Title	Kinetic Resolution of Racemic α-Aminonitriles via Stereoselective N-Acetylation Catalyzed by Lipase in Organic Solvent (Commemoration Issue Dedicated to Professor Shigeo Tanimoto On the Occation of His Retirement)
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NOTE

Kinetic Resolution of Racemic α-Aminonitriles via Stereoselective *N*-Acetylation Catalyzed by Lipase in Organic Solvent

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Lipase-catalysis in organic synthesis has received much attention as an effective method for the preparation of optically active alcohols, carboxylic acids and their derivatives[1]. In particular, asymmetric esterification or transesterification in organic solvents is a method of frequent choice for facile kinetic resolutions of racemic alcohols[2]. In the course of our studies on the kinetic resolution of racemic α-hydroxynitriles (cyanohydrins) via lipase-catalyzed acylation in an organic solvent[3], we were interested in the kinetic resolution of their amino analogues, α-aminonitriles, via stereoselective *N*-acylation. α-Aminonitriles are important key intermediates for the synthesis of amino acids (Strecker synthesis)[4] and are easily prepared from the corresponding cyanohydrins and ammonia[5]. We report here the stereoselective *N*-acetylation of racemic α-aminonitriles catalyzed by lipase in an organic solvent.

Racemic α-aminonitriles **1a-d** were allowed to react with 2,2,2-trifluoroethyl acetate (TFEA) in anhydrous diisopropyl ether in the presence of an immobilized lipase (Scheme). The α-aminonitriles are unique in that they did not react with TFEA at all without the lipase[6], probably due to the strong electron-withdrawing effect of the cyano group at the α-position. As a preliminary experiment, four different lipases[7] including three *Pseudomonas* lipases from different origin and a lipase from *Chromobacterium viscosum* were tested for the *N*-acetylation of **1a.** As shown in Table 1, the enantioselectivity as well as the reaction rate was highly dependent on the lipase, although the total activity or the amount of the active lipase added did not differ significantly from each other[8]. Among the four lipases tested, a lipase from

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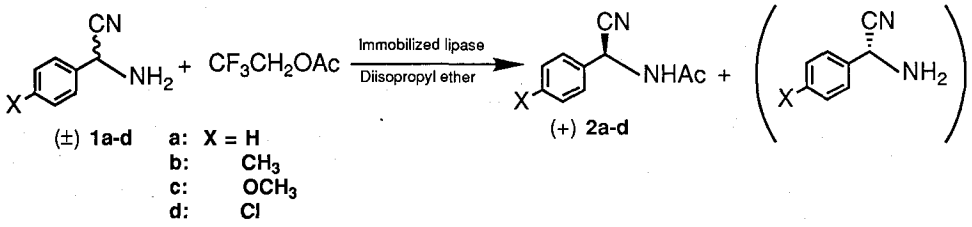

Table 1. Stereoselective *N*-acetylation of **1a** catalyzed by different lipases[a]

origin of lipase	supplier	amount of enzyme (unit)[b]	reaction time (h)	conversion[c] (%)	**2a** e. e.[d] (%)	**2a** abs. config.
Pseudomonas aeruginosa	Toyobo	10.5	44	53	72	*S*
Pseudomonas sp.	Kurita	17.0	147	42	32	*S*
Pseudomonas cepacia	Amano	28.4	147	53	10	*R*
Chromobacterium viscosum	Toyo Jozo	8.9	147	51	70	*S*

[a]Conditions: **1a** (50mg, 0.38mmol), 2,2,2-trifluoroethyl acetate (162mg, 1.14mmol), immobilized lipase powder (38mg), dry diisopropyl ether (10ml), 40℃.
[b]The catalytic activity of the lipases was measured by transesterification of 2,2,2-trifluoroethyl octanoate (TFEO, 50mM) and ethanol (200mM) in dry diisopropyl ether (10ml).[8] One unit of lipase is the amount of immobilized lipase which converts 1 μmol of TFEO to ethyl octanoate per min.
[c]Determined by ^{1}H NMR.
[d]Determined by ^{1}H NMR in the presence of Eu(hfc)$_3$.

