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# Amine Assisted Enzymatic Esterification of 1,2-Diol Monotosylates

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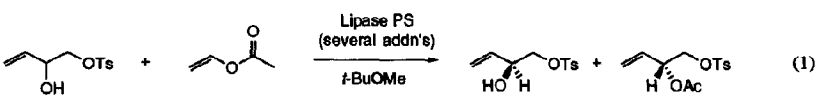
**Abstract:** The enzymatic esterification of 1,2-diol monotosylates in organic solvent under standard conditions often fails to achieve the desired 50% conversion due to enzyme inactivation by acidic contaminants. The inclusion of an amine affords rapid conversion to 50% and provides enhanced enantioselectivity.

Optically active 1,2-diol monotosylates are useful precursors for optically active diols, epoxides, and other interesting single enantiomer materials.[1] Indeed, *S*-3-chloro-2-hydroxypropyl tosylate (1c) has recently been converted into optically pure carnitine, the molecule responsible for human metabolism and transport of long-chain fatty acids through the mitochondrial membrane.[1b,c,2] The attractiveness of these compounds also stems from their rapid preparation in optically active form by many methods. In addition to the reported kinetic resolution of racemic 2-hydroxy-3-butenyl tosylate via Sharpless asymmetric epoxidation,[3] a number of 1,2-diol monotosylates have been resolved expeditiously utilizing enzymatic deacylation of esters of the secondary alcohol substituent.[1,4]

The converse reaction, enzymatic enantioselective esterification of a racemic alcohol in organic solvent, is often preferable to the hydrolysis procedure, since resolution at the alcohol stage avoids the extra step of racemic ester preparation. In addition, the use of organic solvents allows much greater latitude for reaction (and enantioselectivity) optimization. Not surprisingly, reports of enzymatic esterification of 1,2-diol monotosylates have appeared,[1d,e,5] but the optical purities obtained in these procedures often fall short[1d,e] of those obtained in the corresponding deacylation reactions.[1,4]

We chose to examine the enzymatic esterification of 2-hydroxy-3-butenyl tosylate (**1a**) as part of an ongoing effort to prepare four-carbon multifunctional chiral synthons in high optical purity. Our initial enzymatic esterification attempts (Lipase PS-30 [from Amano International Enzyme Co.], vinyl acetate, *t*-butyl methyl ether [TBME]) suffered from apparent enzyme inhibition, with numerous enzyme additions and long reaction times necessary to obtain the desired 50% conversion to acetate **2a**. Changing solvents (acetone, ethyl acetate, THF) did not improve these results.[1,4]




(1)

**1a** **S-1a** **R-2a**

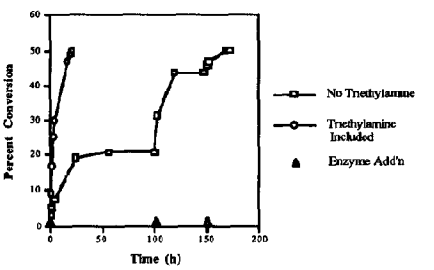
84% ee 90% ee

The source of this apparent "inhibition" became clearer after two further results. First, the enzymatic esterification of highly purified 2-hydroxy-3-butenyl tosylate proceeded uneventfully to 50% conversion, provided that sufficient enzyme was included to afford a reaction time of 24 h or less (if run for longer times, this reaction stalled in a similar manner to the initial result). Second, an aged sample of the substrate **1a** afforded absolutely no product **2a** under identical reaction conditions. These results suggested that an impurity in **1a** whose presence increased with time was interfering with the enzymatic esterification. Identification of this contaminant was approached by adding discrete amounts of several potential substrate-derived impurities to typical enzymatic esterification reactions of purified **1a**. The only deliberate contaminant to have any effect was *p*-toluenesulfonic acid (*p*-TSA), which completely halted the esterification. These results suggested that **1a** spontaneously generated *p*-TSA upon keeping. It is likely that butadiene monoepoxide was concomitantly formed with the *p*-TSA, since the epoxide was qualitatively detected in an older sample of **1a**. The effect of *p*-TSA could be due to either enzyme inhibition or, more simply, to a pH effect. Several indirect results indicated that a pH change due to *p*-TSA was the likely culprit, although this investigation was complicated by the non-aqueous nature of the media. First, a structurally unrelated strong acid, trifluoroacetic acid, demonstrated similar inhibitory behavior upon the enzyme under identical reaction conditions. In contrast, a weaker but related acid, acetic acid, showed no inhibition. Second, as fully elaborated below, the addition of triethylamine to a purposely *p*-TSA-inactivated enzymatic esterification afforded partial recovery of enzyme activity.

The inclusion of a scavenger to provide protection against enzyme inactivation due to adventitious acid was further examined. Carbonate salts gave capricious results, probably due to their insolubility in the reaction mixture. However, tertiary amines gave outstanding results, provided the $pK_a$ of the corresponding conjugate acid was above about 7 (e.g., pyridine is not effective while 4-dimethylaminopyridine is marginally useful). Triethylamine and *N,N*-diisopropylethylamine were particularly effective. The importance of the acid-scavenging amine is indicated in striking fashion in comparing reactions run under identical conditions save the presence, or absence, of triethylamine (Graph I). This beneficial effect of triethylamine was demonstrated for this reaction in numerous organic solvents.

