Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 885–890

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

CHEMO-ENZYMATIC TRANSFORMATIONS IN SENSITIVE SYSTEMS: LIPASE MEDIATED HYDROLYSIS OF VANCOMYCIN ESTERS.

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

Received 19 January 1998; accepted 6 February 1998

Abstract: Recently, an interest in the development of new vancomycin derivatives has been demonstrated. Here, the feasibility of using lipases, particularly those from *Pseudomonas sp.*, for the hydrolysis of vancomycin alkyl esters is demonstrated. Benzyl ester derivatives were more easily cleavable than methyl ester derivatives, resulting in good yields of vancomycin acids without degradation.

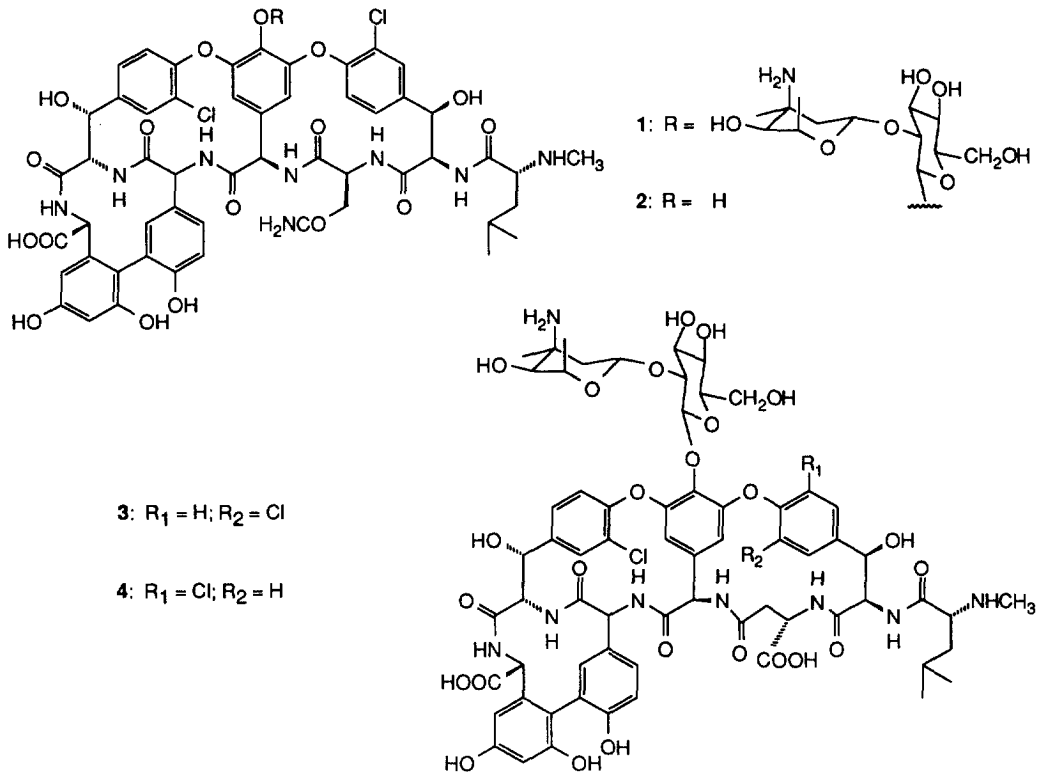
Our continuing interest in exploring chemoenzymatic reactions, especially in the transformation of complex, polyfunctional molecules that display sensitivity to acid, base, oxidation, or reduction, lies rooted in the ability of enzymes to achieve regioselective transformations under extremely mild conditions. Long known for their utility for small scale ester hydrolyses,[1] lipases have become increasingly useful as catalysts for industrial scale reactions. These inexpensive biocatalysts are active in the same organic solvents needed for substrate solubility, produce few by-products, and are generally environmentally benign.[2,3] Previously, we have demonstrated the utility of lipase for the hydrolysis of multifunctional esters, including derivatives of rapamycin,[4] digoxin,[5] and digoxigenin,[6] the synthesis of amides and α-hydroxy amides,[7–9] and the creation of combinatorial libraries.[10] This previous work has shown that lipase displays a variety of transformational capabilities. The suitability of substrates cannot always be predicted, however, and more work is thus required to define the scope and limitations of these versatile enzyme reagents.

