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Stereoselective Hydrolysis of Nitriles and Amides Under Mild Conditions Using a Whole Cell Catalyst

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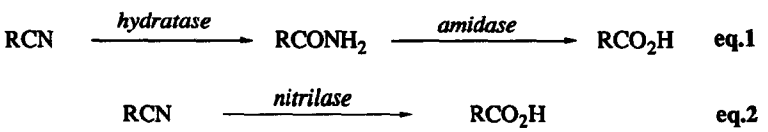
Abstract: An immobilised whole cell *Rhodococcus* sp. (SP 361) has been shown to be an effective catalyst for the stereoselective hydrolysis of both racemic and prochiral nitrile containing compounds. 2-Alkyl-arylacetonitriles **6a-8a** were hydrolysed to (*S*)-acids and (*R*)-amides whereas the closely related substrate **9a** gave the (*R*)-acid. A series of prochiral dinitriles **10a-13a** were hydrolysed to the corresponding (*S*)-acids with e.e.'s 22-84%. Models to account for the stereoselectivity of the enzymic hydrolyses have been proposed.

INTRODUCTION

Organonitriles are versatile intermediates in organic synthesis owing to the ease with which they can be prepared and subsequently transformed.[1] However perhaps the simplest functional group change that a nitrile can undergo *i.e.* hydrolysis to an amide or carboxylic acid is often difficult to achieve due to the harshness of the conditions required (6M HCL - reflux or 2M NaOH - reflux).[2] With this in mind we have sought to develop a mild, catalytic method that would be broadly applicable to a diverse range of nitrile containing compounds.[3] The ability of enzymes to catalyse nitrile hydrolysis is well documented[4] but vastly underexploited, particularly considering the successful application of lipases and esterases for the preparation of chiral building blocks. Enzymic nitrile hydrolysis proceeds *via* one of two pathways (**Scheme 1**), namely a two step process in which the nitrile is firstly converted to an amide (*hydratase*) and subsequently to a carboxylic acid (*amidase*) (**eq. 1**) or alternatively direct hydrolysis of a nitrile to a carboxylic acid (*nitrilase*) (**eq. 2**).[5]



Scheme 1

Little is known about the enzymes involved although they appear to be widely distributed in a range of micro-organisms. A limited number of studies have shown that whole cell preparations containing these enzymes

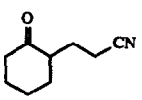
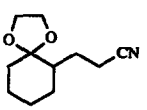
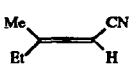
are suitable for the hydrolysis of structurally diverse organonitriles.[6] Indeed we[3] and others[7] have shown that a *Rhodococcus* sp. immobilised onto an ion exchange resin, can efficiently hydrolyse nitriles to both amides and carboxylic acids. This organism contains only the ***hydratase/amidase*** system (**eq. 1**) and not the *nitrilase* enzyme (**eq. 2**). In this paper we report in full the results obtained using racemic and prochiral substrates and propose models to rationalise the observed stereoselectivity.[8]

DISCUSSION

Stereoselective hydrolysis of racemic nitriles:

Initially we chose a range of simple racemic nitriles and exposed them to the *Rhodococcus* SP 361 catalyst under the standard conditions (*i.e.* 5-100 mM concentration in phosphate buffer at pH 7.0)[3] (**Table 1**). Despite obtaining modest to good conversions of the substrates to either the amide and/or carboxylic acid, none of the products showed any optical activity as evidenced by either ^{1}H n.m.r. in the presence of a chiral shift reagent [Eu(hfc)$_3$] or optical rotation measurements. It is interesting to note that the allene nitrile **5** gave only the corresponding amide as the product, albeit in poor yield.

Table 1 Hydrolysis of racemic nitriles (±)-**1-5** with *Rhodococcus* SP361.

Substrate		conc^n./mM	reaction time/h	yield of product amide	acid	ref. for preparation
	**1**	24	2	–*	26	-
	**2**	25	1.5	8	43	9
	**3**	25	120	–*	69	10
	**4**	25	70	–*	12	10
	**5**	47	96	16*	-	11

* Starting material also recovered in these reactions.

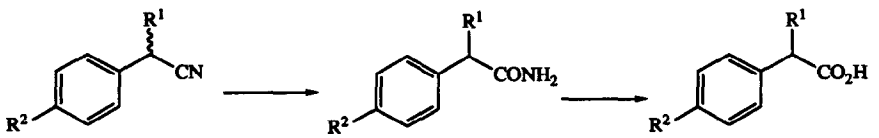
We next turned our attention to a series of 2-alkyl-arylacetonitriles **6a-9a**. The results obtained with these substrates are summarised in **Table 2**. The most surprising aspect of the results presented in **Table 2** is that despite the close similarity between substrates **6a-9a**, the results obtained differ quite markedly. For example;

i) substrates **6a**, **7a**, and **8a** yielded (*R*)-amide and (*S*)-acid whereas **9a** gave (*R*)-acid but *no* amide.

ii) for substrates **6a**, **7a**, and **9a** the recovered nitrile was racemic in all cases whereas for **8a** the recovered nitrile was optically active and could be obtained with an e.e. >95%.

iii) substrate **9a** only underwent hydrolysis at a concentration of 5 mM whereas **6a**, **7a**, and **8a** could be transformed at a concentration of 25 mM.

Table 2; Enantioselective hydrolysis of nitriles (±)-**6a**-**9a** with SP 361



	R^1	R^2		R^1	R^2		R^1	R^2
6a	Me	H	**6b**	Me	H	**6c**	Me	H
7a	Et	H	**7b**	Et	H	**7c**	Et	H
8a	Me	Me	**8b**	Me	Me	**8c**	Me	Me
9a	Me	Bu^i	**9b**	Me	Bu^i	**9c**	Me	Bu^i

substrate	amide			acid			conc.	time
	yield/%	e.e./%	(*R*)/(*S*)[d]	yield/%	e.e./%[b]	(*R*)/(*S*)[e]	mM	h
6a	29	78[c]	(*R*)	41	65	(*S*)	25	12
6a	20	78[c]	(*R*)	45	45	(*S*)	25	20
7a	66	20[a]	(*R*)	NI	-	-	25	25
7a	31	90[a]	(*R*)	22	>98	(*S*)	25	71
8a*		44[a]	(*R*)	32	>95	(*S*)	25	13
8a**	18	>95[a]	(*R*)	41	>95	(*S*)	25	24
9a‡	ND	-	-	12	33	(*R*)	5	30
9a‡	ND	-	-	27	32	(*R*)	5	52
9a‡	ND	-	-	19	35	(*R*)	5	72

NI - not isolated; ND - not detected; ‡ - recovered nitrile was also isolated [51% (30h), 13% (52h)]; *recovered (*R*)-nitrile, 40% yield, 50% e.e. **recovered (*R*)-nitrile, 25% yield, >95% e.e. a) e.e. determined by chiral shift ^1H n.m.r.; b) e.e. determined by chiral shift ^1H n.m.r. on methyl ester; c) e.e. determined by chemical hydrolysis (AcOH/6M H_2SO_4, 1:1) 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.[12,26]; e) absolute configuration determined by comparison of $[\alpha]_D$ value with the literature.[13]

In order to shed further light on these reactions the amides (±)-**7b**, **8b**, and **9b** were prepared from the corresponding nitriles (H_2O_2, 10% NaOH, r.t.)[14] and submitted for hydrolysis by SP 361. In this way it would be possible to examine solely the effect of the amidase on the stereoselectivity of the reaction (**Table 3**).

Table 3. Enantioselective hydrolysis of amides (±)-**7b**-**9b** with SP 361

substrate	amide			acid			conc.	time
	yield/%	e.e./%[b]	(*R*)/(*S*)[c]	yield/%	e.e./%[a]	(*R*)/(*S*)[d]	mM	h
7b	20	>98	(*R*)	25	86	(*S*)	25	145
7b	33	>98	(*R*)	22	80	(*S*)	25	216
8b	75	18	(*R*)	20	>95	(*S*)	25	6
8b	62	51	(*R*)	35	>95	(*S*)	25	15
9b	55	10	(*R*)	29	32	(*S*)	5	6
9b	42	22	(*R*)	51	19	(*S*)	5	12
9b	7	26	(*R*)	60	6	(*S*)	5	24
9b	ND	-	-	79	0	-	5	71

ND - not detected; a) e.e. determined by chiral shift ^{1}H n.m.r. on corresponding methyl ester (CH_2N_2); b) e.e. determined by chiral shift ^{1}H n.m.r. c) absolute configuration determined by chemical hydrolysis to the carboxylic acid then comparison of $[\alpha]_D$ value with the literature.[12,26]; d) absolute configuration determined by comparison of $[\alpha]_D$ value with the literature.[13]

The data in **Table 3** confirm that the amidase is (*S*)-selective yielding, in all cases, (*S*)-acid and (*R*)-amide. Thus the stereoselectivity observed for substrates **6a** -**9a** may be rationalised according to **Scheme 2.**

Scheme 2: Model for the hydrolysis of **6a**-**9a**.

$$RCN \xrightarrow[\text{[(R) or (S) or non-selective]}]{\textit{hydratase}} RCONH_2 \xrightarrow[\text{[(S)-selective]}]{\textit{amidase}} RCO_2H$$

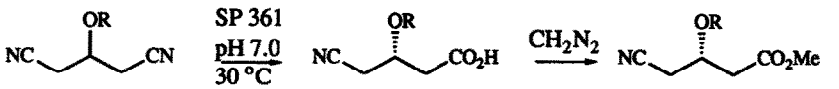
The important feature of the model shown in **Scheme 2** is that all of the observed changes in stereoselectvity are a consequence of the initial *hydratase* reaction. The amidase reaction that follows always shows (*S*)-selectivity. Furthermore the reaction rates of the *hydratase* and *amidase* reactions affect the overall result as follows. Nitriles **6a** and **7a** are hydrolysed rapidly and non-selectively by the *hydratase* to give **6b** and **7b** respectively, both still racemic. Subsequent slow (*S*)-selective hydrolysis catalysed by the *amidase* yields (*S*)-acid and recovered (*R*)-amide. For nitrile **8a** the initial hydrolysis by the *hydratase* proceeds with (*S*)-selectivity yielding recovered (*R*)-nitrile, followed by the (*S*)-selective *amidase* step. Finally for nitrile **9a**, the results can be rationalised by proposing a relatively slow (*R*)-selective *hydratase* reaction followed by a faster (*S*)-selective *amidase* step. The (*R*)-selective *hydratase* step would furnish (*R*)-amide which is not detected since it is immediately consumed by the *amidase* reaction. Since the amidase step is faster, the (*S*)-selectivity would not be apparent and the predominantly (*R*)-amide would be rapidly converted to predominantly (*R*)-acid. The only aspect of the hydrolysis of **9a** that is difficult to explain is the recovery of racemic nitrile from reactions that had not gone to completion. If the *hydratase* step is (*R*)-selective then the recovered nitrile should be of (*S*)-configuration. That this was found not to be the case implies *in situ* racemisation of the nitrile **9a**. Support for this idea comes from the observation that the acid **9c**, recovered from three different reactions, had a constant e.e., implying a dynamic resolution process. However attempts to provide direct evidence for this racemisation

process were unsuccessful. Thus when 2-deuterio-**9a** was prepared and submitted to the reaction conditions there was no evidence for loss of deuterium label from the recovered nitrile. The level of deuterium incorporation in **9a** was judged to be >98% by ^{1}H n.m.r. thereby ruling out any possibility of a kinetic isotope effect that would favour hydrolysis of any residual protio substrate. If racemisation is occurring it is unclear why it does not occur for nitrile **8a**.

