Lipase-Catalyzed Resolutions of both Enantiomers of Ornidazole and Secnidazole†

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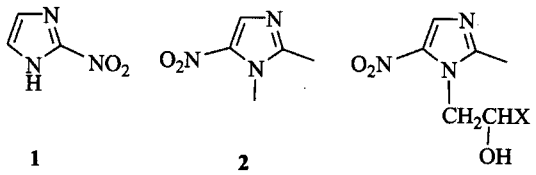
The resolutions of both enantiomers of the ornidazole and secnidazole were achieved with high enantiomeric excesses (*ee* > 99%) by acylation of the corresponding racemates with vinylacetate in the presence of lipase Amano AK (from *Pseudomonas sp.*). The assignments of the absolute configurations of (+) or (−)-ornidazole and secnidazole are described.

Keywords ornidazole, secnidazole, lipase-catalyzed resolution, X-ray

Introduction

The great interest in nitroimidazole was originally aroused by the discovery of the naturally occurring antibiotic Azymycin (**1**) (2-nitroimidazole).[1] Considering their broad spectrum activity against anaerobic microorganisms, several investigations into structure-activity relationships of this class of substances were accomplished, indicating that the 5-nitroimidazoles were more effective than their 4-nitro isomers.[2] The bioassay screening of derivatives of the 5-nitroimidazole resulted in the discoveries of those dimetronidazole (**2**), metronidazole (**3a**, *Flagyl*®, *Clont*®), ornidazole (**3b**, *Tiberal*®) and secnidazole (**3c**, *Flagentyl*®) (Fig. 1), which were found particularly effective in the treatment of *Amoebiasis*, *Giardiasis*, *Trichomoniasis* and bacterial *vaginosis*.[3] In addition, secnidazole (**3c**) and dimetronidazole (**2**) showed an antiamoebic potency ten times higher than metronidazole (**3a**) and ornidazole (**3b**).[4a] Moreover, secnidazole (**3c**) could be rapidly and completely absorbed after oral admistration and has a long terminal elimination half life (17 h to 29 h) than the other imidazole analogues.[4b] The latest developments of those drugs reveal that ornidazole (**3b**) is still a subject of research as antifertility agents in male animals,[5] possibly due to the release of the chlorinated side chain during metabolism.[6,7] Ornidazole (**3b**) was found to inhibit glycolysis within mature spermatozoa in reducing acidosis and glycolytic enzyme activity under anaerobic conditions.[8] If the active enantiomer of the racemate could be used, the dosage might be halved. So the necessity of gaining the optically pure enantiomers of ornidazole and secnidazole is quite obvious.



1 **2**

X=H **3a**
X=CH_2Cl **3b**
X=CH_3 **3c**

Fig. 1 Structures of compounds **1**—**3**.

Lipases are versatile catalysts, as they catalyze a plethora of reactions such as esterification, amidation, and transesterification of esters in addition to the natural reaction of fatty ester hydrolysis. Applications of lipases are wide including production of food addition, chiral intermediates, and pharmaceutical products.[9,10] Herein we wish to report our efforts on acquirement of enantiomerically pure ornidazole (**3b**) and secnidazole (**3c**) via a lipase-catalyzed resolution and the assignments of absolute configurations of these enantiomers (Scheme 1).

Results and discussion

Ornidazole (**3b**) and secnidazole (**3c**) are both the secondary alcohols, the former is a chlorohydrin with the chlorine atom in the terminal position. Chlorohydrins could be separated from their optical antipodes by lipase-catalyzed resolution according to the literature,[11] showing that the lipase from *Pseudomonas sp.* was the best choice of commercial available enzymes.[12] If the substitutents on the two sides of the secondary alcohol differ in size and one side bears a carbocyclic or aromatic system as the largest group, the enantioselectivity would be satisfactory.[13] Besides, to the best of our knowledge, the optically pure sec-

