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Microbial Hydrolysis of Glutaronitrile Derivatives with *Brevibacterium* sp. R 312

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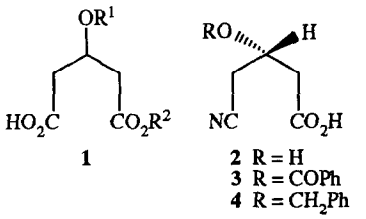
Abstract—The enantiomerically pure (*S*)-cyano acids **3** and **4** can be obtained by biotransformation with *Brevibacterium* sp. R 312 of the corresponding prochiral dinitriles **5** and **6**, respectively. The hydrolysis is probably a two step process involving a nitrile hydratase and an amidase. In connection with these investigations a facile method for the synthesis of racemic 4-cyano-3-hydroxybutanoic acid derivatives was developed.

Introduction

Nitriles can be transformed biocatalytically to the corresponding carboxylic acids or amides under mild conditions. Using racemic substrates these hydrolyses have been shown to be enantioselective.[1] 3-Hydroxyglutaric acid derivatives (**1**) are suitable starting materials for the synthesis of HMG-CoA reductase inhibitors[2] and other natural products.[3] Moreover, 4-cyano-3-hydroxybutanoic acid derivatives represent C_5 building blocks with three different types of functional groups and have considerable synthetic potential. For example, (*R*)-4-cyano-3-hydroxybutanoic acid (*ent*-**2**) has been prepared from isoascorbic acid and used as a starting material for the synthesis of the δ-lactone unit of a HMG-CoA reductase inhibitor.[4]



1

2 R = H
3 R = COPh
4 R = CH_2Ph

5 R = COPh
6 R = CH_2Ph

7 R = COPh
8 R = CH_2Ph

Results and Discussion

Biotransformation of the prochiral dinitriles

The biotransformation of the prochiral 3-hydroxyglutaronitrile derivatives **5** and **6** with *Brevibacterium* sp. R 312 gave the corresponding (*S*)-mono acids **3** and **4**, respectively, in a yield of 70 % and with an enantiomeric excess of >99 %.[5] Benzoic acid was isolated as a minor product in the transformation of **5** into **3**. No evidence for the formation of the corresponding amides was obtained. There are two possibilities for the formation of an acid from a nitrile by a nitrile-hydrolysing enzyme system.[1,6] The first possibility is a two-step process involving initial hydrolysis of **5** and **6** catalysed by a nitrile hydratase to give the cyano-amides **7** and **8**, respectively, as intermediates which are hydrolysed subsequently by an amidase in a second step to produce the acids **3** and **4**. The second possibility is that a nitrilase converts the nitrile to the corresponding acid in one step. Extensive prior studies with both the wild-type *Brevibacterium* sp. R 312[7–10] and derived mutants blocked in either the relevant amidase (strain A4)[11] or the nitrile hydratase (strain 19)[12] have conclusively demonstrated that this bacterium exclusively metabolizes nitriles to equivalent carboxylic acids by the two-step process.

It was of interest to establish the relative contributions of the nitrile hydratase and the amidase in *Brevibacterium* sp. R 312 to the overall conversion of the prochiral dinitriles **5** and **6** into the chiral monoacids **3** and **4** respectively. Previous studies with this bacterium have examined the biotransformation of either racemic or achiral nitriles exclusively.[13] Obviously, in a two-step process the cyano-amides **7** and **8** (in optically active or racemic form) should be intermediates. Therefore the racemic cyano-amides **7** and **8** as well as the cyano-esters **14** and **17** and the cyano-amide **23** have been synthesized and subjected to the biotransformation with *Brevibacterium* sp. R 312.

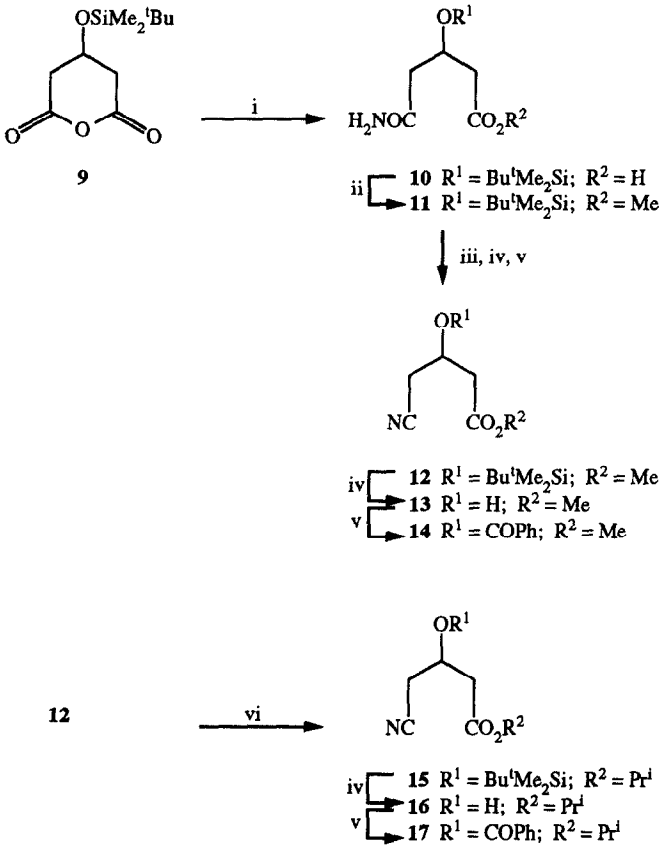
Synthesis of racemic 4-cyano-3-hydroxybutanoic acid derivatives