***Stereoselective hydrolysis of prochiral dinitriles*:**

In order to investigate the stereoselective effect of SP 361 on prochiral substrates a series of protected 3-hydroxyglutaronitrile derivatives **10a-15a** were prepared. This was accomplished *via* protection of the readily available 3-hydroxyglutaronitrile **12a**.[16] When SP 361 was individually challenged with nitriles **10a-15a**, only **15a** failed to undergo hydrolysis (**Table 4**). For substrates **10a-14a**, the nitrile-acids **10b-14b** were obtained with no evidence for the presence of nitrile-amides.

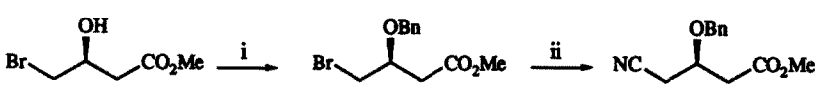
Table 4: Hydrolysis of dinitriles **10a-15a** with SP 361.



10a-15a **10b-14b** **10c-13c**

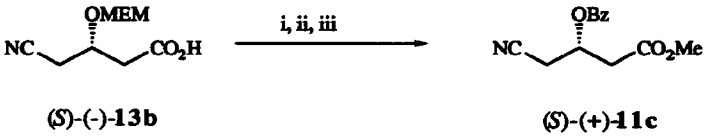
Substrate	R	time/h	product	yield/%	config.	e.e./%
10a	Bn	48	**10b**	73	*S*	83
11a	Bz	48	**11b**	25	*S*	84
12a	H	65	**12b**	52	*S*	22
13a	MEM	44	**13b**	19	*S*	61
14a	Ac	65	**14b**	45	-	0
15a	TBDMS	200	-	-	-	-

The absolute configurations of **10b-13b** were confirmed *via* conversion to their corresponding methyl esters **10c-13c** and subsequent comparison of [a]$_D$ values with compounds of known configuration. Thus the configuration of (*S*)-**10c** was established *via* comparison with (*R*)-**10c** prepared from commercially available (*S*)-(-)-**16** (**Scheme 3**).[17] (*S*)-**11c** and (*S*)-**12c** (after conversion to the 3-*O*-benzoate) were assigned after comparison with a literature report.[18] Finally **13b** was established to be (*S*) as shown in **Scheme 4**.



(*S*)-(-)-**16** (*S*)-(-)-**17** (*R*)-(-)-**10c**

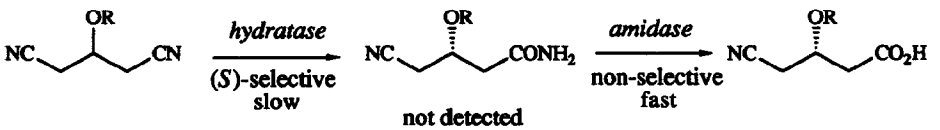
Scheme 3: i, benzyltrichloroacetimidate, CF_3SO_3H, (54%), ii, NaCN, DMSO, (52%).



(*S*)-(-)-**13b** (*S*)-(+)-**11c**

Scheme 4: i. CH_2N_2 ii. Me_2BBr, CH_2Cl_2, -25 °C, (46%) iii. BzCl, pyridine (92%)

All of the products obtained from these enzymic hydrolyses were therefore of (*S*)-configuration. A consideration of the two step hydrolysis responsible for generating the products leads to the model shown in **Scheme 5.**



Scheme 5. Model for the hydrolysis of prochiral dinitriles

Kakeya *et al.*, have carried out related transformation using whole cells of *Rhodococcus butanica* ATCC 2119 and obtained similar results.[18] Contrary to their conclusion however, the presence of an aromatic ring in the substrate is not an absolute requirement for enantioselectivity to be observed. In our work, we have shown that both the MEM protected compound and 3-hydroxyglutaronitrile **12a** itself were hydrolysed to optically active compounds.

CONCLUSIONS

The ability of a whole cell catalyst to carry out stereoselective hydrolyses of nitrile and amide containing compounds has been demonstrated using two different class of substrates. Although trends have emerged governing the exact nature of the stereoselectivity of these reactions, the detailed picture remains unclear. This analysis is complicated by having the two enzymes involved, a *hydratase* and an *amidase*, working in tandem in a whole cell system. To understand explicitly the stereoselectivity at each step it will be necessary to purify the amidase and hydratase enzymes and examine them individually. This goal, and the exploitation of optically active synthons derived from enzymic nitrile hydrolysis, are our current aims.

Acknowledgements:
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EXPERIMENTAL

General procedures;

Unless otherwise stated, all reagents were obtained from commercial suppliers and used without further purification. Light petroleum (b.p. 40-60 °C) and ethyl acetate were distilled prior to use. THF was distilled from sodium wire and benzophenone, while dichloromethane, DMF and DMSO were distilled from calcium hydride. Pyridine was distilled from potassium hydroxide and cyclohexane was distilled from magnesium turnings. All dried solvents were stored over 4 Å molecular sieves under an inert atmosphere of argon. Brine refers to a saturated aqueous solution of sodium chloride.

Reactions were monitored by t.l.c. on Merck Kieselgel 60 F_{254}, 0.25 mm plates. Plates were visualised by alkaline potassium permanganate dip or U.V. (254 nm) light. Preparative column chromatography was performed using silica gel 60H (0.04-0.063 mm/ 230-400 mesh) (Merck 9385). Solvent mixtures are expressed in volume: volume ratios.

250 MHz ^1H and 62.9 MHz ^{13}C NMR spectra were recorded on a Bruker AM250 spectrometer with chemical shifts (δ_H and δ_C) being measured in ppm downfield from tetramethylsilane. Coupling constants (*J*) are given in Hz. IR (υ_{max}) spectra were recorded on a Perkin-Elmer 881 IR grating spectrometer with frequencies measured in wavenumbers (cm^{-1}). High resolution mass spectra (m/z) were recorded at the SERC Mass Spectrometry Centre, Swansea using a BG ZAB-E mass spectrometer.

Polarimetry was carried out using an Optical Activity AA-1000 polarimeter (measurements being made at the sodium D-line) with a 0.5 dm pathlength cell. Concentrations (*c*) are given in g/ 100 ml. Melting points were measured using an Electrothermal melting point instrument and are uncorrected.

All HPLC was run using a Gilson 303 dual pump machine with a UV detector set at 254 nm. Chiral HPLC was performed using a Chiracel OD column at a flow rate of 1 ml/min, with IPA:hexane [10:90] as the solvent system. Chiral shift ^1H NMR spectroscopy experiments were carried out using Tris-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] Eu(III) derivative.

The pH 7.0 phosphate buffer solution used for the biotransformations was prepared using 100 mM solutions of K_2HPO_4 and KH_2PO_4. A CSI AGB 2000 pH meter and combination electrode were used to mix the two phosphate solutions to the required pH.

Methyl esters were made from the corresponding carboxylic acids using diazomethane, as described in "Textbook of Practical Organic Chemistry," 4th edition by A. Vogel.

General Procedure for the Chemical Hydrolysis of Nitriles into Amides.[14]

The substrate was dissolved in a solution of ethanol and aqueous sodium hydroxide (2 M), at room temperature. Hydrogen peroxide solution (30 % w/v) was slowly added and the reaction stirred for 4-72 h (t.l.c. being used to monitor the reaction). Saturated ammonium chloride solution was added and the resulting mixture extracted with ether or ethyl acetate. The combined organic solutions were washed with brine (100 ml), dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation. The required product was then purified by column chromatography.

A General Method for the Chemical Hydrolysis of Amides to the Corresponding Carboxylic Acids.[15]
To a solution of the substrate (0.2-0.7 mmol) in glacial acetic acid (6 ml) was added sulphuric acid (3 ml, 6 M). The mixture was refluxed at 115 °C for 7-20 h., allowed to cool to room temperature and then extracted with ethyl acetate (3 x 50 ml). The organic solution was washed with brine (1 x 20 ml), dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation to afford the crude product. The residual acetic acid was removed by co-evaporation with toluene (3 x 30 ml). No further purification was afforded, unless otherwise stated.

General Procedure for the Enzymic Hydrolysis of Nitriles and Amides.

The substrate was suspended in potassium phosphate buffer (100 mM, pH 7.0). The immobilised enzyme system (SP 361, 1 g/ 100 ml of buffer) was added and the reaction shaken at 220 rpm, 30 °C (t.l.c. being used to monitor the progress of the reaction). The reaction was terminated by filtration of the enzyme (through a celite pad). The aqueous filtrate was basified (pH 10, 2 M NaOH) and extracted with ethyl acetate, ether or chloroform. The combined organic solutions were washed with brine (1 x 50 ml), dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation to afford any unreacted nitrile and/or amide.

The aqueous portion was then acidified (pH 2, 2 M HCl) and again extracted with ethyl acetate, ether or chloroform. The combined organic solutions were washed with brine (1 x 50 ml), dried ($MgSO_4$) and the solvent removed by rotary evaporation to afford the acid product. Quantities of substrate and buffer used, as well as reaction times, purification techniques and yields are given for each individual reaction (below).

Racemic nitriles and Amides;

Enzymic Hydrolysis of (±)-2-Methyl butyronitrile 1.
(±)-2-Methylbutyronitrile (300 mg, 3.61 mmol) yielded (±)-2-methylbutyric acid (96 mg, 26 %) as an orange liquid.
R_f 0.67 (ethyl acetate); $[\alpha]_D^{24}$ 0 (*c* 0.74, $CHCl_3$); Found (CI) $[M + NH_4]^+$: 120.1025 ($C_5H_{14}NO_2$ requires 120.1025); υ_{max} (neat)/cm^{-1} 2400-3550 (OH str), 1704 (CO str), 1415, 1381 (CH def); δ_H (250 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7, Me-CH_2), 1.21 (3H, d, *J* 7, Me-CH), 1.53, 1.74 (1H, septet, *J* 7, CH-H), 2.42 (1H, sextet, *J* 7, CH); δ_C (62.9 MHz, $CDCl_3$) 11.4, 16.3 (Me), 26.5 (CH_2), 40.8 (CH), 182.7 (CO); m/z (CI) 120 ($[M + NH_4]^+$, 100 %), 100 (19), 85 (84), 74 (9), 57 ($[M - COOH]^+$, 15), 39 (14).

Enzymic Hydrolysis of (±)-2-Chloropropionitrile 2.
(±)-2-Chloropropionitrile (500 mg, 5.58 mmol) yielded, after purification by column chromatography (ethyl acetate: petroleum [1:2] as the eluent), (±)-2-chloropropionitrile (25 mg, 5 %) $[\alpha]_D^{25}$ 0 (*c* 0.4, $CHCl_3$), (±)-2-chloropropylamide (48 mg, 8 %) m.p. 75-77 °C [lit.[19] 77-79 °C] $[\alpha]_D^{24}$ 0 (*c* 0.98, $CHCl_3$); e.e. 0 % (chiral shift ^1H NMR spectroscopy) and (±)-2-chloropropanoic acid (259 mg, 43 %).