For three decades vancomycin (**1**) has been the drug of choice for the treatment of Gram-positive infections caused by *Staphylococcus aureus*[11] and bacterial infections in patients allergic to β–lactam antibiotics.[12] Produced by *Amycolatopsis orientalis* (previously *Nocardia orientalis*) and *Streptomyces orientalis*,[13] vancomycin acts by inhibiting the biosynthesis of peptidoglycan in bacterial cell walls.[14] This antibiotic displays no cross resistance with other antibiotics, is relatively free of side effects, and since it is not absorbed from the gastrointestinal tract, can be used to treat Staphylococcal endocarditis, and enterocolitis caused especially by *Clostridium difficile*.[15,16] Recently, the chemistry of vancomycin has become the focus of intense activity due to the emergence of vancomycin-resistant strains of bacteria.[17–19]

The sensitive functionality present in vancomycin poses a challenge toward modification or manipulation of derivatives of this natural product. Aglucovancomycin (**2**), which retains about 75% of the activity of the parent drug,[20] is formed by mild acid hydrolysis at low pH. The therapeutically inactive Crystalline Degradation Product, or CDP, which consists of two closely related conformers (CDP-I, **3**; and CDP-II, **4**) that exist in a

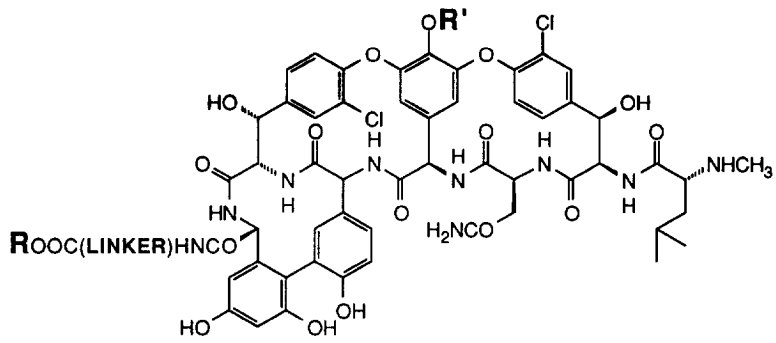
0960-894X/98/$19.00
PII: S0960-894X(98)00109-7

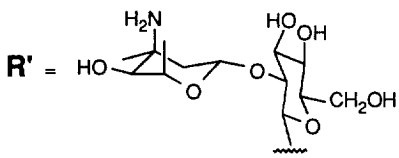
dynamic equilibrium,[21] is produced under physiological conditions by a simple ring rearrangment. The molecule contains several phenols that are susceptible to oxidative conditions, and eight racemizable asymmetric centers. Such a complex, multiply functionalized molecule should be ideally served by the mildness and selectivity commonly observed in lipase transformations. In this communication, we present the use of lipase for synthesis of various vancomycin derivatives.



The lipase utilized (Amano LPL-80, from *Pseudomonas sp.*) was selected for its purity, and was confirmed to be >95% by SDS polyacrylamide gel electrophoresis. The purity of the lipase is critical, since many commercially available enzyme preparations frequently contain other contaminating proteins that can complicate reactions and their analyses.

A number of different ester derivatives, including methyl alkyl and alkoxy esters **5a–5e** and benzyl alkyl and alkoxy esters **6a–6e** were prepared starting from vancomycin, while deglycosylated methyl alkyl esters **8a–8c** and benzyl alkyl esters **9a–9c** were prepared using aglucovancomycin.[22] Hydrolysis of vancomycin ester derivatives was first investigated on a small scale (~2 mg at 2.5 mg/mL, acetonitrile, pH 8 buffer).





