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THE UTILITY OF ENZYMES IN GENERATING MOLECULAR DIVERSITY. LIPASE MEDIATED AMIDATION OF POLYBENZYL ESTERS

Maciej Adamczyk,* John C. Gebler, and Jonathan Grote
Department of Chemistry (D9NM), Abbott Diagnostics Division, Abbott Laboratories, 100 Abbott Park Rd., Abbott Park, IL 60064-3500, U.S.A.

Abstract: The feasibility of using lipases, particularly *Pseudomonas cepacia*, for the synthesis of a diverse molecular library is demonstrated. Twenty-six different compounds were synthesized via lipase-catalyzed reaction of a core diester substrate with several amine partners, resulting in formation of a library of amides which were identified using electrospray mass spectrometry. © 1997 Elsevier Science Ltd.

In recent years, combinatorial chemistry has become extremely popular as a means for generating molecular diversity.[1,2] Previously, Nature served as the source of vast libraries of compounds, from which new lead compounds were identified and modified for the preparation of drug candidates. As great improvements were made in the chemist's ability to separate single compounds from complex mixtures and identify their structures, combinatorial synthesis emerged as the logical next step for more efficient generation of the large families of novel molecules needed for screening in the drug development process.[3]

One interesting approach that had not been reported until recently is the utilization of enzymes to generate combinatorial libraries.[4] Lipases should offer a logical choice of enzyme class, since they combine broad substrate recognition with high efficiency and selectivity for the variety of reaction they catalyze.[5] Recently, lipases have become increasingly attractive as biocatalysts, since they are capable of catalyzing reactions in organic solvents,[6] thus offering an excellent alternative to classical synthetic techniques with respect to the simplicity of product isolation and the regioselectivity observed in the transformation of complex molecules.[7]

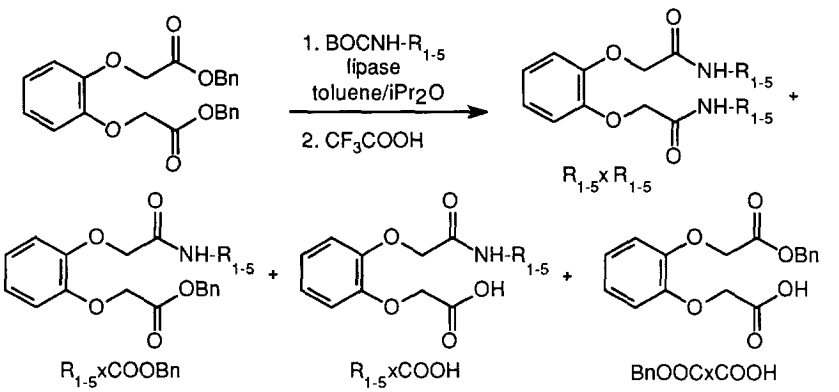
While lipases have been well known as catalysts of ester hydrolysis[8] or transesterification reactions,[9] their alternative utility as amidation catalysts has only recently been exploited. Numerous *Candida antarctica* lipase catalyzed amidation reactions have been described by Gotor and coworkers[10] and Conde and coworkers.[11] Our recent reports on lipase mediated transformations of benzyl esters[12–14] demonstrate that benzyl esters are reactive in amidation and hydrolysis reactions catalyzed by lipase. We envisioned that lipase mediated reactions could be useful for generating molecular diversity. Here, we describe the novel use of a lipase for creation of a library of amides, using electrospray mass spectroscopy (ESMS) to probe product diversity.[15]

Among the various protocols for generating molecular diversity, one method utilizes a core substrate containing multiple sites at which several partners may simultaneously react.[16] In many cases, the reactive partners contain masked functionalities that can be unmasked after coupling to the core substrate, revealing additional reactive sites at which further chemistry can take place. Considerable molecular complexity can thus be built up. In order to test the feasibility for generating molecular diversity in a lipase catalyzed amidation reaction, we envisioned simultaneous transformation of two reactive ester sites with several different amines. Use of an aromatic diester would provide the UV activity essential for easily monitoring of the reaction by TLC or HPLC.

We thus selected dibenzyl 1,2-phenylenedioxydiacetate as a core diester scaffold, and chose monoBOCdiamines[17] as reactive partners for the diester.

A preliminary experiment consisted of testing the reactivity of dibenzyl 1,2-phenylenedioxydiacetate with a single monoBOC diamine. Thus, 10 mg of *Pseudomonas cepacia* lipase was added to a solution of 66 μM of the dibenzyl diester and 73 μM of the monoBOChexanediamine in 1.0 mL 50% toluene/isopropyl ether. The reaction was stirred at ambient temperature in a 2 mL screw-cap vial. After 96 h, the reaction (monitored by HPLC,[18] conversion to amides calculated from the integrations of the starting material and product) showed almost total conversion (>90%) of the starting diester to the bis-amide product, with formation of a slight amount (< 5%) of the mono-amide product. The bis-amide product was identified by coelution of its HPLC peak with the peak from an authentic sample prepared by independent synthesis (bis-acid chloride + amine) and confirmed by ESMS.

We then submitted a mixture of five monoBOC diamines (BOCethylenediamine, BOCdiaminopropane, BOCdiaminopentane, BOCdiaminohexane, and BOCxylylenediamine, 1.1 equiv each) to a *Pseudomonas cepacia* lipase catalyzed reaction with 5 equiv of dibenzyl 1,2-phenylenedioxydiacetate in 50% toluene/isopropyl ether, as shown below. After 96 h, the product mixture was filtered to remove the lipase and evaporated to dryness. After HPLC analysis, the mixture was diluted in dichloromethane, washed with ice-cold dilute HCl to remove unreacted amines, dried, and concentrated to determine the yield of the reaction. The mixture was then treated with trifluoroacetic acid/dichloromethane to remove the BOC groups, the solvents were removed, and the mixture redissolved in acetonitrile for ESMS analysis.