Enzymic Hydrolysis of (±)-2-(2'-cyanoethyl)-cyclohexanone 3.
(±)-2-(2'-Cyanoethyl)-cyclohexanone (300 mg, 1.98 mmol) yielded recovered (±)-2-(2'-cyanoethyl)-cyclohexanone (78 mg, 26 %) $[\alpha]_D^{26}$ 0 (*c* 1, $CHCl_3$) and (±)-3-[1'-(2'-oxo-cyclohexyl)]-propanoic acid (212 mg, 63 %) as a soft white solid.

R_f 0.55 (ethyl acetate); m.p. 55-57 °C [lit.[20] 55 °C]; $[\alpha]_D^{26}$ 0 (*c* 1.1, $CHCl_3$); Found (EI) M^+: 170.0943 ($C_9H_{14}O_3$ requires 170.0943); υ_{max} ($CHCl_3$)/cm^{-1} 2500-3600 (OH str), 1714 (CO str), 1448, 1372 (CH def); δ_H (250 MHz, $CDCl_3$) 1.20-2.50 (13H, m), 10.00 (1H, br s, COOH); δ_C (62.9 MHz, $CDCl_3$) 24.5, 25.0, 27.9, 31.5, 34.1, 42.0 (CH_2), 49.6 (CH), 179.3 (COOH), 212.8 (CO); m/z 170 (EI) (M^+, 5 %), 152 (70), 124 (61), 98 (82), 83 (55), 55 (100).

Enzymic Hydrolysis of (±)-6-(2'-Cyanoethyl)-1,4-dioxospiro [4.5] decane 4.

(±)-6-(2'-Cyanoethyl)-1,4-dioxospiro [4.5] decane (500 mg, 2.56 mmol) yielded recovered (±)-6-(2'-cyanoethyl)-1,4-dioxospiro [4.5] decane (266 mg, 53 %) $[\alpha]_D^{26}$ 0 [*c* 2.3, $CHCl_3$]) and (±)-3-[-6'-(1',4'-dioxospiro [4.5] decane)]-propanoic acid (65 mg, 12%) as a colourless oil.

R_f 0.58 (ethyl acetate), $[\alpha]_D^{26}$ 0 (*c* 1, CHCl3); Found (CI) $[M + H]^+$: 215.1283 ($C_{11}H_{19}O_4$ requires 215.1283); υ_{max} (neat)/cm^{-1} 2400-3600 (OH str), 2938 (CH str), 1703 (CO str), 1443 (CH def); δ_H (250 MHz, $CDCl_3$) 1.10-2.50 (13H, m), 3.90- 4.05 (4H, S, CH_2O x 2), 8.55 (1H, br s, COOH); δ_C (62.9 MHz, $CDCl_3$) 23.6, 23.7, 24.4, 29.1, 32.3, 34.5 (CH_2), 49.6 (CH), 64.5, 64.7 (CH_2O), 110.6 (C), 179.7 (CO); m/z (CI) 215 ($[M + H]^+$, 75 %), 197 (22), 171 (28), 155 (23), 99 (100).

Enzymic Hydrolysis of (±)-3,3-Methylethyl-1-allene nitrile 5.

(±)-3,3-Methylethyl-1-allene nitrile (1.00 g, 9.33 mmol) yielded after purification by mixed solvent recrystallisation (ethyl acetate- petroleum) (±)-3,3-methylethyl-1-allene amide (90 mg, 16 %) as a white solid.

R_f 0.38 (ethyl acetate); m.p. 104-105 °C [lit.[21] 102 °C]; $[\alpha]_D^{20}$ 0 (*c* 1, $CHCl_3$); Found: C, 67.22; H, 8.98; N, 11.00 ($C_7H_{11}NO$ requires C, 67.17; H, 8.86; N, 11.19); Found (CI) $[M + H]^+$: 126.0919 ($C_7H_{12}NO$ requires 126.0919); υ_{max} ($CHCl_3$)/cm^{-1} 3532, 3417 (NH str), 1957 (CC str, allene), 1660, 1552 (Amide I and II); δ_H (250 MHz, $CDCl_3$) 1.03 (3H, t, *J* 7.5, Me-CH_2), 1.79 (3H, d, *J* 3.0, Me), 2.06 (2H, dq, *J* 3.0, *J* 7.5, CH_2), 5.42- 5.50 (1H, m, CH), 5.92 (2H, br s, NH x 2); δ_C (62.9 MHz, $CDCl_3$) 11.9 (Me-CH_2), 18.0 (Me), 26.6 (CH_2), 90.6 (CH), 107.6 (C), 168.8 (CO), 205.8 (=C=); m/z (CI) 126 ($[M + H]^+$, 100 %), 110 (8).

Enzymic Hydrolysis of (±) 2-Phenylpropionitrile 6a.[22]

(R)-(-)-2-Phenylpropylamide 6b **12 h.** (29 %) $[\alpha]_D^{23}$ -55 (*c* 1.1, $CHCl_3$), e.e. 78 % **19.5h** (20 %) $[\alpha]_D^{23}$ -54 (*c* 1, $CHCl_3$); e.e. 78 % (optical rotation) . R_f 0.28 (ethyl acetate: petroleum [1:1]); m.p. 98-99 °C [lit.[23] 97-97.5]; Found (EI) $[M + H]^+$: 150.0919 ($C_9H_{12}NO$ requires 150.0919); ; υ_{max} ($CHCl_3$)/cm^{-1} 3414, 3528 (NH str), 2998 (CH str, aliphatic), 1682, 1588 (Amide I and II), 1493 (CC str, aromatic), 1452, 1379 (CH def, aliphatic), 692 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 1.51 (3H, d, *J* 8.0, Me), 3.59 (1H, q, *J* 8.0, CH), 5.58, 6.23 (1H, br s, NH), 7.18- 7.37 (5H, m, Ph); δ_C (62.9 MHz, $CDCl_3$) 18.3 (Me), 46.6 (CH), 127.3, 127.6, 128.9 (CH, Ph), 141.4 (C, Ph), 177.7 (CO); m/z (EI) 150 ($[M + H]^+$, 18 %), 105 ($[M - CONH_2]^+$, 100), 91 (47), 77 ($[C_6H_5]^+$, 30).

(S)-(+)-2-phenylpropanoic acid 6c **12h** (41 %) $[\alpha]_D^{23}$ +43 (*c* 1.98, $CHCl_3$), e.e. 65 % **19.5h** (45 %) $[\alpha]_D^{23}$ +32 (*c* 1.14, $CHCl_3$) (lit.,[12] $[\alpha]_D^{25}$ +75 (*c* 1.65, $CHCl_3$), (*S*)-(+)-2-phenylpropanoic acid); e.e. 45 % (chiral shift ^1H NMR spectroscopy on the corresponding methyl ester) . R_f 0.65 (ethyl acetate: petroleum [1:1]); Found (EI)

M^+: 150.0681 ($C_9H_{10}O_2$ requires 150.0681); ; υ_{max} (neat)/cm^{-1} 2800- 3400 (OH str), 1707 (CO str), 1603, 1492 (CC str, aromatic), 1414, 1378 (CH def, aliphatic), 727, 698 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 1.57 (3H, d, *J* 7.8, Me), 3.82 (1H, q, *J* 7.8, CH) 7.22- 7.43 (5H, m, Ph), 11.10 (1H, br s, COOH); δ_C (62.9 MHz, $CDCl_3$) 18.1 (Me), 45.5 (CH), 127.4, 127.7, 128.7 (CH, Ph), 139.8 (C, Ph), 180.9 (CO); m/z (EI) 150 (M^+, 13 %), 105 ([M - CO_2H]$^+$, 100), 91 (10), 77 ([C_6H_5]$^+$, 10).

Chemical Hydrolysis of (R)-(-)-2-Phenylpropylamide 6b.

(*R*)-(-)-2-Phenylpropylamide (40 mg, 0.268 mmol, $[\alpha]_D^{23}$ -55 [*c* 1.1, $CHCl_3$]) was refluxed for 22 h in the acid solution. This yielded (*R*)-(-)-2-phenylpropanoic acid as a pale orange oil (40 mg, 0.266 mmol, 99 %). R_f 0.64 (ethyl acetate); $[\alpha]_D^{24}$ -46 (*c* 0.8, $CHCl_3$) (lit.,[12] $[\alpha]_D^{25}$ +75 (*c* 1.65, $CHCl_3$), (*S*)-(+)-2-phenylpropanoic acid); e.e. 78 % (chiral shift ^{1}H NMR on the corresponding methyl ester).

Enzymic Hydrolysis of (±)-2-Phenylbutyronitrile 7a.

***(R)-(-)-2-Phenylbutylamide 7b* 71h** (31 %) as a white solid. $[\alpha]_D^{25}$ - 69 (*c* 1, $CHCl_3$); e.e. 90 % (chiral shift ^{1}H NMR spectroscopy) R_f 0.39 (ethyl acetate: petroleum [1:1]); m.p. 76-77 °C [lit.[24] 80.5-81.5]; Found: C, 73.42; H, 8.23; N, 8.53 ($C_{10}H_{13}NO$ requires C, 73.59; H, 8.03; N, 8.58); Found (CI) [M + NH_4]$^+$: 181.1341 ($C_{10}H_{17}N_2O$ requires 181.1341); υ_{max} ($CHCl_3$)/cm^{-1} 3527, 3411 (NH str), 3190 (CH str, aromatic), 2969 (CH str, aliphatic), 1672, 1583 (Amide I and II), 1491 (CC str, aromatic), 1417, 1380 (CH def, aliphatic); δ_H (100 MHz, $CDCl_3$) 0.89 (3H, t, *J* 6.5, Me), 1.60-2.40 (2H, m, CH_2), 3.29 (1H, t, *J* 8.0, CH), 5.50 (2H, br s, NH x 2), 7.25- 7.40 (5H, m, Ph); δ_C (25 MHz, $CDCl_3$) 12.2 (Me), 26.1 (CH_2), 54.5 (CH), 127.2, 127.9, 128.7 (CH, Ph), 140.0 (C, Ph), 176.3 (CO); m/z (CI) 181 ([M + NH_4]$^+$, 5 %), 164 ([M + H]$^+$, 100).