R' = H

R =	Me	Bn	H	Me	Bn	H	Me	Bn	H
	5a	**6a**	**7a**				**8a**	**9a**	**10a**
	5b	**6b**	**7b**	**5d**	**6d**	**7d**	**8b**	**9b**	**10b**
	5c	**6c**	**7c**	**5e**	**6e**	**7e**	**8c**	**9c**	**10c**

*For **a**, linker = $(CH_2)_5$; for **b**, linker = $(CH_2)_7$; for **c**, linker = $(CH_2)_{10}$; for **d**, linker = $CH_2(OCH_2CH_2)_2$; for **e**, linker = $CH_2(OCH_2CH_2)_3$.

When each of the methyl esters **5a–c** was incubated in a lipase free control reaction for 72 h, no hydrolysis took place. In the presence of lipase, limited hydrolysis (11%) of the terminal methyl ester was observed only for the ester containing longest linking arm (**5c**, Table 1, below). In contrast, the benzyl ester with the longest linking arm (**6c**) hydrolyzed without difficulty (>95%). No other products were observed, attesting to the mildness of the conditions and the selectivity of the lipase. The increased lipophilic nature of the longest chain benzyl ester was thus required for reactivity as a lipase substrate.

Our results with the vancomycin esters differed considerably from other enzyme catalyzed ester hydrolyses described in the literature, in which hydrolysis could be achieved for esters with much shorter chain length linkers (n </= 5).[4–6,23,24] In these shorter chain length cases, an environment exists that is well-suited to the hydrophobic binding pockets of lipase.[23] In the present case, however, several phenolic hydroxyl groups and polar amide bonds are located closely to the ester group, presenting a considerably less lipophilic interface, which renders shorter chain length vancomycin esters unreactive to lipase catalyzed hydrolysis.

Table 1. Hydrolysis of methyl esters **5a–e, 8a–c** and benzyl esters **6a–e, 9a–c** after 72 hrs.

	HPLC Retention Time*		
Ester	**Ester**	**Acid**	**% Conversion**
5a	2.5 min	---	NR
5b	4.7 min	---	NR
5c	8.6 min	2.1 min	11%
6a	8.8 min	---	NR
6b	11.2 min	---	NR
6c	19.3 min	2.1 min	>95%
5d	3.3 min	---	NR
5e	4.8 min	1.7 min	21%
6d	6.1 min	1.6 min	26%
6e	7.9 min	1.7 min	57%
8a	2.6 min	---	NR
8b	3.2 min	---	NR
8c	6.3 min	1.7 min	84%
9a	5.0 min	---	NR
9b	7.5 min	1.6 min	6%
9c	17.8 min	1.7 min	>95%

*Conditions: C18 μBondapak, 254 nm: for **5d–e**, and **6d–e**, 30% CH_3CN/70% 25 mm NH_4OOCH; for **5a–c** and **6a–6c**, 35% CH_3CN/65% 25 mm NH_4OOCH; for esters **8a–c** and **9a–c**, 45% CH_3CN/55% 25 mm NH_4OOCH.

Partial hydrolysis was observed for vancomycin alkoxy benzyl ester **6d**, while alkoxy methyl ester **5d** showed no reaction. Esters **5e** and **6e** showed even greater conversions to the corresponding acid **7e**. Particularly interesting was the fact that the shorter alkoxy benzyl ester **6d** hydrolyzed, while the all-carbon analog with the same number of backbone atoms (**6b**) did not hydrolyze. The presence of an electronegative oxygen atom thus inductively renders this alkoxy carbonyl more electrophilic and more reactive to lipase hydrolysis than in the carbon analog. Such an enhancement in the reactivity of alkoxy ester substrates has been observed,[25,26] and is paralleled by the results we observed in amidation reactions catalyzed by lipase.[7,9]

