Studies on the Chemo- and Enantio-selectivity of the Enzymatic Monoacylations of Amino Alcohols

Liisa T. Kanerva,* Markus Kosonen, Eero Vänttinen, Tuomas T. Huuhtanen and Martti Dahlqvist

Department of Chemistry, University of Turku, SF-20500 Turku, Finland

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The formation of ω-aminoalkyl esters through the lipase PS (*Pseudomonas cepacia*)-catalysed transesterification between carboxylic acid esters **1–6** and ω-amino-1-alkanols [$HO(CH_2)_nNH_2$, $n = 3$–6] in *tert*-amyl alcohol has been studied. For $n = 5$ or 6 the reaction practically leads to the formation of the ester product. The ester/amide ratios decrease with decreasing n so that for $n = 3$ only the amide product is obtained. A moderate kinetic resolution (ca. 50 % e.e. at 50 % conversion) was found in the acylations with two of the racemic starting esters (**3** and **4**).

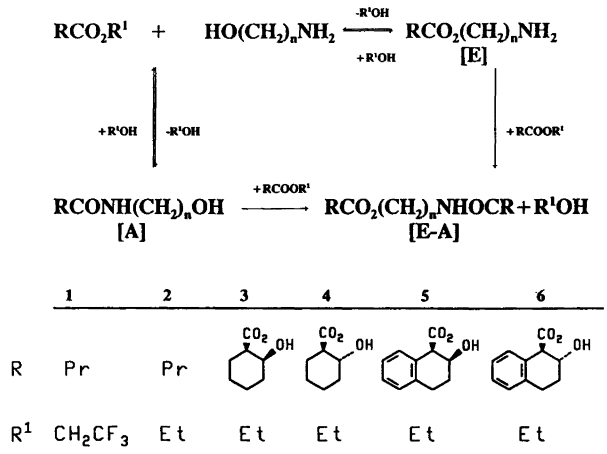
The selective acylation of the HO group of amino alcohols by normal chemical means usually calls for prior protection of the NH_2 group whereas specific *N*-acylations can be more easily performed.[1] On the other hand, the ability of enzymes to act as specific and chiral catalysts has been recognized for many years, and the discovery that hydrolytic enzymes, especially lipases, are catalytically active in a wide variety of anhydrous organic solvents has been of great importance for many synthetic purposes whenever chemo-, regio- or enantio-selectivity is required.[2,3] Accordingly, chemoselective enzymatic *O*-acylations of 6-amino-1-hexanol and *trans*-4-aminocyclohexanol with 2-chloroethyl butyrate have been successfully performed by lipase catalysis in *tert*-amyl alcohol.[3] In addition, acyltransferase enzymes in chloroform are reported to favour *O*-acylation of 3-amino-1-propanol with oleic acid.[4]

The purpose of this work was to study the behaviour of lipase catalysis in the *O*-acylations of ω-amino-1-alkanols with carboxylic acid esters **1–6** (Scheme 1). In the ω-amino-1-alkanols discussed, $HO(CH_2)_nNH_2$, n is in the range 3–6. 2-Aminoethanol was not used as a nucleophile because the acylations of β-amino alcohols have been reported to result in *N*-acylated products only.[4–7] 2,2,2-Trifluoroethyl and ethyl butyrates (compounds **1** and **2**, respectively) were used to study the phenomena involved in enzymatic acylations of amino alcohols in general. Use of the racemic esters **3–6** as acyl donors allowed a study of enantioselectivity in the *O*-acylations of amino alcohols. Enzymatic acylations of 1-hexanol with **2–6** were also studied.

Experimental

Ethyl 2-oxocyclohexanecarboxylate for the preparation of **3** and **4** was purchased from Fluka. The other reagents commercially available were the products of Aldrich. All the reagents were used without further purification except *tert*-amyl alcohol which was dried over molecular sieves (3 Å) before use. Porcine pancreatic and *C. cylindracea* lipases were purchased from Sigma Chemical Co., lipase PS (*Pseudomonas cepacia*) and Amano 30 (*Candida*) from Amano Pharmaceuticals Co. and the other enzymes from Biocatalysts Co.