***(S)-(+)-2-phenylbutyric acid 7c* 71h** (22 %) as a colourless oil. $[\alpha]_D^{26}$ +76.8 (*c* 1, $CHCl_3$)/ $[\alpha]_D^{25}$ +104 (*c* 1.2, toluene) (lit.,[12] $[\alpha]_D^{19}$ +80 (*c* 0.9, toluene), (*S*)-(+)-2-phenylbutyric acid); e.e. > 98 % (Chiral Shift ^{1}H NMR spectroscopy on the corresponding methyl ester); R_f 0.55 (ethyl acetate: petroleum [1:1]); Found (CI) [M + NH_4]$^+$: 182.1181 ($C_{10}H_{16}NO_2$ requires182.1181); υ_{max} (neat)/cm^{-1} 2400- 3600 (OH str), 1707 (CO str), 1600, 1492 (CC str, aromatic), 1412 (CH def, aliphatic), 727, 698 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 0.99 (3H, t, *J* 8.0, Me), 1.73- 1.95, 2.08- 2.26 (1H, m, CH-H), 3.55 (1H, t, *J* 8.0, CH), 7.29- 7.50 (5H, m, Ph), 11.50 (1H, br s, COOH); δ_C (62.9 MHz, $CDCl_3$) 12.1 (Me), 26.4 (CH_2), 53.5 (CH), 127.5, 128.2, 128.7 (CH, Ph), 138.5 (C, Ph), 180.6 (CO); m/z (CI) 182 ([M + NH_4]$^+$, 100 %), 164 (M^+, 40), 119 ([M - COOH]$^+$, 78), 91 (80).

Enzymic Hydrolysis of (±)-2-Phenylbutylamide 7b.

***(R)-(-)-2-Phenylbutylamide 7b* 145 h.** (20 %) $[\alpha]_D^{26}$ -79.5 (*c* 1, $CHCl_3$), e.e. > 98 % **216h** (33 %) $[\alpha]_D^{25}$ -75.5 (*c* 0.8, $CHCl_3$); e.e. > 98 % (chiral shift ^{1}H NMR spectroscopy).

***(S)-(+)-2-Phenylbutyric acid 7c.* 145h** (50 mg, 0.31 mmol, 25 %, $[\alpha]_D^{28}$ +54.8 (*c* 1, $CHCl_3$), e.e. 86 % **216h** (22 %) $[\alpha]_D^{25}$ +75.0 (*c* 1, toluene) (lit.,[12] $[\alpha]_D^{19}$ +80 (*c* 0.9, toluene), (*S*)-(+)-2-phenylbutyric acid); e.e. 80 % (chiral shift ^{1}H NMR spectroscopy on the corresponding methyl ester).

Chemical Hydrolysis of (R)-(-)-2-Phenybutylamide 7b.

(*R*)-(-)-2-Phenybutylamide (104 mg, 0.637 mmol, $[\alpha]_D^{25}$ -69 (*c* 1, $CHCl_3$), e.e. 90 %) was refluxed for 7 h. in the acid solution. Purification by column chromatography (ethyl acetate: petroleum [1:2] as the eluent) afforded (*R*)-(-)-2-phenylbutyric acid (56 mg, 54 %) as a colourless oil.
R_f 0.50 (ethyl acetate: petroleum [1:2]); $[\alpha]_D^{27}$ -60.2 (*c* 1, $CHCl_3$) ($[\alpha]_D^{25}$ +76.8 (*c* 1, $CHCl_3$), (*S*)-(+)-2-phenylbutyric acid

Enzymic Hydrolysis of (±)-2-(4'-Methylphenyl)-propionitrile 8a.[22]
(R)-(+)-2-(4'-methylphenyl)-propionitrile 8a **12.5h**. (40 %) $[\alpha]_D^{25}$ +7.9 (*c* 0.98, $CHCl_3$), e.e. 50 % (optical rotation) **24h** (25 %) $[\alpha]_D^{25}$ +13.1 (*c* 0.88, $CHCl_3$), e.e. >95 %.
(R)-(-)-2-(4'-methylphenyl)-propylamide 8b **12.5h** (23 %) $[\alpha]_D^{25}$ -32.0 (*c* 0.52, $CHCl_3$), e.e. 44 % **24h** (18 %) $[\alpha]_D^{25}$ -49.7 (*c* 1.14, $CHCl_3$); e.e. >95 % (chiral shift ^1H NMR spectroscopy). R_f 0.12 (ethyl acetate: petroleum [1:1]); m.p. 105-106 °C [lit.[25] 102-103]; ; Found (EI) M^+: 163.0970 ($C_{10}H_{13}NO$ requires 163.0997); υ_{max} (nujol)/cm^{-1} 3361, 3199 (NH str), 1648 (CO str); δ_H (100 MHz, $CDCl_3$) 1.50 (3H, d, *J* 8, Me), 2.32 (3H, s, Me-Ph), 3.55 (1H, q, *J* 8, CH), 5.45 (2H, br s, NH x 2), 7.10- 7.20 (4H, m, Ph); δ_C (25 MHz, $CDCl_3$) 18.36 (Me), 20.99 (CH_3-Ph), 46.15 (CH), 127.47, 129.51 (CH, Ph), 136.88, 138.35 (C, Ph), 177.31 (CO); m/z (EI) 163(M^+, 21 %), 149 (5), 119 ([M - COOH]$^+$, 100), 105 (16), 91 ($[MeC_6H_4]^+$, 20), 77 (10), 65 (6).
(S)-(+)-2-(4'-methylphenyl)-propanoic acid 8c **12.5h** (32 %) $[\alpha]_D^{25}$ +46.1 (*c* 1, $CHCl_3$), e.e. >95 % **24h** (41 %) $[\alpha]_D^{25}$ +57.0 (*c* 1, $CHCl_3$)[26]; e.e. >95 % (chiral shift ^1H NMR spectroscopy on the corresponding methyl ester). R_f 0.55 (ethyl acetate: petroleum [1:1]); m.p. 48-49 °C.; Found (EI) M^+: 164.0840 ($C_{10}H_{12}O_2$ requires 164.0837); υ_{max} (nujol)/cm^{-1} 2987 (OH str), 1705 (CO str), 1625, 1513 (CC str, aromatic), 782, 719 (CH def, aromatic); δ_H (100 MHz, $CDCl_3$) 1.48 (3H, d, *J* 8, Me), 2.34 (3H, s, Me-Ph), 3.71 (1H, q, *J* 8, CH), 7.10- 7.28 (4H, m, Ph), 10.75 (1H, br s, COOH); δ_C (25 MHz, $CDCl_3$) 18.18 (Me), 21.05 (Me-Ph), 45.09 (CH), 127.58, 129.45 (CH, Ph), 137.00, 137.12 (C, Ph), 180.88 (CO); m/z (EI) 164 (M^+, 24 %), 119 ([M - COOH]$^+$, 100), 91 ($[MeC_6H_4]^+$, 36), 77 (20), 65 (13), 51 (15), 45 (18), 39 (10).

Enzymic Hydrolysis of (±)-2-(4'-Methylphenyl)-propylamide 8b.
(R)-(-)-2-(4'-methylphenyl)-propylamide 8b **6h** (75 %) $[\alpha]_D^{25}$ -7.9 (*c* 1.06, $CHCl_3$), e.e. 18 % (chiral shift ^1H NMR spectroscopy), **14.5h**. (62 %) $[\alpha]_D^{25}$ -28.1 (*c* 1, $CHCl_3$), e.e. 50 %.
(S)-(+)-2-(4'-methylphenyl)-propanoic acid 8c **6h** (20 %) $[\alpha]_D^{25}$ +66.0 (*c* 0.7, $CHCl_3$), e.e. >95 % (chiral shift ^1H NMR spectroscopy on the corresponding methyl ester), **14.5h** (35 %) $[\alpha]_D^{25}$ +58.9 (*c* 1, $CHCl_3$), e.e. >95 % (chiral shift ^1H NMR spectroscopy on the corresponding methyl ester).

Chemical Hydrolysis of (R)-(-)-2-(4'-Methylphenyl)-propylamide 8b.
(*R*)-(-)-2-(4'-Methylphenyl)-propylamide (43 mg, 0.26 mmol, $[\alpha]_D^{25}$ -49.7 (*c* 1.14, $CHCl_3$); e.e. >95 %) was refluxed for 1 h. in the acid solution. This afforded (*R*)-(-)-2-(4'-methylphenyl)-propanoic acid (41 mg, 0.25 mmol, 95 %) as a white solid. No purification was required. $[\alpha]_D^{25}$ -43.3 (*c* 0.84, $CHCl_3$)[26]; e.e. >95 %; (chiral shift ^1H NMR spectroscopy on the corresponding methyl ester).

Dehydration of (R)-(-)-2-(4'-Methylphenyl)-propylamide 8b.[15]

(*R*)-(-)-2-(4'-methylphenyl)-propylamide (100 mg, 6.13 mmol, $[\alpha]_D^{25}$ -28.1 (*c* 1, $CHCl_3$), e.e. 50 %) was dissolved in toluene (5 ml) and refluxed with P_2O_5 (175 mg, 1.73 mmol) for 4 h. The resulting solution was diluted with water (20 ml) and extracted with chloroform (3 x 50 ml). The organic fractions were combined and washed with brine (30 ml), dried ($MgSO_4$) and the solvent removed by rotary evaporation to yield (*R*)-(+)-2-(4'-methylphenyl)-propionitrile (84 mg, 0.58 mmol, 95%). $[\alpha]_D^{25}$ +7.4; e.e. 50 %; the physical and spectroscopic properties were identical to those of (±)-2-(4'-methylphenyl)-propionitrile.

Enzymic Hydrolysis of (±)-2-(4'-Isobutylphenyl)-propionitrile 9a.[27]
(R)-(-)-2-(4'-isobutylphenyl)-propanoic acid 9c **30h** (12 %) $[\alpha]_D^{28}$ -12.4 (*c* 0.82, $CHCl_3$), e.e. 33 % (chiral shift ^1H NMR spectroscopy on the corresponding methyl ester) **52 h** (27 %) $[\alpha]_D^{25}$ -12.3 (*c* 1.76, $CHCl_3$), e.e. 32 % **72h** (19 %) $[\alpha]_D^{25}$ -15.2 (*c* 1.1, EtOH)/ $[\alpha]_D^{29}$ -14.7 (*c* 1.28, $CHCl_3$) (lit.,[12] $[\alpha]_D^{20}$ -50 (*c* 1, EtOH), e.e. 35 % . R_f 0.39 (ethyl acetate: petroleum [1:4]); m.p. 54-56 °C [lit. 49 °C[12]]; Found (EI) M^+: 206.1307 ($C_{13}H_{18}O_2$ requires 206.1307); υ_{max} ($CHCl_3$)/cm^{-1} 2600-3400 (OH str), 1706 (CO str), 1509 (CC str, aromatic), 1412, 1381 (CH def, aliphatic); δ_H (250 MHz, $CDCl_3$) 0.95 (6H, d, *J* 6.6, $(\underline{Me})_2$-CH), 1.53 (3H, d, *J* 7, $\underline{Me}$-CH(CO_2H), 1.88 (1H, septet, *J* 6.6, $C\underline{H}$-$(Me)_2$), 2.47 (2H, d, *J* 6.6, CH_2), 3.74 (1H, q, *J* 7 $C\underline{H}$-CO_2H), 7.14-7.26 (4H, m, Ph), 10.00 (1H, br s, COOH); δ_C (62.9 MHz, $CDCl_3$) 18.1 (Me), 22.3 ($\underline{Me}_2$-CH), 30.15 (CH), 45.10 ($\underline{C}H$-CO_2H), 45.15 (CH_2), 127.30, 129.4 ($\underline{C}H$, Ph), 137.0, 140.8 ($\underline{C}$, Ph), 180.9 (CO_2H); m/z (EI) 206 (M^+, 72 %), 161 ([M - COOH]$^+$, 100), 119 (55), 107 (40), 91 (92).