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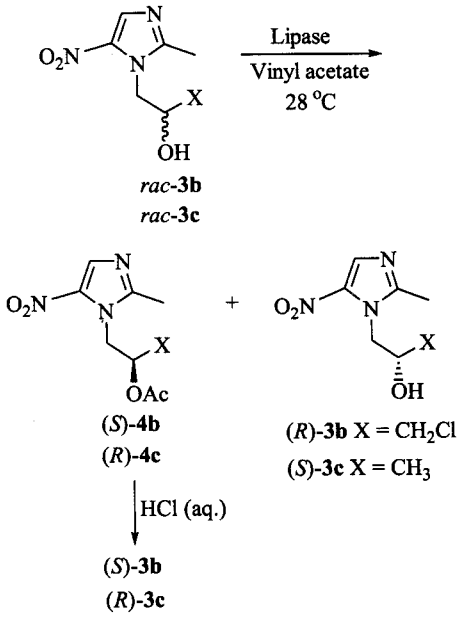
Scheme 1



nidazole in *R* or *S* form has not been isolated yet.

So in our preliminary test, ornidazole (**3b**) was esterfied with vinylacetate employing lipase Amano PS (PSL).[14] As an unsatisfactory result, the reaction was sluggish with low conversion even for a long reaction time (Table 1, Entry 1). Therefore, we turned our attention to other lipase (Amano lipase AK, AKL) from *Pseudomonas sp*. Although the reaction proceeded still slowly, the enantioselectivity was excellent and the conversion was acceptable. Furthermore, we were pleased to find that the resolution of secnidazole employing AKL under these same conditions also gave satisfactory results (Entry 4). However, it was found that the resolution failed, when using Novozym 435 (*Candida antarctica* lipase B, CAL) in our hands, (Entry 3) although it was regarded as the most enantioselective lipase toward the resolution of secondary alcohols.[10]

The absolute configuration of (+)-[or (−)-] ornidazole (**3b**) was established as *R* (or *S*) by comparison its positive (or negative) optical rotation with those reported in literature.[14] The absolute configuration of secnidazole (**3c**) could be deduced by Kazlauskas' rule,[15] which predicts that the enantiomer shown in Fig. 2 proceeds faster than its antipode in the lipaseca-talyzed esterfication of secondary alcohols. **M** and **L** represent large and medium substitutes respectively, for example **M** = CH_3 and **L** = Ph. If the Kazlauskas' rule is applicable to secnidazole (**3c**), the absolute configuration of the acylating secnidazole [(−)-**3c**] should be *R*.

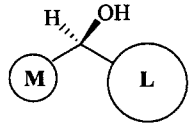


Fig. 2 Kazlauskas' rule for the enantiopreference of lipase from *Pseudomonas sp*.

In order to verify our inference about absolute configuration of (−)-**3c**, the corresponding derivative (−)-**5** was prepared (Scheme 2). As a lucky result, when it was recrystalized from ethyl acetate/petroleum ether, a single crystal of the enantiopure compound could be obtained. The crystal structure of compound (−)-**5** is shown in Fig. 3. Since the anomalous X-ray (copper) scattering of bromine renders the direct determination of the absolute structure, the absolute configuration of compound (−)-**5** was established as R, which meant that the absolute configuration of (−)-**3c** is *R*. Thus the supposed absolute configuration by Kazlauskas' rule was confirmed by X-ray crystallography. This result indicates that the rule is also useful for secondary alcohols bearing a heterocyclic aromatic substituent despite their non-uniform electronic structure.

