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Enantioselective Hydrolysis of Nitriles and Amides Using an Immobilised Whole Cell System

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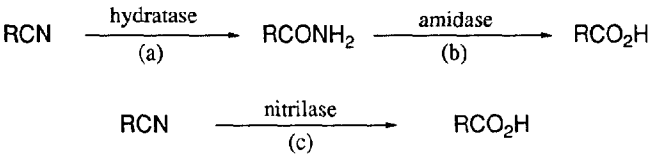
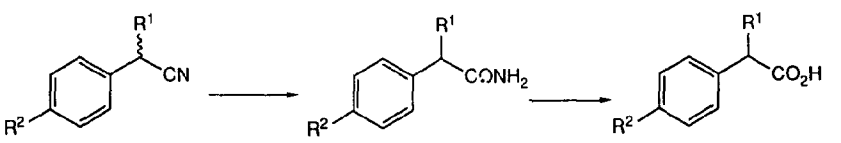
Abstract: An immobilised whole cell catalyst SP361 has been shown to hydrolyse a range of 2-alkyl arylacetonitriles **1a-3a** and amides **2b, 3b** with good to excellent enantioselectivity. The absolute configuration of the derived amides and/or carboxylic acids shows remarkable changes according to the structure of the nitrile substrate.

The enzymic hydrolysis of nitriles to either amides and/or carboxylic acids represents a potentially very useful synthetic method owing to the mildness under which the reactions can be carried out. In a recent communication we described the use of a commercially available immobilised whole cell system that can catalyse the hydrolysis of a wide range of aromatic and aliphatic nitriles and dinitriles.[1] Typically the reactions are carried out in potassium phosphate buffer at pH 7 with a concentration of 5-100 mM over a period of 12-48 hours. The enzyme system (SP 361) is derived from a *Rhodococcus* sp. and is able to catalyse two distinct reactions, namely the hydrolysis of (i) nitriles to amides (hydratase, path a), and (ii) amides to carboxylic acids (amidase path b).[2,3] The SP 361 system does not however appear to contain any nitrilase activity (path c) which is known to be present in other *Rhodococcus* species.[4]



Having established that the SP 361 catalyst is capable of hydrolysing a wide range of substrates[1] we have now turned our attention to the enantioselectivity of these hydrolyses especially with respect to the range of chiral/prochiral substrates that could be usefully transformed and the specific stage at which enantioselection might be occurring (*i.e.* path a or b). Herein we report the results of using racemic nitriles **1a-3a (Scheme 1)** including an unexpected switching of the absolute stereochemisty of the hydrolysis.



1a-3a **1b-3b** **1c-3c**

Scheme 1

The results from substrates **1a-3a** are summarised in **Table 1**. (±)-2-phenylpropionitrile **1a** underwent hydrolysis by SP 361 to give the (*R*)-amide **1b** and (*S*)-acid **1c** both with moderate enantiomeric excesses (78% and 65% respectively). Similarly (±)-2-phenylbutyronitrile **2a** was hydrolysed, albeit more slowly, under the same reaction conditions and gave the corresponding (*R*)-amide **2b** and (*S*)-acid **2c** with greater optical purities (90% and >98% respectively). For both **1a** and **2a** the recovered unreacted nitrile substrate was found to be optically inactive in all cases, implying that the enantioselective resolution had occurred at the amide to carboxylic acid step (*i.e. via* the amidase) and not *via* the hydratase.[5] However, upon incubation of (±)-2-(4'-*iso*-butylphenyl)-propionitrile **3a** with SP 361 the corresponding carboxylic acid **3c** obtained had the (*R*)-configuration with a modest e.e. (32-35%). In addition the reaction had to be run at lower concentration (5 mM) to achieve a good reaction rate and most unexpectedly we could not detect the presence of the corresponding amide **3b**.[4]

Table 1. Enantioselective hydrolysis of nitriles (±)-**1a-3a** with SP 361.