Initial rates (v_0) and the progress of the reactions were determined by taking samples from the reaction mixture at intervals and analysing them by GLC.[8] A gas chromatograph was equipped with a 25 m NB-30 capillary column and with FT-IR and/or a flame ionization detector. GLC/FT-IR serves as an excellent tool with which to analyse the reaction products obtained according to Scheme 1. This is shown in Table 1 where the typical stretching and bending bands in the gas phase are given to the three possible reaction products, the amide (A), the ester (E) and the



Scheme 1.

* To whom correspondence should be addressed.

Table 1. Wavenumber/cm^{-1} for the typical vibrations of the compounds in Scheme 1 in the gas phase.

Compound	*n*	ν(C=O)^{E}	ν(C=O)^{A}	as. ν(C–O–C)	δ(C–N–H)	ν(O–H)
2		1756		1181		
2E	6	1756		1178		
2A	6		1709		1497	
2(E–A)	6	1756	1713	1177	1496	
3		1732		1179		3574
3E	6	1732		1177		
3A	6		1692		1508	
3E	5	1730		1177		
3A	5		1688		1501	
3E	4	1728		1177		
3A	4		1690		1508	
3A	3		1688		1512	
4		1746		1179		3621
4E	6	1744		1173		
4A	6		1694		1501	
4E	5	1748		1173		
4A	5		1697		1507	
4E	4	1732		1177		
4A	4		1697		1508	
4A	3		1697		1510	
5		1740		1163		3547
6		1746		1157		3651

ester–amide (E–A). GLC–MS was also used to identify the products. ^{1}H NMR spectra were recorded at 80 or 400 MHz in $CDCl_3$ solution with $(CH_3)_4Si$ as an internal standard.

Preparation of ethyl 2-hydroxycyclohexanecarboxylate. Ethyl 2-oxocyclohexanecarboxylate (20.0 g, 118 mmol) was reduced with sodium borohydride (2.22 g, 58.7 mmol) in ethanol (200 ml, 0 °C), modifying the method described elsewhere.[9,10] The mixture of **3/4** (64:33 by GLC, yield 92 %) obtained was separated by flash chromatography.[11] In a typical procedure, 2.53 g of the above *cis/trans* mixture was chromatographed using 200 g of silica gel and diethyl ether–hexane (65:35) as the eluant, to yield 1.44 g of **3** (purity 97 % by GLC) and 0.67 g of **4** (purity 99 % by GLC).

Preparation of ethyl 1,2,3,4-tetrahydro-2-hydroxynaphthalene-1-carboxylates. Ethyl 1,2,3,4-tetrahydro-2-oxonaphthalene-1-carboxylate[12] (9.84 g, 54.1 mmol) in ethanol (100 ml, −75 °C) was reduced with sodium borohydride (0.85 g, 22.5 mmol). After work-up, 9.04 g (92 %) of the mixture of **5/6** (87:3 by GLC) were obtained. The *cis*-isomer **5** was purified by double recrystallization from cyclohexane (purity 97 % by GLC, m.p. 62–63 °C). The preparation of pure **6** was unsuccessful.

Enzymatic acylation. tert-Amyl alcohol was used as a solvent because of the poor solubility of ω-amino-1-alkanols in most common organic solvents. Lipase PS from *Pseudomonas cepacia* was used as a catalyst if not otherwise stated. A solution of ω-amino-1-alkanol or 1-hexanol (0.15 or 0.5 M) and one of the substrates **1–6** (0.1 M) in *tert*-amyl alcohol was added to a known amount of the lipase (100 mg ml^{-1}). After sonication the mixture was shaken at 40 °C throughout the course of the reaction. Usually, the reaction products were not isolated, but were analysed by GLC. Under the reaction conditions, no reaction in the absence of the enzyme was detected.