Pseudomonas aeruginosa (Toyobo) exhibited the highest catalytic activity and stereoselectivity toward **1a.** Despite the total catalytic activity of twice as much as that of Toyobo enzyme, very slow reaction as well as low stereoselectivity was observed for a lipase from *Pseudomonas cepacia* (Amano), which is so far one of the most frequently used biocatalysts for organic synthesis[9]. The preliminary experiment revealed that the stereoselectivity of the reaction was also affected by the choice of the acylating reagent and that TFEA served as a better acylating reagent with respect to the enantiomeric ratio (E=37)[10] than isopropenyl acetate (E=19) for the reaction with the Toyobo enzyme. Thus the lipase from *Ps. aeruginosa* (Toyobo) and TFEA were used for the reaction on a preparative scale.

The results of the stereoselective *N*-acetylation of **1a–d** were summarized in Table 2. The reaction was monitored with ^{1}H NMR and was stopped by filtering the enzyme powder when the proper conversion was attained. The filtrate was washed with dilute HCl to remove the unreacted amines **1a–d**, and the products **2a–d** were purified by column chromatography. The e. e. of **2a–d** was determined by ^{1}H NMR, using a chiral shift reagent, Eu(hfc)$_3$. The absolute configuration of **2a** was found to be *S* by comparing the sign of the optical rotation with that reported[11]. The absolute configuration of **2b–d** was tentatively assigned as *S* by correlating the configuration to the sign of optical rotation and to the relative intensity of two ^{1}H NMR peaks observed for *N*-acetyl proton in the presence of the chiral shift reagent. In the case of the corresponding oxygen analogues, (*S*)-cynohydrin acetates, the ^{1}H NMR

Table 2. Kinetic resolution of (±)-**1a-d** via lipase-catalyzed *N*-acetylation[a]

α-amino nitriles **1a-d**		reaction time (h)	conversion[b] (%)	Isolated yield of **2** (%)	$[\alpha]_D^{25}$ [c] (deg)	e. e. of **2**[d] (%)
1a	X=H	19	40	40	+16.0	90
1b	CH_3	56	43	42	+24.5	86
1c	CH_3O	80	50	44	+30.5	90
1d	Cl	147	39	24	+32.6	87

[a]Typical reaction conditions : α-aminoacetonitriles **1a** (6.0mmol), 2,2,2-trifluoroethyl acetate (18.0mmol), immobilized lipase powder (1.3g), dry diisopropyl ether (100ml), 40℃.
[b]Determined by ^1H NMR (200MHz).
[c]c=1.0, $CHCl_3$.
[d]Determined by ^1H NMR in the presence of Eu(hfc)$_3$.

peak (OAc) in lower magnetic field was invariably larger[3], and this correlation holds for the *N*-acetyl proton of (*S*)-**2a** (see Experimental). The *S* configuration of **2a, c** and **2d** was also confirmed by converting them to the corresponding phenylglycines (6*N* HCl, reflux, 24 h) and by comparing the optical rotation of the amino acids with those reported[12),13)].