The anhydride **9**[2a] was chosen as a suitable starting material for a route which allows the facile preparation of compounds **7**, **8**, **14**, **17** and **23**. Following this scheme

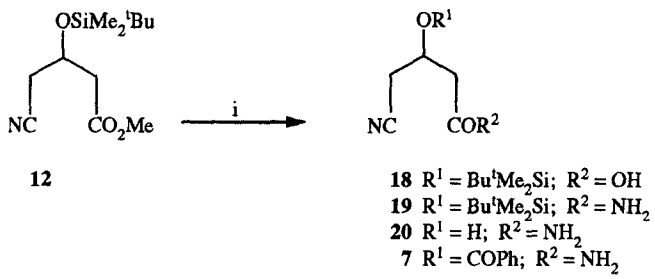
it is possible to introduce different types of protecting groups for the hydroxy function and to prepare carboxylic esters and amides of the 4-cyano-3-hydroxybutanoic acid.

The synthesis of the carboxylic esters **14** and **17** is summarized in Scheme I. The anhydride **9** was opened with ammonium acetate[14] in acetone–water to give the amido acid **10** which was converted into the corresponding methyl ester **11** by reaction with diazomethane. Dehydration of the amide **11** under neutral conditions employing triphenyl phosphine–tetrachloromethane–triethylamine in refluxing dichloromethane[15] gave the cyano ester **12** as a key intermediate in high yield. Subsequent deprotection of **12** with tetrabutylammonium fluoride in tetrahydrofuran and benzoylation of the product **13** yielded the cyano ester **14** as one of the target compounds. Transesterification of the methyl ester **12** with titanium(IV) isopropoxide in dichloromethane in a modified procedure to that described in the literature[16] gave the corresponding isopropyl ester **15**. This ester was subjected to a deprotection–benzoylation procedure to give the cyano isopropyl ester **17**.

For the preparation of the cyano-amide **7** the carboxylic ester function of the nitrile **12** was hydrolysed with one equivalent of sodium hydroxide to obtain the cyano-acid **18** in high yield. The latter compound was subsequently transformed (via chlorination with oxalyl chloride in the presence of a catalytic amount of pyridine) to the corresponding carboxylic acid chloride; reaction of this acid chloride with ammonia furnished the cyano-amide **19**. After deprotection of **19**, the corresponding alcohol **20** was benzoylated to give the target compound **7** in 42 % yield. The low yield of the last step was due to the reactivity of the primary amide function towards benzoyl chloride[17] to give *N*-benzoylated side products (Scheme II).

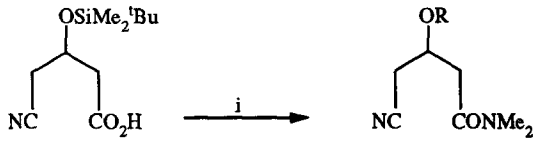


Scheme I. i) NH_4OAc, H_2O-Me_2CO, r.t.; ii) CH_2N_2, Et_2O-MeOH, r.t.; iii) Ph_3P-CCl_4-NEt_3, CH_2Cl_2, reflux; iv) Bu_4NF, THF, r.t.; v) PhCOCl, pyridine, 0 °C ⇒ r.t.; vi) $Ti(OPr^i)_4$, CH_2Cl_2, r.t.



Scheme II. i) NaOH, MeOH, H_2O, r.t.; ii) $(COCl)_2$, cat. pyridine, CH_2Cl_2, r.t. ⇒ 30-40 °C then NH_3, MeOH, 0 °C ⇒ r.t.; iii) Bu_4NF, THF, r.t.; iv) PhCOCl, pyridine, 0 °C ⇒ r.t.

The preparation of the cyano-amide **23** from the cyano-acid **18** followed the above-mentioned sequence except that aqueous dimethylamine was used for the amidation step instead of ammonia (Scheme III).

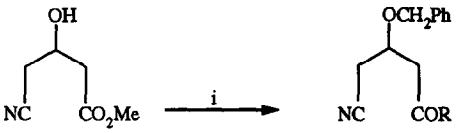


18

21 R = Bu^tMe_2Si
22 R = H
23 R = COPh

Scheme III. i) $(COCl)_2$, cat. pyridine, CH_2Cl_2, r.t. ⇒ 30-40 °C then $NHMe_2$, H_2O, 0 °C ⇒ r.t.; ii) Bu_4NF, THF, r.t.; iii) PhCOCl, pyridine, 0 °C ⇒ r.t.

The crucial step in the synthesis of the benzyl protected cyano amide **8** was the acid-catalysed benzylation[18] of the β-hydroxy-cyano ester **13** with benzyl 2,2,2-trichloroacetimidate in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate which gave the benzylated compound **24** in moderate yield. Base-catalysed hydrolysis of the cyano-ester **24** followed by a chlorination–amidation sequence yielded the target compound **8** (Scheme IV).



13

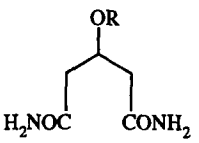
24 R = OMe
25 R = OH
8 R = NH_2

Scheme IV. i) Benzyl 2,2,2-trichloroacetimidate, cat. $CF_3SO_3SiMe_3$, CH_2Cl_2, 0 °C ⇒ r.t.; ii) NaOH, MeOH, H_2O, r.t.; iii) $(COCl)_2$, cat. pyridine, CH_2Cl_2, r.t. ⇒ 30-40 °C then NH_3, H_2O, 0 °C ⇒ r.t.