On the other hand, while alkoxy methyl ester **5e** showed slightly greater hydrolysis than its corresponding alkyl methyl ester (**5c**), alkoxy benzyl ester **6e** showed lower conversion than its all-carbon analog (**6c**). These results have profound implications on the now popular utility of polyoxyethylene chains for enhancing substrate hydrophilicity.[27] Despite the inductive effect of oxygen, the results indicate that increasingly greater numbers of oxygens apparently result in a polarity or conformational change that renders this vancomycin substrate less susceptible to binding and hydrolysis by lipase.

The reactivity of the deglycosylated molecules paralleled that of the glycosylated species. In the cases of deglycosylated esters **8a** and **9a**, no enzyme catalyzed hydrolysis was observed. Limited hydrolysis was observed for ester **9b**. In contrast to vancomycin methyl ester **5c**, however, deglycoslylated methyl ester **8c** showed considerably improved reactivity. These results demonstrate that the polar, hydrophilic groups present in a nonproximal environment of the molecule, especially those capable of hydrogen bonding, can play a pivotal role in determining whether a particular substrate will be susceptible to binding and hydrolysis by lipase. As we noted previously, they further underscore the importance of running control reactions.

Additionally, in order to demonstrate the preparative utility of the method, we performed a hydrolysis on 100 mg of the longest chain vancomycin benzyl ester derivative **6c**, and obtained the desired acid in 94% yield and excellent purity.[28]

In conclusion, we have found that Amano LPL-80 lipase tolerates a variety of different long-chain vancomycin substrates, but shows a clear preference for more lipophilic benzyl esters over methyl esters. While hydrolysis by lipase is strongly dependent on the local topography, enhanced reactivity can be globally affected by the absence of polar, hydrophilic groups, even those situated remotely from the reactive site, as demonstrated by the greater susceptibility of aglucovancomycin esters to hydrolysis by lipase. Additionally, oxygen atoms contained in the linking arm can provide an inductive effect which enhances reactivity. This chemo-enzymatic strategy was used to cleanly hydrolyze a vancomycin substrate on a scale useful for demonstrating synthetic utility, resulting in >90% yield without glycoside cleavage or rearrangement. The yields obtained and the mildness and selectivity observed in lipase catalyzed transformations demonstrate that such a strategy will prove useful for other complex, glycosylated molecules.

References and Notes

1. For a recent review, see Fang, J.-M.; Wong, C.-H. *Synlett* **1994**, 393.
2. Klibanov, A. M. *CHEMTECH* **1986**, *16*, 354.
3. Waldmann, H., Sebastian, D. *Chem. Rev.* **1994**, *94*, 911, and references therein.
4. Adamczyk, M.; Gebler, J.; Grote, J. *Tetrahedron Lett.* **1995**, *36*, 6987.
5. Adamczyk, M.; Gebler, J. C.; Grote, J. *J. Org. Chem.* **1995**, *60*, 3557.
6. Adamczyk, M., Gebler, J. C., Mattingly, P. G. *Tetrahedron Lett.* **1994**, *35*, 1019.
7. Adamczyk, M.; Grote, J. *Tetrahedron Lett.* **1996**, *37*, 7913.
8. Adamczyk, M.; Grote, J. *Tetrahedron: Asymmetry* **1997**, *8*, 2099.
9. Adamczyk, M.; Grote, J.; Rege, S. *Tetrahedron: Asymmetry* **1997**, *8*, 2509.
10. Adamczyk, M.; Gebler, J. C.; Grote, J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1027.
11. Kirby, W. M. M. *Rev. Infect. Dis.* **1981**, *3*, 5236.
12. Ingerman, M. J.; Santoro, J. *Infect. Dis. Clin. N. Am.* **1989**, *3*, 641.
13. Lechevalier, M. P.; Prauser, H.; Labeda, D. P.; Ruan, J. S. *Int. J. Syst. Bacteriol.* **1986**, *36*, 29.
14. Harris, T. M.; Harris, C. M.; Kannan, R.; Waltho, J. P.; Skelton, N. J.; Williams, D. *J. Am. Chem. Soc.* **1988**, *110*, 2946, and references cited therein.
15. Crossley, K.; Loesch, D.; Landesman, B.; Mead, K.; Chern, M.; Strate, R. *J. Infect. Dis.* **1979**, *139*, 273.

