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Enzymatic resolution of 2,2,2-trifluoro-1-arylethylamine derivatives by *Pseudomonas fluorescens* lipase in organic solvents

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Abstract

Amination of phenyl trifluoromethyl ketone with ammonium formate (Leuckart-Wallach reaction) in a one-pot reaction gave 2,2,2-trifluoro-1-phenylethylamine (**3a**) in high yield (81%). Other fluorinated 1-arylethylamine derivatives were obtained from their corresponding aryl ketones in moderate to good yields (55–81%). Enzymatic resolution of racemic **3a** was done with *Pseudomonas fluorescens* lipase (Amano lipase AK) via enantioselective alcoholysis of its chloroacetamide (**4a**) with *n*-amyl alcohol in diisopropyl ether giving good enantioselectivity (*E*-value = 44). Other amines also were resolved by lipase AK under similar conditions (*E*-values = 25 to >100).

Keywords: Lipase; Enantioselective deacylation; Trifluoromethyl amine; Leuckart-Wallach reaction; Ammonium formate

1. Introduction

The trifluoromethyl group is a highly important substituent in organic chemistry. As compared to non-fluorinated analogues, its powerful electron-withdrawing ability and small size produce significant changes in the chemistry of substituted compounds [1]. Effects reported include stabilization of small rings, as well as changes in regioselectivity and reactivity. Many performance chemicals benefit from the presence of a trifluoromethyl group. The high lipophilicity of such groups produces active pharmaceutical and agrochemical compounds with improved transport characteristics in vivo, enabling lower dose rates [2,3]. Substituted polymers that show enhanced stability, resistance to chemicals, and flame retardance have been synthesized from trifluoromethylated precursors. The intensity and wash fastness of dyes are improved by the presence of CF_3. The synthesis of optically active chiral fluoroorganic compounds, which have important roles in bio-chemical research and the development of medicines, is one of the most fascinating aspects of modern organofluorine chemistry because of fluorine's unique influence on biological activity [4,5]. These compounds also have been used to produce materials such as liquid crystals. Optically active α-trifluoromethylated amine, in particular, is a useful building block in pharmaceutical synthesis because the introduction of fluorine atoms to its β-carbon atom group often confers special physico-chemical and biological properties. The development of novel methods for synthesizing optically active α-trifluoromethylated amine is of great interest to researchers. Recent reports on the preparation of optically active α-trifluoromethylated amines mainly involve: (1) resolution of racemates with optically active acids [6]; (2) a stereoselective-proton [1,3] shift via the asymmetric alkylation of imines bearing a suitable chiral auxiliary [7–9]; (3) enantioselective reduction of *O*-benzyloxime by means of BH_3-oxazaborolidine complexes [10]. In this context, the establishment of new synthetic methodology for preparing optically active fluorine-containing amines is of great interest.

The chemo-enzymatic approach to asymmetric synthesis is increasingly being used in synthetic strategies, and the use of enzymes as routine chiral catalysts for asymmetric synthesis is well documented [11–14]. Lipase releases fatty acids non- or regiospecifically from the outer 1- and 3-positions of acylglycerols. Moreover, it catalyzes the

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asymmetric hydrolysis and esterification of a wide range of substrates. This ability has gained it the attention of synthetic chemists because lipase requires no additional cofactors, is readily available, and easily handled. Many recent examples of enzymatic resolution of non-fluorinated amines have been reported [15–17]. To our knowledge, however, little effort has been made to resolve racemic 2,2,2-trifluoro-1-arylethylamines enzymatically.

As reported elsewhere [18], phenyl trifluoromethyl ketone can be used as the starting material for the synthesis of 2,2,2-trifluoro-1-phenylethylamine (**3a**) by reductive amination (Leuckart-Wallach reaction). Moreover, the amine **3a** could be resolved by enenaioselective alcoholysis of its activated chloroacetamide by *Pseudomonas fluorescens* lipase. In amplification of our reported preliminary results, we here describe a convenient synthetic method for various 2,2,2-trifluoro-1-arylethylamine derivatives by reductive amination in organic solvent of the corresponding aryl a-trifluoromethyl ketones and resolution via enantioselective alcoholysis of their chloroacetamides by *P. fluorescens* lipase (lipase AK).

2. Experimental

2.1. Materials and methods

All the starting materials were obtained commercially and used without purification. ^{1}H NMR spectra were recorded with tetramethylsilane (TMS) as the internal standard at 90 MHz with a Hitachi R-90H FT spectrometer and at 299.95 MHz with a Varian INOVA-300 FT spectrometer. ^{19}F NMR spectra were recorded with the same spectrometers with hexafluorobenzene as the internal standard, respectively at 84.7 and 282.22 MHz. Mass spectra (70 eV) were measured with a Shimadzu QP-5000 instrument. High-resolution mass spectra were measured with a JEOL JMS-SX102A MS spectrometer and optical rotation values with a JASCO DIP-370 polarimeter. Melting points, determined in a glass capillary tube on a heating block, were uncorrected. Lipase AK (*P. fluorescens*, Amano, 18,500 units/g), lipase PS (*Pseudomonas cepacia*, Amano, 30,000 units/g), lipase LIP (*Pseudomonas aeruginosa*, Toyobo, 0.5 units/mg), lipase PLC (*Alcaligenes* sp., Meito, 30,000 units/g), lipase SL (*P. cepacia*, Meito, 60,000 units/g), lipase TL (*Pseudomonas stutzeri*, Meito, 50,000 units/g), lipase SP 435 (*Candida antarctica*, Novo Nordisk, 7000 PLU/g).

