



Lipase Catalysed Synthesis of Optically Enriched α -Haloamides

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Abstract—An efficient lipase catalysed synthesis of optically enriched α -halogenated amides with concomitant optical enrichment of the starting α -haloesters is described. *Candida antarctica* lipase (CAL) was found to be a better catalyst over porcine pancreatic lipase (PPL) and *Candida cylindracea* lipase (CCL). The effect of different organic solvents was also studied. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

The synthesis of optically active amides is an area of growing interest in synthetic organic chemistry,¹ since amide bonds are present in a large number of compounds exhibiting different biological activities.^{2–5} Synthesis of these compounds have mainly been achieved by classical chemical reactions which generally involve the generation of reactive carboxy derivative, either an acid chloride or anhydride, followed by aminolysis with amine.⁶ However, the conversion of esters to amides has some limitations as the commonly used reagents, that is sodium methoxide, sodium hydride, sodium metal, butyl lithium, and so forth, often interfere with other functional groups present in the reacting species.^{7–12} Besides these, the growing environmental consciousness and increasing demand of the chiral amides and acids led us to employ the biocatalytic procedures for carrying the amidation.

Due to their low cost and tolerance towards a variety of organic molecules, we explored the possibility of using lipases for carrying the aminolysis of esters. The use of lipases to catalyse amide bond formation is a mild and an interesting alternative to conventional methods using proteases, because lipases can act as catalysts in low hydrated (dry) organic solvents exhibiting wide substrate preference, high enantioselectivity and very low amidase activity.

α -Haloamides constitute an important class of compounds, they are used as starting materials for a wide variety of compounds, such as α -lactams, dioxopiper-

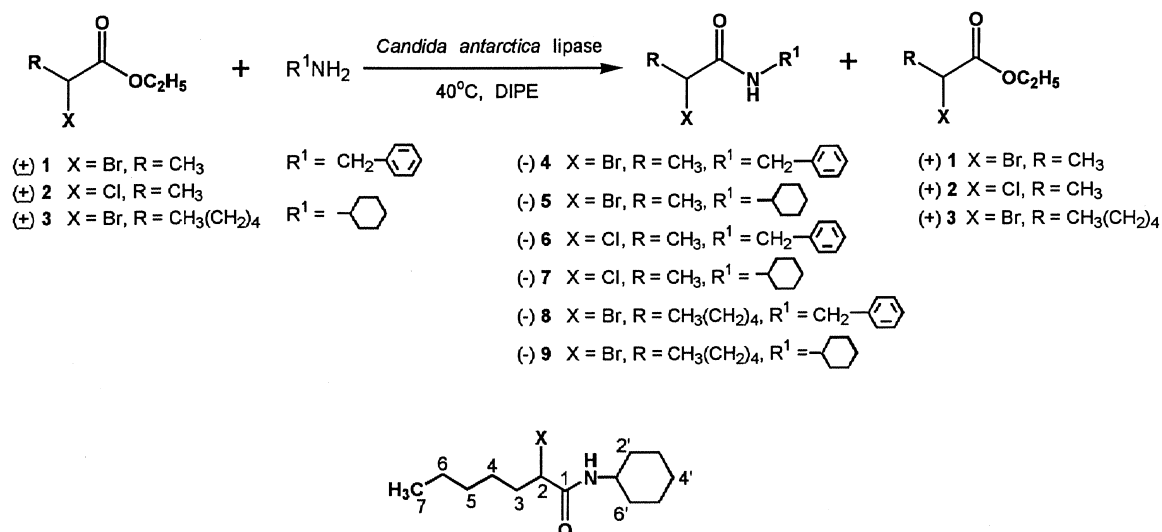
azines, oxazolidines and depsipeptides.^{13–16} Halogenated amides in optically pure form are also being used as herbicides.