The lipase showed fairly good stereoselectivity for all the α-aminonitriles **1 a-d**, giving optically active (*S*)-amides **2 a-d** with 86 to 90% e. e. The stereochemical preference of the lipase for the α-aminonitriles was in good agreement with that observed for the corresponding oxygen analogue, mandelonitrile[14]. Considering that efficient kinetic resolution of chiral amines has not yet been performed successfully by lipases[15], the present lipase served as an effective catalyst for the preparation of optically active amines.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were measured on a Varian VXR-200 spectrometer (200 MHz). Solvent was $CDCl_3$ with TMS as an internal standard. Infrared spectra were recorded on a Hitachi 215 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed on a Yanako MT-5 apparatus. Melting points are uncorrected. Diisopropyl ether was distilled over CaH_2 and stored over molecular sieves 4 Å. 2,2,2-Trifluoroethyl acetate (TFEA) was prepared from acetyl chloride and 2,2,2-trifluoroethanol [neat, 0℃ to room temp., bp 79℃, 74%]. The substrates **1 a-d** were prepared from the corresponding cyanohydrins by the reported procedure[5]. The lipases are generous gifts from the companies as follows: *Pseudomonas aeruginosa* TE3285 (Toyobo Co., Ltd.; Osaka, Japan); *Pseudomonas cepacia* (Amano Pharmaceutical Co., Ltd.; Nagoya, Japan); *Pseudomonas* sp. KWI-56 (Kurita Water Industries Ltd.; Atsugi, Japan); *Chromobacterium viscosum* (Toyo jozo Co., Ltd.; Tokyo, Japan). The lipases were used after immobilization as described below.

Immobilization of lipase.

The lipase (10 mg) and sucrose (75 mg) were dissolved in 20mM Tris–HCl buffer (pH 8.0, 5 ml). Hyflo Super-Cel (Johns-Manville International Corp., 1 g) was added to the solution, and the mixture was stirred at 0℃ for 15min and lyophilized. The lyophilized powder was slightly moistened by exposing the powder to humid air (37 ℃, 24 h). This operation was essential for high catalytic activity of the immobilized lipase in an organic solvent.

Preparation of (*S*)-(+)-*N*-(1-cyano-1-phenyl)methyl acetamide (2a);

Typical procedure. 2-Amino-2-phenylacetonitrile (**1a**) (1.0 g, 7.6 mmol) and 2,2,2-trifluoroethyl acetate (3.2 g, 23 mmol) were dissolved in dry diisopropyl ether (100 ml). The immobilized lipase (1.3 g) was added to the solution. The suspention was stirred for 19h at 40 ℃. The reaction conversion was checked by ^{1}H NMR [CH proton for **1a** (δ=4.92, s), and CH and NH proton for the amide **2a** (δ=6.09–6.23, m)] and was found to be 40%. The reaction mixture was filtrated and the lipase powder was washed three times with diisopropyl ether. The combined filtrates were washed with 2*N* HCl and brine. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The crude product was chromatographed on silica gel [dichloromethane (19): ethyl acetate (1)] to afford (*S*)-(+)-*N*-(1-cyano-1-phenyl)methyl acetamide (**2a**) as colorless crystals (531 mg, 40% yield); mp 100.3 ℃; $[\alpha]_D^{25}$=+16.0° (*c* 1.00, $CHCl_3$) [lit[11]. $[\alpha]_D^{25}$=−21.65° (*c* 1.4, $CHCl_3$) for the *R* isomer with 100 % e.e.]; 90 % e.e. [^{1}H NMR with Eu(hfc)$_3$: δ (NHCOC*H*$_3$) 5.48 (*R*, minor) and 5.80 (*S*, major)]; ^{1}H NMR δ=2.07 (s, 3H, CH_3CO), 6.09–6.23 (m, 2H, CH and NH), 7.41–7.52 (m, 5H$_{arom}$); IR (KBr) 2200 (C≡N) and 1640 (C=O) cm^{-1}; ^{13}C NMR δ =22.57 (*C*H$_3$CO), 44.11 (CH), 117.55 (C≡N), 127.04, 129.33, 129.51, 133.12, and 168.9 (C=O); Anal. Calcd. for $C_{10}H_{10}N_2O$: C, 68.95; H, 5.78; N, 16.08 %. Found: C, 69.04; H, 5.85; N, 15.76 %.

(*S*)-(+)-*N*-[1-cyano-1-(4-methylphenyl)]methyl acetamide (2b).