2.2. Synthesis of racemic fluorinated arylethylamines

The representative procedure: A mixture of the corresponding ketone (10 mmol) and ammonium formate (50 mmol) was heated at 120 °C for 2 h, stirred for 4 more hours at 180 °C, and then diluted with ethyl acetate (10 ml). The organic layer was washed with distilled water (2×10 ml) and dried over sodium sulfate, after which it was evaporated in vacuo. The residue was subjected to capillary GC to determine the conversion [DB-17, 0.25 mm × 30 m, oven temperature from 80 °C (initial time 15 min) to 200 °C at a heating rate of 5 °C/min, FID, He, 1.0 ml/min, J&W Scientific]. Retention times are: **1a**, 4.1 min; **2a**, 30.9 min; **3a**, 13.0 min; **1b**, 6.5 min; **2b**, 33.5 min; **3b**, 13.8 min; **1c**, 18.1 min; **1d**, 12.4 min; **2d**, 34.3 min; **3d**, 9.8 min; **1e**, 6.8 min; **2e**, 37.5 min; **3e**, 22.8 min; **1f**, 8.3 min; **2f**, 38.0 min; **3f**, 18.0 min; **1g**, 6.5 min; **2g**, 35.8 min; **3g**, 14.9 min; **1h**, 32.5 min; **2h**, 55.3 min; **3h**, 48.5 min; **1i**, 48.5 min; **2i**, 65.8 min; **3i**, 47.2 min. The residue was then dissolved in 8N aqueous sodium hydroxide and methanol (1/1, v/v, 20 ml) which isolated only (*R*, *S*)-**2i**. Next, the mixture was heated at 60 °C for 3 h and was treated with ethyl acetate, and then the organic phase was concentrated in vacuo. Purification of the residue by silica gel column chromatography and elution with dichloromethane–hexane (1/1, v/v) gave fluorinated arylethylamine derivatives.

2.2.1. 2,2,2-Trifluoro-1-phenylethylamine (3a)

81% yield; colorless oil; MS *m*/*z* (ion, relative intensity, %): 176 ([M^+H], 75), 159 (M^+–NH_2, 50), 107 ([M^+H]–CF_3, 100); IR (KBr) ν_{max} (cm^{-1}): 3400, 3020, 1600, 1500, 1450, 1260, 1160, 860, 760; ^{1}H NMR δ (CD_3COCD_3, TMS): 1.83 (2H, br-s, NH_2), 4.45 (1H, q, $J = 7.1$ Hz, CH), 7.40–7.65 (5H, m, Ar–H); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 85.9 (d, $J = 7.1$ Hz, CF_3).

2.2.2. 2,2-Difluoro-1-phenylethylamine (3b)

56% yield; colorless oil; MS *m*/*z* (ion, relative intensity, %): 157 (M^+, 8), 107 ([M^+H]–CHF_2, 100), 141 (M^+–NH_2, 8); IR (KBr) ν_{max} (cm^{-1}): 3390, 1490, 1450, 1200, 1070, 840; ^{1}H NMR *d* (CD_3COCD_3, TMS): 2.39 (2H, br-s, NH_2), 4.82 (1H, br, CH), 5.76 (1H, dt, $J = 54.6$ and 4.6 Hz, CHF_2), 7.25–7.40 (5H, m, Ar–H); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 34.2 (dd, $J = 54.6$ and 7.1 Hz, CHF_2); high resolution-MS revealed: 157.0695. Calcd. for $C_{12}H_{11}F_3N$: 157.0703.

2.2.3. 2,2,3,3,3-Pentafluoro-1-phenylpropylamine (3e)

76% yield; colorless oil; MS *m*/*z* (ion, relative intensity, %): 226 ([M^+H], 2), 209 (M^+–NH_2, 100), 107 ([M^+H]–CF_2CF_3, 33); IR (KBr) ν_{max} (cm^{-1}): 3420, 1210, 1190, 1130, 1030, 720, 700; ^{1}H NMR *d* (CD_3COCD_3, TMS): 2.47 (2H, br-s, NH_2), 5.10 (1H, dd, $J = 14.9$ and 7.7 Hz, CH), 7.20–7.55 (5H, m, Ar–H); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 36.3 (2F, dq, $J = 275.4$ and 14.9 Hz, CF_2CF_3), 80.6 (3F, s, CF_2CF_3); high resolution-MS revealed: 226.0650. Calcd. for $C_{12}H_{11}F_3N$: 226.0655 [M^+H].

2.2.4. 2,2,2-Trifluoro-1-(4-methoxyphenyl)ethylamine (3f)

69% yield; colorless oil; MS *m*/*z* (ion, relative intensity, %): 206 ([M + H], 36), 137 ([M^+H]–CF_3, 100), 109 (M^+–CF_3–HCN, 32); IR (KBr) ν_{max} (cm^{-1}): 3420, 1510,

1250, 1170, 1120, 1030, 820; ^{1}H NMR *d* (CD_3COCD_3, TMS): 2.64 (2H, br-s, NH_2), 3.80 (3H, s, OCH_3), 4.93 (1H, q, J = 6.8 Hz, CH), 6.91 (2H, d, J = 8.5 Hz, Ar–H), 7.38 (2H, d, J = 8.5 Hz, Ar–H); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 83.4 (3F, s, CF_3); high resolution-MS revealed: 206.0779. Calcd. for $C_{12}H_{11}F_3N$: 206.0793 [M^+H].

*2.2.5. 2-Chloro-2,2-difluoro-1-phenylethylamine (**3g**)*

78% yield; colorless oil; MS *m/z* (ion, relative intensity, %): 192 ([M^+H], 25), 175 (M^+–NH_2, 8), 137 ([M^+H]–Cl–HF, 35), 107 ([M + H]–CF_2Cl, 100); IR (KBr) ν_{max} (cm^{-1}): 3400, 1200, 1120, 1070, 1020, 980, 700; ^{1}H NMR *d* (CD_3COCD_3, TMS): 2.76 (2H, br-s, NH_2), 5.05 (1H, br, CH), 7.20–7.50 (5H, m, Ar–H); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 96.3 (1F, dd, J = 166.5 and 7.4 Hz, CF_2Cl), 99.4 (1F, dd, J = 166.5 and 7.4 Hz, CF_2Cl); high resolution-MS revealed: 192.0388. Calcd. for $C_{12}H_{11}F_3N$: 192.0392 [M^+H].