For the reactions of **3** or **4** with 6-amino-1-hexanol or 1-hexanol, the separation of the unchanged starting ester and the ester product E were performed by flash chromatography.[11,13] Accordingly, the reaction between racemic **3** (668 mg, 3.88 mmol) and 6-amino-1-hexanol (0.15 M) in 40 ml of *tert*-amyl alcohol at 47 % conversion produces 263 mg (1.53 mmol) of (+)-**3** (50 % e.e. of the 1*R*,2*S*[9] enantiomer) and 261 mg (1.07 mmol) of the corresponding (−)-6-amino-hexyl ester (51 % e.e. of the 1*S*,2*R* enantiomer {purity (% by GLC), $[\alpha]_D^{25}$ (*c* 2.6, $CHCl_3$): 94, +12.6° and 93, −10.1°, respectively}. A similar reaction of racemic **4** (660 mg, 3.83 mmol) at 49 % conversion yielded 352 mg (2.04 mmol) of (+)-**4** (50 % e.e. of the 1*S*,2*S*[14] enantiomer) and 346 mg (1.42 mmol) of the corresponding (−)-6-amino-hexyl ester (46 % e.e. of the 1*R*,2*R* enantiomer) {purity (% by GLC), $[\alpha]_D^{25}$ (*c* 3.5, $CHCl_3$): 94, +20.0 and 93 and −14.6°, respectively}. On the other hand, the acylation of racemic **3** with 1-hexanol at 50 % conversion gave (+)-**3** (e.e. 50 %, purity 97 %) and the corresponding (−)-hexyl ester (e.e. 44 %, purity 98 %). A similar reaction of **4** gave (+)-**4** (e.e. 48 %, purity 99 %) and the corresponding (−)-hexyl ester (e.e. 48 %, purity 98 %).

Table 2. Chemoselectivity of lipases in the alcoholysis (E) and aminolysis (A) of ethyl butyrate (0.1 mol l^{-1}) (1) with 1-hexanol and 1-hexylamine, respectively, and (2) with 6-amino-1-hexanol in *tert*-amyl alcohol at 40 °C.

Lipase	$c_{nucleophile}$/mol l^{-1}	(1)			(2)		
		$(v_0)_E$[a]	$(v_0)_A$[a]	$(v_0)_E/(v_0)_A$	$(v_0)_E$[a]	$(v_0)_A$[a]	$(v_0)_E/(v_0)_A$
Lipase PS	0.15	185	27.1	7	102	3.0	34
(*Pseudomonas cepacia*)	0.5	137	24.1	6	74.7	2.7	27
Mucor miehei	0.15	110	5.4	20	3.3	1.0	3
	0.5	106	4.7	23	1.7	0.47	4
PPL	0.15	21.5	5.0	4	10.8	2.8	4
	0.5	15.7	4.2	4	9.3	1.9	5
Candida cylindracea	0.15	4.8	0.25	19	[b]	[b]	
	0.5	3.7	0.16	23	0.03	[b]	
Amano 30	0.15	3.4	0.70	5	0.09	[b]	
(*Candida*)	0.5	5.7	0.23	25	0.05	[b]	
Rhizopus javanicus	0.5	[b]	[b]		[b]	[b]	
Candida lypolytica	0.5	[b]	[b]		[b]	[b]	

[a] μmol h^{-1} g^{-1}. [b] No reaction within 6 days.

Determination of e.e. The enantiomeric excesses were determined by converting the ethyl, aminohexyl and hexyl esters to the corresponding methyl esters by acid-catalysed transesterification in methanol. The enantiomeric methyl protons of the methyl esters thus obtained were determined in the presence of $Eu(hfc)_3$ by ^1H NMR spectroscopy.

Results and discussion

Of the enzymes listed in Table 2, only a bacterial lipase from *Pseudomonas cepacia* (lipase PS) showed high activity and *O/N* ratio in the acylation of 6-amino-1-hexanol with **2** in *tert*-amyl alcohol. Chemoselectivity of this enzyme depends on the acylating agent. Thus, the chemoselectivity ratio, v_0^E/v_0^A, for the acylation of 6-amino-1-hexanol (0.15 M) with **2** is 34 while the values of the ratios are 61 and 42 with **3** and **4**, respectively. At the higher amino alcohol concentration (0.5 M) the chemoselectivity is somewhat lower. Moreover, some lipases are considerably inhibited by the presence of amines. Thus, in the presence of both 1-hexanol (0.5 M) and 1-hexylamine (0.5 M) and under the conditions at which no aminolysis is detectable, the values of v_0/mol min^{-1} g^{-1} for the lipase PS-, *Mucor miehei*- and porcine pancreatic lipase-catalysed 1-hexanolyses of ethyl butyrate are only 52, 2.4 and 5.2, respectively. On the other hand, if the same enzymes are first incubated for 14 days in *tert*-amyl alcohol, 0.15 M with respect to 1-hexylamine, followed by the separation and washing with the solvent, the catalytic activities are as shown in Table 2.