These observations encouraged us to synthesise α -haloamides using lipases as catalysts. Gotor et al.¹⁷ have earlier reported the *Candida cylindracea* lipase (CCL) catalysed enantioselective aminolysis of the esters of (\pm)-2-chloropropanoic acid with aliphatic as well as aromatic amines, but the reactions were carried out at 2 and 60 °C with aliphatic and aromatic amines, respectively. In order to overcome this problem, we attempted aminolysis at a relatively more standard temperature (40 °C) using porcine pancreatic lipase (PPL) and *Candida antarctica* lipase (CAL) in different organic solvents, that is DMF, tetrahydrofuran, acetonitrile, hexane, cyclohexane and diisopropyl ether. Of these, CAL in diisopropyl ether (DIPE) was found to be a better choice over PPL as no significant reaction was observed with PPL.

Results and Discussion

We carried out the aminolysis of racemic α -haloesters, namely (\pm)-ethyl 2-bromopropanoate (**1**), (\pm)-ethyl 2-chloropropanoate (**2**) and (\pm)-ethyl 2-bromoheptanoate (**3**) with benzylamine and cyclohexylamine to obtain the corresponding enantiomerically enriched α -halogenated amides, that is (–)-*N*-benzyl 2-bromopropanamide (**4**), (–)-*N*-cyclohexyl 2-bromopropanamide (**5**), (–)-*N*-benzyl 2-chloropropanamide (**6**), (–)-*N*-cyclohexyl 2-chloropropanamide (**7**), (–)-*N*-benzyl 2-bromoheptanamide (**8**) and (–)-*N*-cyclohexyl 2-bromoheptanamide (**9**) (Scheme 1). It has been observed that the amide synthesis was faster with benzylamine as compared to that with cyclohexylamine (Table 1).

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Scheme 1. Lipase catalysed synthesis of α -haloamides.

The best conversion of (\pm)-esters to amides was observed by using CAL in diisopropyl ether, but no reaction occurred in DMF, chloroform and acetonitrile. With PPL also, no significant reaction was observed. The fact that both the amides as well as the unreacted esters were found to be optically active indicated enantioselection in CAL-catalysed aminolysis. It is noteworthy to mention that no amidation was observed when reaction was carried out under identical conditions, but without the addition of CAL and also no CAL-catalysed reaction was observed when phenyl or ethyl group was present at the α -position of the starting esters.

All the esters and amides were fully characterized from their spectral data. The three amides viz. (–)-*N*-cyclohexyl 2-bromopropanamide (**5**), (–)-*N*-benzyl 2-bromoheptanamide (**8**) and (–)-*N*-cyclohexyl 2-bromoheptanamide (**9**) have been synthesised for the first time. While the amide **4** is reported in literature in the racemic form,¹⁸ the amides **6** and **7** have been synthesised by Gotor et al.¹⁷ in optically enriched form by reaction with *C. cylindraceae* lipase (CCL). Since the optical rotation values of our samples of the compounds **6** and **7** were found to be higher than those reported by Gotor et al.¹⁷ (Table 1), we expect enhanced enantioselectivity with CAL in comparison to CCL-catalysed aminolysis. However, like CCL, CAL also prefers the *S*-enantiomer of the racemic

α -halo esters, thus giving in all the cases the corresponding (*S*)-amide. Quite interestingly, no product corresponding to the nucleophilic substitution of α -halogen atom by the amines was observed during the course of these reactions. Further work on the exact ee determination of the α -haloamides and esters is in progress and would be communicated separately.

In conclusion, we have developed a novel, efficient and environmentally benign method affording optically enriched α -halogenated amides and esters. This method may find general utility towards the synthesis of analogous compounds in optically enriched form as it is difficult to synthesise such compounds by purely chemical means in enantiomerically pure form due to the labile nature of the α -proton. CAL was found to be a better enzyme over PPL and CCL for carrying out the aminolysis of α -halogenated esters. No nucleophilic substitution of halides with amines was observed and optically enriched amides were exclusively obtained in high yields (80–92%).