*2.2.6. 2,2,2-Trifluoro-1-(1-naphthyl)ethylamine (**3h**)*

55% yield; colorless oil; MS *m/z* (ion, relative intensity, %): 226 ([M^+H], 53), 157 ([M^+H]–CF_3, 85), 129 (M^+–CF_3–HCN, 100); IR (KBr) ν_{max} (cm^{-1}): 3400, 3050, 2970, 1600, 1510, 1160, 1120, 800, 780; ^{1}H NMR *d* (CD_3COCD_3, TMS): 2.56 (2H, br-s, NH_2), 6.02 (1H, q, J = 6.8 Hz, CH), 7.50–8.50 (7H, m, Ar–H); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 87.2 (d, J = 6.8 Hz, CF_3); high resolution-MS revealed: 226.0844. Calcd. for $C_{12}H_{11}F_3N$: 226.0844 [M^+H].

*2.2.7. N-[2,2,2-Trifluoro-1-(indol-3-yl)ethyl]formamide (**2i**)*

51% yield; colorless columns from dichloromethane–ethyl acetate (1:1); mp 139–140 °C; MS *m/z* (ion, relative intensity, %): 242 (M^+, 66), 222 (M^+–HF, 46), 173 (M^+–CF_3, 51), 118 (M^+–CF_3–CO–HCN, 82); IR (KBr) ν_{max} (cm^{-1}): 3300, 3030, 1650, 1620, 1530, 1460, 1270, 1120, 740; ^{1}H NMR *d* (CD_3COCD_3, TMS): 6.23 (1H, q, J = 7.5 Hz, CH), 7.10–7.70 (5H, m, 2′, 4′, 5′, 6′, 7′-H), 7.90–8.15 (1H, m, NH), 8.22 (1H, s, CHO), 10.50 (1H, br, 1′-NH); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 90.0 (s, CF_3); Anal. Found: C, 54.60; H, 3.78; N, 11.35%. Calcd. for $C_{11}H_9F_3N_2O$ (%): C, 54.53; H, 3.75; N, 11.57.

*2.2.8. 2,2,2-Trifluoro-1-(indol-3-yl)ethylamine (**3i**)*

89% yield; colorless needles from dichloromethane–ethyl acetate (1:1); mp 130–131 °C; MS *m/z* (ion, relative intensity, %): 214 (M^+, 55), 145 (M^+–CF_3, 100), 118 (M^+–CF_3–HCN, 83); IR (KBr) ν_{max} (cm^{-1}): 3350, 3150, 3050, 1450, 1260, 1110, 1010, 900, 740; ^{1}H NMR *d* (CD_3COCD_3, TMS): 2.30 (2H, br-s, NH_2), 4.84 (1H, q, J = 7.7 Hz, CH), 7.10–7.60 (4H, m, 2′, 5′, 6′, 7′-H), 7.75 (1H, m, 4′-H), 10.35 (1H, br, 1′-NH); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 87.4 (d, J = 7.7 Hz, CF_3); Anal. Found: C, 56.34; H, 4.25; N, 12.82%. Calcd. for $C_{10}H_9F_3N_2$ (%): C, 56.06; H, 4.24; N, 13.08.

2.3. Chloroacetylation of amines

Chloroacetamides were obtained with chloroacetic anhydride as the acyl reagent. General procedures: Chloroacetic anhydride (920 mg, 5.4 mmol) and 4-(dimethylamino)pyridine (DMAP, 60 mg, 0.5 mmol) were added to a mixture of racemic amine (500 mg, 1.8 mmol) in dicholoromethane (20 ml) and pyridine (0.1 ml) at 0 °C. This mixture was stirred at room temperature for 5 h, and then the solvent was removed in vacuo. The residue was dissolved in ethyl acetate (20 ml), washed successively with 1N hydrochloric acid (20 ml), 5% sodium hydrogen carbonate (20 ml), and brine (20 ml), and then dried over anhydrous sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography, and eluted with dichloromethane–hexane, giving the corresponding chloroacetamide.

*2.3.1. N-[2,2,2-Trifluoro-1-phenylethyl]chloroacetamide (**4a**)*

87% yield; colorless oil; MS *m/z* (ion, relative intensity, %): 252 ([M^+H], 19), 232 ([M^+H]–HF, 56), 183 ([M^+H]–CF_3, 10), 176 ([M^+H]–COCHCl, 40), 156 ([M^+H]–HF–COCHCl, 83), 107 ([M^+H]–CF_3–COCHCl, 100); IR (KBr) ν_{max} (cm^{-1}): 1770, 1540, 1270, 1250, 1190, 1140, 1030, 950; ^{1}H NMR *d* (CD_3COCD_3, TMS): 4.52 (2H, s, $COCH_2Cl$), 6.40 (1H, q, J = 6.9 Hz, CH), 7.45–7.65 (5H, m, Ar–H); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 87.9 (d, J = 6.9 Hz, CF_3).

*2.3.2. N-[2,2-Difluoro-1-phenylethyl]chloroacetamide (**4b**)*

76% yield; colorless oil; MS *m/z* (ion, relative intensity, %): 234 ([M^+H], 3), 214 ([M^+H]–HF, 48), 183 ([M^+H]–CHF_2, 22), 138 ([M^+H]–HF-COCHCl, 83), 107 ([M^+H]–CHF_2–COCHCl, 100); IR (KBr) ν_{max} (cm^{-1}): 1770, 1500, 1410, 1280, 1150, 1020, 860, 700; ^{1}H NMR *d* (CD_3COCD_3, TMS): 4.15 (2H, s, $COCH_2Cl$), 5.95 (1H, br, CH), 6.00 (1H, dt, J = 64.5 and 4.4 Hz, CHF_2), 7.25–7.40 (5H, m, Ar–H); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 34.6 (dd, J = 64.5 and 9.9 Hz, CHF_2).