R^1	R^2	substrate	amide			acid			conc.	time
			yield/%	e.e./%	(*R*)/(*S*)[d]	yield/%	e.e./%[b]	(*R*)/(*S*)[e]	mM	
Me	H	**1a**	29	78[c]	(*R*)	41	65	(*S*)	25	12
Me	H	**1a**	20	78[c]	(*R*)	45	45	(*S*)	25	20
Et	H	**2a**	66	20[a]	(*R*)	NI	-	-	25	25
Et	H	**2a**	31	90[a]	(*R*)	22	>98	(*S*)	25	71
Me	Bu^i	**3a**‡	ND	-	-	12	33	(*R*)	5	30
Me	Bu^i	**3a**‡	ND	-	-	27	32	(*R*)	5	52
Me	Bu^i	**3a**‡	ND	-	-	19	35	(*R*)	5	72

NI - not isolated; ND - not detected; ‡ - recovered nitrile was also isolated [51% (30h), 13% (52h)]; a) e.e. determined by chiral shift ^{1}H n.m.r.; b) e.e. determined by chiral shift ^{1}H n.m.r. on methyl ester; c) e.e. determined by chemical hydrolysis (AcOH/6M H_2SO_4, 1:1, reflux 22h) to carboxylic acid then as for b); d) absolute configuration determined by chemical hydrolysis to the carboxylic acid then comparison of $[\alpha]_D$ value with the literature.[5]; e) absolute configuration determined by comparison of $[\alpha]_D$ value with the literature.[6]

In an attempt to shed further light on these processes, the racemic amides **2b** and **3b** were chemically prepared by hydrolysis of the corresponding nitriles **2a** and **3a** (H_2O_2, 10% NaOH, EtOH). The results of challenging amides **2b** and **3b** with SP 361 are given in **Table 2**. Amide **2b** was hydrolysed with high selectivity to give the (*S*) - acid **2c** (86% e.e.) and recovered (*R*)-amide **2b** (> 98% e.e., $[\alpha]_D$ = -79.5, c=1 in $CHCl_3$). These results are consistent with those obtained with the racemic nitrile **2a** and confirm that the enantioselectivity is due to action of the amidase. With the racemic amide **3b**, at 5 mM concentration, the acid **3c** obtained from the reaction now had the (*S*)-configuration with an e.e. of up to 32% dependent upon the time of the reaction. Accordingly the recovered amide **3b** was of the (*R*)-configuration (maximum e.e. = 26%). Thus although hydrolysis of the nitrile **3a** gave the (*R*)-acid as the major isomer, the corresponding amide **3b** produced the (*S*)-acid predominantly.

Clearly the nitrile **3a** exhibits anomalous behaviour when compared with nitriles **1a** and **2a**. This is all the more remarkable since **1a** and **3a** differ only by the presence of a *p-iso*-butyl group. In an attempt to rationalise the results obtained from all the substrates we propose the two following **schemes 1** and **2**.

Table 2. Enantioselective hydrolysis of amides (±)-**2b** and **3b** with SP 361.

R^1	R^2	substrate	amide			acid			conc.	time
			yield/%	e.e./%[c]	(*R*)/(*S*)[d]	yield/%	e.e./%[b]	(*R*)/(*S*)[e]	mM	h
Et	H	**2b**	20	>98[c]	(*R*)	25	86	(*S*)	25	145
Et	H	**2b**	33	>98[c]	(*R*)	22	80	(*S*)	25	216
Me	Bu^i	**3b**	55	10[c]	(*R*)	29	32	(*S*)	5	6
Me	Bu^i	**3b**	42	22[c]	(*R*)	51	19	(*S*)	5	12
Me	Bu^i	**3b**	7	26[a]	(*R*)	60	6	(*S*)	5	24
Me	Bu^i	**3b**	ND	-	-	79	0	-	5	71

ND - not detected; a) e.e. determined by $[\alpha]_D$; b) e.e. determined by chiral shift ^1H n.m.r. on corresponding methyl ester (CH_2N_2); c) e e. determined by chiral shift ^1H n.m.r. d) absolute configuration determined by chemical hydrolysis to the carboxylic acid then comparison of $[\alpha]_D$ value with the literature.[5]; e) absolute configuration determined by comparison of $[\alpha]_D$ value with the literature.[6]

Scheme 1: Pathway for **1a, 2a, 1b** and **2b**

$$\text{RCN} \xrightarrow[\textit{fast}]{\text{hydratase [non-selective]}} \text{RCONH}_2 \xrightarrow[\textit{slow}]{\text{amidase [(S)-selective]}} \text{RCO}_2\text{H}$$

Scheme 2: Pathway for **3a** and **3b**

$$\text{RCN} \xrightarrow[\textit{slow}]{\text{hydratase [(R)-selective]}} \text{RCONH}_2 \xrightarrow[\textit{fast}]{\text{amidase [(S)-selective]}} \text{RCO}_2\text{H}$$

