Pergamon

Tetrahedron Letters, Vol. 37, No. 44, pp. 7913-7916, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01792-3

Pseudomonas cepacia Lipase Mediated Amidation of Benzyl Esters

Maciej Adamczyk* and Jonathan Grote

Department of Chemistry (D9NM), Abbott Diagnostics Division, Abbott Laboratories, 100 Abbott Park Rd, Abbott Park, IL 60064-3500, USA

Abstract: Although lipases have been known for some time as catalysts useful for regioselective transesterification or hydrolysis, studies regarding their use as ester amidation catalysts are limited. We have found that the lipase from *Pseudomonas cepacia* catalyzes amidation reactions with *benzyl* esters, producing high yields of amides with a variety of different amines. Copyright © 1996 Elsevier Science Ltd

Regioselective transformation of polyfunctional compounds is a challenging problem in organic synthesis,[1] especially in the cases where a structure that is sensitive to acid, base, oxidation, or reduction limits the choice of reagents needed to accomplish a particular transformation.[2] In recent years, lipases have become attractive as biocatalysts capable of achieving regioselective reactions under extremely mild (near neutral pH) conditions; they can be used in a wide variety of organic solvents and do not require a coenzyme for activity.[3] Lipases combine a broad substrate recognition with high efficiency and selectivity for the type of reaction they catalyze, and thus offer an excellent alternative to classical organic techniques in the selective transformation of complex molecules.[4]

Although lipases are now well established as catalysts capable of accomplishing transesterification[5] and ester hydrolysis,[6] their more atypical use in amidation reactions has been limited. While Klibanov and co-workers have studied the utility of enzymes in organic solvents[7] and first noted the non-aqueous aminolysis reaction of methyl butyrate with butylamine using porcine pancreatic lipase,[8] several reports of *Candida antarctica* lipase catalyzed amidation reactions using different types of methyl and ethyl esters have been described by Gotor and co-workers.[9] Recently, Conde and co-workers have described additional *Candida antarctica* lipase catalyzed amidations with a variety of glutamate diesters, including a benzyl ester.[10] Our recent studies of digoxin[11] and digoxigenin[12] ester hydrolyses demonstrated that benzyl esters can be regioselectively cleaved in cases where the corresponding methyl esters are unreactive. We believed that the lipase-mediated transformation of benzyl esters to amides would also have important practical implications in the regioselective transformation of complex organic molecules containing different ester functionalities.

Initially, we studied a number of commercially available lipases for their ability to catalyze the amidation reaction between benzyl acetate and phenethylamine. The lipases tested included Amano PS-30

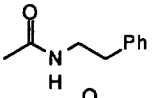
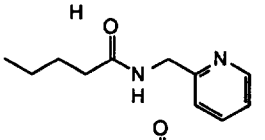
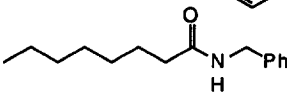
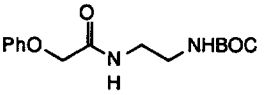
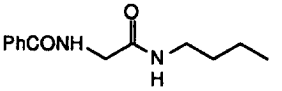
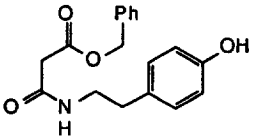
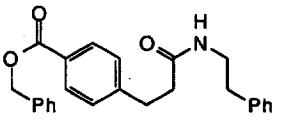
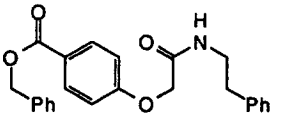
(originally Lipase P-30, from *Pseudomonas cepacia*), Amano LPL-80, from *Pseudomonas sp.*, Amano AY-30, from *Candida cepacia*, Amano MAP-10, from *Mucor sp.*, Amano AK, from *Pseudomonas sp.*, Altus lipase CR, from *Candida rugosa*, and Aldrich lipase Type VIII, from *Candida cylindracae*. All reactions were carried out as described below in isopropyl ether (including a control study). Amano lipase PS-30 was selected for further study based on its ability to achieve benzyl ester amidation. Other enzymes failed to catalyze the reaction under the conditions tested.