*2.3.3. N-[2,2,3,3,3-Pentafluoro-1-phenylpropyl]chloroacetamide (**4e**)*

89% yield; colorless oil; MS *m/z* (ion, relative intensity, %): 302 ([M^+H], 7), 183 ([M^+H]–CF_2CF_3, 67), 107 ([M^+H]–CF_2CF_3–COCHCl, 16), 105 (M^+–CF_2CF_3–$COCH_2Cl$, 100); IR (KBr) ν_{max} (cm^{-1}): 1770, 1500, 1410, 1280, 1150, 1020, 860, 700; ^{1}H NMR *d* (CD_3COCD_3, TMS): 4.15 (2H, s, $COCH_2Cl$), 6.26 (1H, dd, J = 14.9 and 8.4 Hz, CH), 7.20–7.45 (5H, m, Ar–H); ^{19}F NMR *d* (CD_3COCD_3, C_6F_6): 38.4 (2F, dq, J = 277.9 and 14.9 Hz, CF_2), 80.1 (3F, t, CF_3).

2.3.4. N-[2,2,2-Trifluoro-1-(4-methoxyphenyl)ethyl]-chloroacetamide (4f)

78% yield; colorless oil; MS *m*/*z* (ion, relative intensity, %): 282 ([M^+H], 23), 189 (M^+–NHCOCH$_2$Cl, 35), 137 ([M^+H]–CF$_3$–COCHCl, 100); IR (KBr) ν_{max} (cm^{-1}): 1780, 1620, 1520, 1190, 1140, 1030, 830; ^{1}H NMR *d* (CD$_3$COCD$_3$, TMS): 3.82 (3H, s, OCH$_3$), 4.16 (2H, s, COCH$_2$Cl), 6.12 (1H, d, J = 6.6 Hz, CH), 6.93 (2H, d, J = 8.8 Hz, Ar–H), 7.40 (2H, d, J = 8.8 Hz, Ar–H); ^{19}F NMR *d* (CD$_3$COCD$_3$, C$_6$F$_6$): 85.8 (3F, s, CF$_3$).

2.3.5. N-[2-Chloro-2,2-difluoro-1-phenylethyl]-chloroacetamide (4g)

69% yield; colorless oil; MS *m*/*z* (ion, relative intensity, %): 268 ([M^+H], 3), 232 (M^+–Cl, 32), 183 ([M^+H]–CF$_2$Cl, 25), 156 (M^+–Cl–COCHCl, 35), 107 ([M^+H]–CF$_2$Cl–COCHCl, 100); IR (KBr) ν_{max} (cm^{-1}): 1780, 1200, 1150, 1020, 980, 700; ^{1}H NMR *d* (CD$_3$COCD$_3$, TMS): 4.19 (2H, s, COCH$_2$Cl), 6.24 (1H, t, J = 8.8 Hz, CH), 7.20–7.55 (5H, m, Ar–H); ^{19}F NMR *d* (CD$_3$COCD$_3$, C$_6$F$_6$): 99.0 (2F, s, CF$_2$Cl).

2.3.6. N-[2,2,2-Trifluoro-1-(1-naphthyl)ethyl]-chloroacetamide (4h)

76% yield; colorless oil; MS *m*/*z* (ion, relative intensity, %): 302 ([M^+H], 54), 233 ([M^+H]–CF$_3$, 12), 209 (M^+–NHCOCH$_2$Cl, 24), 157 ([M^+H]–CF$_3$–COCHCl, 100); IR (KBr) ν_{max} (cm^{-1}): 1770, 1270, 1170, 1130, 1050, 1000, 800; ^{1}H NMR *d* (CD$_3$COCD$_3$, TMS): 4.58 (2H, s, COCH$_2$Cl), 6.60 (1H, q, J = 7.5 Hz, CH), 7.20–8.20 (7H, m, Ar–H), 8.38 (1H, d, J = 7.5 Hz, NH); ^{19}F NMR *d* (CD$_3$COCD$_3$, C$_6$F$_6$): 88.9 (d, J = 7.5 Hz, CF$_3$).

2.3.7. N-[2,2,2-Trifluoro-1-(indol-3-yl)ethyl]-chloroacetamide (4i)

91% yield; colorless oil; MS *m*/*z* (ion, relative intensity, %): 290 (M^+, 30), 221 (M^+–CF$_3$, 27), 235 (M^+–CO–HCN, 100), 157 (M^+–CF$_3$–COCHCl, 41); IR (KBr) ν_{max} (cm^{-1}): 1680, 1540, 1520, 1310, 1260, 1120, 830; ^{1}H NMR *d* (CD$_3$COCD$_3$, TMS): 4.20 (2H, s, COCH$_2$Cl), 6.14 (1H, q, J = 7.5 Hz, CH), 7.05–7.80 (5H, m, Ar–H), 8.25 (1H, d, J = 7.5 Hz, NH), 10.54 (1H, br, 1′-NH); ^{19}F NMR *d* (CD$_3$COCD$_3$, C$_6$F$_6$): 90.15 (d, J = 7.5 Hz, CF$_3$).

2.4. Lipase-catalyzed alcoholysis of racemic chloroacetamide derivatives

Lipase (25 mg) was added to a mixture of (*R*, *S*)-racemic chloroacetamide (1.5 mg), *n*-butanol (25 μl), and molecular sieves 4 Å (30 mg) in diisopropyl ether (3 ml). After being stirred on a reciprocal shaker at 120 rpm and 30 °C for an adequate period, the mixture was filtered, and the filtrate evaporated in vacuo. The residue was subjected to gas chromatography (GC) to determine the conversion and enantiomeric excess. GC was done in combination with a flame ionization detector and a Chiraldex B-PN (0.25 mm × 30 m, He, 1.0 ml/min, Astec). Retention times were: (*S*)-**4a**, 8.1 min; (*R*)-**4a**, 8.3 min; (*S*)-**3a**, 6.7 min; (*R*)-**3a**, 6.9 min (130 °C); (+)-**4b**, 12.2 min; (−)-**4b**, 12.5 min; (+)-**3b**, 5.8 min; (−)-**3b**, 6.0 min (140 °C); (+)-**4e**, 10.1 min; (−)-**4e**, 10.9 min; (*R*, *S*)-**3e**, 9.5 (120 °C); (+)-**4f**, 19.3 min; (−)-**4f**, 19.6 min; (+)-**3f**, 12.4 min; (−)-**3f**, 12.8 min (140 °C); (+)-**4g**, 16.9 min; (−)-**4g**, 17.2 min; (+)-**3g**, 13.1 min; (−)-**3g**, 13.4 min (130 °C); (+)-**4h**, 12.8 min; (−)-**4h**, 13.5 min; (+)-**3h**, 12.8 min; (−)-**3h**, 13.5 min (130 °C); (+)-**4i**, 12.8 min; (−)-**4i** 13.5 min; (+)-**3i**, 12.8 min; (−)-**3i**, 13.5 min (130 °C);