Conclusion

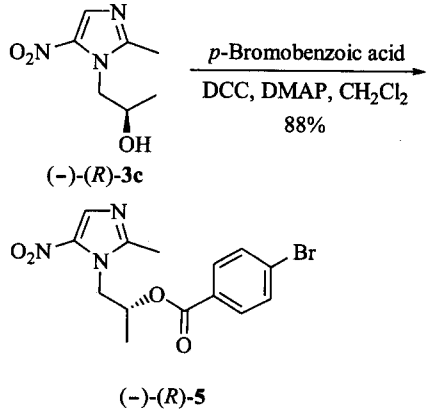
In summary optically pure ornidazole and secnidazole were obtained in a medium scale with high enantiomeric excessrs (*ee* > 99%) by enzymatic resolutions of their racemates. The bioactivity test is in progress, which will reveal the relationships between configuration and bioactivities of these two chiral drugs.

Table 1 Enzymatic esterfication of ornidazole (**3b**) and secnidazole (**3c**)

Entry	Compd	Lipase	Time (d)	Conv. (%)	Product	Yield[a] (%)	*ee* (%)
1	*rac*-**3b**	PSL	20	14	—	—	—
2	*rac*-**3b**	AKL	18	44	(−)-(*S*)-**3b**	43.0	95 (>99)[b]
					(+)-(*R*)-**3b**	52.2	94 (>99)[b]
3	*rac*-**3b**	CAL	10	5	—	—	—
4	*rac*-**3c**	AKL	10	44	(−)-(*R*)-**3c**	37.5	94 (>99)[b,c]
					(+)-(*S*)-**3c**	51.8	90 (>99)[b,c]

[a] Isolated yield; [b] enantiomeric excess (*ee*) after crystallization; [c] determined by chiral HPLC analysis of secnidazole acetate.

Scheme 2



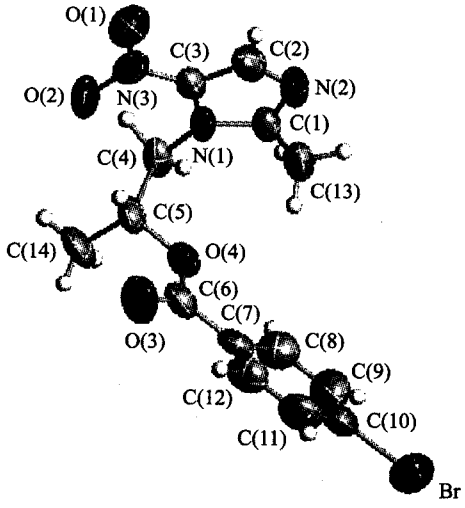


Fig. 3 Crystal structure of compound (-)-(*R*)-**5**.

Experimental

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AM 300 spectrometer. Chemical shifts for ^{1}H NMR were reported as δ-values relative to TMS as internal standard in $CDCl_3$. ^{13}C NMR spectra were calibrated to δ 77.1 of the $CDCl_3$ triplet. Low-resolution mass spectra were recorded on an Finigan-4201 spectrometer, HRMS on an Finigan MAT-8430 spectrometer and IR spectra on an FTS-185 spectrometer. Elemental analyses were performed on an EA-MOD 7106 instrument. Optical rotations were measured on a Perkin-Elimer 241 MC polarimeter. Melting points were not corrected.

Racemic ornidazole (**3b**) and secnidazole (**3c**) were purchased from Zhejiang East-Asia Pharmaceutical Chemical Co. Ltd. Vinylacetate was distilled prior to use. Other solvents were used without further purification. Purification of reaction products was carried out by flash chromatography on a silica gel column in 300—400 mesh.

General procedure for the enzymatic resolutions of ornidazole and secnidazole

The suspension of ornidazole or secnidazole (60 mmol), 4 g of lipase Amano AK and 100 mL of vinyl acetate was stirred at 28 ℃ in the darkness.[17] For monitoring the reaction each time, 0.1 mL of quota was taken from the suspension and submitted to HPLC analysis [determined by HPLC on YWG C18 with methanol/water (55:45); $t_{alcohol}$ = 7.65 min, $t_{acetate}$ = 10.32 min for ornidazole; $t_{alcohol}$ = 5.60 min, $t_{acetate}$ = 8.74 min for secnidazole]. Whole reaction was terminated, when 44% conversion was reached. The solution was filtered and evaporated under reduced pressure to yield a residue. Purification of the residue by chromatography on a silica gel column [eluent: CH_2Cl_2 to CH_2Cl_2/acetone (4:1)] gave corresponding alcohols and acetates.

