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Resolution of Methyl Nonactate by *Rhodococcus erythropolis* under Aerobic and Anaerobic Conditions

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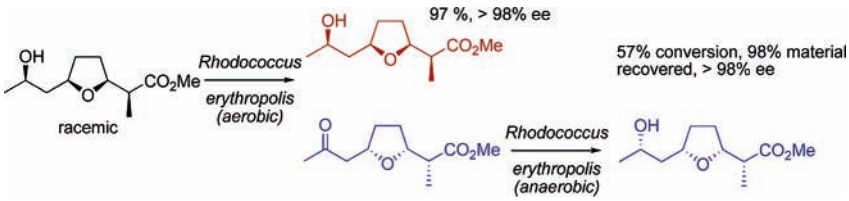
ABSTRACT



An efficient resolution of methyl nonactate is reported by biotransformation in shake flask cultures of *Rhodococcus erythropolis*. The equilibrium of the reaction redox system can be manipulated by switching from aerobic to anaerobic growth, thereby generating both enantiomers of the target in excellent yield and enantiomeric purity.

The majority of pharmaceuticals either are natural products, are made from natural products, or have structures that are inspired by those of natural products.[1] Unfortunately, there are a number of well-known problems typically associated with natural product leads. First among these are issues related to scale as shown, for example, in the development of Taxol and discodermolide. Second, there are no generally applicable solutions to preparing the analogues of a natural product that are needed to optimize its activity and "drug-gability". Each new natural product scaffold comes with its own set of synthetic challenges, and the strategies for analogue production are usually developed on a case-by-case basis. The situation is unfortunate, as the very properties[2] of natural products, such as topological complexity and stereochemistry, that give this group of compounds their diversity are those that complicate synthesis.

We have been interested in finding straightforward methods to include topologically complex natural product building blocks into combinatorially prepared compound libraries. The desire to do this comes from a need to meld the complexity of natural products with the analogue generation capabilities of diversity-oriented synthesis so that high diversity, large number, natural product-like libraries can be efficiently generated. Of course, the use of natural product precursors for library generation is not new.[3] Typically, however, such precursors "lock" the library into one enantiomeric series (D-carbohydrate or L-amino acid) expanded into a minimal number of diastereoisomers.

Nonactin[4] is an ionophore antibiotic produced by *Streptomyces griseus* that we have been interested in for some time (Figure 1).[5−9] Nonactin is a cyclic tetrameric species composed of two units of (+)-nonactate ((+)-**2**) and two

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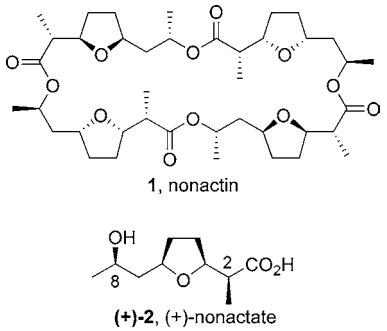
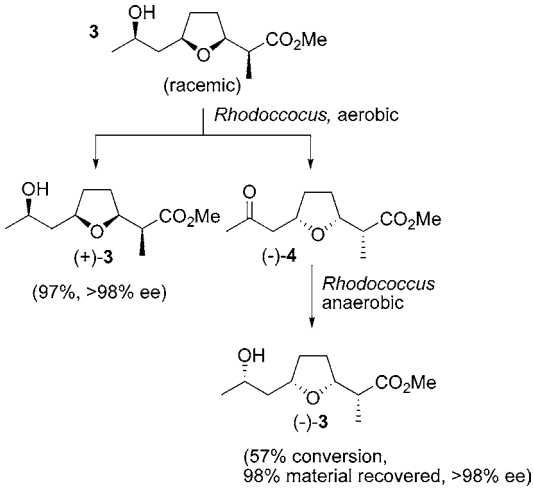


Figure 1. Nonactin, a macrotetrolide natural product, is composed of both enantiomers of the hydroxy acid nonactate.

units of (−)-nonactate ((−)-**2**) arranged (+)-(−)-(+)-(−) around the macrocycle so that nonactin is achiral. Nonactate represents an outstanding natural product derived building block ("bio-block") for use in combinatorial synthesis, having a quite complex stereochemistry contained in a relatively small molecule with functionality that can be orthogonally diversified in a straightforward fashion. Nonactate can be epimerized at both the C-2 and C-8 positions giving easy access to four diastereoisomeric building blocks in each enantiomeric series from a common intermediate, itself available on a 100 g scale. The most direct route to the common intermediate methyl nonactate, **3**, is through methanolysis of nonactin which delivers racemic material. The production of homochiral nonactate, in both enantiomeric series, however, poses a significant hurdle to the further exploration of **2** as a building block for library synthesis. We report herein an interesting solution to the problem using *Rhodococcus* under aerobic and anaerobic conditions to afford (+)-**3** and (−)-**3** in greater than 98% ee and >90% yield from the racemate.