Enzymic Hydrolysis of (±)-2-(4'-Isobutylphenyl)-propylamide 9b.
(R)-(-)-2-(4'-isobutylphenyl)-propylamide 9b **6h** (55 %) $[\alpha]_D^{28}$ -6.2 (*c* 1.93, $CHCl_3$); e.e. 10 % (chiral shift ^1H NMR spectroscopy); **12h** (42 %) $[\alpha]_D^{28}$ -7.8 (*c* 1.46, $CHCl_3$), e.e. 22 %; **24 h** (7 %) $[\alpha]_D^{28}$ -9.1 (*c* 1.44, $CHCl_3$), e.e. 26 %.

(S)-(+) 2-(4'-isobutylphenyl)-propanoic acid 9c **6h** (29 %) $[\alpha]_D^{27}$ +17.8 (*c* 2.06, EtOH) (lit.,[12] $[\alpha]_D^{20}$ -50 (*c* 1, EtOH), (*R*)-(-)-2-(4'-isobutylphenyl)-propanoic acid); e.e. 32 % (chiral shift ^1H NMR spectroscopy on the corresponding methyl ester); **12h** (51 %) $[\alpha]_D^{28}$ +10.3 (*c* 1.46, $CHCl_3$), e.e. 19 % **24h** (60 %) $[\alpha]_D^{27}$ +2.5 (*c* 1.63, $CHCl_3$), e.e. 6 % **71h** (79 %) $[\alpha]_D^{27}$ 0 (*c* 2.7, $CHCl_3$), e.e. 0 %.

Chemical Hydrolysis of (R)-(-)-2-(4'-Isobutylphenyl)-propylamide 9b.
(*R*)-(-)-2-(4'-Isobutylphenyl) propylamide (83 mg, 0.404 mmol, $[\alpha]_D^{28}$ -7.8 (*c* 1.46, $CHCl_3$), e.e. 22 %), was refluxed for 15 h in the acid solution. This yielded (*R*)-(-)-2-(4'-Isobutylphenyl)-propanoic acid as a pale yellow oil (60 mg, 72 %).
R_f 0.61 (ethyl acetate: petroleum [1:2]); $[\alpha]_D^{27}$ -10.3 (*c* 1.2, $CHCl_3$) ($[\alpha]_D^{28}$ -12.4 (*c* 0.82, $CHCl_3$).

Preparation of (±)-2-Deutero-2-(4'-isobutylphenyl)-propionitrile.
Sodium methoxide (20 mg, 0.37 mmol) was added to a vigorously stirred solution of (±)-2-(4'-isobutylphenyl)-propionitrile (500 mg, 2.67 mmol) in 1,4-dioxane (5 ml) and deuterium oxide (5 ml), at room temperature. After 65 h, the mixture was extracted with chloroform (2 x 50 ml). The combined organic solutions were washed with

brine (1 x 10 ml), dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation to afford a colourless liquid (380 mg, 2.0 mmol, 76 %).
δ_H (250 MHz, $CDCl_3$) 0.95 (6H, d, *J* 7, $(CH_3)_2$-CH), 1.63 (3H, s, Me-CD), 1.89 (1H, septet, *J* 7, C<u>H</u>-$(CH_3)_2$), 2.50 (2H, d, *J* 7, C<u>H</u>$_2$-Ph), 7.20 (4H, m, Ph); δ_C (62.9 MHz, $CDCl_3$) 21.3 (Me-CD), 22.3 (Me x 2), 30.1 (<u>C</u>H-$(CH_3)_2$), 30.6 (t, CD), 45.0 (CH_2), 121.8 (CN), 126.5, 129.1 (<u>C</u>H, Ph), 134.4, 141.7 (<u>C</u>, Ph).

Enzymic Hydrolysis of (±)-2-Deutero-2-(4'-isobutylphenyl)-propionitrile.
(±)-2-Deutero-2-(4'-isobutylphenyl)-propionitrile (239 mg, 1.27 mmol) was incubated with SP 361 (2.5 g) in the phosphate buffer (250 ml, 5 mM substrate concentration) for 27 h. On work up (as previously described), the substrate (81 mg, 0.43 mmol, 34 %, $[\alpha]_D^{24}$ -1.9 [*c* 1.62, $CHCl_3$]) was recovered with no proton loss of deuterium as shown by ^{1}H NMR spectroscopy. The corresponding carboxylic acid was also isolated (50 mg, 0.24 mmol, 19 %) and was again shown by ^{1}H NMR spectroscopy to have no loss of deuterium.
$[\alpha]_D^{22}$ -16.2 (*c* 1, $CHCl_3$); δ_H (60 MHz, $CDCl_3$) 1.00 (6H, d, *J* 7, $(CH_3)_2$-CH), 1.70 (3H, s, <u>Me</u>-CD), 1.95- 2.25 (1H, m, C<u>H</u>-$(CH_3)_2$), 2.65 (2H, d, *J* 7, C<u>H</u>$_2$-Ph), 7.40- 7.55 (5H, m, Ph).

Preparation of 3-O-Substituted Glutaronitriles.

3-Hydroxyglutaronitrile 12a.[16] b.p. 123-138 °C, 0.01 mmHg (lit.,[16] 155-160 °C, 0.4 mmHg); Found (CI) $[M + NH_4]^+$: 128.0824 ($C_5H_{10}N_3O$ requires 128.0824); υ_{max} (neat)/cm^{-1} 3454 (OH str, broad), 2976, 2938 (CH str), 2258 (CN str), 1418, 1365 (CH def); δ_H (250 MHz, $(CD_3)_2CO$) 2.52- 2.60 (4H, m, CH_2 x 2), 4.10- 4.21 (1H, m, CH), 4.68 (1H, br s, OH); δ_C (62.9 MHz, $(CD_3)_2CO$) 25.0 (CH_2 x 2), 63.4 (CH), 116.8 (CN x 2); m/z (CI) 128 ($[M + NH_4]^+$, 100 %), 101 (15), 84 (2), 56 (3).

Preparation of 3-O-(Benzyl)-glutaronitrile 10a.
A suspension of sodium hydride (80 % in mineral oil) (1.30 g, 42.9 mmol) in DMF (20 ml) was slowly added to a stirred solution of 3-hydroxyglutaronitrile (4.30 g, 39 mmol) in DMF (40 ml), under an inert atmosphere of nitrogen, at 0 °C. After 5 mins, benzyl bromide (8.00 g, 5.56 ml, 47 mmol) was slowly added. The reaction was stirred for a further 30 mins at 0 °C and then at room temperature for 18 h. The resulting brown mixture was poured into a saturated aqueous ammonium chloride solution (600 ml) and extracted into ethyl acetate (4 x 200 ml). The combined organic solutions were dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation to yield a brown liquid (15.46 g). Purification by column chromatography (ethyl acetate: petroleum [1:3] as the eluant) afforded the required product as a pale brown oil (2.04 g, 10.2 mmol, 26 %).
R_f 0.55 (ethyl acetate: petroleum [1:3]); Found (EI) M^+: 200.0950 ($C_{12}H_{12}N_2O$ requires 200.0950);.υ_{max} (neat)/cm^{-1} 3036 (CH str, aromatic), 2937 (CH str, aliphatic), 2254 (CN str), 1455, 1495 (CC str, aromatic), 1418, 1351 (CH def aliphatic), 1094 (CO str, ether), 600, 743 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 2.66 (4H, d, *J* 5.8, CH_2 x 2), 3.97 (1H, qu, *J* 5.8, CH), 4.65 (2H, s, Ph-C<u>H</u>$_2$), 7.32- 7.39 (5H, m, Ph); δ_C (62.9 MHz, $CDCl_3$) 22.9 (CH_2 x 2), 70.2 (CH), 72.4 (Ph-C<u>H</u>$_2$), 116.2 (CN x 2), 128.1,128.5, 128.7 (<u>C</u>H, Ph), 136.4 (<u>C</u>, Ph); m/z (EI) 200 (M^+, 10 %), 107 (20), 91 ($[C_6H_5CH_2]^+$, 100), 79 (38), 65 (30), 54 (18).

Preparation of 3-O-(Benzoyl)-glutaronitrile 11a.

Benzoyl chloride (1.41 g, 1.16 ml, 10 mmol) was slowly added to a stirred solution of 3-hydroxyglutaronitrile (1.0 g, 9.1 mmol) in pyridine (20 ml), at room temperature under an inert atmosphere of nitrogen. After 2 h, the reaction was poured into a mixture of hydrochloric acid (150 ml, 2 M) and ice, with the product being extracted into ethyl acetate (3 x 200 ml). The combined organic solutions were washed with hydrochloric acid (1 x 50 ml, 2 M), water (1 x 50 ml), brine (1 x 50 ml) and dried ($MgSO_4$). After filtration, the solvent was removed by rotary evaporation to yield a red/ orange solid (2.05 g). Purification by column chromatography (ethyl acetate: petroleum [1:3] as the eluant) afforded the product as a pale yellow solid (1.86 g, 8.70 mmol, 96 %).

R_f 0.10 (ethyl acetate: petroleum [1:3]); m.p. 82-83 °C; Found (EI) M^+: 214.0751 ($C_{12}H_{10}N_2O_2$ requires 214.0742); υ_{max} (nujol)/cm^{-1} 2256 (CN str), 1720 (C=O str), 713 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 3.02 (4H, d, *J* 6.0, CH_2 x 2), 5.49 (1H, qu, *J* 6.0, CH), 7.45- 7.53 (2H, m, Ph), 7.60- 7.68 (1H, m, Ph), 8.05- 8.13 (2H, m, Ph); δ_C (62.9 MHz, $CDCl_3$) 22.3 (CH_2 x 2), 64.7 (CH), 114.8 (CN x 2), 128.1 (C, Ph), 128.7, 130.0, 134.2 (CH, Ph), 164.9 (CO); m/z (EI) 214 (M^+, 36 %), 122 (60), 105 ($[C_5H_5CO]^+$, 100), 77 (28).

Preparation of 3-O-(Methoxyethoxymethyl)-glutaronitrile 13a.

Sodium hydride (80 % in mineral oil) (0.30 g, 10 mmol) was added to a stirred solution of 3-hydroxyglutaronitrile (1.0 g, 9.1 mmol) in THF (40 ml), at 0 °C under an inert atmosphere of nitrogen. After 5 mins, MEM-Cl (1.37 g, 1.25 ml, 11 mmol) was added dropwise and stirring was continued for a further 45 mins. The reaction was poured into water (150 ml) and the product extracted into ether (3 x 150 ml). The combined organic solutions were dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation to yield an orange liquid (1.19 g). Purification by column chromatography (ethyl acetate: petroleum [1:1] as the eluant) followed by high vacuum drying (0.05 mmHg, room temperature), to remove the residual MEM-Cl, afforded the required product as a pale yellow oil (0.75 g, 3.81 mmol, 42 %).

