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USE OF LIPASE FOR REGIOSELECTIVE ONE POT AMIDATION AND HYDROLYSIS

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Abstract: A study of the reactivity of esters with oxygenated linkers in lipase catalyzed transformations demonstrates that enhanced reactivity is observed in amidation reactions, but diminished reactivity is observed in hydrolyses using the same lipase. Two regioselective transformations (amidation and hydrolysis) can thus be achieved with diesters in the same pot using a single catalyst, effectively demonstrating lipase's versatility. © 1999 Elsevier Science Ltd. All rights reserved.

The ability of enzymes to promote regioselective transformations in complex, polyfunctional molecules continues to motivate our interest in lipase. Long known as catalysts for research scale ester hydrolyses,[1] lipases have become increasingly recognized as catalysts that perform well in industrial scale reactions. These inexpensive and environmentally benign biocatalysts are hydrolytically active in many organic solvents needed for substrate solubility, as long as an adequate amount of water is present.[2,3] By combining broad substrate recognition with a high selectivity for the type of reaction they catalyze, lipases offer an excellent alternative to classical synthetic methods.[4]

Recently, we have demonstrated the utility of lipases as biocatalysts capable of regioselective amide bond formation in compounds containing multiple esters under extremely mild conditions.[5,6] The observation that α-alkoxy and α-hydroxy esters showed enhanced reactivity in amidation reactions relative to non-oxygenated ester substrates[5,7,8] and the ability of lipase to catalyze hydrolysis reactions offered a unique opportunity to study the previously unexplored ability of a lipase to regioselectively catalyze amidation and hydrolysis reactions on diester substrates in a single pot. Such a novel process would be of great synthetic value for the transformation of molecules containing multiple ester functionalities. We were interested in studying substrates containing oxygenated linkers in lipase catalyzed transformations, since such linkers are now used to enhance the hydrophilicity of molecules to which they are attached.[9,10]

First, we tested the relative reactivity of pairs of monoesters with oxygenated linkers and their aliphatic analogs in amidation and hydrolysis reactions using the same lipase (Amano P-30; see Table 1, below). While the esters containing oxygenated linkers did show enhanced reactivity compared with their aliphatic analogs in lipase catalyzed amidation reactions, we found that the same oxygenated substrates demonstrated *slower* lipase-catalyzed hydrolysis to their corresponding acids relative to the analogous aliphatic esters. Thus, benzyl phenoxyacetate underwent amidation more rapidly than benzyl phenylacetate (entry 1 vs 2), but benzyl phenylacetate hydrolyzed more rapidly (entry 3 vs 4). Similarly, benzyl 8-N-CBz-amino-3,6-dioxyoctanoate

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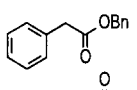
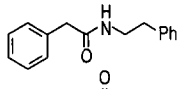
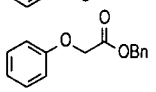
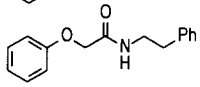
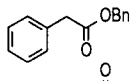
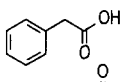
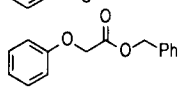
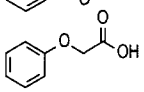
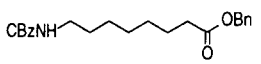
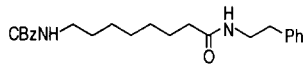
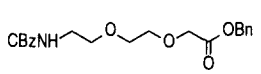
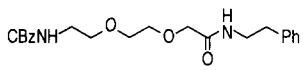
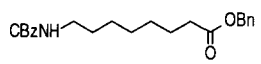
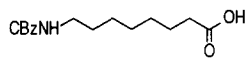
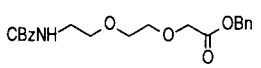
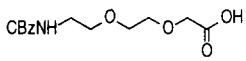
Table 1. Amidation and hydrolysis of benzyl esters containing oxygenated and aliphatic linkers.

Entry	Reaction Type	Substrate	Product	% Conversion 24 h	48 h	168 h
1	A			31	49	78
2	A			97	99	99
3	H			99	99	99
4	H			35	52	70
5	A			11	16	25
6	A			56	87	96
7	H			89	97	99
8	H			5	17	24

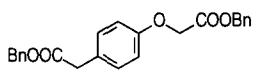
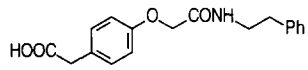
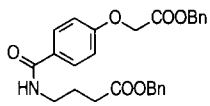
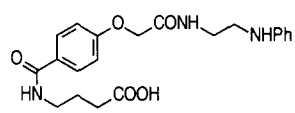
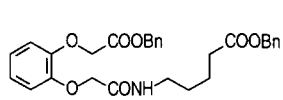
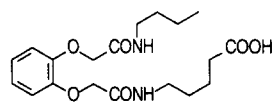
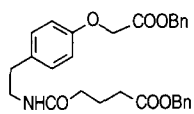
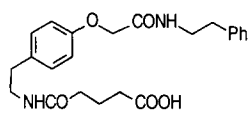
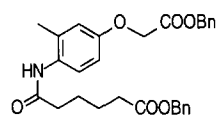
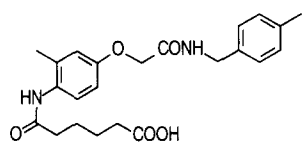
A = amidation; H = hydrolysis

