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# Enzymic Hydrolysis of Prochiral Dinitriles

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**Abstract:** A series of prochiral 3-hydroxyglutaronitrile derivatives **1-5** has been enzymically hydrolysed to the corresponding nitrile-carboxylic acids **1b-5b** with enantiomeric excesses ranging from 22-84%. In all cases the products were of the (*S*)-configuration.

The ability of enzymes to hydrolyse nitriles to the corresponding amides and/or carboxylic acids is well known but has only recently begun to be exploited as a potentially useful synthetic transformation.[1-4] The mild conditions required for the enzymic hydrolysis (pH 7.0, 30 °C, phosphate buffer) contrast strongly with the classical non-enzymic processes (6M HCl-reflux or 2M NaOH-reflux) and thus offer the possibility of reducing the unwanted side reactions that often accompany this reaction. In addition the enzyme mediated process may proceed with desirable enantio-, regio-, or chemoselectivity.

We have previously shown that an immobilised whole cell system from *Rhodococcus* sp. is capable of hydrolysing a wide range of structurally diverse nitriles.[5] This immobilised catalyst (SP 361) contains both hydratase (RCN to $RCONH_2$) and amidase ($RCONH_2$ to $RCO_2H$) enzymes (**eq. 1**) but lacks nitrilase activity (RCN to $RCO_2H$) (**eq. 2**).[6]

$$RCN \xrightarrow{\text{hydratase}} RCONH_2 \xrightarrow{\text{amidase}} RCO_2H \qquad \textbf{eq. 1}$$

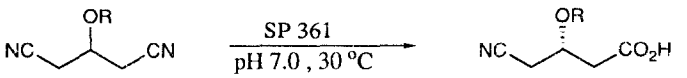
$$RCN \xrightarrow{\text{nitrilase}} RCO_2H \qquad \textbf{eq. 2}$$

In the preceding paper[7] we have shown that the SP 361 catalyst is capable of resolving racemic nitriles to the corresponding optically active amides and carboxylic acids. In this communication we report the enantioselective hydrolysis of prochiral dinitriles.

A series of protected 3-hydroxyglutaronitrile derivatives **1-6** was prepared *via* protection of the readily available 3-hydroxyglutaronitrile.[8] Upon subjection of **1-6** to SP 361 under standard conditions[9] only **6** failed to undergo hydrolysis. Substrates **1-5** were converted to the nitrile-carboxylic acids **1b-5b** with no evidence for the presence of nitrile-amides (**Table 1**).

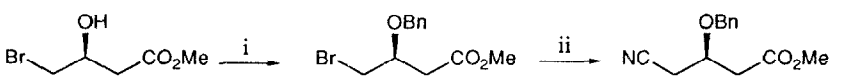
The enantiomeric excesses and absolute configurations of the products **1b-5b** were determined as follows. (*S*)-(-)-Methyl-3-hydroxy-4-bromobutanoic acid **7** (98% e.e.)[10] was benzylated to give (*S*)-**8** {$[\alpha]_D$ = -12.6 (c = 1, $CHCl_3$)} which was converted to the nitrile (*R*)-(-)-**9** {$[\alpha]_D^{25}$ = -11.3 (c = 1.1, $CHCl_3$)} (**Scheme 1**). Treatment of **1b** (derived from the enzyme hydrolysis) with $CH_2N_2$ gave (*S*)-(+)-**9** {$[\alpha]_D^{26}$ = + 9.1 (c = 1.1, $CHCl_3$). The enantiomeric excess was established to be 84% *via* chiral HPLC analysis.[11]

**Table 1**: Hydrolysis of dinitriles **1-6** with SP 361




**1-6** **1b-6b**

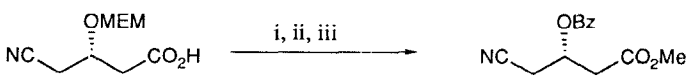
| Substrate | R | time/h | product | yield/% | config. | e.e./% |
|---|---|---|---|---|---|---|
| **1** | Bn | 48 | **1b** | 73 | *S* | 83 |
| **2** | Bz | 48 | **2b** | 25 | *S* | 84 |
| **3** | H | 65 | **3b** | 52 | *S* | 22 |
| **4** | MEM | 44 | **4b** | 19 | *S* | 61 |
| **5** | Ac | 65 | **5b** | 45 | - | 0 |
| **6** | TBDMS | 200 | - | - | - | - |