(+)-(R)-1-Chloro-3-(2-methyl-5-nitro-imidazol-1-yl)-propan-2-ol [(+)-(*R*)-**3b**]

Yellow oil, 6.90 g, yield 52.2%. Crystallization of the oil from ethyl acetate/petroleum ether (1:1) afforded a white prismy crystal (5.80 g), m.p. 93.0—94.0 ℃ (Lit.[14] 92 ℃); $[\alpha]_D^{20}$ +62.3 (*c* 1.0, CH_2Cl_2) [Lit.[14] $[\alpha]_D^{20}$ +65.5 (*c* 0.99, CH_2Cl_2)]; *ee* >99% [determined by HPLC on Chiral OB-H with hexane/*i*-PrOH (95:5); t_R = 5.48 min (major), t_S = 5.58 min (minor)]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.81 (s, 1H), 4.65 (d, J = 11.4 Hz, 1H), 4.25—4.19 (m, 2H), 4.08 (d, J_{XB} = 5.4 Hz, 1H), 3.80—3.66 (ABX, J_{AB} = 11.7 Hz, J_{XB} = 5.4 Hz, 2H), 2.52 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 151.880, 138.182, 132.133, 69.780, 49.839, 46.880, 14.456; IR (KBr) ν: 3167, 2980, 1535, 1362, 1272, 1142, 829, 521 cm^{-1}; MS (70 eV) *m/z* (%): 173 (45.72), 172 (44.07), 112 (73.13), 95 (42.42), 81 (100.00), 80 (41.76), 54 (54.75), 53 (93.29). Anal. calcd for $C_7H_{10}ClN_3O_3$: C 38.28, H 4.59, N 19.13; found C 38.49, H 4.63, N 19.39.

(-)-(S)-Chloro-acetic acid 1-methyl-2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl ester [(-)-(*S*)-**4b**]

Yellow solid, 6.77 g, yield 43.0%. Crystallization of the solid from ethyl acetate/petroleum ether (1:1) afforded a white prismy crystal (2.67 g), m.p. 113.5—114.5 ℃, $[\alpha]_D^{20}$ −63.5 (*c* 1.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.95 (s, 1H), 5.42—5.39 (m, 2H), 4.79—4.73 (m, 1H), 4.53—4.50 (m, 1H), 3.85—3.80 (m, 2H), 2.55 (s, 3H), 2.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.297, 151.003, 138.508, 133.098, 70.673, 46.975, 43.311, 20.447, 14.267; IR (KBr) ν: 3128, 2980, 1743, 1524, 1194, 1051, 743, 475 cm^{-1}; MS (70 eV) *m/z* (%): 261 (M^+, 25.96), 219 (53.45), 173 (30.71), 137 (26.75), 135 (66.35), 111 (22.23), 53 (28.04), 43 (100.00). Anal. calcd for $C_9H_{12}ClN_3O_4$: C 41.31, H 4.62, N 16.06; found C 41.18, H 4.58, N 16.35.