Prepared from 2-amino-2-(4-methylphenyl)acetonitrile (**1b**) in 43 % conversion yield. Column chromatogaphy [dichloromethane (19); ethyl acetate (1)] gave **2b** as colorless crystals: (42 %); mp 158.7–159.2 ℃; $[\alpha]_D^{25}$=+24.5° (*c* 1.00, $CHCl_3$); 86 % e.e. [^{1}H NMR with Eu(hfc)$_3$; δ (NHCOC*H*$_3$) 5.78 (minor) and 6.14 (major)], the absolute configuration was assigned as *S* by correlating the configuration to the sign of optical rotation and to the relative intensity of the two ^{1}H NMR peaks shown above; ^{1}H NMR δ=2.06 (s, 3H, CH_3CO), 2.38 (s, 3H, CH_3), 5.96–6.10 (m, 2H, CH and NH), 7.22–7.39 (m, 4H$_{arom}$); IR (KBr) 2200 (C≡N) and 1640 (C=O) cm^{-1}; ^{13}C NMR δ =21.14 (CH_3), 22.69 (*C*H$_3$CO), 43.90 (CH), 117.63 (C≡N), 126.96, 129.99, 130.20, 139.65, and 169.41 (C=O); Anal. Calcd. for $C_{11}H_{12}N_2O$: C, 70.19; H, 6.42; N, 14.88%. Found: C, 70.40; H, 6.40; N, 14.90%.

(*S*)-(+)-*N*-[1-cyano-1-(4-methoxyphenyl)]methyl acetamide (2c).

Prepared from 2-amino-2-(4-methoxypheny)acetonitrile (**1c**) in 50 % conversion yield. Column chromatography [dichloromethane (19): ethyl acetate (1)]

gave **2c** as colorless crystals: (44%); mp 161.1–161.2 ℃; $[\alpha]_D^{25}=+30.5°$ (*c* 1.00, $CHCl_3$); 90% e.e. [^{1}H NMR with Eu(hfc)$_3$; δ (NHCOC*H*$_3$) 5.14 (minor) and 5.46 (major)], the absolute configuration was assigned to be *S* by the same correlation as for (*S*)-(+)-**2b**; ^{1}H NMR δ=2.05 (s, 3H, CH_3CO), 3.83 (s, 3H, CH_3O), 6.01–6.96 (m, 2H, CH and NH), 6.92–7.42 (2×d, *J*=8.8 Hz, 4H$_{arom}$); IR (KBr) 2200 (C≡N) and 1640 (C=O); ^{13}C NMR δ=22.74 (*C*H$_3$CO), 43.66 (CH), 55.42 (CH_3O), 114.69, 117.67 (C≡N), 125.14, 128.48, 160.44, and 169.24 (C=O); Anal. Calcd. for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72%. Found: C, 64.81; H, 5.86; N, 13.67%.

(*S*)-(+)-*N*-[1-cyano-1-(4-cholorophenyl)]methyl acetamide (2 d).

Prepared from 2-amino-2-(4-chlorophenyl)acetonitrile (**1d**) in 39% conversion yield. Column chromatography [dichloromethane (19): ethyl acetate (1)] gave **2d** as colorless crystals: (24%); mp 129.1–129.2 ℃; $[\alpha]_D^{25}=+32.6°$ (*c* 1.00, $CHCl_3$); 87% e.e. [^{1}H NMR with Eu(hfc)$_3$; δ (NHCOC*H*$_3$) 5.68 (minor) and 6.20 (major)]; the absolute configuration was assigned to be *S* by the same correlation as for (*S*)-(+)-**2b**; ^{1}H NMR δ=2.07 (s, 3H, CH_3CO), 6.28 (m, 2H, CH and NH), 7.41 (m, 4H$_{arom}$); IR (KBr) 2200 (C≡N) and 1640 (C=O); ^{13}C NMR δ=22.68 (*C*H$_3$CO), 43.48 (CH), 117.16 (C≡N), 128.41, 129.54, 131.69, 135.66, and 169.65 (C=O). Anal. Calcd. for $C_{10}H_9ClN_2O$: C, 57.57; H, 4.35; N, 13.43%. Found: C, 57.73; H, 4.38; N, 13.12%.

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