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Nitrile Hydratase Enzymes in Organic Synthesis: Enantioselective Synthesis of the Lactone Moiety of the Mevinic Acids

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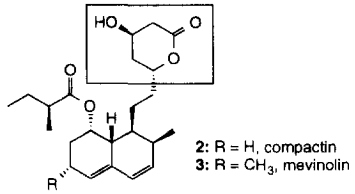
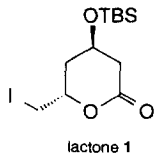
Abstract: (*R*)-4-Hydroxy-5-cyanopentene (-)-**9**, a known precursor of the protected lactone moiety of the mevinic acids **1**, has been prepared in 9 steps from (*S*)-3-(benzyloxy)-4-cyanobutanoic acid **5** (88% e.e.), which was obtained by the asymmetric 2 step hydrolysis of 3-benzyloxyglutaronitrile **4** involving the successive activity of a *nitrile hydratase* and an *amidase* enzyme. Copyright © 1996 Elsevier Science Ltd

The enzyme-catalysed hydrolysis of nitriles to amides and/or carboxylic acids has recently emerged as a synthetically useful transformation.[2] By employing whole cell systems that contain the required *nitrile hydratase* (RCN to $RCONH_2$) and *amidase* ($RCONH_2$ to RCO_2H) enzymes it is possible to catalyse the hydrolysis a wide range of substrates,[3] under mild conditions (*e.g.* pH = 7.5, 30 °C). Additionally, these two enzymes have been shown to be stereoselective resulting in their application for kinetic resolutions[4] and asymmetric transformations.[5] We now report the application of this methodology to the synthesis of the protected lactone **1** which is a precursor to the δ-lactone pharmacophore of the mevinic acids.[6] Both of the mevinic acids, compactin **2** and mevinolin **3**, are effective hypocholesterolemic agents that inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoA reductase), the rate-limiting enzyme in the human *de novo* cholesterol biosynthetic pathway.[7]

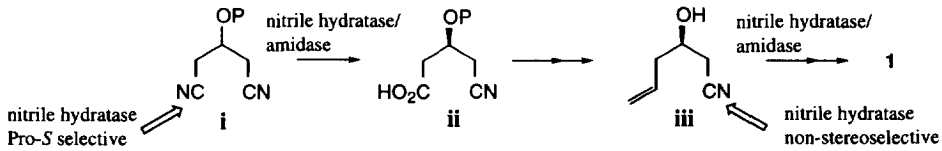


2: R = H, compactin
3: R = CH_3, mevinolin



lactone **1**

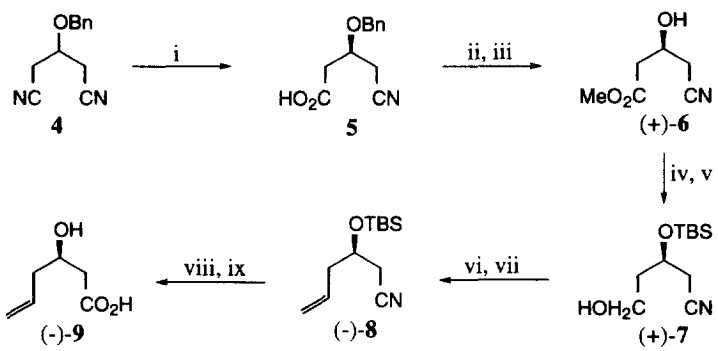
The synthetic route chosen was based on the observation that *nitrile hydratases* from *Rhodococcus* sp. and *Brevibacterium* sp., catalysed hydrolysis of the pro-*S* nitrile group of protected 3-hydroxyglutaronitrile derivatives **i** (Scheme 1). In both cases, the end product from the biotransformation was the (*S*)-cyanoacid **ii** resulting from the successive activities of a *nitrile hydratase* and *amidase* enzyme. Elaboration of nitrile **ii** to the β-hydroxynitrile **iii**, followed by a second use of the dual enzyme system (which would be predicted to be non-stereoselective based on previous studies), would provide a known precursor to the lactone **1** (Scheme 1).



Scheme 1: Strategy for use of a nitrile hydratase/amidase in the synthesis of lactone **1**.

We have previously reported the asymmetric hydrolysis of 3-(benzyloxy)glutaronitrile **4**[8] on a small scale to give the 3-(*S*)-cyano acid **5** using either an immobilised whole-cell preparation (SP 361) of a *Rhodococcus* sp. (73% yield; 83% e.e.)[5] or freshly prepared cells of *Brevibacterium* R312 (65% yield; 99% e.e.).[9] The requirement for large quantities of the acid **5** prompted us to select the use of a whole-cell preparation of *Brevibacterium* R312 and develop a procedure for carrying out the nitrile hydrolysis reaction on a multi-gram scale. Thus, to a washed-cell suspension (175 ml; 0.18g wet weight ml^{-1}) of the bacterium harvested in late log-phase was added **4** (7g, final substrate concentration = 4% w/v). After incubation in an orbital shaker (30 °C, 200 rpm) for 24h and subsequent extraction of the aqueous medium, 4.55g of the 3-*S*-acid **5** (65% yield, 88% e.e.) was recovered from the reaction mixture.[10]

Having prepared the acid **5** on a multi-gram scale we now proceeded to establish a route for its conversion to the hydroxyacid (-)-**9** which is a known[11] precursor of the lactone **1** (Scheme 2).