shows more rapid lipase catalyzed amidation than benzyl 8-N-CBz-aminooctanoate (entry 5 vs 6), but slower lipase-catalyzed hydrolysis than its aliphatic analog (entry 7 vs 8). A typical amidation experiment consisted of combining 30 mg lipase with a solution of 66 μM of the benzyl ester and 132 μM (2 equiv) of the amine in 1.0 mL of anhydrous isopropyl ether and stirring in a 2 mL screw-cap vial at ambient temperature, while hydrolysis reactions typically involved the combination of 30 mg lipase with 66 μM of the benzyl ester in 1.0 mL 70% CH_3CN/pH 8.0 buffer. Reactions were monitored by HPLC (8 x 100 mm radial compression uBondapak column, CH_3CN : 0.05% CF_3COOH/H_2O mixtures, 2 mL/min, UV detection at 220 nm), and conversions to amides or acids calculated from the integrations of the starting material and product. As in our previous work,[11] we ran appropriate controls (the reaction mixtures described above containing no lipase), which demonstrated that no reaction occurred under these conditions without lipase.

These results indicate that an increasingly greater number of oxygens present in a substrate proximal to a benzyl ester render substrates with oxygenated linkers *less* susceptible to binding and hydrolysis by lipase. The lipase thus displays two different catalytic natures depending on the reaction and its environment. Such information about the relative reactivities of monoesters has great practical value on its own and demonstrates feasibility for a regioselective one pot diester amidation and hydrolysis. We were careful, however, to not assume that such information would be simply additive, since the intermediate hydrolysis substrate, actually an amido ester rather than a diester, could prove to be not susceptible to binding and hydrolysis by lipase.[11,12]

We then synthesized diester substrates containing both an alkoxy benzyl ester and an aliphatic benzyl ester, and subjected them successively to amidation conditions using a variety of amines and hydrolysis conditions *in the same pot*. For example, treatment of benzyl 4-(carboxybenzylmethyloxy)phenylacetate (entry 1, Table 2 below) with phenethylamine and P-30 lipase in organic solvent resulted in regioselective amidation of the alkoxy ester. Subsequent addition of buffer (and acetonitrile for solubility) to the reaction mixture led to hydrolysis of the remaining alkyl ester, producing a good yield of the amide acid from the diester.[13] Typically, one pot lipase catalyzed amidation/hydrolysis reactions were initially run as described above for an amidation, adding up to 66% toluene when needed for solubility. When the amidation was judged complete by analytical HPLC (72–96 h), 70% CH_3CN/pH 8.0 buffer was added, and the hydrolysis reaction was stirred for 72–96 h. Isolation of the product by preparative reversed phase HPLC and spectral characterization[13] identified the amide acid products shown in Table 2.

Table 2. One pot amidation and hydrolysis reactions.

Entry	Starting Diester	Amide Acid	Yield
1			82%
2			78%
3			91%
4			88%
5			80%

The preference of lipase for esters with oxygenated linkers in amidation reactions is due to the inductive effect of the neighboring oxygen, which renders the ester carbonyl more electrophilic. What force could overwhelm this inductive effect and in fact retard the hydrolysis of these esters relative to their aliphatic counterparts? We theorize that in an aqueous environment the oxygens in the linking arm can participate in hydrogen bonding to nearby unbound water molecules, and that the resultant polar "hydrated" oxygenated chain

is not preferred for binding to the hydrophobic regions of lipase.[14] In these cases, the aliphatic substrates are considerably more hydrophobic, and are preferred for interaction with the hydrophobic binding pocket of lipase. Conversely, in the amidation environment, the only water present is tightly bound to the lipase, and without the encumbrance of hydration from free water molecules, adjacent oxygens in the linker inductively render esters more reactive to a lipase catalyzed process than their aliphatic analogs.

In conclusion, we have shown that lipases can be useful for achieving chemoenzymatic transformations under mild conditions, but also have demonstrated that substrate preferences can vary dramatically depending on the environment utilized in a particular lipase catalyzed transformation. Additionally, while oxygenated linkers may provide molecules with enhanced hydrophilicity, they can also render neighboring esters less susceptible to lipase catalyzed hydrolysis. Such knowledge can be useful in predicting the regioselectivity in molecules containing multiple ester functionalities, and can also yield the successful realization of multiple lipase catalyzed transformations in a single pot with a single enzyme. Such knowledge will undoubtedly prove useful in medicinal chemistry and the synthesis of polyfunctional natural products.

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13. A solution of 33 mg of the diester (Table 2, Entry 3) and 13 μL of butylamine in 1.0 mL diisopropyl ether was stirred with 30 mg lipase for 96 h. HPLC indicated >95% conversion to a new product. After adding 1.0 mL acetonitrile and 500 μL pH 8 buffer, the mixture stirred for an additional 72 h. HPLC indicated conversion of the amide ester into an amide acid. Purification by reversed-phase preparative HPLC produced 23 mg (91%) of the phenoxyamide aminovaleric acid as a white solid: ^{1}H NMR (CD_3CN) δ 7.24 (br s, 1H), 7.18 (br s, 1H), 6.98 (s, 4H), 4.53 (s, 4H), 3.24 (m, 4H), 2.91 (br s, 1H), 2.28 (t, 2H, J = 7.0 Hz), 1.55–1.26 (m, 8H), 0.91 (t, 3H, J = 7.2 Hz); ^{13}C NMR (CD_3CN) δ 169.9, 162.5, 149.0, 123.7, 118.4, 116.1, 69.4, 39.5, 39.2, 34.1, 32.3, 29.6, 22.8, 20.7, 14.0; ESMS $(M + H)^+$ at 381.2; HPLC (35% CH_3CN/65% 0.05% CF_3COOH T_R 4.9 min (>98%).
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