16. Hook, E. W., III; Johnson, W. D., Jr. *Am. J. Med.* **1978**, *65*, 411.
17. Walsh, C. T.; Fisher, S. L.; Park, I. S.; Prahalad, M.; Wu, Z. *Chem. Biol.* **1996**, *3*, 21.
18. Sundram, U. N.; Griffin, J. H.; Nicas, T. I. *J. Am. Chem. Soc.* **1996**, *118*, 13107, and references therein.
19. Neu, H. C. *Science* **1992**, *257*, 1064.
20. Marshall, F. J. *J. Med. Chem.* **1965**, *8*, 18.
21. Harris, C. M.; Kopecka, H.; Harris, T. M. *J. Am. Chem. Soc.* **1983**, *105*, 6915.
22. Vancomycin was reacted with methyl amino ester hydrochlorides in the presence of HBTU[29] to produce methyl esters **5a–e**, while the benzyl esters **6a–e** were prepared using benzyl amino ester p-toluenesulfonates. The benzyl alkylamino ester p-toluenesulfonate salts were prepared from commercially available alkylamino acids using a previously described method.[30,31] Alkoxyamino esters were used to synthesize vancomycin conjugates **5d–e** and **6d–e** containing alkoxy linkers. Aglycone derivatives were prepared by reacting the aglycone (1 equiv) with 5 equiv dicyclohexylcarbodiimide, 5 equiv of the amine, 2 equiv of hydroxybenztriazole, and 2 equiv of triethylamine or diisopropylethylamine in anhydrous DMSO (20 mL/mmol).
23. Lemke, K.; Lemke, M.; Theil, F. *J. Org. Chem.* **1997**, *62*, 6268, and references therein.
24. We also observed no reaction for vancomycin benzyl and methyl esters with shorter linking arms.
25. Wirz, B.; Spurr, P. *Tetrahedron: Asymmetry* **1995**, *3*, 669.
26. Fuganti, C.; Rosell, C. M.; Servi, S.; Tagliana, A.; Terreni, M. *Tetrahedron: Asymmetry* **1992**, *3*, 383.
27. For recent examples, see; Johnson, G. M.; Abarella, J. P.; Petry, C. *Bioconj. Chem.* **1997**, *8*, 447; Koyama, Y.; Umehara, M.; Mizuno, A.; Itaba, M.; Yasukouchi, T.; Natsume, K.; Suginaka, A.; Watanabe, K. *Bioconj. Chem.* **1996**, *7*, 298.
28. 100 mg of vancomycin benzyl ester **6e** was dissolved in 50% acetonitrile/50% pH 8 phosphate buffer and reacted with 100 mg of lipase (conventional tube rotator, ambient temperature). After 72 hrs, the reaction showed > 95% conversion by HPLC. The solution was purified by preparative HPLC using the conditions described above. Lyophilization provided 91 mg (94%) of a white solid: ESMS: $(M + H)^+$ at 1633.7, $(M + Na)^+$ at 1654.8, $(M + 2H)^{++}$ at 817.1.
29. Griffin, J. H.; Sundram, U. N. *J. Org. Chem.* **1995**, *60*, 1102.
30. Greenstein, J. P.; Winitz, M. Chemistry of the Amino Acids; Krieger: Malabar, 1984, Vol. 2, p 939.
31. Zervas, L.; Winitz, M.; Greenstein, J. P. *J. Org. Chem.* **1957**, *22*, 1515.