(+)-(S)-1-(2-Methyl-5-nitro-imidazol-1-yl)-propan-2-ol [(+)-(*S*)-**3c**]

Yellow oil, 5.75 g, yield 51.8%. Crystallization of

the oil from ethyl acetate/petroleum ether (1:1) afforded a white prismy crystal, m.p. 70—71 ℃, $[\alpha]_D^{20}$ + 83.6 (*c* 1.1, CH_2Cl_2); *ee* > 99% (determined by its acetate); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.76 (s, 1H), 4.55—4.50 (m, 1H), 4.17—4.01 (AB, J_{AB} = 13.5 Hz, 2H), 2.51 (s, 3H), 1.36 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 151.489, 132.067, 132.041, 66.756, 53.337, 20.803, 14.535; IR (KBr) ν: 3377, 2981, 1533, 1425, 1265, 1189, 827, 487 cm^{-1}; MS (70 eV) *m/z* (%): 138 (48.35), 112 (84.52), 81 (46.27), 54 (39.89), 53 (100.00), 52 (40.51), 45 (48.98), 42 (39.37). Anal. calcd for $C_7H_{11}N_3O_3$: C 45.40, H 5.99, N 22.69; found C 45.67, H 6.00, N 22.75; HRMS calcd for $C_7H_{11}N_3O_3$ 185.08004, found 185.08109.

(−)-(*R*)-*Acetic acid 1-methyl-2-(2-methyl-5-nitro-imidazol-1-yl)-ethyl ester* [(−)-(*R*)-**4c**]

Yellow solid, 5.10 g, yield 37.5%. Crystallization of the solid from ethyl acetate/petroleum ether (1:1) afforded a white prismy crystal (4.17 g), m.p. 99.0—100.5 ℃, $[\alpha]_D^{20}$ − 48.7 (*c* 0.8, CH_2Cl_2); *ee* > 99% [determined by HPLC on Chialpak As$^+$ with hexane/*i*-PrOH (80:20); t_R = 14.42 min (major), t_S = 16.07 min (minor)]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.94 (s, 1H), 5.27—5.23 (m, 1H), 4.64—4.24 (AB, J_{AB} = 14.4 Hz, 2H), 2.53 (s, 3H), 1.96 (s, 3H), 1.36 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.540, 150.698, 132.848, 132.827, 69.291, 49.946, 20.719, 17.486, 14.304; IR (KBr) ν: 3122, 2995, 1732, 1533, 1367, 1192, 825, 489 cm^{-1}; MS (70 eV) *m/z* (%):228 (M$^+$, 29.91), 139 (24.43), 111 (23.64), 101 (23.04), 53 (37.58), 52 (23.22), 43 (100.00), 42 (36.24). Anal. calcd for $C_9H_{13}N_3O_4$: C 47.57, H 5.77, N 18.49; found C 47.48, H 5.69, N 18.82; HRMS calcd for $C_9H_{13}N_3O_4$ 227.09061, found 227.09059.

(−)-(*S*)-1-*Chloro*-3-(2-*methyl*-5-*nitro-imidazol*-1-*yl*)-*propan*-2-*ol* [(−)-(*S*)-**3b**]

Compound (−)-(*S*)-**4b** (5.5 g) was dissolved in HCl (50 mL). The mixture was stirred for 18 h at room temperature, and neutralized with 25% aqueous ammonia (50 mL) to exactly pH = 7. The solution was extracted three times with ethyl acetate (100 mL). After drying over Na_2SO_4, the organic phase was evaporated under reduced pressure to give a yellow solid (4.1 g, yield 89%). Crystallization from ethyl acetate/petroleum ether (1:1) provided a white prismy crystal (−)-(*S*)-**3b** (3.0 g), m.p. 92.5—93.5 ℃, $[\alpha]_D^{20}$ − 65.6 (*c* 1.0, CH_2Cl_2) [Lit.[14] $[\alpha]_D^{20}$ − 67.8 (*c* 0.99, CH_2Cl_2)]; *ee* > 99% [determined by HPLC on Chiral OB − H with hexane/*i*-PrOH (95:5); t_R = 5.48 min (minor), t_S = 5.58 min (major)]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.81 (s, 1H), 4.65 (d, J = 10.8 Hz, 1H), 4.22—4.16 (m, 3H), 3.79—3.66 (m, 2H), 2.51 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 151.894, 138.213, 132.202, 69.858, 49.876, 46.923, 14.515; IR (KBr) ν: 3169, 2980, 1535, 1362, 1272, 1142, 830, 521 cm^{-1}; MS (70 eV) *m/z* (%): 173 (51.60), 172 (49.89), 112 (73.78), 81 (99.70), 80 (42.59), 54 (56.08), 53 (100.00), 52 (44.07). Anal. calcd for $C_7H_{10}ClN_3O_3$: C 38.28, H 4.59, N 19.13; found C 38.18, H 4.58, N 19.51.