Recognizing that significant changes in enzymatic properties,[13] including alterations in ester/acid enantioselectivity,[14] regioselectivity,[15] prochirality,[16] and protease-catalyzed amidation,[17] can be affected simply by changing the solvent utilized in the reaction, we investigated the effect of other solvents on this lipase-catalyzed amidation reaction. Hexane and tetrahydrofuran were studied in addition to isopropyl ether.[18] In hexane, the lipase rapidly aggregated, producing low yields of the desired products.[19] On the other hand, reactions performed in tetrahydrofuran resulted in a well-dispersed lipase, but also produced low yields of the desired products. We believe that the tetrahydrofuran is stripping away the "essential water" required for the active conformation of the lipase, resulting in adverse conformational changes in the lipase structure.[3,8,20]

We subsequently examined a variety of benzyl esters and different amines to study the utility of lipase PS-30 in isopropyl ether. As Table 1 indicates, good to excellent conversions to amides were achieved in one to seven days. While unsubstituted amines such as benzylamine or phenethylamine reacted smoothly, other types of amines containing polar functional groups (entries 2 and 6) produced slightly lower yields of amides. In the case of substrates containing two benzyl ester groups (entries 6-8), only one monoamide product was obtained. This example illustrates the great potential of Amano PS-30 lipase for achieving regioselective amidations. The fact that protective groups incorporated into either the benzyl ester substrate or amine (entries 4 or 5) were accomodated by the enzymatic reaction has important practical implications, particularly in the multistep synthesis of complex organic molecules.

A typical experiment consisted of combining 10 mg lipase with a solution of 66 μM of the benzyl ester and 200 μM (3 equivalents) of the amine in 1.0 mL isopropyl ether and stirring in a 2 mL screw-cap vial at ambient temperature. Reactions were monitored by HPLC (8 x 100 mm radial compression uBondapak column, 50% CH3CN: 50% 0.05% CF3COOH/H2O, 2 mL/min, UV detection at 220 nm), and conversions to amides calculated from the integrations of the starting material and product. As in our previous work,[11] we ran appropriate controls (the above reaction mixture containing no lipase), which demonstrated that no reaction occurred under these conditions without lipase. The products were identified by coelution of their HPLC peaks with the peaks from authentic samples prepared by independent synthesis and confirmed by mass spectral analysis (electrospray mass spectrometry). In all cases, benzyl alcohol was also produced in the reaction, and conveniently served as an internal control, since its integrations were proportional to that of the amide product.

Table 1. Reactions of benzyl esters with amines catalyzed by Amano PS-30 lipase.

Entry	Ester	Amine	Product	% Conversion to Amide 24 hr	72 hr	168 hr
1	benzyl acetate	phenethylamine		51	75	88
2	benzyl valerate	2-aminomethyl-pyridine		23	37	51
3	benzyl octanoate	benzylamine		68	79	91
4	benzyl phenyl-oxyacetate	BOCethylene-diamine		75	91	>99
5	N-benzoylglycine benzyl ester	butylamine		16	37	66
6	dibenzyl malonate	tyramine		52	62	78
7	benzyl 4-(carboxy-benzyl)phenyl-propionate	phenethylamine		19	33	51
8	benzyl 4-(carboxy-benzyl) phenoxy-acetate	phenethylamine		17	29	59

Additionally, the amidation reaction described in entry 8 (Table 1 above) was scaled up, producing the indicated product in good yield and purity comparable to the small scale reaction.[21]

In conclusion, we have found that Amano lipase PS-30, isolated from *Pseudomonas cepacia*, is a synthetically useful enzyme for the transformation of benzyl esters to amides. This amidation method

accomodates a variety of different amines, including those with protective groups and other functionalities, and demonstrates regioselectivity with some dibenzyl esters.