Experimental

Melting points were determined on a Mettler FP62 instrument and are uncorrected. The IR spectra were recorded on a Perkin-Elmer RX/FT-IR spectro-

Table 1. *Candida antarctica* lipase (CAL) catalysed enantioselective aminolysis of α -haloesters by benzylamine and cyclohexylamine in diisopropyl ether at 40 °C

α -Haloamide	Time (h)	$[\alpha]_D^{25}$ value of the product amide	Yield (%)	Mp (°C)	Recovered ester	$[\alpha]_D^{25}$ value of the recovered ester	Yield (%)
4	14	–10.2	80	93–94 (lit.: 93.5–94.5) ¹⁸	1	+ 6.7	44
5	20	–6.0	85	128–129	1	+ 7.1	29
6	48	–8.6 (lit.: –4.3, ee74%) ¹⁷	85	77–78 (lit.: 64–66) ¹⁷	2	+ 4.5	22
7	60	–8.9 (lit.: –4.6, ee30%) ¹⁷	88	102–104 (lit.: 102–104) ¹⁷	2	+ 6.3	36
8	28	–14.7	87	55–57	3	+ 10.0	67
9	36	–25.0	92	97–99	3	+ 8.9	45

photometer. The optical rotations were measured with Bellingham Stanley AD 220 polarimeter. The ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AC-300 spectrometer at 300 and at 75 MHz, respectively, using TMS as internal standard. EI mass spectra were recorded with a Jeol AX 505 W mass spectrometer at 70 eV, while electrospray ionization (ESI) mass spectra were recorded with Esquire LC-00141 Bruker Daltonik GmbH. The enzyme, porcine pancreatic lipase (PPL, Type II) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used after keeping in vacuo over P_2O_5 for 24 h. The *C. antarctica* lipase, immobilised on accurel was gifted by Novo Nordisk Co. and used as such. Analytical TLCs were performed on precoated Merck silica gel 60F₂₅₄ plates; the spots were detected either under UV light or by charring with 4% alcoholic sulphuric acid. Solvent systems used were: **A** (ethyl acetate/petroleum ether, 1:19) and **B** (ethyl acetate:petroleum ether, 1:49). Reactions were monitored on Shimadzu LC-10AS HPLC instrument with SPD-10A UV-vis detector and Shimpack CLC-ODS (4.6×150 mm) reverse phase column; solvent system used was methanol/water at the flow rate of 1 mL/min.

Chemical method for the preparation of racemic amides

To a solution of the racemic α -halogenated ester (2.0 mmol) and the amine (2.0 mmol) in anhydrous toluene (5 mL) was added anhydrous aluminium chloride in catalytic amounts. The heterogeneous solution was kept for refluxing and progress of the reaction monitored by TLC. On completion, the reaction mixture was concentrated and poured over crushed ice. The aqueous solution was extracted with chloroform and the organic layer dried over Na_2SO_4 , solvent evaporated and the residue was subjected to column chromatography to afford the racemic amide.

General method for the aminolysis of esters using *C. antarctica* lipase

To a solution of the racemic α -halogenated esters **1–3** (5.0 mmol) and appropriate amine (2.5 mmol) in anhydrous diisopropyl ether (30 mL) was added immobilized *C. antarctica* lipase (300 mg). The mixture was incubated at 40 °C and progress of the reaction was monitored by TLC. When whole of the amine was consumed, the reaction was quenched by filtering off the enzyme. The organic solvent from the filtrate was evaporated in vacuo and the residue so obtained was subjected to column chromatography using petroleum ether/ethyl acetate as eluent yielding, both the amide and the unreacted ester in optically enriched forms.

(–)-*N*-Cyclohexyl 2-bromopropanamide (**5**). It was obtained as a white crystalline solid (497 mg) in 85% yield, mp 128–129 °C; R_f : 0.45 (solvent A); $[\alpha]_D^{25}$ –6.0 (c 0.42 in CHCl_3); EIMS, m/z (% rel. int.): 235 $[\text{M}+2]^+$ (12), 233 $[\text{M}]^+$ (11), 192 (10), 190 (10), 154 $[\text{M}-\text{Br}]^+$ (100), 152 (68), 109 (11), 107 (11), 98 (17), 83 (53), 67 (17) and 56 (59); ^1H NMR (CDCl_3): δ 1.12–1.24 (m, 4H, $2\times\text{CH}_2$), 1.31–1.44 (m, 2H, CH_2), 1.60–1.79 (m, 4H, $2\times\text{CH}_2$), 1.90 (d, $J=7.1$ Hz, 3H, CH_3), 3.69–3.79 (m,

1H, C-1'H), 4.39 (q, 1H, C-2H) and 6.28 (brs, 1H, NH); ^{13}C NMR (CDCl_3): δ 23.34 (CH_3), 24.68 (C-3', C-5'), 25.49 (C-4'), 32.73 (C-2', C-6'), 45.86 (C-2), 48.88 (C-1') and 168.27 (C=O); IR (Nujol): 3273 (NH), 2925, 1648 (C=O), 1561, 1462, 1378, 1251, 1197 and 1070 cm^{-1} .