(+)-(*R*)-1-(2-*Methyl*-5-*nitro-imidazol*-1-*yl*)-*propan*-2-*ol* [(−)-(*R*)-**3c**]

The procedure was run in the same manner as described in (−)-(*S*)-**3b**, $[\alpha]_D^{20}$ − 93.5 (*c* 0.9, CH_2Cl_2); *ee* > 99% [according to the *ee* of (*R*)-(−)-**4c**]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.75 (s, 1H), 4.55—4.50 (m, 1H), 4.20—4.15 (m, 1H), 4.00—3.95 (m, 1H), 2.51 (s, 3H), 1.36 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 151.491, 132.075, 132.053, 66.784, 53.367, 20.810, 14.545; IR (KBr) ν: 3245, 2978, 1537, 1428, 1270, 1192, 830, 486 cm^{-1}; MS (70 eV) *m/z* (%): 141 (41.26), 138 (48.32), 124 (24.61), 112 (100.00), 111 (35.15), 94 (18.68), 81 (28.86), 53 (21.80). Anal. calcd for $C_7H_{11}N_3O_3$: C 45.40, H 5.99, N 22.69; found C 45.73, H 6.08, N 22.65; HRMS calcd for $C_7H_{11}N_3O_3$ 185.08004, found 185.07644.

(−)-(*R*)-4-*Bromo-benzoic acid* 1-*methyl*-2-(2-*methyl*-5-*nitro-imidazol*-1-*yl*)-*ethyl ester* [(−)-(*R*)-**5**]

To a mixture of (−)-(*R*)-**3c** (556 mg, 3 mmol), 4-bromobenzoic acid (663 mg, 3.3 mmol) and 4-(dimethylamino)pyridine (25 mg) in 50 mL of CH_2Cl_2 were added 1,3-dicyclohexylcarbodiimide (680 mg, 3.3 mmol) in three portions. The resulting suspension was stirred at room temperature overnight. The reaction mixture was filtered over celite. The organic phase was washed with 2% HCl, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated under reduced pressure to give a residue. Purification of the residue by chromatography on a silica gel column eluting with ethyl acetate/petroleum ether (1:1) gave the subtitle compound as white solid (974 mg, yield 88%), m.p. 125.5—126.0 ℃; $[\alpha]_D^{20}$ − 237.7 (*c* 1.2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.91 (s, 1H), 7.75 (dd, J = 8.7, 1.8 Hz, 1H), 7.57 (dd, J = 8.7, 1.8 Hz, 1H), 5.57—5.51 (m, 1H), 4.49—4.46 (AB, J_{AB} = 14.7 Hz, 2H), 2.46 (s, 3H), 1.49 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 164.632, 133.136, 131.884, 130.849, 128.613, 129.089, 69.952, 50.047, 17.746, 14.404; IR (KBr) ν: 2988, 1719, 1527, 1365, 1262, 1193, 755, 463 cm^{-1}; MS (70 eV) *m/z* (%): 323 (17.14), 321 (17.40), 185 (94.40), 183 (100.00), 157 (17.77), 155 (18.55), 76 (19.75), 53 (28.72); HRMS calcd for $C_{14}H_{14}BrN_3O_4(M^+) - NO_2$ 321.02386, found 321.02464.