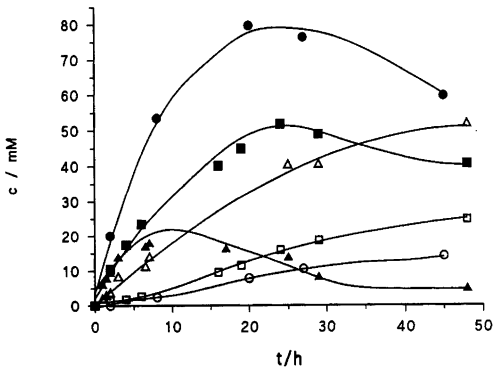


Fig. 1. The formation of the ester (filled symbols) and amide (open symbols) products with time for the lipase PS-catalysed reactions between ethyl butyrate and $HO(CH_2)_nNH_2$: $n = 3$ (▲ and △), $n = 4$ (■ and □), and $n = 6$ (● and ○).

That the lipase PS-catalysed monoacylations proceed *O*-selectively is obvious from the data shown in Fig. 1 and Table 3. Thus, independent of the structure of ω-amino-1-alkanol the ester product E is always the primary product. However, the final monoacylation product in the case of 3-amino-1-propanol is the *N*-acylated compound A. This is due to intramolecular acyl transfer which, in the case of *O*-monoacylated β- and γ-amino alcohols, is energetically favourable through cyclic intermediates.[15–17] The existence of such intermediates becomes less favourable with increasing *n* in ω-amino-1-alkanol. Thus, the half-life of the 4-aminobutyl butyrate was approximately 3 days; for the 5-aminopentyl and 6-aminohexyl esters no reaction was noted. In spite of this, there is a concentration maximum even for the formation of 6-aminohexyl butyrate (Fig. 1). The reversibility of lipase-catalysed transesterifications in organic solvents is an explanation of this behaviour. Thus, the concentration of E starts to decrease at the point where slow enzymatic aminolysis (leading to A) begins to disturb the equilibrium concentration of **2** with respect to the much

Table 3. Enzymatic acylation of ω-amino-1-alkanols (*c*/mol l^{-1}) with carboxylic acid esters (0.1 mol l^{-1}) by lipase PS (100 mg ml^{-1}) in *tert*-amyl alcohol.

Substrate	ω-Amino-1-alkanol	*c*	Yield of product (%)[a]			Time/h
			E	A	E–A	
1	$HO(CH_2)_6NH_2$	0.5	100			2
	$HO(CH_2)_4NH_2$	0.5	80	10		1
2	$HO(CH_2)_5CH_3$	0.1	50			30
		0.5	80			30
	$HO(CH_2)_6NH_2$	0.5	80	8	4	25
	$HO(CH_2)_4NH_2$	0.5	50	15	1	25
	$HO(CH_2)_3NH_2$	0.5	20	20 (50)[b]		10
	$HO(CH_2)_6NHCO_2Et$	0.5	80			50
3	$HO(CH_2)_5CH_3$	0.15	55			400
	$HO(CH_2)_6NH_2$	0.15	45	3		300
		0.5	50	1	2	800
	$HO(CH_2)_5NH_2$	0.15	40	3		150
	$HO(CH_2)_4NH_2$	0.15	25	6		150
		0.5	20	5		200
	$HO(CH_2)_3NH_2$	0.15	10	10 (40)[c]		20
		0.5		35		300
	$HO(CH_2)_6NHCO_2Et$	0.5	35			140
4	$HO(CH_2)_5CH_3$	0.15	60			400
	$HO(CH_2)_6NH_2$	0.15	50	2		300
		0.5	60	2	2	800
	$HO(CH_2)_5NH_2$	0.15	40	3		300
	$HO(CH_2)_4NH_2$	0.15	40	10		150
		0.5	30	5		150
	$HO(CH_2)_3NH_2$	0.15	3	5 (45)[c]		5
		0.5		50		300
	$HO(CH_2)_6NHCO_2Et$	0.5	50			140

