Reversible Michael Reaction−Enzymatic Hydrolysis: A New Variant of Dynamic Resolution

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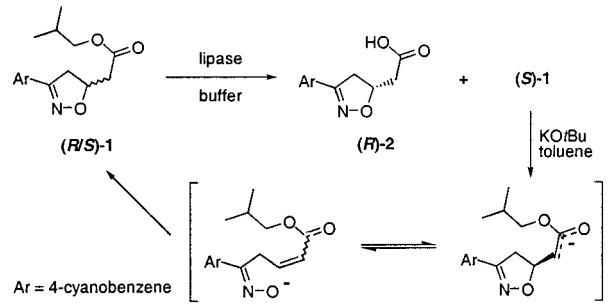
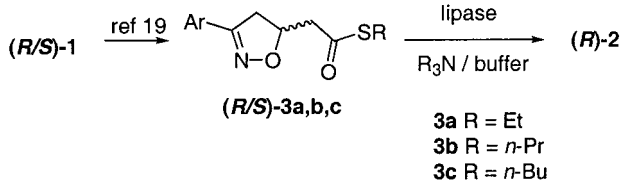
Enzymatic resolution of racemates into enantiomerically enriched compounds is a valuable and popular technique whose value extends from the efficient preparation of complex compounds to the preparative production of pure enantiomers.[1,2] However, simple kinetic enzymatic resolutions are restricted to a maximum yield of 50% per enantiomer. More useful is the coupling of racemization with resolution, known as a dynamic resolution.[3,4] The benefits are two-fold: the need to remove or recycle (after racemization) the undesired isomer is eliminated, and if both enantiomers are substrates for the enzyme, enantioselectivity remains constant due to continuous racemization of the less reactive enantiomer.[5,6] Since racemization conditions are infrequently compatible with enzyme activity, dynamic resolutions are uncommon.[7,8] We report here the combination of enzymatic resolution with a Michael−retro-Michael tandem[9] to achieve racemization, an unprecedented means to produce dynamic kinetic resolution.

Single enantiomers of 3-aryl-4,5-dihydroisoxazol-5-ylacetic acid derivatives are important cores of a series of non-peptide platelet GPIIb/IIIa antagonists.[10] This work in this area has led to roxifiban,[11,12] our leading candidate in development as therapy for a range of cardiovascular disorders arising from undesired platelet adhesion. The aryl isoxazoline **1** (Scheme 1) is resolved by the lipase *Pseudomonas cepacia* (Amano PS-30) in pH 8 phosphate buffer to produce (*R*)-**2** in 93% available yield and 95% enantiomeric excess (ee_p).[13,14] Of note is that the unreactive *S* isomer can be subsequently racemized by conditions as mild as sodium bicarbonate in 1:1 methanol:acetonitrile. In addition, when **1** or **3b** was stirred in methanol-*d* with catalytic methoxide, only the side-chain methylene group was deuterated. The simplest explanation for this observation, as well as for the facile racemization,[15] would be equilibrium between the enolate and the enone/oxime anion structures,[16] (see Scheme 1 and further discussion in Supporting Information). Unfortunately, reaction conditions permitting racemization and enzymatic resolution for **1** were incompatible, precluding a dynamic resolution and requiring the recycling of (*S*)-**1** for efficiency.

Scheme 1. Kinetic Enzymatic Resolution of (*R*/*S*)-**1**



Scheme 2. Dynamic Enzymatic Resolution Route to (*R*)-**2**



Drueckhammer and co-workers have recently demonstrated that thioesters enhance the acidity of the α-protons when compared to corresponding oxoesters, sometimes increasing the racemization rate sufficiently to lead to dynamic enzymatic resolution under mild reaction conditions.[17,18] As part of a study of new syntheses of roxifiban, we discovered that the conversion of **1** to a wide variety of thioesters **3** is efficient.[19] In turn, some of these thioesters, in the presence of phosphate buffer, amine, lipase PS-30, and surfactant, could be hydrolyzed to the acid (*R*)-**2** in >90% ee_p and yields as high as 89%, clear evidence of a dynamic resolution (Scheme 2).