R_f 0.20 (ethyl acetate: petroleum [1:1]); Found (CI) $[M + NH_4]^+$: 216.1348 ($C_9H_{18}N_3O_3$ requires 216.1348); υ_{max} (neat)/cm^{-1} 2936 (CH str), 2254 (CN str), 1456, 1419, 1363 (CH def), 1044, 1112 (C-O str); δ_H (250 MHz, $CDCl_3$) 2.74- 2.78 (4H, m, CH_2 x 2), 3.31 (3H, s, OMe), 3.52- 3.57 (2H, m, CH_2O), 3.73- 3.81 (2H, m, CH_2O), 4.13- 4.21 (1H, m, CH), 4.82 (2H, s, OCH_2O); δ_C (62.9 MHz, $CDCl_3$) 23.4 (CH_2 x 2), 58.9 (OMe), 67.8 (OCH_2), 69.2 (CH), 71.5 (OCH_2), 95.3 (OCH_2O), 116.0 (CN x 2); m/z (CI) 216 ($[M + NH_4]^+$, 100 %), 199 ($[M + H]^+$, 8), 128 (5), 106 (5), 94 (15), 89 ($[CH_3OCH_2CH_2OCH_2]^+$, 25), 58 (10), 45 (12).

Preparation of 3-O-(Acetyl)-glutaronitrile 14a.

Acetic anhydride (3.06 g, 2.83 ml, 30 mmol) was slowly added to a stirred solution of 3-hydroxyglutaronitrile (3.00 g, 27.3 mmol) in pyridine (50 ml), at 0 °C under an inert atmosphere of nitrogen. After 5 mins, the reaction was allowed to warm to room temperature and stirred for a further 3 h. The mixture was poured into hydrochloric acid (300 ml, 2 M) and extracted into ethyl acetate (3 x 300 ml). The combined organic solutions were washed with brine (1 x 100 ml), dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation to afford a red/ orange solid (3.74 g). Purification by column chromatography (ethyl acetate: petroleum [1:1] as the eluant) yielded the product as a pale yellow solid (3.55 g, 23.4 mmol, 86 %).

R_f 0.55 (ethyl acetate: petroleum [1:1]), m.p. 42-43 °C [lit.[28] 45]; Found (EI) $[M + H]^+$: 153.0664 ($C_7H_9N_2O_2$ requires 153.0664); υ_{max} (nujol)/cm^{-1} 2257 (CN str), 1751 (CO str); δ_H (250 MHz, $CDCl_3$) 2.12 (3H, s, Me),

2.81 (4H, d, *J* 6.0, CH_2 x 2), 5.20 (1H, qu, *J* 6.0, CH); δ_C (62.9 MHz, $CDCl_3$) 20.5 (Me), 22.2 (CH_2 x 2), 64.1 (CH), 115.1 (CN x 2), 169.4 (CO); m/z (EI) 195 (30), 170 (10), 153 ([M + H]$^+$, 25 %), 135 (10), 107 (10), 93 ([M - CH_3CO]$^+$, 45), 66 (11), 61 (15), 43 (100).

Preparation of 3-O-(tert-Butyldimethylsilyl)-glutaronitrile 15a.

Imidazole (1.24 g, 18.2 mmol) and *tert.* butyldimethylsilyl chloride (2.74 g, 18.2 mmol) were added to a stirred solution of 3-hydroxyglutaronitrile (1.0 g, 9.1 mmol) in DMF (25 ml), at room temperature. After 24 h, the reaction mixture was poured into a solution of saturated aqueous ammonium chloride (300 ml) and extracted into ether (4 x 100 ml). The combined organic solutions were washed with saturated aqueous ammonium chloride (1 x 50 ml), brine (1 x 50 ml) and then dried ($MgSO_4$). After filtration, the solvent was removed by rotary evaporation to yield an orange oil (2.91 g). Purification by column chromatography (ethyl acetate: petroleum [1:6] as the eluant) afforded the required product as an off white solid (1.65 g, 7.40 mmol, 81 %).

R_f 0.34 (ethyl acetate: petroleum [1:5]); m.p. 33-34 °C; Found (CI) [M + NH_4]$^+$: 242.1689 ($C_{11}H_{24}N_3OSi$ requires 242.1688); υ_{max} (nujol)/cm^{-1} 2253 (CN str); δ_H (250 MHz, $CDCl_3$) 0.15 (6H, s, $Si-Me_2$), 0.91 (9H, s, Si-C-$(Me)_3$), 2.63 (4H, d, *J* 5.0, CH_2 x 2), 4.25 (1H, qu, *J* 5.0, CH); δ_C (62.9 MHz, $CDCl_3$) - 4.8 ($Si-Me_2$), 17.8 (Si-C), 25.5 (Si-C-$(\underline{Me})_3$), 25.9 (CH_2 x 2), 64.8 (CH), 116.0 (CN x 2); m/z (CI) 242 ([M + NH_4]$^+$, 100 %), 167 (5), 132 (2), 91 (15), 74 (10).

Enzymic Hydrolysis of 3-Hydroxyglutaronitrile 12a.

3-Hydroxyglutaronitrile (3.60 g, 32.7 mmol) was dissolved in the buffer solution (330 ml), to afford a substrate concentration of 99 mM, and incubated for 65 h. The work up required the continuous extraction of the acidified aqueous solution using ether (350 ml, 48 h). Purification by column chromatography (ethyl acetate: petroleum [1:1] as the eluant) afforded (*S*)-3-hydroxy-4-cyanobutanoic acid **12b**(2.18 g, 16.9 mmol, 52 %) as a colourless oil.

R_f 0.44 (ethyl acetate + 2 % acetic acid); $[\alpha]_D^{25}$ 0 (*c* 1, EtOH); Found (EI) [M + H]$^+$: 130.0504 ($C_5H_8NO_3$ requires 130.0504); υ_{max} (neat)/cm^{-1} 3441 (OH str, alcohol), 3200- 2500 (OH str, acid), 2256 (CN str), 1715 (CO str), 1416, 1369 (CH def); δ_H (250 MHz, $(CD_3)_2CO$) 2.60- 2.76 (4H, m, CH_2 x 2), 4.29- 4.39 (1H, m, CH), 6.95 (1H br s, OH); δ_C (62.9 MHz, $(CD_3)_2CO$) 25.7 ($\underline{C}H_2$-CN), 41.2 ($\underline{C}H_2$-COOH), 64.9 (CH), 118.5 (CN), 172.7 (CO); m/z (EI) 130 ([M + H]$^+$, 2 %), 112 ([M - OH]$^+$, 15), 107 (30), 91 (60), 71 (79), 60 (30), 43 (100).

Treatment of the product with diazomethane yielded the corresponding methyl ester, (*S*)-methyl-(3-hydroxy-4-cyano)-butanoate **12c**, in quantitative yield. No purification of the colourless oil was required.

R_f 0.29 (ethyl acetate: petroleum [1:1]); $[\alpha]_D^{25}$ 0 (*c* 1.04, EtOH); Found (EI) [M + H]$^+$: 144.0660 ($C_6H_{10}NO_3$ requires 144.0660); υ_{max} (neat)/cm^{-1} 3470 (OH str), 2962 (CH str), 2254 (CN str), 1728 (CO str), 1440 (CH def); δ_H (250 MHz, $CDCl_3$) 2.56- 2.68 (4H, m, CH_2 x 2), 3.62 (3H, s, OMe), 3.80 (1H, br s, OH), 4.20- 4.31 (1H, m, CH); δ_C (62.9 MHz, $CDCl_3$) 25.1 ($\underline{C}H_2$-CN), 40.1 ($\underline{C}H_2$-COOMe), 52.0 (OMe), 63.9 (CH), 117.4 (CN), 171.6 (CO); m/z (EI) 144 ([M + H]$^+$, 64 %), 126 ([M - OH]$^+$, 17), 112 ([M + H - OH - OMe]$^+$, 58), 103 (69), 94 (25), 74 (37), 71 (88), 61 (40), 43 (100).

Enzymic Hydrolysis of 3-O-(Benzyl)-glutaronitrile 10a.

3-*O*-(Benzyl)-glutaronitrile (300 mg, 1.50 mmol) was suspended in the buffer solution (300 ml), to afford a substrate concentration of 5 mM, and incubated for 48 h. Purification by activated charcoal treatment yielded (*S*)-(+)-3-*O*-(benzyl)-4-cyanobutanoic acid **10b** (240 mg, 1.10 mmol, 73 %), as a white solid.

R_f 0.58 (ethyl acetate); m.p. 37-39 °C; $[\alpha]_D^{27}$ +9.6 (*c* 3.4, $CHCl_3$); Found (EI) M^+: 219.0895 ($C_{12}H_{13}NO_3$ requires 219.0895); υ_{max} (nujol)/cm^{-1} 3200- 2600 (OH str), 2254 (CN str), 1712 (CO str), 1495 (CC str, aromatic), 746, 698 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 2.71- 2.82 (4H, m, CH_2 x 2), 4.03- 4.18 (1H, m, CH), 4.63 (2H, s, Ph-$\underline{C}H_2$), 6.38 (1H, br s, COOH), 7.25- 7.38 (5H, m, Ph); δ_C (62.9 MHz, $CDCl_3$) 23.0 ($\underline{C}H_2$-CN), 38.7 ($\underline{C}H_2$-COOH), 71.1 (CH), 72.3 (Ph-$\underline{C}H_2$), 116.9 (CN), 127.9, 128.1, 128.5 ($\underline{C}$H, Ph), 137.0 ($\underline{C}$, Ph), 174.2 (CO); m/z (EI) 219 (M^+, 7 %), 181 (7), 107 (31), 91 ($[C_6H_5CH_2]^+$, 100), 79 (20), 65 (17).

Treatment of the product with diazomethane yielded the corresponding methyl ester, (*S*)-(+)-methyl-(3-*O*-[benzyl]-4-cyano)-butanoate **10c**, in quantitative yield. No purification of the colourless oil was required.

R_f 0.75 (ethyl acetate); $[\alpha]_D^{26}$ +9.1 (*c* 1.1, $CHCl_3$) (lit.,[18] $[\alpha]_D^{21}$ +12.0 ($CHCl_3$), (*S*)-(+)-methyl-(3-*O*-[benzyl]-4-cyano)-butanoate); e.e. 84 % (chiral HPLC: R_t (*R*) 18.9 min, R_t (*S*) 25.9 min); Found (EI) $[M + H]^+$: 234.1130 ($C_{13}H_{16}NO_3$ requires 234.1130); υ_{max} (neat)/cm^{-1} 3034 (CH str, aromatic), 2957 (CH str, aliphatic), 2252 (CN str), 1735 (CO str), 1603, 1494 (CC str, aromatic), 1437, 1352 (CH def, aliphatic), 739, 699 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 3.20- 3.45 (4H, m, CH_2 x 2), 3.69 (3H, s, OMe), 4.08- 4.18 (1H, m, CH), 4.62 (2H, s, Ph-$\underline{C}H_2$), 7.26- 7.34 (5H, m, Ph); δ_C (62.9 MHz, $CDCl_3$) 23.0 ($\underline{C}H_2$-CN), 38.7 ($\underline{C}H_2$-COOMe), 51.9 (OMe), 71.2 (CH), 72.3 (Ph-$\underline{C}H_2$), 116.9 (CN), 127.8, 128.1, 128.5 ($\underline{C}$H, Ph), 137.1 ($\underline{C}$, Ph), 170.5 (CO); m/z (EI) 234 ($[M + H]^+$, 26 %), 181 (11), 107 (62), 91 ($[C_6H_5CH_2]^+$, 100).