2.5. Resolution of fluorinated 1-arylethylamine derivatives by Pseudomonas fluorescens lipase

2.5.1. (S)-2,2,2-Trifluoro-1-phenylethylamine: (S)-3a and (R)-2,2,2-trifluoro-1-phenylethylamine: (R)-3a

Lipase AK (2.5 g) and (*R*, *S*)-**4a** (150 mg) were added to a mixture of *n*-amyl alcohol (1.0 ml), molecular sieves 4 Å (0.5 g), and diisopropyl ether (50 ml). This suspension was stirred at 120 rpm for 17 h at 30 °C on a reciprocal shaker, after which it was treated as described above. Silica-gel column chromatography with *n*-hexane/ethyl acetate (95:5, v/v) as the eluent gave (*R*)-*N*-(2,2,2-trifluoro-1-phenylethyl)chloroacetamide: (*R*)-**4a** [68 mg, 45% yield; $[\alpha]_D^{25}$ −63.3° (c = 3.4 ethanol)]. Further elution with hexane/ethyl acetate (80:20, v/v) gave (*S*)-**3a** [39 mg, 37% yield, 85% e.e.; $[\alpha]_D^{25}$ +19.2° (c = 1.9 ethanol)]. Chloroacetamide (*R*)-**4a** (68 mg) was added to a solution of 3N aqueous hydrochloric acid (5 ml), and the whole was stirred at reflux for 24 h. Distilled water (5 ml) was added, and the mixture was neutralized with saturated aqueous sodium hydrogen carbonate solution. The aqueous layer was separated and treated twice with ethyl acetate. The organic phases were combined, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [eluted with 80:20 (v/v) *n*-hexane:ethyl acetate], giving the amine (*R*)-**3a** [39 mg, 82% yield, >99% e.e.; $[\alpha]_D^{25}$ −23.0° (c = 1.9 ethanol) {[7], $[\alpha]_D^{25}$ −17.72° (c = 3.44 ethanol), 81% e.e.}].

2.5.2. (+)-2,2-Difluoro-1-phenylethylamine: (+)-3b and (−)-2,2-difluoro-1-phenylethylamine: (−)-3b

Compounds (+)-**3b** and (−)-**3b** were prepared as above (reaction period, 15 h). (−)-**4b** [68 mg, 45% yield, >99% e.e., $[\alpha]_D^{20}$ −80.4° (c = 3.4, ethanol)] and (+)-**3b** [50 mg, 50% yield, 89% e.e., $[\alpha]_D^{20}$ +13.0° (c = 2.5, ethanol)]. Chemical hydrolysis of (−)-**4b** (60 mg) gave (−)-**3b** [35 mg, 87% yield, 99% e.e., $[\alpha]_D^{20}$ −14.5° (c = 1.7, ethanol)].

2.5.3. (+)-2,2,3,3,3-Pentafluoro-1-phenylpropylamine: (+)-3e and (−)-2,2,3,3,3-pentafluoroethyl-1-phenylethylamine: (−)-3e

Compounds (+)-**3e** and (−)-**3e** were prepared as above (reaction period, 90 h). (−)-**4e** [50 mg, 33% yield, >99% e.e.,

$[\alpha]_D^{20}$ −49.3° (c = 2.5, ethanol)] and (+)-**3e** [41 mg, 45% yield, 75% e.e., $[\alpha]_D^{20}$ +15.5° (c = 2.0, ethanol)]. Chemical hydrolysis of (−)-**4e** (50 mg) gave (−)-**3e** [29 mg, 78% yield, >99% e.e., $[\alpha]_D^{20}$ −20.9° (c = 1.5, ethanol)].

*2.5.4. (+)-2,2,2-Trifluoro-1-(4-methoxyphenyl)ethylamine: (+)-**3f** and (−)-2,2,2-trifluoro-1-(4-methoxyphenyl)-ethylamine: (−)-**3f***

Compounds (+)-**3f** and (−)-**3f** were prepared as above (reaction period, 45 h). (−)-**4f** [68 mg, 45% yield, 96% e.e., $[\alpha]_D^{20}$ −94.6° (c = 3.4, ethanol)] and (+)-**3f** [58 mg, 53% yield, 91% e.e., $[\alpha]_D^{20}$ +28.0° (c = 2.9, ethanol)]. Chemical hydrolysis of (−)-**4f** (68 mg) gave (−)-**3f** [44 mg, 89% yield, 94% e.e., $[\alpha]_D^{20}$ −29.3° (c = 2.2, ethanol)].

*2.5.5. (+)-2-Chloro-2,2-difluoro-1-phenylethylamine: (+)-**3g** and (−)-2-chloro-2,2-difluoro-1-phenylethylamine: (−)-**3g***

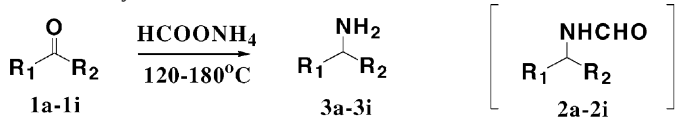
Compounds (+)-**3g** and (−)-**3g** were prepared as above (reaction period, 24 h). (−)-**4g** [80 mg, 53% yield, 80% e.e., $[\alpha]_D^{20}$ −48.8° (c = 4.0, ethanol)] and (+)-**4g** [45 mg, 42% yield, >99% e.e., $[\alpha]_D^{20}$ +15.0° (c = 2.3, ethanol)]. Chemical hydrolysis of (−)-**4g** (80 mg) gave (−)-**3g** [40 mg, 70% yield, 80% e.e., $[\alpha]_D^{20}$ −11.3° (c = 2.0, ethanol)].