To optimize this unique resolution, we examined the reaction of a set of common lipases upon various thioesters **3**. Thioesters are rarely used as substrates for enzymatic resolution, and the best choice of R was not predictable.[20] Of the enzymes and thioesters screened, only the combination of PS-30 with the

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Table 1. Resolution of Thioesters with *P. cepacia* Lipase under the Optimized Reaction Conditions[a]

thioester	time (h)	% conversion	ee_p % (*R*)
3a	45.3	85	95.5
3b	22.1	>99	97.6
3b[b]	70.2	94	96.8
3c	47.5	99	97.3

[a] 0.18 M in 0.65 N sodium dihydrogen phosphate buffer, 40 °C, pH = 9.2 ± 0.3 (maintained by the periodic addition of 6 N NaOH), 2.0 equiv of 25% trimethylamine in H_2O, Triton X-100 as surfactant and lipase PS-30 (the last two charged at 0.1 of the substrate weight).

n-alkyl thioesters: ethyl, *n*-propyl and *n*-butyl, produced an acceptable enantioselectivity and reaction rate. In contrast to the *O*-isobutyl ester **1**,[13] α- or *β*-branched thioesters, such as *i*-propyl, *s*-butyl, *i*-butyl, etc, were either not hydrolyzed by this enzyme, or in low conversion and poor ee_p. Evidently, the sulfur atom exerts influence upon the hydrolysis beyond electronic effects.

We found no racemization of the *n*-propyl thioester **3b** in 40 °C phosphate buffer of pH 9.25 with surfactant until amines were added. In contrast to the frequently selected base trioctylamine,[17,18,20d−f] trimethylamine was superior; only 15.2% racemization occurred after 161 h for the former compared to 49.7% racemization after 90.5 h for trimethylamine. This may be a function of trioctylamine's lower water solubility.[21] This system only minimally hydrolyzed any of the thioesters **3** (<1%) over 2 days, but once lipase was also added, this combination possessed high enantioselectivity (Table 1) and, furthermore, would not racemize the product (*R*)-**2**. When conducted with **3b** at 0.64 mol scale, the reaction was >99.0% complete after 47 h in 98.8% ee. Ethanol recrystallization raised this to 99.7% ee in 89% overall yield. The addition of 10% v/v toluene dramatically increased the racemization rate for all thioesters. For example, **3b** was 49% racemized after only 24 h. However, with PS-30 present, the lipase was inhibited under these biphasic conditions and after 70 h, only a 94% conversion in 96.8% ee_p had occurred.

A useful gauge for a resolution is the "enantiomeric ratio" (*E*), a measure of the intrinsic enantioselectivity of a particular enzymatic resolution.[6,22] For nondynamic resolutions with large *E*, the ee_p remains high only at low conversions. In contrast, resolution combined with racemization decouples optical purity from the extent of the reaction; the ee_p is only dependent upon the value of *E*.[23] To determine *E* for **3b**, it was reacted under the optimized conditions but without trimethylamine so as to stop racemization, and the reaction was halted after 7.0 h. Isolation of the unreacted thioester and analysis of the product **2** indicated 22.7% conversion (c) and 96.5% ee_p, respectively. Using the formula $E = (\ln[1 - c(1 + ee_p)])/(\ln[1 - c(1 - ee_p)])$[6] to determine *E* as 74.1 under these nonracemizing conditions, a maximum ee_p of 97.3% is predicted[28] under dynamic resolution conditions. This is in good agreement with the value of 97.6% ee measured at >99% hydrolysis after 22.1 h when PS-30 was added to an otherwise identical reaction mixture.[24]

Several aspects of this dynamic resolution underscore the efficiency of the reaction. The solubility of **3b** is measured as 7.0×10^{-3} g/mL after 5 h of reaction, (the particles of **3b** are slow to establish equilibrium with solution). The combination of this low solubility with the 90.5 h racemization half-life would suggest that at least several half-lives would be required to achieve reaction completion. Yet rapid resolution to (*R*)-**2** (sometimes as brief as 20 h) in high ee_p still occurs. The heterogeneity of the reaction complicates measurements, but the fast and highly enantioselective reaction suggests the lipase expeditiously hydrolyzes any solubilized (*R*)-**3b**. Another indication of a rapid enantiospecific reaction is that the ratio of (*S*) to (*R*) thioester **3b** gradually increased to 7−8:1 once the reaction was ∼80% complete, suggesting a significantly higher enzyme-catalyzed hydrolysis rate versus racemization rate (otherwise comparable rates would have soon equilibrated the **3b** enantiomers). Typical dynamic resolutions require racemization rates to at least approximate that of the enzymatic reaction of the fast reacting isomer to attain acceptable ee_p values.[5] These examples demonstrate, that if the lipase's enantioselectivity is high and the reaction conditions properly selected, the optical purity does not degrade even when the desired rate order of racemization and hydrolysis is reversed.

Enzymatic dynamic resolution has been conducted using a fundamentally different racemization mechanism (retro-Michael/Michael reactions) from those previously known for resolutions. Any system capable of facile, reversible cleavage two bonds distal from an ester may be a candidate for resolution by this combination of reactions. Much as the use of thioesters has increased the scope of dynamic resolutions, this new combination of racemization with enzymatic resolution adds a new dimension.

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Supporting Information Available: Expanded discussion of isoxazoline racemization mechanisms, the chiral LC methods, and detailed experimental for conducting the enzymatic resolution of **3b** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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