[a]According to GC. [b]The final product after 50 h. [c]The final product after 300 h.

faster enzymatic alcoholysis (leading to E) (Scheme 1). In support of this, if the reaction is stopped by filtering off the enzyme or if ethanol is removed as it is formed using molecular sieves (4 Å), the concentration of 6-aminohexyl butyrate stays at the equilibrium level corresponding to 80 % yield of E (Table 2). The monoacylations of ω-amino-1-alkanols by **3** and **4** follow a course very similar to that shown in Fig. 1.

The results of the present work (Table 3) clearly show that the yields of the products E–A always stay negligible (0–4 %). It is possible to prepare ω-aminoalkyl esters E through the enzymatic reactions between the esters **1**–**4** and ω-amino-1-alkanols with $n > 3$. The yields of E vary from 20 to 80 % depending on *n* in the amino alcohol and on the substrate. These yields can be somewhat increased by shifting the equilibrium of the alcoholysis reaction (Scheme 1) more to the products. One way to do this is to increase the nucleophile concentration with respect to the substrate concentration (Table 2). However, if the *O*-acylations of ω-amino-1-alkanols with $n = 5$ or higher are considered reaction times increase considerably. In the case of lower ω-amino-1-alkanols the opposite effect of nucleophile concentration is observed. This is reasonable because the lower the amino alcohol concentration the faster the enzymatic reaction (Table 3), and any effect which favours enzymatic *O*-acylation with respect to the spontaneous $O \rightarrow N$ acyl migration must have a positive effect on the yields of E.

Another way of shifting the equilibrium of the alcoholysis reaction is to use activated esters. Thus, the yield of 4-aminobutyl butyrate can be increased from 40 to 80 % by replacing **2** with **1** as a substrate (Table 3). However, the use of activated esters is not always possible, for example, if a modification of a natural product with an amino alcohol is under way.

Evidently due to steric reasons, enzymatic acylations of ω-amino-1-alkanols or 1-hexanol with **5** and **6** were unsuccessful. It is also worth mentioning that a 86:14 mixture of **6**/**5** was always obtained by amine-catalysed isomerization[18] of the *trans*- or *cis*-isomer in the presence of amino alcohols.

One of the most important synthetic benefits of lipase catalysis is enantioselectivity. There are two asymmetrically substituted carbon atoms in compounds **3** and **4**, and the present results clearly demonstrate enantioselectivity in addition to chemoselectivity of lipase PS. Thus, at ca. 50 % conversion the enantiomeric excesses of 50 % for the unchanged (+)-**3** and (+)-**4** and of 51 and 46 % for the corre-

sponding (−)-6-aminohexyl esters were obtained for the reactions of racemic **3** and **4** with 6-amino-1-hexanol, respectively (see the Experimental section). In the lipase PS-catalysed reactions of the same racemates with 1-hexanol at 50 % conversion, the e.e. values were 50 and 48 % for (+)-**3** and (+)-**4** and 44 and 48 % for the corresponding (−)-hexyl carboxylates, respectively. Thus, it can be concluded that the NH_2 group of 6-amino-1-hexanol does not have an effect on the enantioselectivity of the enzyme. It is also obvious that the enzyme cannot discriminate between the *cis*- and *trans*-isomers of ethyl 2-hydroxycyclohexanecarboxylates.

Conclusions

Our results clearly demonstrate that, independent of the substrate, lipase PS quite chemoselectively leads to the formation of *O*-acylated ω-amino-1-alkanols with **1–4**. However, because of fast spontaneous $O \rightarrow N$ acyl migrations in *O*-monoacylated β- and γ-amino alcohols, $n > 3$ in $HO(CH_2)_nNH_2$ is required so that the ω-aminoalkyl ester obtained is stable enough to be separated from the reaction mixture. Because of the reversibility of enzymatic reactions, the preparation of *O*-acylated amino alcohols must be more or less kinetically controlled to obtain the optimum yield of the ester product E. The enantioselectivity of the enzyme also plays a role in these reactions. The enzyme does not accept the esters **5** and **6** as substrates.

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