$R_1 = (CH_2)_2NH_2$; $R_2 = (CH_2)_3NH_2$; $R_3 = (CH_2)_5NH_2$; $R_4 = (CH_2)_6NH_2$; $R_5 = p\text{-}CH_2C_6H_4CH_2NH_2$.
All products are trifluoroacetic acid salts.

HPLC analysis showed that <5% starting material remained after 96 h. The yield of the reaction, calculated using the average molecular weight of the bis-amide products, was 93%. Although HPLC product determination was not useful due to unresolvable peaks, ESMS analysis identified the presence of twenty-six different products, including all of the fifteen desired bis-amides, five mono-amide mono-esters, and slight amounts of the five mono-amide mono-acid products. As shown in Table 1 below, the $(M+H)^+$ peak is observed for each product.

Studies utilizing a mono-amide and bis-amide (singly charged and doubly charged, respectively) showed that each molecular type produced a very different ionization pattern in ESMS. Using the purified mono-amide and bis-amide obtained from a reaction of BOCxylenediamine with the diester, mixtures containing different relative molar quantities of the two compounds were prepared, subjected to treatment with trifluoroacetic acid, and assessed by ESMS. In all cases, the bis-amide displayed a signal intensity of ~6% relative to that of the mono-amide. The intensities of the mono-amide signals thus appear ~16× larger than those of the bis-amide signals in the ESMS for equivalent molar quantities. The bis-amides S and T (peaks at 423 and 429), however,

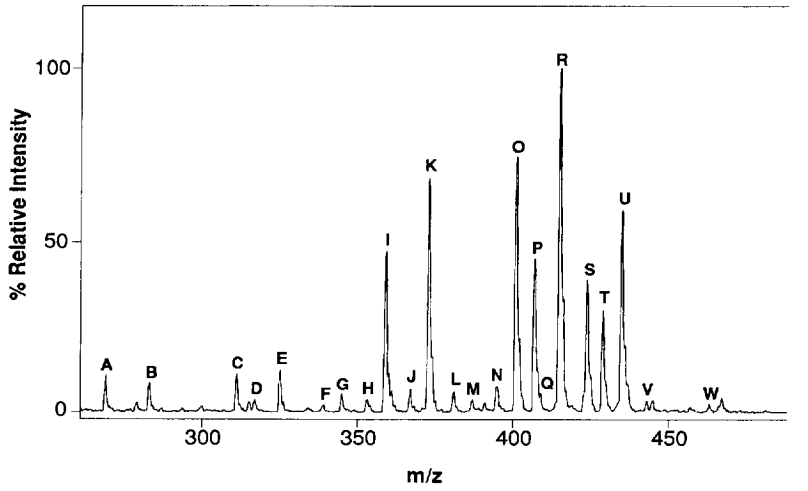


Table 1. Mass of each $(M + H)^+$ peak labeled in mass spectrum above.

Peak	Mass	Product(s)	Peak	Mass	Product(s)
A	269	R_1xCOOH	M	387	R_1xR_5
B	283	R_2xCOOH	N	395	R_3xR_3
C	311	R_1xR_1, R_3xCOOH	O	401	R_2xR_5, R_3xCOOBn
D	317	BnOOCxCOOH	P	407	BnOOCxCOOBn (SM)
E	325	R_1xR_2, R_4xCOOH	Q	408	R_3xR_4
F	338	R_2xR_2	R	414	R_4xCOOBn
G	345	R_5xCOOH	S	423	R_4xR_4
H	353	R_1xR_3	T	429	R_3xR_5
I	359	R_1xCOOBn	U	435	R_5xCOOBn
J	367	R_1xR_4, R_2xR_3	V	443	R_4xR_5
K	373	R_2xCOOBn	W	463	R_5xR_5
L	381	R_2xR_4			

show stronger signals than other bis-amides, indicative of the preference of lipase for more hydrophobic substances. Similar inequities in product distribution have already been reported in non-enzymatic experiments.[2] While four of the bis-amide products are isobaric with another compound (peaks 311, 325, 367, and 401), the pairs of products were easily resolved via MS-MS using a triple quadrapole instrument, which clearly

demonstrated the presence of both products given in the Table. The acids probably result from the inclusion of traces of water present in the hygroscopic monoprotected amine substrates, since a control reaction of the diester with lipase showed no hydrolysis. Also, a control experiment (treatment of the diester with the amine mixture in the absence of lipase)[13] showed only slight formation of some of the amide products.

In conclusion, we have found that *Pseudomonas cepacia* lipase has broad substrate specificity for the transformation of benzyl esters to amides. The products formed in the lipase mediated reaction contained all of the expected amides (with a somewhat greater reactivity being observed for more hydrophobic substances), as well as some side-products, thus validating its use in generating molecular diversity. In principle, this library could be further elaborated in combinatorial fashion by subsequent reaction with several different active esters, anhydrides, etc., further increasing molecular diversity. This amidation reaction demonstrates that a variety of different amines, including those with protective groups or other functionalities, can be utilized in a lipase catalyzed combinatorial style reaction with dibenzyl esters, generating a library of amide products which can be easily isolated, identified, and deprotected for further synthetic manipulation and diversification.

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18. 8 x 100 mm radial compression uBondapak column, 60% CH_3CN: 40% 0.05% CF_3COOH/H_2O, 2 mL/min, UV detection at 220 nm.

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