3. Results and discussion

The phenyl a-fluorinated ketones, starting materials **1a**, **1b**, **1c**, **1e**, **1f** and **1g**, were prepared from fluorinated esters and the Grignard reagent in good yields (55–91%) [19]. 1-Naphthyl trifluoromethyl ketone **1b** was prepared from naphthalene, trifluoroacetic anhydride, and $AlCl_3$ (54% yield) [20], and the 3-indolyl trifluoromethyl ketone **1c** from indole and trifluoroacetic anhydride (90% yield) [21] according to the reported procedures. The racemic fluorinated arylethylamines **3a**–**3i** were synthesized by direct reductive amination (Leuckart-Wallach reaction) [22] of the corresponding ketones **1a**–**1i** (Table 1). Treatment of the phenyl trifluoromethyl- **1a**, pentafluoroethyl- **1e**, chlorodifluoromethyl- **1g**, and 4-methoxyphenyl trifluoromethyl- **1f**, ketones with ammonium formate gave the corresponding amines (**3a**, **3e**, **3f** and **3g**) in high yields (>90% yield, as determined by GC). Difluoromethylated amine **3b** was prepared from ketone **1b** under the same reaction conditions, but the yield was only 68%, and formamide **2b** was produced as an intermediate in the reaction mixture. The corresponding reaction of fluoromethylated ketone **1c** yielded complicated products, possibly due to the hydrogen fluoride (HF) generated attacking the substrate. With 1-naphthyl α-trifluoromethyl ketone **1h**, the reaction proceeded slowly, as compared with that of the corresponding phenyl ketone **1a**, providing the amine **3h** in moderate yield (68%). In contrast, a high yield (83%) of the trifluoromethyl indol-3-yl formamide **2h** was obtained in the reaction of the indol-3-yl α-trifluoromethyl ketone **1h**, but the desired amine **3h** was not detected in the mixture. The formamide **2h** that was isolated was smoothly converted to amine **3h** by hydrolysis with aqueous sodium hydroxide.

Earlier experiments on the lipase-catalyzed kinetic resolution of trifluoromethylated alcohols in anhydrous organic solvents prompted this investigation of the acetylation of amines in anhydrous solvents. The *P. cepacia* (Amano PS), *P. fluorescens* (Amano AK), and *C. antarctica* (Novo SP435) lipases did not catalyze the acetylation of amines with various acetyl donors in diisopropyl ether, which previously we have selected for lipase-catalyzed acetylation.

Table 1
Reaction of aryl fluorinated ketones with ammonium formate[a]



1a-1i 3a-3i 2a-2i

a; $\mathbf{R_1}$ =Ph, $\mathbf{R_2}$ =CF_3 **b**; $\mathbf{R_1}$ =Ph, $\mathbf{R_2}$ =CHF_2 **c**; $\mathbf{R_1}$ =Ph, $\mathbf{R_2}$ =CH_2F
d; $\mathbf{R_1}$ =Ph, $\mathbf{R_2}$ =CH_3 **e**; $\mathbf{R_1}$ =Ph, $\mathbf{R_2}$ =CF_2CF_3 **f**; $\mathbf{R_1}$ =4-MeOPh, $\mathbf{R_2}$ =CF_3
g; $\mathbf{R_1}$ =Ph, $\mathbf{R_2}$ =CF_2Cl **h**; $\mathbf{R_1}$ =1-Nap, $\mathbf{R_2}$ =CF_3 **i**; $\mathbf{R_1}$ =Indol-3-ly, $\mathbf{R_2}$ =CF_3

Entry	Ketone (yield[b], %)	Amide (%)	Amine (%)
1	**1a**: trace	**2a**: 4	**3a**: 95
2	**1b**: trace	**2b**: 39	**3b**: 68
3	**1c**:	Complex mixture	
4	**1d**: 29	**2d**: 68	**3d**: trace
5	**1e**: trace	**2e**: 3	**3e**: 95
6	**1f**: 2	**2f**: 3	**3f**: 93
7	**1g**: trace	**2g**: 5	**3g**: 92
8	**1h**: 23	**2h**: 5	**3h**: 68
9	**1i**: 15	**2i**: 83	**3i**: trace

[a] Reactions were performed at 120 °C for 2 h then at 180 °C for 4 h. Aryl ketone (10 mmol) and ammonium formate (50 mmol) were used.
[b] Chemical yields were determined by GC.

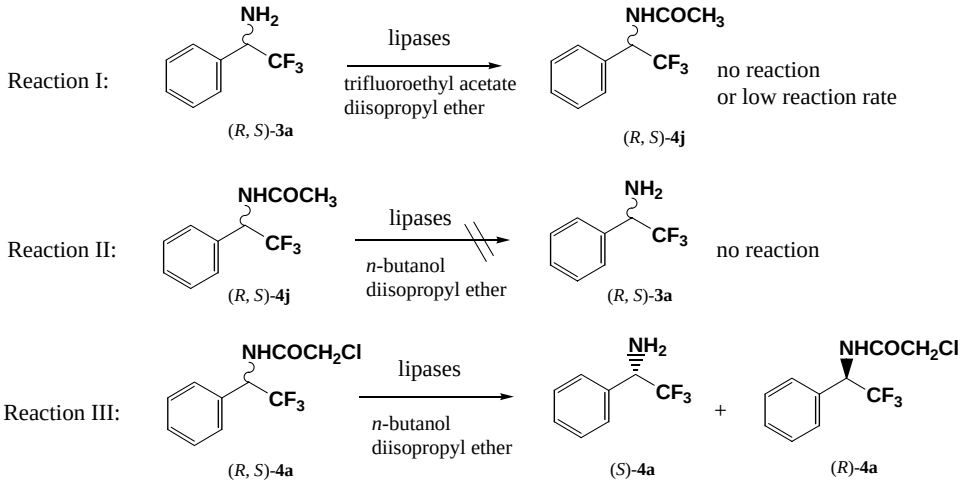


Fig. 1. Enantoselective alcoholysis of (*R*, *S*)-**4a** by lipases.

