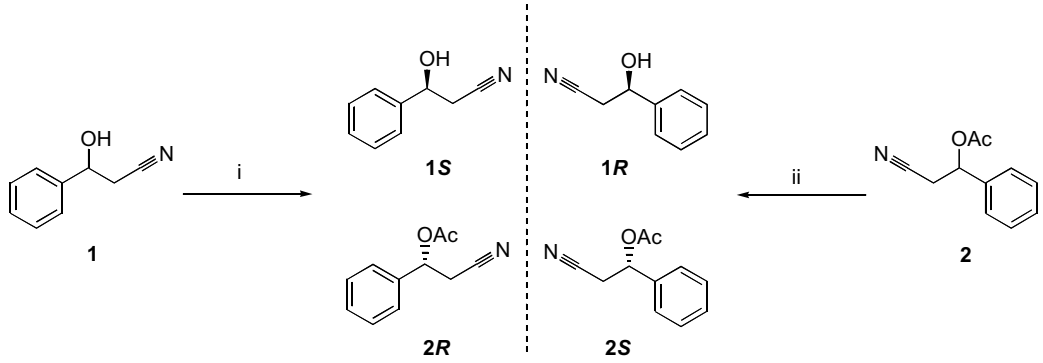
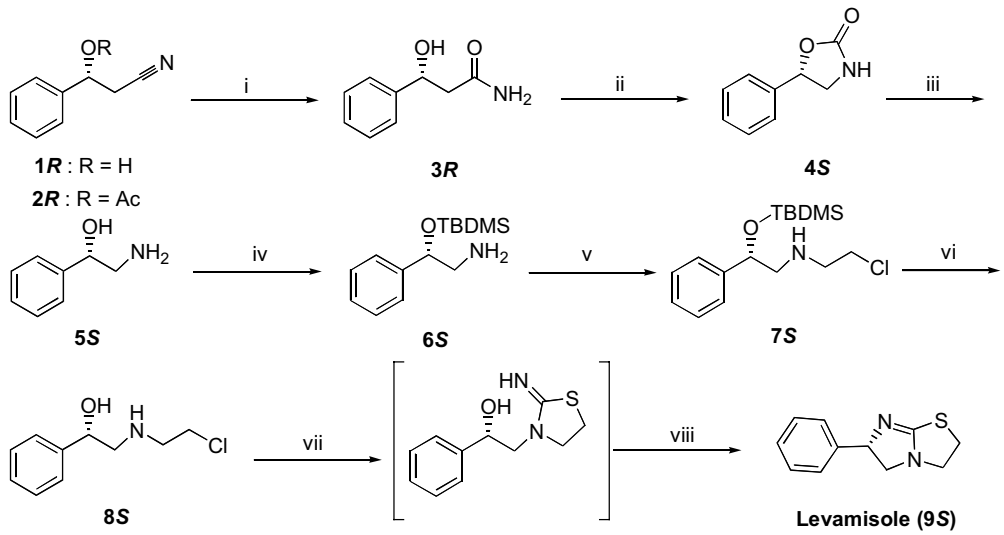


Scheme 1. Reagents and conditions: (i) immobilized *Pseudomonas cepacia* (PS-C) lipase, vinyl acetate, diisopropyl ether; (ii) immobilized *P. cepacia* lipase, phosphate buffer (pH = 7.2).



Scheme 2. Reagents and conditions: (i) H_2O_2, aq NH_3; (ii) $Pb(OAc)_4$, pyridine; (iii) NaOH, EtOH–H_2O, reflux; (iv) TBDMS–Cl, imidazole; (v) 1,2-dichloroethane, K_2CO_3, 18-crown-6; (vi) TBAF, THF; (vii) KSCN, EtOH–H_2O; (viii) $SOCl_2$, CH_2Cl_2.