Homochiral nonactate has been prepared in the past by total synthesis on a number of occasions,[10−12] although the routes are not short and cannot compete on scale with the fermentation process. Wang and Metz reported two options for the resolution of nonactate.[13] Oxidation of racemic **3** was accomplished using Jones' reagent. Baker's yeast was used to enantioselectively reduce **4** to generate (−)-**3** (20%, 94.3% ee), (+)-8-*epi*-**3** (40%, 94.5% ee), and unreacted (−)-**4** (23%, 97% ee) (Scheme 1). While (−)-**3** was obtained directly, conversion of (+)-8-*epi*-**3** to (+)-**3** (oxidation−reduction or Mitsunobu) adds steps to the process. Wang and Metz also reported the HPLC separation of diastereoisomeric mandelate

Scheme 1. *Rhodococcus*-Catalyzed Resolution



derivatives of **3**. While we have had some success in adapting this method to a larger scale, the maximum throughput was too limited for our needs and the removal of the mandelate auxiliary adds steps. We therefore sought a more efficient and scalable process.

The use of enzyme-catalyzed kinetic resolution has met with much success in the field of organic synthesis.[14] We initially expended some quite significant effort in screening an extensive panel of lipase, protease, and esterase enzymes against a range of nonactate derivatives. A number of enzyme−substrate pairs did show some selectivity. Unfortunately, the best selectivity we could achieve was not great ($E = 5$).[15]

Given our long interest in *Streptomyces* and their metabolic capabilities, it was only natural for us to become interested in the related Gram-positive, high G+C% *Rhodococci*.[16] The *Rhodococci* have a much simpler morphology and life cycle than the *Streptomyces* yet have a very promiscuous metabolic capability. They are important in environmental remediation and also biotechnology. For example, the phenylacetate catabolism of *Rhodococcus* sp. strain RHA1 has been exploited for the degradation of aromatic compounds and has been extensively studied.[17] *Rhodococci* have also found use in resolution reactions. Wang et al. report the use of *Rhodococcus* sp. strain AJ270 as a whole cell catalyst with stereoselective nitrilase activity and have prepared α-methylated serine and isoleucine derivatives.[18] Kroutil's group demonstrated that *Rhodococcus ruber* DSM44541 could catalyze asymmetric hydrogen transfer.[19]

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In this latter example, the microorganism was used as a lyophilized powder. Oxidation reactions were driven using an excess of acetone as the ultimate hydrogen acceptor; reductions were driven using 2-propanol as the hydrogen donor. Very good conversion and outstanding enantioselectivity were observed for a range of secondary alcohol substrates.

We obtained methyl nonactate from a methanolysis of nonactin.[20] Our original screening showed that *R. erythropolis* and *R. opaca* but neither *R. luteus* nor *R. rhodochrous* could convert **3** in aerobic shake flask cultures into a less polar compound which upon isolation and characterization was shown to be **4**. The remaining **3** starting material was recovered and shown to be enantiomerically enriched (∼80% ee). Through optimization, we discovered that the enantioselective oxidation was only catalyzed during log-phase growth of the organism. Two solutions for increasing enantioselectivity were found. First, the low initial enantioselectivity was a result of incomplete conversion of (−)-**3** into (−)-**4**. Lowering the concentration of **3** from 1 mg/mL (∼76% ee) to 0.8 mg/mL (90% ee) and then 0.6 mg/mL (>98% ee) significantly increased the enantioselectivity of the process by increasing the conversion.

An alternate solution was found by extending the time the culture was in log-phase growth by reducing the incubation temperature from 30 to 16 °C. This had little effect upon the rate of conversion but allowed us to achieve >98% ee at 1 mg/mL substrate concentration. One of the particular advantages of using *Rhodococcus* is the ability to grow the strain at temperatures down to 4 °C. We have in hand, therefore, an efficient route to homochiral (+)-**3**.

Our attention then turned to obtaining (−)-**3** and recycling the remaining ketone, (−)-**4**. Analysis of the product ketone showed that it too was generated with excellent stereoselectivity (>98% ee). Unfortunately, it is well precedented that chemical reduction of **4** with a wide variety of reductants primarily affords 8-*epi*-**3**. Given that *R. erythropolis* was capable of the enantioselective oxidation reaction we reasoned that the reverse reaction should afford (−)-**3** if the equilibrium could be driven backward. We chose to grow the *Rhodococcus* strain under anaerobic conditions where the need for NADH recycling would drive a terminal reduction much like the conversion of pyruvate to lactate and acetaldehyde to ethanol in classic anaerobic fermentations. We were happy to find that (−)-**4** was readily reduced to (−)-**3** with excellent stereoselectivity and in 98% yield.

Overall, treatment of the racemic **3** with *Rhodococcus* under aerobic conditions generated (−)-**4**, leaving (+)-**3** as unreacted starting material in high yield and high stereochemical purity. Treatment of the recovered (−)-**4** with *Rhodoccocus* under anaerobic conditions generated (−)-**3** in high yield and high stereochemical purity. We have shown this process to be reliable on a gram scale. We have processed 5 g of (±)-**3** in four incubations. As (+)-**3**, (−)-**3**, and their C-2 and C-8 epimers are now available in scale, the *Rhodoccus*-mediated resolution is the key step in the development of **2** as an outstanding "bio-block" for the generation of combinatorial libraries that encompass great stereochemical diversity.

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Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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