Enzymic Hydrolysis of 3-O-(Acetyl)-glutaronitrile 14a.

3-*O*-(Acetyl)-glutaronitrile (400 mg, 2.63 mmol) was suspended in the buffer solution (150 ml), to afford a substrate concentration of 17.5 mM, and incubated for 65 h. Purification by column chromatography (ethyl acetate: petroleum [1:1] as the eluant) afforded (±)-3-*O*-(acetyl)-4-cyanobutanoic acid **14b** (199 mg, 1.20 mmol, 45 %) as a white solid.

R_f 0.57 (ethyl acetate); m.p. 62-64 °C; $[\alpha]_D^{27}$ 0 (*c* 1.85, $CHCl_3$); Found (CI) $[M + NH_4]^+$: 189.0875 ($C_7H_{13}N_2O_4$ requires 189.0875); υ_{max} (nujol)/cm^{-1} 3400- 2500 (OH str), 2254 (CN), 1736 (CO str); δ_H (250 MHz, $CDCl_3$) 2.09 (3H, s, $\underline{Me}$-CO), 2.75- 2.92 (4H, m, CH_2 x 2), 5.26- 5.36 (1H, m, CH), 10.02 (1H, br s, COOH); δ_C (62.9 MHz, $CDCl_3$) 20.7 ($\underline{Me}$-CO), 22.5 ($\underline{C}H_2$-CN), 37.1 ($\underline{C}H_2$-COOH), 65.0 (CH), 115.7 (CN), 170.0 (Me-$\underline{C}$O), 174.4 (COOH); m/z (CI) 189 ($[M + NH_4]^+$, 100 %), 140 (4), 129 ($[M + H - CH_3CO]^+$, 3), 85 (2), 79 (2), 70 (2), 61 (1), 43 ($[CH_3CO]^+$, 1).

Treatment of the product **14c** with diazomethane yielded the corresponding methyl ester, (±)-methyl-(3-*O*-[acetyl]-4-cyano)-butanoate in quantitative yield. No purification of the colourless oil was required.

R_f 0.76 (ethyl acetate); e.e. 0 % (chiral shift ^1H NMR spectroscopy); δ_H (250 MHz, $CDCl_3$) 2.05 (3H, s, $\underline{Me}$-CO), 2.75- 2.95 (4H, m, CH_2 x 2), 3.76 (3H, s, COOMe), 5.26- 5.37 (1H, m, CH).

Enzymic Hydrolysis of 3-O-(tert-Butyldimethylsilyl)-glutaronitrile 15a.

3-*O*-(*tert.* Butyldimethylsilyl)-glutaronitrile (300 mg, 1.34 mmol) was suspended in the buffer solution (350 ml), to afford a substrate concentration of 3.8 mM, and incubated for 835.5 h. Upon work up, only substrate was isolated (210 mg, 0.94 mmol, 70 %).

Enzymic Hydrolysis of 3-O-(Benzoyl)-glutaronitrile 16a.

3-*O*-(Benzoyl)-glutaronitrile (320 mg, 1.50 mmol) was suspended in the buffer solution (300 ml), to afford a substrate concentration of 5 mM, and incubated for 48 h. Purification by column chromatography (ethyl acetate: petroleum [1:1] as the eluant) afforded (*S*)-(+)-3-*O*-(benzoyl)-4-cyanobutanoic acid **11b** (90 mg, 0.39 mmol, 25 %) as a white solid. Benzoic acid (110 mg, 0.90 mmol, 60 %) was also isolated.

R_f 0.32 (ethyl acetate: petroleum [1:1]); m.p. 93-94 °C; $[\alpha]_D^{24}$ +32.4 (*c* 1.08, $CHCl_3$); Found (EI) M^+: 233.0690 ($C_{12}H_{11}NO_4$ requires 233.0688); υ_{max} (nujol)/cm^{-1} 3400- 2800 (OH str), 2246 (CN str), 1718 (CO str), 712 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 2.85- 3.11 (4H, m, CH_2 x 2), 5.53- 5.66 (1H, m, CH), 7.39- 7.55 (2H, m, Ph-H x 2), 7.59- 7.67 (1H, m, Ph-H), 8.01- 8.10 (2H, m, Ph-H x 2), 8.91 (1H, br s, COOH); δ_C (62.9 MHz, $CDCl_3$) 22.7 (CH_2-CN), 37.2 (CH_2-COOH), 65.5 (CH), 115.7 (CN), 128.6 (CH, Ph), 128.9 (C, Ph), 129.9, 133.8 (CH, Ph), 165.4 (Ph-CO), 174.4 (COOH); m/z (EI) 233 (M^+, 2 %), 122 ($[C_6H_5COOH]^+$, 43), 105 ($[C_6H_5CO]^+$, 100), 77 ($[C_6H_5]^+$, 57), 51 (34), 41 (16).

Treatment of the product with diazomethane yielded the corresponding methyl ester, (*S*)-(+)-methyl-(3-*O*-[benzoyl]-4-cyano)-butanoate **11c**, in quantitative yield. No purification of the colourless oil was required.

R_f 0.80 (ethyl acetate: petroleum [1:1]); $[\alpha]_D^{22}$ +35.6 (*c* 0.95, $CHCl_3$) (lit.,[18] $[\alpha]_D^{22}$ +46.6 ($CHCl_3$), (*S*)-(+)-methyl-(3-*O*-[benzoyl]-4-cyano)-butanoate); e.e. 84 % (chiral shift ^1H NMR spectroscopy); Found (CI) $[M + NH_4]^+$: 265.1190 ($C_{13}H_{17}N_2O_4$ requires 265.1188); υ_{max} (neat)/cm^{-1} 3080 (CH str, aromatic), 2960 (CH str, aliphatic), 2255 (CN str), 1723 (CO str), 1602 (CC str, aromatic), 1440, 1385 (CH def, aliphatic), 713 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 2.88- 3.05 (4H, m, CH_2 x 2), 3.71 (3H, s, OMe), 5.50- 5.60 (1H, m, CH), 7.42- 7.53 (2H, m, Ph-H x 2), 7.55- 7.66 (1H, m, Ph-H), 8.00- 8.10 (2H, m, Ph-H); δ_C (62.9 MHz, $CDCl_3$) 22.7 (CH_2-CN), 37.3 (CH_2-COOMe), 52.1 (OMe), 65.8 (CH), 115.8 (CN), 128.5 (CH, Ph), 129.0 (C, Ph), 129.8 (CH, Ph), 133.6 (CH, Ph), 165.2 (Ph-CO), 169.5 (COMe); m/z (CI) 265 ($[M + NH_4]^+$, 72 %), 248 ($[M + H]^+$, 24), 202 (5), 185 (3), 140 (2), 122 (17), 105 ($[C_6H_5CO]^+$, 100), 94 (3), 78 ($[C_6H_6]^+$, 4).

Enzymic Hydrolysis of 3-O-(Methoxyethoxymethyl)-glutaronitrile 13a.

3-*O*-(Methoxyethoxymethyl)-glutaronitrile (300 mg, 1.52 mmol) was suspended in the buffer solution (300 ml), to afford a substrate concentration of 5 mM, and incubated for 115.5 h. Purification by column chromatography (ethyl acetate as the eluant) afforded (*S*)-(-)-3-*O*-(methoxyethoxymethyl)-4-cyanobutanoic acid **13b** (64 mg, 0.29 mmol, 19 %) as a colourless oil.

R_f 0.48 (ethyl acetate + 2 % acetic acid); $[\alpha]_D^{25}$ -2.8 (*c* 1.21, $CHCl_3$); Found (CI) $[M + NH_4]^+$: 235.1294 ($C_9H_{19}N_2O_5$ requires 235.1294); υ_{max} (neat)/cm^{-1} 3500- 2900 (OH str), 2936 (CH str), 2254 (CN str), 1720 (CO str), 1413, 1365 (CH def); δ_H (250 MHz, $CDCl_3$) 2.60- 2.89 (4H, m, CH_2 x 2), 3.37 (3H, s, OMe), 3.50- 3.60 (2H, m, CH_2-OMe), 3.65- 3.72 (1H, m, OCH-H), 3.78- 3.84 (1H, m, OCH-H), 4.20- 4.30 (1H, m, CH), 4.78- 4.85 (2H, m, O-CH_2-O), 9.10 (1H, br s, COOH); δ_C (62.9 MHz, $CDCl_3$) 23.7 (CH_2-CN), 38.8 (CH_2-COOH), 58.8 (OMe), 67.5 (O-CH_2), 70.1 (CH), 71.5 (O-CH_2), 95.4 (O-CH_2-O), 116.6 (CN), 174.5 (CO); m/z

(CI) 235 ([M + NH_4]$^+$, 8 %), 218 ([M + H]$^+$, 8), 159 (3), 142 (2), 106 (5), 89 ([$CH_3OCH_2CH_2OCH_2$]$^+$, 100), 73 (15), 59 ([$CH_3OCH_2CH_2$]$^+$, 44), 45 (38).

Treatment of the product with diazomethane yielded the corresponding methyl ester, (*S*)-(-)-methyl-(3-*O*-[methoxyethoxymethyl]-4-cyano)-butanoate **13c**, in quantitative yield. No purification of the colourless oil was required.

R_f 0.59 (ethyl acetate + 2 % acetic acid); $[\alpha]_D^{22}$ -6.1 (*c* 1.76, $CHCl_3$); Found (CI) [M + NH_4]$^+$: 249.1450 ($C_{10}H_{21}N_2O_5$ requires 249.1450); υ_{max} (neat)/cm^{-1} 2935 (CH str), 2253 (CN str), 1738 (CO str), 1440, 1376 (CH def); δ_H (250 MHz, $CDCl_3$) 2.55- 2.90 (4H, m, CH_2 x 2), 3.32 (3H, s, CH_2O-Me), 3.45- 3.55 (2H, m, CH_2-OMe), 3.61- 3.68 (4H, m, COOMe + OCH-H), 3.72- 3.80 (1H, m, OCH-H), 4.17- 4.27 (1H, m, CH), 4.70- 4.80 (2H, m, O-CH_2-O); δ_C (62.9 MHz, $CDCl_3$) 23.6 (CH_2-CN), 38.8 (CH_2-COOMe), 51.8 (COOMe), 58.9 (CH_2O-Me), 67.5 (OCH_2), 70.2 (CH), 71.6 (OCH_2), 95.4 (O-CH_2-O), 116.9 (CN), 170.4 (CO); m/z (CI) 249 ([M + NH_4]$^+$, 100 %), 232 ([M + H]$^+$, 26), 156 ([M - $CH_3OCH_2CH_2O$]$^+$, 99), 126 ([M - $CH_3OCH_2CH_2OCH_2O$]$^+$, 5), 106 (17), 94 (22), 89 ([$CH_3OCH_2CH_2OCH_2$]$^+$, 8), 82 (8), 73 (9), 58 (22), 44 (16).