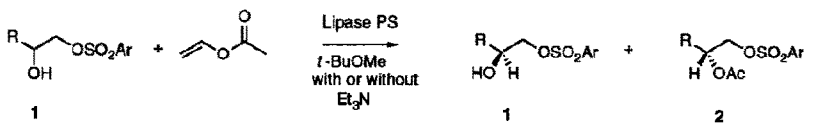
Graph I

**Enzymatic Esterification of 1a**



The moderate optical purities observed in the initial reaction compounded the enzyme inactivation phenomenon (eq 1). The preparative usefulness of the reaction would be severely limited if the already marginal enantioselectivity (E value[6] of 50) was degraded by enantiorandom esterification catalyzed solely by the amine. Fortunately, blank reactions without enzyme indicated that non-enzymatic acylation was very minimal both in the presence of tertiary amines (<1% acylation in 24 h) and even for secondary and primary amines (<5% acylation in 48 h), although the latter led to more by-products. Indeed, instead of affording reduced optical purities, the enantioselectivity of the enzymatic esterification of **1a** was clearly enhanced by inclusion of the amine. The presence of triethylamine, besides affording a much faster reaction, consistently afforded both *S*-**1a** and *R*-**2a** of ≥96% ee[7] and E values of >200. This effect was not limited to **1a**. Several other 1,2-diol monosulfonates **1** were prepared and submitted to the enzymatic esterification protocol with and without triethylamine (Table 1).[8] In all cases the amine accelerated the reaction and eliminated multiple enzyme feeds, and, to differing extents, enhanced the enantioselectivity of the esterification.

Table 1




**1** **1** **2**

**a**, R = $CH{=}CH_2$, Ar = *p*-$CH_3Ph$
**b**, R = $CH_3$, Ar = *p*-$CH_3Ph$
**c**, R = $CH_2Cl$, Ar = *p*-$CH_3Ph$
**d**, R = $CH_2CH_3$, Ar = *p*-$CH_3Ph$
**e**, R = $CH{=}CH_2$, Ar = Ph

| Substrate | Conditions[a] | Enzyme Amount (# of additions) | %ee, **1**[b,d] | %ee, **2**[c,d] | E Value |
|---|---|---|---|---|---|
| **1a** | A | 30 mg/mmol (3) | 84% | 90% | 50 |
| | B | 10 mg/mmol (1) | 98% | 96% | >200 |
| **1b** | A | 17.5 mg/mmol (5) | 99.6% | 90% | 111 |
| | B | 2.5 mg/mmol (1) | 99% | 93% | 145 |
| **1c** | A | 80 mg/mmol (6) | 68% | 90% | 39 |
| | B | 5 mg/ mmol (1) | >99% | 92% | >120 |
| **1d** | A | 50 mg/mmol (5) | 62% | 60% | 7 |
| | B | 5 mg/mmol (1) | 98% | 80% | 60 |
| **1e** | A | 30 mg/mol (3) | 49% | 96% | 89 |
| | B | 10 mg/mmol (1) | >99% | 96% | >200 |

(a) Conditions: A; Substrate, vinyl acetate (3 equiv), lipase PS-30 (amounts indicated) in TBME at room temperature. B; as in A with 0.1 equiv triethylamine added. (b) Optical purities determined by either HPLC using a CHIRALCEL OB chiral column (DIACEL Chemical Industries, Ltd.)with UV detection (**1a**, **1b**, **1e**), HPLC analysis on a Hypersil silica column (Hewlett Packard) of the corresponding MTPA ester (**1c**), or conversion to the acetonide of the corresponding diol and GC analysis using a CYCLODEX-B (J&W Scientific) column (**1d**). (c) Optical purities of **2** obtained by initial acidic hydrolysis to **1** and further analysis as indicated for **1**. (d) Absolute configurations are known for **1b** and **2b**,[1a] **1c** and **2c**,[4a] and **1d** and **2d**.[1a] The absolute configuration of **1e** was determined as per **1a**.[7]

Further investigation indicated that these results are endemic to this class of compounds, presumably due to their predisposition to generate a sulfonic acid *in situ*, and that there is no effect for species which do not possess this strong acid-generating propensity. In particular, triethylamine had no effect on either the rate or enantioselectivity of the enzymatic esterifications of several species (e.g., 1-phenyl-2-propanol, 4-phenylthio-2-butanol) structurally unrelated to the 1,2-diol monotosylates.

Thus, many examples of an important class of chiral synthons, 1,2-diol monotosylates, are now available in high enantiomeric excess via a reproducible enzymatic esterification protocol utilizing the inclusion of an amine to scavenge any adventitious acid. The presence of the amine allowed rapid enzymatic reactions, avoided reaction stalling and multiple enzyme feeds, and afforded enhanced enantioselectivities, allowing the simple preparation of several of these species, most notably 2-hydroxy-3-butenyl tosylate, in high optical purity.

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7. For optical purity determination, see Table I. The absolute configuration of **1a** was determined by conversion to 1-butene-3,4-diol of known absolute configuration: Crawford, R. J.; Lutener, S. B.; Cockroft, R. D. *Can J. Chem.* **1976**, *54*, 3364.

8. Adequate characterization was obtained for all new compounds.

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