(–)-*N*-Benzyl 2-bromoheptanamide (**8**). It was obtained as a white crystalline solid (648 mg) in 87% yield, mp 55–57 °C; R_f : 0.30 (solvent B); $[\alpha]_D^{25}$ –14.7 (c 0.27 in CHCl_3); EIMS, m/z (% rel. int.): 218 $[\text{M}-\text{Br}]^+$ (100), 162 (6), 160 (6), 149 (17), 117 (6), 106 (15), 91 (93), 77 (4) and 55 (9); ESI, m/z (% rel. int.): 298 $[\text{M}+\text{H}]^+$ (100) and 300 $[\text{M}+\text{H}+2]^+$ (100); ^1H NMR (CDCl_3): δ 0.89 (t, $J=6.7$ Hz, 3H, CH_3), 1.23–1.52 (m, 6H, C-4H, C-5H and C-6H), 1.99–2.20 (m, 2H, C3H), 4.34 (dd, $J=5.3$ and 8.0 Hz, 1H, C-2H), 4.47 (d, $J=4.8$ Hz, 2H, $\text{C}_6\text{H}_5\text{CH}_2$), 6.76 (brs, 1H, NH) and 7.25–7.37 (m, 5H, $\text{C}_6\text{H}_5\text{CH}_2$); ^{13}C NMR (CDCl_3): δ 13.92 (CH_3), 22.40, 26.91 and 30.97 (C-4, C-5, C-6), 35.93 (C-3), 44.11 (NH- CH_2), 51.95 (C-2), 127.66, 128.51 and 128.79 (C-2', C-3', C-4', C-5' and C-6'), 137.64 (C-1') and 168.80 (C=O); IR (Nujol): 3290 (NH), 2924, 1644 (C=O), 1455, 1377, 1182 and 1031 cm^{-1} .

(–)-*N*-Cyclohexyl 2-bromoheptanamide (**9**). It was obtained as a white crystalline solid (667 mg) in 92% yield, mp 97–99 °C; R_f : 0.40 (solvent B); $[\alpha]_D^{25}$ –25.0 (c 0.42 in CHCl_3); EIMS, m/z (% rel. int.): 210 $[\text{M}-\text{Br}]^+$ (100), 168 (5), 141 (22), 130 (32), 128 (12), 98 (17), 83 (56) and 56 (46); ESI, m/z (% rel. int.): 290 $[\text{M}+\text{H}]^+$ (100) and 292 $[\text{M}+\text{H}+2]^+$ (100); ^1H NMR (CDCl_3): δ 0.89 (t, $J=6.8$ Hz, 3H, CH_3), 1.28–1.44 (m, 12H, $6\times\text{CH}_2$), 1.68–1.75 (m, 2H, CH_2), 1.93–2.14 (m, 4H, $2\times\text{CH}_2$), 3.71–3.78 (m, 1H, C-1'H), 4.29 (dd, $J=5.2$ Hz and 8.0 Hz, 1H, C-2H) and 6.29 (brs, 1H, NH); ^{13}C NMR (CDCl_3): δ 14.27 (CH_3), 22.75, 25.03, 25.83, 27.17 and 31.33 (C-4, C-5, C-6, C-3', C-4' and C-5'), 33.06 (C-2' and C-6'), 36.31 (C-3), 49.16 (C-1'), 52.85 (C-2) and 168.16 (C=O); IR (Nujol): 3283 (NH), 2924, 1645 (C=O), 1556, 1462, 1378, 1181 and 1091 cm^{-1} .

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