Preparation of (S)-(+)-Methyl-(3-O-[benzoyl]-4-cyano)-butanoate 11c.

Benzoyl chloride (0.089 ml, 0.77 mmol) was slowly added to a stirred solution of (*S*)-methyl-(3-hydroxy-4-cyano)-butanoate **12c** 100 mg, 0.70 mmol) in pyridine (5 ml), at room temperature. After 16 h. the reaction was poured into a mixture of hydrochloric acid (50 ml, 2 M) and ice, the product being extracted into ethyl acetate (3 x 50 ml). The combined organic solutions were washed with hydrochloric acid (1 x 20 ml), water (1 x 20 ml), brine (1 x 20 ml) and dried ($MgSO_4$). Removal of the drying agent by filtration, followed by removal of the solvent by rotary evaporation afforded an orange oil (250 mg). Purification by column chromatography (ethyl acetate: petroleum [1:3] as the eluant) yielded the required product as a colourless oil (148 mg, 0.60 mmol, 86 %).

R_f 0.54 (ethyl acetate: petroleum [1:1]); $[\alpha]_D^{26}$ +9.0 (*c* 1, $CHCl_3$) (lit.,[18] $[\alpha]_D^{22}$ +46.6 ($CHCl_3$), (*S*)-(+)-methyl-(3-*O*-[benzoyl]-4-cyano)-butanoate); e.e. 22 % (chiral shift ^{1}H NMR spectroscopy).

Preparation of (S)-(-)-Methyl-(3-O-[benzyl]-4-bromo)-butanoate 17.[17]

Benzyl-2,2,2-trichloroacetimidate (1.41 g, 1.04 ml, 5.58 mmol) was slowly added to a stirred solution of (*S*)-(-)-methyl-(3-hydroxy-4-bromo)-butanoate **16** (1.00 g, 5.08 mmol) in dichloromethane (15 ml) and cyclohexane (15 ml), at room temperature under an inert atmosphere of argon. Trifluoromethane sulfonic acid (0.05 ml, 0.57 mmol) was added and the reaction was left to stir for 6 h. The resulting precipitate was removed by filtration with the filtrate being washed with saturated sodium bicarbonate solution (2 x 20 ml) and water (1 x 20 ml). The organic layer was dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation to afford an off white solid (1.98 g). Purification by column chromatography (ethyl acetate: petroleum [1:8] as the eluant) yielded the required product as a colourless oil (780 mg, 2.72 mmol, 54 %).

R_f 0.32 (ethyl acetate: petroleum [1:8]); $[\alpha]_D^{23}$ -12.6 (*c* 1, $CHCl_3$); Found (CI) [M + NH_4]$^+$: 304.0568 ($C_{12}H_{19}{}^{79}BrNO_3$ requires 304.0548); υ_{max} (neat)/cm^{-1} 3032 (CH str, aromatic), 2953 (CH str, aliphatic), 1733 (CO str), 1506 (CC str, aliphatic), 1440 (CH def, aliphatic), 743 (CH def, aromatic); δ_H (250 MHz, $CDCl_3$) 2.60-2.81 (2H, m, CH_2-COOMe), 3.47- 3.55 (2H, m, CH_2-Br), 3.68 (3H, s, OMe), 4.06- 4.17 (1H, m, CH), 4.64 (2H,

q, *J* 11, Ph-C$\underline{H}_2$), 7.26- 7.38 (5H, m, Ph); δ_C (62.9 MHz, $CDCl_3$) 34.0 (CH_2-Br), 38.5 ($\underline{C}H_2$-COOMe), 51.8 (OMe), 72.3 (Ph-$\underline{C}H_2$), 75.0 (CH), 127.8, 128.0, 128.2 ($\underline{C}$H, Ph), 137.8 ($\underline{C}$, Ph), 171.3 (CO); m/z (CI) 306 ([M + NH_4]$^+$, 67 %), 304 ([M + NH_4]$^+$, 68), 287 ([M + H]$^+$, 33), 289 ([M + H]$^+$, 32), 108 (100), 91 ([$C_6H_5CH_2$]$^+$, 44).

Preparation of (R)-(-)-Methyl-(3-O-[benzyl]-4-cyano)-butanoate 10c.

Sodium cyanide (150 mg, 3.06 mmol) was added to a stirred solution of (*S*)-(-)-methyl-(3-*O*-[benzyl]-4-bromo)-butanoate **17c** (780 mg, 2.72 mmol) in DMSO (20 ml), at room temperature under an inert atmosphere of argon. After 4 h, water (75 ml) was added and the mixture was extracted with ether (3 x 50 ml). The combined organic solutions were washed with water (1 x 30 ml), hydrochloric acid (1 x 30 ml, 1 M) and brine (1 x 30 ml). After drying ($MgSO_4$) and filtration, the solvent was removed by rotary evaporation to yield a colourless oil (536 mg). Purification by column chromatography (ethyl acetate: petroleum [1:3] as the eluant) yielded the required product as a colourless oil (328 mg, 1.41 mmol, 52 %).

R_f 0.20 (ethyl acetate: petroleum [1:3]); $[\alpha]_D^{25}$ -11.3 (*c* 1.1, $CHCl_3$) (lit.,[18] $[\alpha]_D^{21}$ +12.0 ($CHCl_3$), (*S*)-(+)-methyl-(3-*O*-[benzyl]-4-cyano)-butanoate).

Deprotection of (S)-(-)-Methyl-(3-O-[methoxyethoxymethyl]-4-cyano)-butanoate 13c.[29]

Dimethyl boron bromide (0.49 ml, 1.34 mmol) was slowly added to a solution of (*S*)-(-)-methyl-(3-*O*-[methoxyethoxymethyl]-4-cyano)-butanoate B (154 mg, 0.67 mmol) in dichloromethane (8 ml), at -50 °C under an inert atmosphere of argon. The mixture was stirred for 30 min and then allowed to warm to room temperature over a 30 min period. THF (10 ml) and saturated sodium bicarbonate solution were added with the mixture being diluted with ether (30 ml). The organic solution was washed with water (1 x 20 ml) and brine (1 x 20 ml), dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation to yield an orange-red liquid (70 mg). Purification by column chromatography (ethyl acetate as the eluant) afforded (*S*)-methyl-(3-hydroxy-4-cyano)-butanoate (44 mg, 0.31 mmol, 46 %).

Preparation of (S)-(+)-Methyl-(3-O-[benzoyl]-4-cyano)-butanoate 11c.

Prepared by the same method as above using (*S*)-methyl-(3-hydroxy-4-cyano)-butanoate (44 mg, 0.31 mmol), pyridine (3 ml) and benzoyl chloride (48 mg, 0.04 ml, 0.34 mmol). Purification afforded the required product (70 mg , 0.28 mmol, 92 %).

R_f 0.57 (ethyl acetate: petroleum [1:1]); $[\alpha]_D^{25}$ +24.3 (*c* 1.4, $CHCl_3$) (lit.,[ref] $[\alpha]_D^{22}$ +46.6 ($CHCl_3$), (*S*)-(+)-methyl-(3-*O*-[benzoyl]-4-cyano)-butanoate); e.e. 61 % (chiral shift ^{1}H NMR spectroscopy).

REFERENCES

1. 'The Chemistry of the Cyano Group', Wiley Interscience, New York, 1970, pp. 77-86
2. Vogel's 'Textbook of Practical Organic Chemistry', 4th Ed., Longman, pp. 478-479..
3. M.A. Cohen, J. Sawden, and N.J. Turner, *Tetrahedron Lett.*, 1990, **31**, 7223.

4. D.B. Harper, *Biochem. J.*, 1977, **165**, 309; L.A. Thompson, C.J. Knowles, E.A. Linton, and J.M. Wyatt, *Chem. Br.*, 1988, 900; M. Kobayashi, T. Nagasawa, and H. Yamada, *Appl. Microbiol. Technol.*, 1988, **29**, 231.
5. E.A. Linton and C.J. Knowles, *J. Gen. Microbiol.*, 1986, **132**, 1493.
6. C. Bengis-Garber and A.L. Gutman, *Tetrahedron Lett.*, 1988, **29**, 2589; P.A. Vaughan, C.J. Knowles, and P.S.J. Cheetham, *Microbiol. Technol.*, 1989, **11**, 815; D. Bianchi, A. Bosetti, P. Cesti, G. Franzosi, and S. Spezia, *Biotechnol. Lett.*, 1991, **13**, 241.
7. P. Honicke-Schmidt and M.P. Schneider, *J. Chem. Soc., Chem. Commun.*, 1990, 648; N. Klempier, A. de Raadt, 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.
8. Preliminary accounts of this work have been published; M.A. Cohen, J.S. Parratt, and N.J. Turner, *Tetrahedron Asymmetry*, 1992, **3**, 1543; J. Crosby, J.S. Parratt, and N.J. Turner, *Tetrahedron Asymmetry*, 1992, **3**, 1547.
9. Purchased from Aldrich Chemical Co.
10. W. Carruthers and R.C. Moses, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1625.
11. P.M. Greaves, S.R. Landor, and D.R.J. Laws, *J. Chem. Soc. (C)*, 1968, 291.
12. K. Yamamoto, K. Otsubo, and K. Oishi, European Patent Application, No. 0 348 901(A2), 1989.
13. Dictionary of Organic Compounds (5th edition), Chapman and Hall.
14. C.R. Noller, *Org. Syn.* Coll. Vol. II, 586.
15. H. Kakeya, N. Sakai, T. Sugai, and H. Ohta, *Tetrahedron Lett.*, 1991, **32**, 1343.
16. F. Johnson, J.P. Panella, and A.A. Carlson, *J. Org. Chem.*, 1962, **27**, 2241.
17. Purchased from Berk Chemicals Ltd., P.O. Box 56, Priestley Road, Basingstoke, Hampshire RG24 9QB.
18. H. Kakeya, N. Sakai, A. Sano, M. Yokoyama, T. Sugai, and H. Ohta, *Chem. Lett.*, 1991, 1823.
19. G. Stork, W.S. Worrall, *J. Am. Chem. Soc.*, 1962, **82**, 4315.
20. H.T. Openshaw and R. Robinson, *J. Chem. Soc.*, 1937, 941.
21. P.M. Greaves, P.D. Landor, S.R. Landor, and O. Odyck, *Tetrahedron*, 1974, **30**, 1427.
22. O.H. Oldenziel. D. van Leusen, and A.M. van Leusen, *J. Org. Chem.*, 1977, **42**, 3114.
23. W.A. Bonner, *J. Am. Chem. Soc.*, 1952, **74**, 1034.
24. K. Pettersson, *Arkiv. for Kemi*, 1956, **10**, 283.
25. H. Rupe and F. Wiederkehr, *Helv. Chim. Acta*, 1924, **7**, 654.
26. G. Guanti, E. Narisano, T. Podgorski, S. Thea, and A. Williams, *Tetrahedron*, 1990, **46**, 7081.
27. Supplied by Cheminor, India.
28. F. Johnson, J.P. Panella, A.A. Carlson, and D.H. Hunneman, *J. Org. Chem.*,1962, **27**, 2473.
29. Y. Guindon, C. Yoakim, and H.E. Morton, *J. Org. Chem.*, 1984, **49**, 3912.