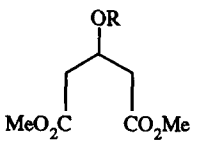
Biotransformation of the racemic 4-cyano-3-hydroxybutanoic acid derivatives

The racemic cyano amide **7** was subjected to transformation by *Brevibacterium* sp. R 312. After 6 h the cyano-acid (*S*)-**3** was isolated in 36 % yield (enantiomeric excess 83 %) from the organic extracts together with benzoic acid (12 %) and a trace of starting material. Evaporation of the aqueous phase and subsequent treatment with diazomethane yielded the diamide **26** (7 %) but no dimethyl glutarates **28** or **29**. Trace amounts of the diamide **26** could also be isolated from a biotransformation involving the prochiral dinitrile **5**.

When the racemic cyano-amide **8** was biotransformed under the same conditions for 6 h, the cyano-acid (*S*)-**4** was produced in 37 % yield (*e.e.* 53 %) as well as starting material (8 %). From the aqueous phase the diamide **27** was isolated in 7 % yield; no dimethyl glutarate **30** was found.



26 R = COPh
27 R = CH_2Ph



28 R = H
29 R = COPh
30 R = CH_2Ph

The racemic tertiary cyano-amide **23** was not a substrate for *Brevibacterium* sp. R 312, while the corresponding cyano esters **14** and **17** were decomposed by the whole-cell system to a range of unidentified materials.

Although the results of hydrolysis of the prochiral dinitriles **5** and **6** do not allow us to unambiguously distinguish between the two-step/one step pathways the isolation of (*S*)-cyano-acids from the corresponding racemic cyano amides is fully consistent with a two-step process and leads us to the following conclusions. As first suggested by Arnaud *et al.*[7-10,13] on the basis of the biotransformation of a wide range of achiral and racemic nitriles, the hydrolysis of the dinitriles **5** and **6** probably takes place, at least in part, by a two-step process involving a nitrile hydratase and an amidase.[19] If so, both steps must be enantiocomplementary to give the cyano acids (*S*)-**3** and (*S*)-**4** respectively. This would result from either both enzymes being (*S*)-selective or one of the two enzymes being (*S*)-selective but the other non-selective. In the postulated two-step sequence the overall enantioselectivity of the hydrolysis of the prochiral dinitriles **5** and **6** must be largely determined by the first step of the pathway (the conversion of the dinitrile to the corresponding cyano-amide catalysed by a nitrile hydratase) which must be rate-determining and more enantioselective than the second faster step (catalysed by an amidase). This conclusion derives from the observation that conversion of the prochiral dinitriles **5** and **6** to the corresponding nitrile-carboxylic acids occurs in high yield and enantiomeric excess. This result demands that the first hydrolysis be highly stereoselective since a poorly stereoselective nitrile hydratase catalysed hydrolysis would severely limit the final yield of the highly optically active nitrile-carboxylic acid products.

This paper describes novel biotransformations of prochiral dinitriles by *Brevibacterium* sp. R 312; prior studies with racemic nitriles have suggested that the enzymes present in this bacterium typically undertake an initial non-selective hydrolysis to yield racemic amides followed by a selective hydrolysis to yield (*S*)-acid and resolved (*R*)-amide.[13]

The identification of the diamides **26** and **27** could be considered as further evidence for a two-step process.[20] It is noteworthy that the mass balance of both reactions involving the cyano amides is poor: it can be purported that the diamides **26** and **27** are intermediates which can be further metabolized, possibly to the corresponding glutaric acids which are broken down to give volatile products.

Experimental

FT-IR spectra were recorded on a Nicolet Magna-IR spectrometer 550. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer operating at 300 and 75 MHz, respectively. Chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hz. Mass spectra were recorded on a Kratos Profile HV 3 spectrometer. Melting points were determined on a Gallenkamp apparatus. Elemental analyses were performed by Butterworth Laboratories Ltd, Teddington, Middlesex, U.K. Flash chromatography was performed on silica gel 60 (230–400 mesh). Tetrahydrofuran was distilled from sodium wire and benzophenone. Dichloromethane was distilled from CaH_2. Tetrachloromethane was distilled from P_2O_5. Anhydrous pyridine was purchased from Aldrich.

Cultivation of Brevibacterium *sp. R 312*

Brevibacterium sp. R 312 (CBS 717-73) was cultivated on a medium composed of glucose (10 g), KH_2PO_4 (6.8 g), Na_2HPO_4 (7.1 g), NH_4Cl (5.0 g), $MgSO_4$ x 7 H_2O (0.5 g), $FeSO_4$ x 7 H_2O (0.01 g) and thiamine-hydrochloride (0.002 g) per litre of distilled water adjusted to pH 7. $FeSO_4$ and thiamine-hydrochloride were made up as stock solutions and added via sterilised millipore filters (0.22 μm) to the previously autoclaved medium. An inoculum of *Brevibacterium* sp. R 312 was grown in 100 mL of medium at 28 °C for 2 days in an orbital shaker (200 rpm). This suspension was aseptically transferred to 1 L of medium and grown for a further 2 days until an optical density A_{600} of 1.0 was attained. The organism was harvested by centrifugation (10,000 *g* for 20 min at 5 °C), washed twice with 0.1 M KH_2PO_4–Na_2HPO_4 buffer (pH 7) and finally resuspended in fresh buffer at a concentration of 0.18 g wet weight cells per mL of buffer.