In **Scheme 1** we rationalise the results simply on the basis of a non-specific hydratase followed by an (*S*)-specific amidase. The amidase reactions occur with high specificity allowing recovery of both amide and acid with high optical activities. The situation for substrates **3a** and **3b** is shown in **Scheme 2**. In this case we propose that the hydratase exhibits (*R*)-selectivity followed by the (*S*)-selective amidase. Thus with the racemic nitrile **3a**, the isolation of (*R*)-acid **3c** having 32-35% e.e. is a consequence of initial (*R*)-selectivity during nitrile to amide conversion followed by a fast amide to acid hydrolysis wherein the low (*S*)-selectivity of the amidase is not manifested in the product. Both the enantiomeric excess and absolute configuration of the product **3c** are determined by the hydratase enzyme. This rationalisation is consistent with the observation that hydrolysis of amide **3b** to acid **3c** is faster [**Table 2**: 24 h reaction yields recovered **3b** in 7% yield and **3c** in 60% yield] than hydrolysis of nitrile **3a** to acid **3c** [**Table 1**: 30 h reaction yields recovered nitrile **3a** in 51% yield and acid **3c** in 12% yield]. Both reactions were carried out under the same conditions with 5 mM concentration of substrate. Finally the constant e.e. of the product **3c** from three separate reactions (30, 52, and 72 hours) suggests possibly that *in situ* racemisation of the unreacted (*S*)-nitrile during the hydratase reaction is occurring. Such a process would eventually convert all racemic nitrile **3a** to optically active (*R*)-

acid **3c**. This process of racemisation might also be occurring for substrates **1a** and **2a** since in all experiments involving these two substrates, the recovered nitrile was found to be optically inactive. However attempts to probe the detail of this racemisation process have been inconclusive. Firstly, when substrate **3a**, with deuterium at C-2, was incubated with SP 361 under the usual conditions, no appreciable wash out of deuterium in the recovered nitrile could be detected. Secondly, incubation of **3a** with SP 361 in a deuteriated buffer medium was attempted but no enzyme activity was detected under these conditions. Despite the absence of direct evidence for an enzyme catalysed racemisation step it is worth noting that such a process has been detected in related nitrile hydrolysing systems.[7]

In summary we have shown that the immobilised whole system SP 361 is a useful enzyme for the preparation of optically active 2-alkyl aryl amides and carboxylic acids. In addition we have begun to uncover the effects of substrate structure on both the enantioselectivity and absolute stereochemistry of the hydrolysis and are continuing efforts in this direction in order to develop a more accurate model for hydrolysis of nitriles and amides using the SP 361 catalyst.

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References and Notes

1. M.A. Cohen, J. Sawden and N.J. Turner, *Tetrahedron Lett.* , 1990, **31**, 7223.
2. The catalyst SP361 is derived from a *Rhodococcus* sp. CH5 and is immobilised on an ion-exchange resin.
3. For other reports using a related immobilised enzyme system see; N. Klempier, A. de Raadt, K. Faber, and H. Griengl, *Tetrahedron Lett.*, 1991, **32**, 341; P. Hönicke-Schmidt, and M.P. Schneider, *J. Chem. Soc., Chem. Commun.*, 1990, 648.
4. M. Kobayashi, N. Yanaka, T. Nagasawa, and H. Yamada, *Tetrahedron Lett.*, 1990, **46**, 5587; *n.b.* the absence of nitrilase activity in SP 361 has only recently come to our attention and therefore previous statements regarding SP 361 (reference 1) should be modified in line with this observation.
5. In two recent reports the (*S*)-acid **3c** has been obtained from racemic nitrile **3a** using (a) *Rhodococcus butanica* ATCC 21197 (13% yield, 87% e.e.); H. Kakeya, N. Sakai, T. Sugai, and H. Ohta, *Tetrahedron Lett.*, 1991, **32**, 1343, and (b) *Acinetobacter* sp. strain AK226 (23% yield, 95% e.e.); K. Yamamoto, Y. Ueno, K. Otsubo, K. Kawakami and K.-I. Komatsu, *Appl. Envir. Microb.*, 1990, **56**, 3125. See also N. Murakami, European Patent Application, No. 0 356 912 (**A2**), 1989; K. Yamamoto, K. Otsubo, and K. Oishi, European Patent Application, No. 0 348 901 (**A2**), 1989.
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