References

1. Waldmann, H., Sebastian, D. *Chem. Rev.* **1994**, *94*, 911-937, and references therein.
2. Adamczyk, M., Gebler, J. C., and Mattingly, P. G. *Tet. Lett.* **1994**, *35*, 1019-1022, and references therein.
3. Klibanov, A. M. *CHEMTECH* **1986**, *16*, 354-359.
4. Wu, S.-H., Guo, Z.-W., and Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 1990-1995.
5. For a recent review, see Fang, J.-M.; Wong, C.-H. *Synlett* **1994**, 393-402.
6. Whitesides, G.M.; Wong, C.-H. *Angew. Chem., Int. Ed. Eng.* **1985**, *24*, 617-718; *Enzymes as Catalysts in Organic Synthesis*, Schneider, M., Ed; Reidel: Dordrecht, FRG, 1986.
7. Klibanov, A.M. *Acc. Chem. Res.* **1990**, *23*, 114-120, and references therein.
8. Zaks, A.; Klibanov, A.M. *Proc. Natl. Acad. Sci.* **1985**, *82*, 3192-3196.
9. For leading references: β-keto esters: Garcia, M. J.; Rebolledo, F.; Gotor, V.*Tetrahedron* **1994**, *23*, 6935-6940; β-hydroxy esters: Garcia, M. J.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymmetry* **1993**, *4*, 2199-210; β-aryl esters: Gotor, V.; Menendez, E.; Mouloungui, Z.; Gaset, A. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2453-6; α,β-unsaturated esters: Sanchez, V.; Rebolledo, F.; Gotor, V. *Synlett* **1994**, 529-30; α-halo esters: Quiros, M.; Sanchez, V. M.; Brieva, R.; Rebolledo, F.; Gotor, V.*Tetrahedron: Asymmetry* **1993**, *4*, 1105-12; diesters: Astorga, C.; Rebolledo, F.; Gotor, V. *J. Chem. Soc., Perkin Trans. 1* **1994**, 829-32.
10. Chamorro, C.; Gonzalez-Muniz, R.; Conde, S.*Tetrahedron: Asym.* **1995**, *6*, 2343-2352.
11. Adamczyk, M.; Gebler, J.C.; Grote, J. *J. Org. Chem.* **1995**, *60*, 3557-60.
12. Adamczyk, M.; Gebler, J.C.; Grote, J. *Tetrahedron Lett.* **1995**, *36*, 6987-90.
13. Klibanov, A.M. *Trends Biochem. Sci.* **1989**, *14*, 141; Dordick, J.S. *Enzyme Microb. Technol.* **1989**, *11*, 194.
14. Tawaki, S.; Klibanov, A.M. *J. Am. Chem. Soc.* **1992**, *114*, 1882-1884, and references therein.
15. Rubio, E.; Fernandez-Mayorales, A.; Klibanov, A.M. *J. Am. Chem. Soc.* **1991**, *113*, 695-696.
16. Terradas, F.; Teston-Henry, M.; Fitzpatrick, P.A.; Klibanov, A.M. *J. Am. Chem. Soc.* **1993**, *115*, 390-396, and references therein.
17. Kitaguchi, H.; Fitzpatrick, P.A.; Huber, J.E.; Klibanov, A.M. *J. Am. Chem. Soc.* **1989**, *111*, 3094-5.
18. The solvents used were anhydrous grade (Aldrich) and used as received.
19. Zaks, A.; Klibanov, A.M. *J. Am. Chem. Soc.* **1986**, *108*, 2767-2768, footnote 5.
20. Faber, S.; Riva, S. *Synthesis* **1992**, 895-910.
21. A solution of 520 mg of the dibenzyl ester and 0.68 mL benzylamine in 8.0 mL isopropyl ether was stirred with 520 mg PS-30 lipase for 96 hrs. HPLC showed < 5% starting ester.The reaction mixture was filtered to remove the lipase, and the lipase was washed with 20 mL ethyl acetate. The filtrate and washings were washed 2 x 10 mL with 1N HCl, dried over MgSO4, and concentrated. Column chromatography (EtOAc / hexane mixtures) produced 489 mg (91%) of the monoamide shown in Table 1 as a white solid: HPLC >98% (retention time 5.08 min, 60% CH_3CN / 40% 0.05% CF_3COOH); ^1H NMR ($CDCl_3$) δ 8.05 (d, 2H, J=8.9 Hz), 7.48-7.13 (m, 10H), 7.87 (d, 2H, J=8.9 Hz), 6.55 (br s, 1H), 5.35 (s, 2H), 4.51 (s, 2H), 3.61 (q, 2H, J=6.9 Hz), 2.82 (t, 2H, J=7.0 Hz); ^{13}C NMR (CDCl3) δ 167.4, 165.9, 160.8, 138.4, 136.1, 132.0, 128.8, 128.7, 128.6, 128.3, 128.2, 126.7, 124.1, 114.3, 67.2, 66.6, 40.4, 35.5; ESMS (M + H)+ at 390. Crystallization from ethyl acetate / hexane provided fine white needles with mp 107-108°.

(*Received in USA* 15 *July* 1996; *revised* 10 *September* 1996; *accepted* 11 *September* 1996)