Microbial hydrolysis of the nitriles—general procedure

A suspension (30 mL) of the above-described culture of *Brevibacterium* sp. R 312 in a 250 cm^3 conical flask was treated with the nitrile (150 mg) and then incubated at 30 °C with reciprocal shaking (200 rpm). After 6 h the cells were removed by centrifugation and the resultant supernatant solution was adjusted to pH 10 with 2.5 M NaOH. The aqueous solution was extracted with EtOAc (3 x 20 ml). The organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give unchanged starting material. The basic aqueous solution was acidified to pH 5 with glacial acetic acid and extracted with EtOAc (3 x 20 mL). The organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography over silica gel (10 g) with hexane–ethyl acetate–acetic acid (10 : 10 : 0.2) as eluant to give benzoic acid and the acids (*S*)-**3** and (*S*)-**4**, respectively.

The acidic aqueous phase was evaporated. The residue was extracted with boiling methanol (3 x 10 cm^3). The MeOH extracts were combined and treated with an excess of diazomethane. The excess of diazomethane was destroyed using glacial acetic acid. The solvents were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (10 g) with EtOAc–EtOH (7 : 3) to give the diamides **26** and **27**, respectively.

Microbial hydrolysis of 5

From the acidic aqueous extract (*S*)-**3** (115 mg, 70 %) and benzoic acid (13 mg, 15 %) were isolated. The acid was converted to its corresponding methyl ester by reaction with diazomethane in the usual manner; mp 33–34 °C (from diethyl ether–hexane); $[\alpha]_D^{25}$ + 46.0° (c 1.0, $CHCl_3$) (lit.[5a] + 46.6°); thus the product has the (*S*)-configuration; *e.e.* >99 % was determined by ^1H NMR in the presence of Eu(hfc)$_3$. All other analytical data are in agreement with those of the corresponding racemic compound **14**.

Microbial hydrolysis of 6

From the acidic aqueous extract (*S*)-**4** (115 mg, 70 %) was isolated as a colourless oil. All analytical data are in agreement with those for the racemic compound **25**.

The acid was converted to their corresponding methyl ester by reaction with diazomethane in the usual manner; $[\alpha]_D^{25}$ + 12.1° (c 1.0, $CHCl_3$) lit.[5b] $[\alpha]_D^{25}$ –11.3° (c 1.1, $CHCl_3$) for the (*R*)-enantiomer; thus the product has the (*S*)-configuration; *e.e.* >99 %). The *e.e.* of the methyl ester was determined by HPLC on the chiral phase Chiracel OD with hexane-2-propanol as the mobile phase (flow rate 1 mL/min).

Microbial hydrolysis of 7

From the acidic aqueous extract (*S*)-**3** (57 mg, 36 %) and benzoic acid (9 mg, 12 %) were isolated. The acid was converted to their corresponding methyl ester by reaction with diazomethane in the usual manner; $[\alpha]_D^{25}$ + 36.0° (c 1.0, $CHCl_3$); *e.e.* 83 %. From the acidic aqueous phase the diamide **26** (12 mg, 7 %) was isolated; mp ›150 °C (decomp., from EtOH–EtOAc); (found C, 57.0; H, 5.5; N, 11.0. $C_{12}H_{14}N_2O_4$ requires C, 57.6; H, 5.6; N, 11.2 %); δ_H (300 MHz, [^2H]$_6$-DMSO) 2.48–2.64 (4 H, m, CH_2), 5.58 (1H, m, *J* 6.5, CH), 6.84 (2 H, s, NH_2), 7.44 (2 H, s, NH_2) and 7.50–8.00 (5 H, m, Ph); m/z 251 (M^+ + 1, 3 %), 250 (M^+, 1) 192 (8), 145 (11), 129 (29), 128 (29), 122 (20), 112 (24), 111 (14), 105 (100), 85 (30) and 77 (90); (found M^+, 250.09846. $C_{12}H_{14}N_2O_4$ requires M, 250.09536).

Microbial hydrolysis of 8

From the acidic aqueous extract (*S*)-**4** (55 mg, 37 %) was isolated. The acid was converted to their corresponding methyl ester by reaction with diazomethane in the usual manner; $[\alpha]_D^{20}$ + 5.2° (c 2.0, $CHCl_3$); *e.e.* 53 %. From the acidic aqueous phase the diamide **27** (11 mg, 7 %) was isolated as an amorphous solid; δ_H (300 MHz, [^2H]$_6$-DMSO) 2.28 (2 H, dd, *J* 14, 5, CH_2), 2.40 (2 H, dd, *J* 14, 7.5, CH_2), 4.21 (1 H, m, CH), 4.50 (2 H, s, CH_2O), 6.78 (2 H, s, NH_2) and 7.28 (7 H, super-imposed s and m, NH_2 and Ph).