(*S*)-(-)-**7** (*S*)-(-)-**8** (*R*)-(-)-**9**

**Scheme 1**: i. benzyltrichloroacetimidate, $CF_3SO_3H$, (54%), ii, NaCN, DMSO, (52%).

Attempts to assign the configuration of **2b-5b** *via* a similar approach failed due to the inability to convert **7** to the corresponding 3-*O*-derivative and thus alternative routes were sought. The methyl ester of **2b** $\{[\alpha]_D^{22} = +35.6$ (c = 0.95, $CHCl_3$) was assigned as (*S*) *via* comparison with a literature report[12] and the e.e. was determined by $^1H$ n.m.r. (250 MHz) in the presence of Eu (hfc)$_3$. Similarly **3b** was treated with $CH_2N_2$ followed by PhCOCl to give (*S*)-(+)-**9** (e.e. 22%). Finally assignment of the MEM protected compound **4b** was carried out as shown in **scheme 2**.

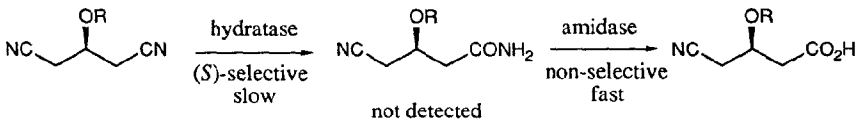



(*S*)-(-)-**4b** (*S*)-(+)-**9**

**Scheme 2**: i. $CH_2N_2$ ii. $Me_2BBr$, $CH_2Cl_2$, -25 °C, (46%) iii. BzCl, pyridine (92%).

It is interesting to note that whereas 3-hydroxyglutaronitrile **3** gave **3b** with 22% enantiomeric excess, the corresponding 3-*O*-acetyl compound **5** was biotransformed to **5b** with 0% e.e. In a related study using

*Rhodococcus butanica* ATCC 2119 on a narrower range of compounds. Kakeya *et al.*,[12] concluded that an aromatic ring was essential for enantioselectivity with this class of substrate. Our results suggest that the picture is more complicated and it may well be that hydrogen bonding interactions between the substrate and residues at the active site are more important. On the basis of the results obtained we tentatively propose the mechanism shown in **scheme 3** to rationalise the observed stereoselectivity. Thus it is suggested that the first hydratase step is (*S*)-selective and slow followed by a second amidase step that is non-selective and fast. The relative rates of the two processes would account for the absence of any nitrile-amide.




**Scheme 3**

In summary we have shown that a range of optically active nitrile-carboxylic acid containing compounds **1b-5b** can be obtained *via* enzymic hydrolysis of nitriles under mild conditions. We are currently exploring the application of these derived synthons.

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**References and Notes**

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6. For reports using a closely related catalyst see A. de Raadt, N. Klempier, K. Faber, and H. Griengl, *Tetrahedron Lett.*, 1991, **32**, 341; A. de Raadt, N. Klempier, K. Faber, and H. Griengl, *J. Chem. Soc., Perkin Trans. 1*, 1992, 137; P. Honicke-Schmidt and M.P. Schneider, *J. Chem. Soc., Chem. Commun.*, 1990, 648.
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9. Typical procedure for the hydrolysis of **1-6**; the substrate was dissolved (or suspended) in potassium phosphate buffer (100 mM, pH 7.0) giving a final concentration of 5-100 mM. The immobilised enzyme (SP 361, 1g per 100 ml of buffer) was added and the reaction shaken at 220 r.p.m., 30 °C. After completion the reaction was terminated by filtration of the enzyme through a celite pad. The aqueous filtrate was basified (pH 10, 2M NaOH) and extracted with ethyl acetate or

ether (3 x 100 ml) to remove any unreacted substrate. The aqueous portion was then acidified (pH 2, 2M HCl) and again extracted with ethyl acetate or ether (3 x 100 ml). The combined organic solutions were washed with brine (1 x 100 ml), dried ($MgSO_4$) and the solvent removed by rotary evaporation to afford the acid product.

10. Purchased from Berk Chemicals Ltd., P.O. Box 56, Priestley Road, Basingstoke, Hampshire RG24 9QB.
11. Chiral HPLC was performed using a Chiracel OD column with a flow rate of 1 ml/min using isopropyl alcohol:hexane (1:9) as the mobile phase.
12. H. Kakeya, N. Sakai, A. Sano, M. Yokoyama, T. Sugai, and H. Ohta, *Chem. Lett.*, 1991, 1823.