(*R*)-3-phenyl-3-hydroxypropanamide (**3*R***)[14] $[\alpha]_D^{30} = +32.1$ (*c* 1.0, EtOH) without any loss in optical purity in almost quantitative yield. The resulting amide has been subjected to Hoffmann-like rearrangement in the presence of $Pb(OAc)_4$ in pyridine to afford (*S*)-5-phenyl-1,3-oxazolidine-2-one (**4*S***)[14] $[\alpha]_D^{29} = +51.4$ (*c* 1.1, $CHCl_3$) in about 80% yield without any racemization or inversion at the chiral center. In principle, in Hoffmann rearrangement the isocyanate formed is hydrolyzed with external nucleophile like water to yield amine after the rearrangement. Here, in this process the vicinal hydroxyl group acts as a nucleophile on the isocyanate resulting in the cyclization to form an oxazolidinone ring system. *N*-Alkylation at this stage (**4*S***) using various reagents under different conditions has not been successful. Hence, **4*S*** has been hydrolyzed in aqueous-alcoholic NaOH to provide (*S*)-2-amino-1-phenyl-1-ethanol (**5*S***)[14] in 79% yield. Alkylation of the amino alcohol (**5*S***) resulted in the formation of both *N*-alkylation and *O*-alkylated product. To overcome this problem, alcohol function has been protected with TBDMSCl (**6*S***) and then refluxed in 1,2-dichloroethane in the presence of K_2CO_3 and 18-crown-6 to afford *N*-(2-chloroethyl)-2-*tert*-butyldimethylsilyloxy-2-phenylethanamine (**7*S***)[14] $[\alpha]_D^{27} = +47.1$ (*c* 1.0, $CHCl_3$) in 60% yield (two steps). Deprotection of the protecting group resulted in the formation of *N*-(2-chloroethylamino)-1-phenyl-1-ethanol (**8*S***) $[\alpha]_D^{27} = +31.0$ (*c* 1.1, MeOH), which on coupling with KSCN in refluxing ethanol afforded 2-imino-3-(2*S*-hydroxy-2-phenylethyl)thiazolidine a precursor for levamisole as described by Achiwa and co-workers.[5] This precursor without purification has been transformed into the target molecule (**9*S***) $[\alpha]_D^{29} = -51.2$ (*c* 1.1, $CHCl_3$) lit.[5] $[\alpha]_D^{27} = -49.0$ (*c* 1.0,

Table 1. Enzymatic hydrolysis of 3-acetyloxy-3-phenylpropanenitrile in phosphate buffer

Entry	Lipases[a]	Time (h)	Alcohol		Acetate	
			Yield[b]%	ee[c]%	Yield[b]%	ee[c]%
1	PS-C	14	41	>99	40	>99
2	PS-D	80	40	>99	41	>99

[a] *Pseudomonas cepacia* lipase immobilized on modified ceramic particles (PS-C), *P. cepacia* lipase immobilized on diatomite (PS-D).
[b] Isolated yields.
[c] Determined by chiral HPLC (chiral OJ-H column; Diacel) employing hexane–isopropanol (90:10) as mobile phase at 0.5 mL/min and monitored by UV (254 nm).

$CHCl_3$) on treating with $SOCl_2$ in CH_2Cl_2 with double inversion at the chiral center in about 50% yield.

In summary, a simple, practical and highly enantioselective synthesis of levamisole has been accomplished by employing (*R*)-3-acetoxy-3-phenylpropanenitrile and (*R*)-3-hydroxy-3-phenylpropanenitrile obtained by both enzymatic transesterification and hydrolysis processes.