(RS)-3-tert-Butyldimethylsilyloxyglutaric acid monoamide 10

To a stirred solution of the anhydride **9** (5.84 g, 24 mmol) in He_2CO (60 ml) was added NH_4OAc (3.70 g, 48 mmol) and water (2 cm^3). After stirring for 3 h glacial acetic acid (8 cm^3) was added. The solution was concentrated under reduced pressure to a volume of *ca* 10 cm^3 and diluted with EtOAc (50 cm^3). This solution was washed with water (2 x 50 cm^3) and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure and co-distillation with toluene (2 x 50 cm^3) gave the monoamide **10** as colourless crystals (6.14 g, 98 %); mp 119–120 °C (from diethyl ether–hexane) (found: C, 49.0; H, 8.6; N, 5.0. $C_{11}H_{23}NO_4Si$ requires 0.5 H_2O C, 48.9; H, 8.7; N, 5.2 %); ν_{max} (KBr)/cm^{-1} 3419 (NH), 1699 (CO), 1662 (CO) and 1605 (CO); δ_H (300 MHz; $CDCl_3$) 0.12 (6 H, s, $SiMe_2$), 0.89 (9 H, s, Bu^t), 2.50 (1 H, dd, *J* 15, 5, CH_2), 2.60 (1 H, dd, *J* 15, 5, CH_2), 2.62 (2 H, d, *J* 6.5, CH_2), 4.52 (1 H, m, *J* 6.5, CH) and 6.23 (2 H, br s, NH_2); δ_C (75 MHz; $CDCl_3$), 174.10 (CO), 174.01 (CO), 66.39 (CH), 43.14 (CH_2), 41.55 (CH_2), 25.70 (CH_3), 17.89 (C), –4.95 ($SiMe_2$) and –4.97 ($SiMe_2$); m/z 262 (M^+ + 1, 6 %), 244 (2), 204 (21), 186 (21), 145 (14) and 112 (100).

Methyl (RS)-3-t-butyldimethylsilyloxyglutarate monoamide 11

A solution of the acid **10** (5.9 g, 22.6 mmol) in diethyl ether (50 cm^3) and MeOH (10 cm^3) was treated at room temperature with diazomethane. Excess diazomethane was destroyed by adding glacial acetic acid. The solvents were removed under reduced pressure to give the methyl ester **11** as colourless crystals (6.14 g, 100 %); mp 58–60 °C (found: C, 52.5; H, 9.5; N, 5.1. $C_{12}H_{25}NO_4Si$ requires C, 52.3; H, 9.15; N, 5.1 %); ν_{max} (KBr)/cm^{-1} 3406 (NH), 3149 (NH), 1732 (CO) and 1684 (CO); δ_H (300 MHz; $CDCl_3$) 0.49 and 0.51, respectively (3 H, 2 s, $SiMe_2$), 0.67 and 0.69 (3 H, 2 s, $SiMe_2$), 0.832 and 0.835 (9 H, 2 s, Bu^t), 2.39 (1 H, dd, *J* 15, 5.5, CH_2), 2.51 (1 H, ddd, *J* 15, 5, 1.5, CH_2), 2.54 (2 H, dd, *J* 6, 1.5, CH_2), 3.63 and 3.65 (3 H, 2 s, OCH_3), 4.49 (1 H, quintet, *J* 6, CH), 6.09 (1 H, br s, NH_2) and 6.21 (1 H, 2 br s, NH_2); δ_C (75 MHz, $CDCl_3$) 172.98 (CO), 172.34 (CO), 66.53 (CH), 51.56 (OMe), 43.50 (CH_2), 41.58 (CH_2), 25.64 (CH_3), 17.82 (C), –4.90 ($SiMe_2$) and –5.10 ($SiMe_2$); m/z 276 (M^+ + 1, 1 %), 260 (2), 218 (50), 112 (70) and 83 (100).

Methyl (RS)-3-tert-butyldimethylsilyloxy-4-cyanobutanoate 12

To a solution of the amide **11** (5.28 g, 19.2 mmol) in dichloromethane (100 cm^3) was added triphenylphosphine (7.08 g, 27 mmol), tetrachloromethane (3.54 g, 23 mmol), and triethylamine (2.32 g, 23 mmol). The solution was refluxed for 3.5 h. The solvent was removed under reduced pressure. The residue was treated with EtOAc–hexane (100 cm^3, 1 : 2) and the resulting crystals were filtered off. The crystals were washed with EtOAc–hexane (2 x 50 cm^3, 1 : 2). The combined filtrates were evaporated and the filtration procedure was repeated. The filtrate was evaporated to dryness. Flash chromatography over silica gel (200 g) using hexane–EtOAc (5 : 1) as eluant gave the nitrile **12** as a colourless oil (4.16 g, 84 %), bp 130–140 °C/0.3 mmHg (bath temp., Kugelrohr); mp 31–32 °C (found: C, 55.95; H, 9.3; N, 5.5. $C_{12}H_{23}NO_3Si$ requires C, 56.0; H, 9.0; N, 5.4 %). ν_{max} (KBr)/cm^{-1} 2244 (CN) and 1745 (CO); δ_H (300 MHz; $CDCl_3$) 0.08 (3 H, s, $SiMe_2$), 0.13 (3 H, s, $SiMe_2$), 0.88 (9 H, s, Bu^t), 2.61 (4 H, m, CH_2), 3.69 (3 H, s, OMe) and 4.47 (1 H, m, *J* 6, CH); δ_C (75 MHz; $CDCl_3$) 170.60 (CO), 117.05 (CN), 65.32 (CH), 51.78 (OMe), 41.55 (CH_2), 26.21 (CH_2) 25.54 (CH_3), 17.83 (C), –4.88 ($SiMe_2$) and –5.00 ($SiMe_2$); m/z 258 (M^+ + 1, <1 %), 242 (4), 200 (94), 158 (57) and 89 (100).