Only *P. aeruginosa* (Toyobo LIP) catalyzed the acetylation of (*R*, *S*)-**3a** in isopropyl ether with trifluoroethyl acetate (reaction I, Fig. 1), acetylation proceeding at an exceedingly slow rate, and prolonged reaction times (>7 days) being required for any reasonable substrate conversion. The bulky and low affinity trifluoromethyl group reduced the reactivity of lipases [23]. Lipase-catalyzed enantioselective alcoholysis of acetamide derivatives in organic solvents therefore was investigated. Lipases did not significantly catalyze the reaction of *N*-(2,2,2-trifluoro-1-phenylethyl)acetamide with *n*-butanol (reaction II). As lipase-catalyzed reactions were improved with the activated choloroacetates of trifluoromethylated secondary alcohols [23], we extended our experiments to primary fluorinated arylethylamine **3a**. The above lipases were screened for enantioselecitve alcoholysis of racemic chloroacetamide (*R*, *S*)-**4a** (reaction III). Enzymatic alcoholysis for **4a** with selected lipases are shown Table 2. Of those lipases, AK and PS had very high catalytic activity and enantioselectivity in the alcoholysis of (*R*, *S*)-**4a** (Fig. 1), AK having the highest *E*-value. For example, alcoholysis of (*R*, *S*)-**4a** with *n*-butanol in diisopropyl ether led to 42% conversion within 17 h, and an *E*-value of 42 [24]. Reactions with five other lipases (LIP, SL, TL, PLC, SP435) were also examined, but there was no improvement in enantioselectivity ($E < 10$) (Table 2). Enzymatic hydrolysis of (*R*, *S*)-**4a** in aqueous solution (pH 7, 0.1 M phosphate buffer) was also done, but enantioselectivity was slightly decreased as compared to that found for alcoholysis in organic solvents. We also confirmed that no reaction occurred in the absence of lipase.

Because the obtained *E*-value of 42 can be considered just acceptable for asymmetric synthesis, we studied the dependence of enantioselectivity on the nature of the solvent in more detail (Table 3). Diisopropyl ether proved the best solvent for lipase AK-catalyzed alcoholysis. The highest *E*-value was obtained with the polar solvent dichloroethane, but the reactivity was lower than with isopropyl ether. For optimization of enantioselectivity, attention was paid to the structure of the alcohol chain in the alcoholysis. Of the four straight chain alcohols, the highest *E*-value and substrate conversion were with *n*-amyl alcohol. The others, *n*-propyl, *n*-butyl, and *n*-hexyl alcohol, had slightly lower selectivities and reactivities.

To determine the effect of the fluorine atom(s) on the alcoholysis activity of lipase AK, four fluorinated 1-phenylethylamines with different fluoroalkyl groups (**4a**, CF_3–; **4b**, CHF_2–; **4e**, CF_3CF_2–; **4g**, CF_2Cl–) were synthesized and subjected to lipase-catalyzed enantioselective alcoholysis with *n*-butanol in diisopropyl ether (Table 4). This lipase showed similar substrate conversions for **4a** (42%) and **4b** (38%) during a reaction period of 17 h, but different enantioselectivities (*E*-value) of 42 (**4a**) and 82 (**4b**). In

Table 2
Lipase-catalyzed alcoholysis of racemic 2,2,2-trifluoro-1-phenylethylchloroacetamide (*R*, *S*)-**4a** with *n*-butanol in diisopropyl ether[a]

Lipase[b]	Time (h)	Conversion (%)[c]	Amine produced (%e.e.) (configuration)	E[d]
AK	17	42	91 (*S*)	42
PS	17	32	89 (*S*)	26
LIP	17	42	61 (*S*)	6
SL	17	40	55 (*S*)	5
TL	10	44	3 (*S*)	1
PLC	17	75	29 (*S*)	5
SP435	17	54	54 (*S*)	9

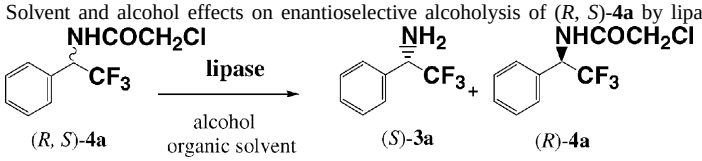
[a] Lipase (25 mg) was added to a mixture of (*R*, *S*)-**4a** (1.5 mg), *n*-butanol (25 μl), and molecular sieves 4 Å (30 mg) in diisopropyl ether (3 ml) at 30 °C.

[b] AK: *Pseudomonas fluorescens* lipase, PS: *Pseudomonas cepacia* lipase, LIP: *Pseudomonas aeruginosa* lipase, SL: *Pseudomonas cepacia* lipase, TL: *Pseudomonas stutzeri* lipase, AY: *Candida rugosa* lipase, PLC: *Alcaligenes* sp. lipase, SP435: *Candida antarctica* lipase.

[c] Determined by HPLC.

[d] *E*-values were calculated by the method of Chen et al. [24]. $E = \ln[1 - \text{conversion}\{1 + \text{e.e.(amine)}\}]/\ln[1 - \text{conversion}\{1 - \text{e.e.(amine)}\}]$.