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14. **1*R***: IR (Neat) 3438, 3046, 3015, 2954, 2923, 2892, 2238, 1115, 1092 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.67 (d, 2H, $J = 7.23$ Hz), 3.15 (br s, 1H), 4.94 (t, 1H, $J = 5.78$), 7.35 (s, 5H); Mass (EI) 147 (M^+), 121, 107, 105, 91, 79, 77. **2*R***: mp 121–124 °C; IR (KBr) 3054, 3008, 2961, 2923, 2254, 1738, 1238, 1200, 1046 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.15 (s, 3H), 2.86 (d, 2H, $J = 5.52$ Hz), 5.94 (t, 1H, $J = 5.52$ Hz), 7.35 (s, 5H); Mass (EI) 189, 162, 149, 130, 120, 107, 77. **3*R***: mp 101–103 °C; IR (KBr) 3377, 3300, 3177, 2961, 2900, 2761, 1623, 1431, 1392, 1331, 1046 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 2.41–2.70 (m, 2H), 4.93–5.11 (m, 1H), 7.20–7.40 (m, 5H); Mass (EI) 165, 141, 120, 105, 91, 77, 59. **4*S***: mp 92–94 °C; IR (Neat) 3262, 2933, 2902, 2839, 1718, 1239, 1082, 972 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.55 (t, 1H, $J = 8.2$ Hz), 3.99 (t, 1H, $J = 8.2$ Hz), 5.60 (t, 1H, $J = 7.3$ Hz), 6.67 (br s, 1H), 7.28–7.40 (m, 5H); Mass (EI) 163 (M^+), 118, 107, 105, 91, 79, 77. **5*S***: mp 57–59 °C; lit.[15] $[\alpha]_D^{29} = +45.5$ (*c* 1.5, EtOH); $[\alpha]_D^{23} = +47.9$ (*c* 1.5, EtOH); IR (Neat) 3412, 3349, 3318, 2941, 2902, 2862, 1082, 1004 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.78 (dd, 1H, $J_1 = 7.7$ Hz, $J_2 = 12.6$ Hz), 2.98 (dd, 1H, $J_1 = 3.8$ Hz, $J_2 = 12.6$ Hz), 4.58 (dd, 1H, $J_1 = 3.8$ Hz, $J_2 = 7.7$ Hz), 7.21–7.30 (m, 5H); Mass (EI) 106, 90, 78, 77, 51. **6*S***: $[\alpha]_D^{28} = +67.4$ (*c* 1.1, $CHCl_3$); IR (Neat) 3373, 3059, 3027, 2957, 2925, 2886, 2855, 1247, 1098, 1058 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ −0.16 (s, 3H), 0.00 (s, 3H), 0.86 (s, 12H), 2.74 (d, 2H, $J = 5.6$ Hz), 4.57 (t, 1H, $J = 5.6$ Hz), 7.15–7.27 (m, 5H); Mass (EI) 251 (M^+), 236, 222, 194, 155, 141, 102, 91, 77, 73. **7*S***: IR (Neat) 2941, 2910, 2870, 2847, 1741, 1247, 1105, 1059, 1035 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ −0.19 (s, 3H), 0.00 (s, 3H), 0.84 (s, 12H), 3.23–3.31 (m, 3H), 3.57–3.65 (m, 1H), 4.13–4.20 (m, 2H), 4.88 (t, 1H, $J = 6.2$ Hz), 7.19–7.30 (m, 5H); Mass (EI) 264 (M^+-CH_2Cl), 222, 155, 141, 102, 77, 73, 44. **8*S***: mp 135–137 °C; IR (Neat) 3357, 3027, 2988, 2925, 2847, 1710, 1451, 1255, 1082, 1051 cm^{-1}; ^1H NMR (200 MHz, DMSO (D_6)) δ 3.22–3.42 (m, 2H), 3.46–3.58 (m, 1H), 3.70–3.82 (m, 1H), 4.21–4.30 (m, 2H), 4.76–4.85 (m, 1H), 5.30 (br s, 1H), 7.22–7.39 (m, 5H); Mass (EI) 200, 107, 101, 79, 77, 56, 51. **9*S***: ^1H NMR (300 MHz, $CDCl_3$) δ 3.26–3.60 (m, 3H), 3.65–3.80 (m, 2H), 4.04 (t, 1H, $J = 8.75$ Hz), 5.48 (t, 1H, $J = 8.23$ Hz), 7.24–7.33 (m, 5H); Mass (EI) 178 ($M^+-C_2H_4$), 132, 105, 91, 77, 42.
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