Methyl (RS)-4-cyano-3-hydroxybutanoate 13

A solution of the nitrile **12** (0.865 g, 3.37 mmol) in tetrahydrofuran (5 cm^3) was treated with an 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (4 cm^3, 4 mmol) and stirred at room temperature for 20 min. The solution was concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel (20 g) using petrol ether–EtOAc (1 : 1) as eluant to give the hydroxy nitrile **13** as an oil (0.454 g, 94 %); ν_{max} (film)/cm^{-1} 3460 (OH), 2250 (CN) and 1736 (CO); δ_H (300 MHz; $CDCl_3$) 2.62 (4 H, m, CH_2), 3.71 (3 H, s, OMe) and 4.33 (1 H, m, *J* 6.5, CH); δ_C (75 MHz; $CDCl_3$) 171.82 (CO), 117.04 (CN), 64.06 (CH), 52.11 (OMe), 39.93 (CH_2) and 25.10 (CH_2); m/z 144 (M^+ + 1, 4 %), 127 (13), 112 (40) and 103 (100); (found: M^+ + H, 144.06607. $C_6H_9NO_3$ requires M + H, 144.06583).

Methyl (RS)-3-benzoyloxy-4-cyanobutanoate 14

A solution of the hydroxy nitrile **13** (0.454 g, 3.17 mmol) in pyridine (5 cm^3) was treated under stirring and cooling (ice water) with benzoyl chloride (0.703 g, 5 mmol). After 15 min the cooling bath was removed and the stirring was continued for a further 15 min. The mixture was diluted with EtOAc (30 cm^3) and washed with a saturated aqueous solution of $NaHCO_3$ (10 cm^3) and water (2 x 10 cm^3). After drying ($MgSO_4$) the solvents were removed under reduced pressure by co-distillation with toluene. The residue was purified by flash chromatography over silica gel (35 g) with hexane–EtOAc (4 : 1) as eluant to give the benzoyloxy nitrile **14** as a colourless oil (0.735 g, 94 %); (found: C, 63.4; H, 5.0; N, 5.6. $C_{12}H_{13}NO_4$ requires C, 63.15; H, 5.3; N, 5.7 %); ν_{max} (film)/cm^{-1} 2256 (CN), 1736 (CO) and 1611 (CO); δ_H (300 MHz; $CDCl_3$) 2.97 (4 H, m, CH_2), 3.72 (3 H, s, OMe) and 5.58-8.10 (5 H, m, Ph). δ_C (75 MHz; $CDCl_3$) 169.55 (CO), 165.23 (CO), 133.66 (CH-Ph), 129.82 (CH-Ph), 129.01 (C-Ph), 128.55 (CH-Ph), 115.88 (CN), 65.81 (CH), 52.15 (OMe), 37.30 (CH_2) and 22.70 (CH_2); m/z 247 (M^+, 4 %), 210 (2), 122 (60), 105 (79) and 83 (100).

Isopropyl (RS)-3-tert-butyldimethylsilyloxy-4-cyanobutanoate 15

A solution of the nitrile **12** (0.270 g, 1.05 mmol) in dichloromethane (10 cm^3) was treated with titanium(IV)

isopropoxide (0.600 g, 2.1 mmol) and stirred for 48 h at room temperature. The reaction mixture was diluted with chloroform (30 cm^3) and washed with 2 N hydrochloric acid (10 cm^3), saturated aqueous solution of $NaHCO_3$, and water (10 cm^3). After drying ($MgSO_4$) the solvent was removed under reduced pressure. The residue was purified by flash chromatography over silica gel (10 g) using petrol ether (40–60°)–diethyl ether (4 : 1) as eluant to give unchanged **12** (0.043 g, 16 %) and the isopropyl ester **15** as a colourless oil (0.236 g, 79 %); bp 190 °C/0.5 mmHg (bath temp., Kugelrohr); (found: C, 58.5; H, 10.0; N, 5.05. $C_{14}H_{27}NO_3Si$ requires C, 58.9; H, 9.5; N, 4.9 %. ν_{max} (film)/cm^{-1} 2252 (CN) and 1732 (CO); δ_H (300 MHz; $CDCl_3$) 0.09 (3 H, s, $SiMe_2$), 0.13 (3 H, s, $SiMe_2$), 0.88 (9 H, s, Bu^t), 1.23 (6 H, d, *J* 6.5, Me), 2.57 (2 H, dd, *J* 6, 3, CH_2), 2.61 (2 H, dd, *J* 5, 3, CH_2), 4.35 (1 H, m, *J* 6.5, CH) and 5.00 (1 H, m, *J* 6.5, CH); δ_C (75 MHz; $CDCl_3$) 169.69 (CO), 117.18 (CN), 68.37 (CH), 65.31 (CH), 41.98 (CH_2), 26.14 (CH_2), 25.57 (Me), 21.78 (Me), 21.76 (CH_3), 17.84 (C), –4.90 ($SiMe_2$) and –4.93 ($SiMe_2$); m/z 286 (M^+ + 1, <1 %), 228 (52), 226 (66), 186 (100), 144 (90) and 101 (80).