Table 3
Solvent and alcohol effects on enantioselective alcoholysis of (*R*, *S*)-**4a** by lipase AK[a]



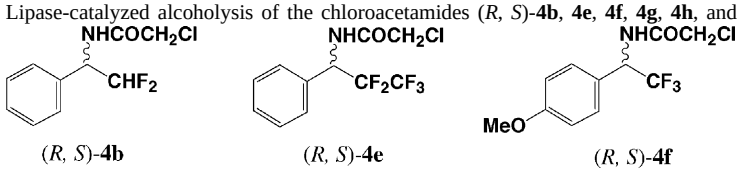
(*R*, *S*)-**4a** (*S*)-**3a** (*R*)-**4a**

Solvent	Alcohol	Time (h)	Conversion (%)[b]	Amine produced (%e.e.) (configuration)	*E*
Isopropyl ether	*n*-Propyl	17	44	90 (*S*)	40
Isopropyl ether	*n*-Butyl	17	42	91 (*S*)	42
Isopropyl ether	*n*-Amyl	17	48	89 (*S*)	44
Isopropyl ether	*n*-Hexyl	17	48	88 (*S*)	39
Heptane	*n*-Amyl	10	44	85 (*S*)	25
Toluene	*n*-Amyl	17	24	83 (*S*)	14
Dichloroethane	*n*-Amyl	17	27	95 (*S*)	57

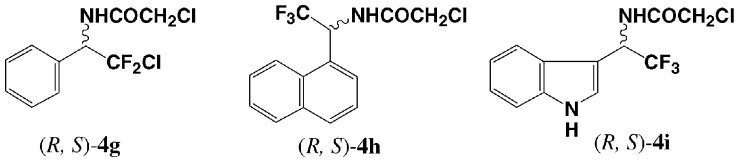
[a] Lipase (25 mg) was added to a mixture of (*R*, *S*)-**4a** (1.5 mg), the test alcohol (25 μl), and molecular sieves 4 Å (30 mg) in organic solvent (3 ml) at 30 °C.
[b] Determined by GC.

general, lipase-catalyzed acetylation of the non-fluorinated phenylethylamine (**3d**) with ethyl acetate provided good stereoselective resolution ($E > 100$). Enantioselectivity was markedly affected by the number of fluorine atoms on the alkyl group of phenylethylamine. Moreover, compounds **4e** and **4g** which have larger substituents were resolvable in good enantioselectivity by lipase AK ($E = 25$, **4e**; $E > 100$, **4g**) with lower substrate conversion, more than 45 h being needed to achieve a reasonable conversion. To determine how the structures of the aromatic rings affect lipase AKs ability to catalyze the alcoholysis of acetamides, three substrates (4-methoxyphenyl, **4f**; 1-naphthyl, **4h**; indole-3-yl, **4i**) were subjected to alcoholysis, and the enantioselectivity and reactivity were monitored. The methoxy group on the phenyl ring of **4f** decreased lipase reactivity as compared to the unsubstituted compound **4a**, but had a similar *E*-value on enantioselectivity ($E = 44$, **4a**; $E = 54$, **4f**). Compounds **4h** (1-naphthyl) and **4i** (indol-3-yl), bearing sterically hindered aromatic rings, did not react with lipase AK, even over a 65 h period. The reaction was markedly slower, and enantioselectivity was not examined further.

Table 4
Lipase-catalyzed alcoholysis of the chloroacetamides (*R*, *S*)-**4b**, **4e**, **4f**, **4g**, **4h**, and **4i** with *n*-amyl alcohol in diisopropyl ether[a]



(*R*, *S*)-**4b** (*R*, *S*)-**4e** (*R*, *S*)-**4f**



(*R*, *S*)-**4g** (*R*, *S*)-**4h** (*R*, *S*)-**4i**

Compound	Time (h)	Conversion (%)[b]	Amine produced (%e.e.)	*E*
(*R*, *S*)-**4b**	17	38	96	82
(*R*, *S*)-**4e**	45	15	91	25
(*R*, *S*)-**4f**	17	26	95	54
(*R*, *S*)-**4g**	17	22	>99	>100
(*R*, *S*)-**4h**	65	–	–[c]	–
(*R*, *S*)-**4i**	65	–	–[c]	–

[a] Lipase (25 mg) was added to a mixture of racemic chloroacetamide (1.5 mg), *n*-amyl alcohol (25 μl), and molecular sieves 4 Å (30 mg) in diisopropyl ether (3 ml) at 30 °C.
[b] Determined by GC.
[c] Not detected.

Our findings show that a biocatalyst can be a valuable agent for the separating of an enantiomeric mixture. To this end, preparative scale enzymatic separations of **4a** (150 mg) were done by alcoholysis of acetamides with *n*-amyl alcohol (1.0 ml) in diisopropyl ether (50 ml) in the presence of lipase AK (2.5 g). After incubation for 17 h, the products were isolated by silica gel chromatography, giving the amine (*S*)-**3a** with 85% e.e. in 37% yield and the non-reacted acetamide (*R*)-**4a** in 45% yield. Hydrolysis of (*S*)-**4a** with 3 M HCl gave the amine (*S*)-**3a** with >99% e.e. in 82% yield. A similar procedure readily provided the enantiomers of **3b**, **3e**, **3f**, and **3g** in good enantiomeric excess, as described in materials and methods, respectively: (+)-**3b**, 89% e.e.; (−)-**3b**, 99% e.e.; (+)-**3e**, 75% e.e.; (−)-**3e**, >99% e.e.; (+)-**3f**, 91% e.e.; (−)-**3f**, 94% e.e.; (+)-**3g**, >99% e.e.; (−)-**3g**, 80% e.e. Determinations of the absolute configurations of the two enantiomers of alcohols **3b**, **3e**, **3f**, and **3g** are in progress.

4. Conclusions

We showed that, among the chosen enzymes, the *P. fluorescens* lipase (Amano AK) is suitable for the alcoholysis of fluorinated arylethyl chloroacetamides **4a**, **4b**, **4f**, and **4g** at rates compatible with those in the preparative application. Enantioselectivities of the reactions were good to excellent ($E = 25$ to >100) for the four racemic chloroacetamides. Lipase AK, however, did not catalyze the alcoholysis of compounds **4h** and **4i** which have large-size aromatic rings. Further studies are required to investigate these enzymatic reactions in detail and to answer the questions raised by the findings of present research.

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