X-Ray analyses gave the crystallographic data for (−)-(*R*)-**5**: formula $C_{14}H_{14}BrN_3O_4$, formula weight: 368.19; temperature: 293(2) K; wavelength 7.1073×10^{-2} nm; crystal system: monoclinic, $P2_1$; unit cell dimensions: $a = 0.6513(2)$ nm, $b = 0.9088(3)$ nm, $c = 1.3373(4)$ nm, $\beta = 98.523°$, $V = 0.7829(4)$ nm^3; $Z = 2$, calculated density = 1.562 Mg/m^3; $F(000) = 372$, $\mu = 2.646$ mm^{-1}; final R indices $[I > 2\sigma(I)]$, $R_1 = 0.0688$, $wR_2 = 0.1725$; R indices (all data), $R_1 = 0.1098$, $wR_2 = 0.2107$.

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References and notes

1 (a) Beaman, A. G.; Tautz, W.; Gabriel, T.; Duschinsky, R. *J. Am. Soc. Chem.* **1965**, *87*, 389.
(b) Maeda, K.; Osato, T.; Umezawa, H. *J. Antibiot. Ser. A* **1953**, *6*, 182.

2 Butler, K.; Howes, H. L.; Lynch, J. E.; Pirie, D. K. *J. Med. Chem.* **1967**, *10*, 891.

3 (a) Hoffer, M.; Grunberg, E. *J. Med. Chem.* **1974**, *17*, 1019.
(b) Richle, R.; Scholer, H. J.; Angehrn, P.; Fernex, M.; Hummler, H.; Jeunet, F.; Schaerer, K.; Schuepbach, M.; Schwartz, D. E. *Arzneim.-Forsch.* **1978**, *28* I, 612.

4 (a) Mata-Cardenas, B. D.; Vargas-Villarreal, J.; Gonzalez-Garza, M. T.; Said-Fernandez, S. *Pharm. Sci.* **1996**, *2*, 513.
(b) Gillis, J. C.; Wiserman, L. R. *Drugs* **1996**, *51*, 621.

5 Oberlaender, G.; Yeung, C. H.; Cooper, T. G. *J. Reprod. Fertil.* **1994**, *100*, 551.

6 Schwartz, D. E.; Jordan, J. C.; Vetter, W.; Oesterhelt, G. *Xenobiotica* **1979**, *9*, 571.

7 Jones, A. R.; Davies, P.; Edwards, K.; Jackson, H. *Nature* **1969**, *224*, 83.

8 Bone, W.; Jones, N. G.; Kamp, G.; Yeung, C. H.; Cooper, T. G. *J. Reprod. Fertil.* **2000**, *118*, 127.

9 Gandhi, N. N.; Patil, N. S.; Sawant, S. B.; Joshi, J. B.; Wangikarp, P. P.; Mukesl, D. *Catal. Rev.* **2000**, *42*, 439.

10 Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Syhthesis: Regio- and Stereoselective Biotransformations*, Wiley-VCH, **1999**.

11 Chen, C.-S.; Liu, Y.-C.; Marsella, M. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2559.

12 Ader, U.; Schneider, M. P. *Tetrahedron: Asymmetry* **1992**, *3*, 201.

13 Faber, K. *Biotansformations in Organic Chemistry*, 4th edn., Springer, Berlin, **1995**.

14 Skupin, R.; Cooper, T. G.; Froehlich, R.; Prigge, J.; Haufe, G. *Tetrahedron: Asymmetry* **1997**, *8*, 2453.

15 Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656.

16 The lipase from *Pseudomonas cepacia* was formerly categorized with other lipases from this family as *Pseudomonas sp*. Lipase Amano PS was formerly known as lipase P (classified as *Pseudomonas fluorescens*, until **1989**). Product information Amano Pharmaceuticals Co.

17 Originally colorless aqueous solution of ornidazole became slightly yellow because of its photochemical rearrangement,[18] when it was exposed to daylight.

18 Pfoertner, K.-H.; Daly, J. J. *Helv. Chim. Acta* **1987**, *70*, 171.

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