Isopropyl (RS)-4-cyano-3-hydroxybutanoate ***16***

A solution of the isopropyl ester **15** (0.400 g, 1.4 mmol) in tetrahydrofuran (10 cm^3) was treated with a 1 M solution of tetrabutylammonium fluoride (2 cm^3, 2 mmol) and stirred for 20 min at room temperature. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography over silica gel (10 g) using hexane–EtOAc (1 : 1) as eluant to give the hydroxy ester **16** as an oil (0.185 g, 77 %); ν_{max} (film)/cm^{-1} 3470 (OH), 2253 (CN) and 1727 (CO); δ_H (300 MHz; $CDCl_3$) 1.23 (6 H, d, *J* 6.5, Me), 2.59 (4 H, m, CH_2), 3.63 (1 H, br s, OH), 4.31 (1 H, m, CH) and 5.03 (1 H, septet, *J* 6.5, CH); δ_C (75 MHz; $CDCl_3$) 171.03 (CO), 116.90 (CN), 68.95 (CH), 40.31 (CH_2), 25.03 (CH_2) and 21.70 (Me); m/z 172 (M^+ + 1, <1 %), 156 (6), 130 (12) and 112 (100); (found: M^+ + H 172.09800. $C_8H_{13}NO_3$ + H requires 172.09737).

Isopropyl (RS)-3-benzoyloxy-4-cyanobutanoate ***17***

An ice-cold solution of the hydroxy ester **16** (0.155 g, 0.91 mmol) in pyridine (3 cm^3) was treated with benzoyl chloride (0.210 g, 1.5 mmol). After 10 min the cooling bath was removed and stirring was continued at room temperature for 2 h. The reaction mixture was diluted with EtOAc (20 cm^3) and washed with a saturated aqueous solution of $NaHCO_3$ (10 cm^3) and water (2 x 10 cm^3). After drying ($MgSO_4$) the solvent was removed under reduced pressure. The residue was purified by flash chromatography over silica gel (10 g) with petrol ether (40–60°)–EtOAc (4 : 1) as eluant to give the benzoate **17** as an oil (0.225 g, 90 %); (found: C, 65.4; H, 6.3; N, 5.1. $C_{15}H_{17}NO_4$ requires C, 65.4; H, 6.2; N, 5.1 %); ν_{max} (film)/cm^{-1} 2262 (CN), 1743 (CO) and 1604 (CO); δ_H (300 MHz; $CDCl_3$) 1.21 (3 H, d, *J* 6.5, Me), 1.24 (3 H, d, *J* 6.5, Me), 2.93 (4 H, m, CH_2), 5.04 (1 H, m, *J* 6.5, CH), 5.59 (1 H, m, CH) and 7.40–8.06 (5 H, m, Ph); δ_C (75 MHz; $CDCl_3$) 168.55 (CO), 165.24 (CO), 133.64 (CH-Ph), 129.82 (CH-Ph), 129.03 (C-Ph), 128.52 (CH-Ph), 115.87 (CN), 68.89 (CH), 65.92 (CH), 37.96 (CH_2), 22.74 (CH_2), 21.71 (Me) and 21.67 (Me); m/z 275 (M^+, 4 %), 234 (8), 216 (12), 122 (35) and 105 (100).

(RS)-3-tert-Butyldimethylsilyloxy-4-cyanobutanoic acid ***18***

A solution of the methyl ester **12** (1.54 g, 6 mmol) in MeOH (15 cm^3) was treated with a solution of NaOH (0.26 g, 6.5 mmol) in MeOH (10 cm^3) and water (0.5 cm^3). The mixture was left at room temperature for 62 h and the solvent was removed under reduced pressure. The remaining residue was dissolved in water (20 cm^3) and extracted with EtOAc (2 x 20 cm^3). The water phase was acidified with acetic acid and then extracted with EtOAc (3 x 20 cm^3). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel (50 g) using petrol ether (40–60°)–EtOAc–glacial acetic acid (10 : 10 : 0.2) as eluant to give the acid **18** as colourless crystals (1.34 g, 92 %); mp 51–52 °C (from diethyl ether–hexane) (found: C, 54.2; H, 8.4; N, 5.6. $C_{11}H_{21}NO_3Si$ requires C, 54.3; H, 8.7; N, 5.8 %); ν_{max} (KBr)/cm^{-1} 3400–2400 (COOH), 2256 (CN) and 1710 (CO); δ_H (300 MHz; $CDCl_3$) 0.10 (3 H, s, $SiMe_2$), 0.14 (3 H, s, $SiMe_2$), 0.89 (9 H, s, Bu^t), 2.63 (2 H, dd, *J* 6, 2.5, CH_2), 2.67 (2 H, d, *J* 6, CH_2), 4.38 (1 H, m, *J* 6, CH) and 10.40 (1 H, br s, CO_2H); δ_C (75 MHz; $CDCl_3$) 176.20 (CO), 116.87 (CN), 65.09 (CH), 41.50 (CH_2), 26.16 (CH_2), 25.45 (Me), 17.84 (C), –4.87 ($SiMe_2$) and –5.00 ($SiMe_2$); m/z 244 (M^+ + 1, <1 %), 186 (13), 144 (19) and 83 (100).