Scheme 2: i) *Brevibacterium* R312; ii) CH_2N_2, Et_2O; iii) H_2, $Pd(OH)_2$, $BF_3{\cdot}OEt_2$, CCl_3CONH_2, MeOH; iv) TBSCl, Imidazole, DMF; v) $LiBH_4$, THF; vi) *o*-iodoxybenzoic acid, DMSO; vii) PPh_3CH_3Br, BuLi, THF; viii) TBAF, THF; ix) *Rhodococcus* sp. SP 361, phosphate buffer.

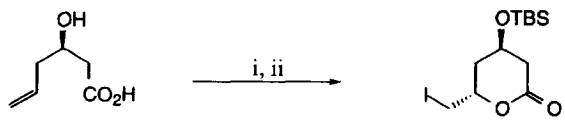
Methylation of the acid **5** with excess diazomethane gave the corresponding ester in excellent yield (93%). Use of chiral HPLC (Chiracel OD; hexane : 2-propanol) revealed the e.e. to be 88%. The absolute configuration of the ester was found to 3*S* by comparison of its rotation $[\alpha]_D^{21}$ + 11 (*c* 1.1 in $CHCl_3$) with that of optically pure (*R*)-methyl 3-(benzyloxy)-4-cyanobutanoate $[\alpha]_D^{25}$ - 11 (*c* 1.1 in $CHCl_3$), available in 3 steps from commercially available (*S*)-(-)-methyl 3-hydroxy-4-bromobutanoate.

Exchange of the 3-*O*-protecting group was found to be necessary to avoid subsequent problems with elimination of the benzyloxy group under basic conditions. However, removal of the benzyl protecting group by hydrogenation proved to be problematical giving variable yields of the hydroxyester (+)-**6**. After considerable experimentation, the optimal conditions were found to be $Pd(OH)_2$ in the

presence of BF_3 etherate and a catalytic (7 mol%) amount of 2,2,2-trichloroacetamide[12] which gave reproducible yields of (+)-**6** (74%). Conversion of the alcohol (+)-**6** to its benzoate ester (BzCl, pyridine, 65%) allowed the e.e. to be confirmed as 88.7% by chiral shift ^{1}H NMR [Eu(hfc)$_3$].

Treatment of the alcohol (+)-**6** with *tert*-butyldimethylsilyl (TBS) chloride gave the silyl ether in near quantitative yield (99.5%) which was treated with $LiBH_4$ in THF at reflux[13] to achieve chemoselective reduction of the ester to the corresponding alcohol (+)-**7** in the presence of the nitrile function (63%). Again chiral shift ^{1}H NMR [Eu(hfc)$_3$] was used to confirm that the e.e. was unchanged at 89%. Oxidation of (+)-**7** was carried out using the novel oxidant *o*-iodoxybenzoic acid[14] in DMSO, which furnished the product aldehyde (86%) and unchanged alcohol **7** (13%), giving a yield of 99% based on recovered starting material. Wittig methylenation to introduce the required olefin was found be somewhat variable although yields of up to 74% of **8** could be obtained.

The 3-hydroxy group of (-)-**8** was liberated quantitatively by use of TBAF, allowing chiral shift ^{1}H NMR [Eu(hfc)$_3$] to be performed (e.e. = 88%). Finally, the nitrile group was hydrolysed to the corresponding acid (-)-**9** (86%) by a second non-stereoselective application of the extremely mild conditions provided by using the two-step *nitrilehydratase/amidase* biotransformation catalysed by the immobilised cell preparation SP 361. That no racemisation had occurred at the sensitive 3*S* position was established by correlation of $[\alpha]_D^{27}$ - 25 (*c* 1.0 in $CHCl_3$) measured for (-)-**9** with a previously determined value $[\alpha]_D$ - 26.5 (*c* 2.1 in $CHCl_3$)[11] for material of 78% e.e. Completion of the synthetic route from (-)-**9** to the silyl protected lactone **1** has already been published by Knight and co-workers.[11] Thus, selective silyl protection, using TBSCl, of the 3-hydroxy group of (-)-**9** (74%), followed by iodolactonisation (I_2, $NaHCO_3$) yielded the TBS protected iodo-lactone **1** in good yield (84% yield) as a 3:1 mixture of diastereomers (50% d.e.), (Scheme 3).



(-)-**9** lactone **1**

Scheme 3: i) TBSCl, Imidazole, DMF; K_2CO_3, MeOH; ii) $NaHCO_3$, I_2, CH_3CN.

Methylation of the acid (-)-**9** generated by the pathway shown in Scheme 2 (*vide supra*) using excess diazomethane in ether afforded the corresponding methyl ester (83%). This allowed further confirmation of the absolute stereochemistry by polarimetry, $[\alpha]_D^{29}$ - 11 (*c* 0.9 in $CHCl_3$) which was in good agreement with a literature value[15] of $[\alpha]_D$ - 13 (*c* 1.3 in $CHCl_3$) for authentic material of 97% e.e. Additionally, use of chiral shift ^{1}H NMR [Eu(hfc)$_3$] revealed the e.e. to be 89%, identical to that of the starting material (+)-**6**.

In conclusion, a formal synthesis of the silyl protected lactone **1** has been successfully completed in 12 steps starting from the readily prepared 3-benzyloxyglutaronitrile **4**, demonstrating both the synthetic utility of the intermediate **5** and the use of *nitrile hydratase/amidase* enzymes in the enantioselective synthesis of natural products.

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