(RS)-3-tert-Butyldimethylsilyloxy-4-cyanobutanoic acid amide ***19***

A solution of the acid **18** (0.890 g, 3.65 mmol) in dichloromethane (10 cm^3) was treated with pyridine (5 drops) and then with a 2 M solution of oxalyl chloride in CH_2Cl_2 (2.5 cm^3, 5 mmol). The solution was stirred for 45 min at room temperature, then for 30 min at 30–40 °C, and finally for a further 15 min at room temperature. This solution was dropped into an ice-cold 2 M solution of ammonia in MeOH (20 cm^3). The reaction mixture was stirred for 10 min at room temperature and then concentrated under reduced pressure to a volume of *ca* 10 cm^3. The residue was filtered. The filtrate was diluted with EtOAc (40 cm^3) and washed with water (3 x 20 cm^3). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure to give the amide **19** as colourless crystals (0.845 g, 95 %); mp 88–89 °C (from diethyl ether–hexane); (found: C, 52.85; H, 8.7; N, 10.9. $C_{11}H_{22}N_2O_2Si.0.5\ H_2O$ requires C, 52.6; H, 9.2; N, 11.1 %); ν_{max} (KBr)/cm^{-1} 3440 (NH), 3433 (NH), 2249 (CN), 1686 (CO) and 1622 (CO); δ_H (300 MHz; $CDCl_3$) 0.11 (3 H, s, $SiMe_2$), 0.15 (3 H, s, $SiMe_2$), 0.90 (9 H, s, Bu^t), 2.52 (2 H, d, *J* 6, CH_2), 2.62 (2 H, dd, *J* 6, 1.5, CH_2), 4.40 (1 H, m, *J* 6, CH), 5.68 (1 H, br s, NH_2) and 5.79 (1 H, br s, NH_2); δ_C (75 MHz; $[^2H_6]$-DMSO)

171.13 (CO), 118.41 (CN), 65.51 (CH), 42.43 (CH_2), 25.73 (CH_2), 17.65 (C), –4.90 ($SiMe_2$) and –5.12 ($SiMe_2$); m/z 243 (M^+ + 1, 2 %), 227 (15), 185 (94), 116 (45) and 74 (100).

(RS)-4-Cyano-3-hydroxybutanoic acid amide 20

A solution of the amide **19** (0.843 g, 3.5 mmol) in tetrahydrofuran (15 cm^3) was treated with an 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (5 cm^3, 5 mmol) and stirred for 20 min at room temperature. The solvent was removed under reduced pressure. The residue was purified by flash chromatography over silica gel (30 g) using EtOAc–EtOH (4 : 1) as eluant to give the hydroxy amide as colourless crystals **20** (0.421 g, 94 %); mp 116–117 °C (from EtOH–EtOAc); (found: C, 46.1; H, 6.1; N, 22.0. $C_5H_8N_2O_2$ requires C, 46.9; H, 6.3; N, 21.9 %); ν_{max} (KBr)/cm^{-1} 3470 (NH, OH), 3433 (NH, OH), 3214 (NH, OH), 2250 (CN), 1687 (CO) and 1662 (CO); δ_H (300 MHz; [$^2H_6$]-DMSO) 2.23 (1 H, dd, *J* 15, 5, CH_2), 2.33 (1 H, dd, *J* 15, 7.5, CH_2), 2.57 (1 H, dd, *J* 15, 7.5, CH_2), 2.69 (1 H, dd, *J* 15, 4.5, CH_2), 4.10 (1 H, m, CH), 5.41 (1 H, d, *J* 5, OH), 6.87 (1 H, br s, NH_2) and 7.38 (1 H, br s, NH_2); δ_C (75 MHz; [$^2H_6$]-DMSO) 171.86 (CO), 118.82 (CN), 63.38 (CH), 41.82 (CH_2) and 25.26 (CH_2); m/z 129 (M^+ + 1, 3 %), 101 (16), 88 (39), 72 (21) and 59 (100).

(RS)-3-Benzoyloxy-4-cyanobutanoic acid amide 7

An ice-cold solution of the hydroxy amide **20** (0.250 g, 1.95 mmol) in pyridine (7 cm^3) was treated dropwise with a solution of benzoyl chloride (0.422 g, 3 mmol) in pyridine (2 cm^3). After 5 min the cooling bath was removed and stirring was continued for 40 min at room temperature. Then the mixture was cooled (0–5 °C) and treated with a further portion of benzoyl chloride (0.127 g, 1 mmol) in pyridine (1 cm^3). After 5 min the cooling bath was removed and stirring was continued for a further 30 min. The reaction mixture was diluted with EtOAc (30 cm^3) and washed with a saturated aqueous solution of $NaHCO_3$ (10 cm^3) and water (2 x 10 cm^3). The organic phase was dried ($MgSO_4$) and the solvents were removed under reduced pressure. The residue was purified by flash chromatography over silica gel (30 g) using EtOAc as eluant to give the benzoate 7 as colourless crystals (0.190 g, 42 %); mp 141–141.5 °C (from EtOAc–hexane); (found: C, 61.8; H, 5.0; N, 12.0. $C_{12}H_{12}N_2O_3$ requires C, 62.1; H, 5.2; N, 12.1 %); ν_{max} (KBr)/cm^{-1} 3467 (NH), 3362 (NH), 3184 (NH), 2249 (CN), 1729 (CO), 1690 (CO) and 1618 (CO); δ_H (300 MHz; [$^2H_6$]-DMSO) 2.60 (1 H, dd, *J* 15, 5, CH_2), 2.73 (1 H, dd, *J* 15, 7.5, CH_2), 3.10 (1 H, dd, *J* 15, 5, CH_2), 3.12 (1 H, dd, *J* 15, 5, CH_2), 5.51 (1 H, m, CH), 6.99 (1 H, br s, NH_2) and 7.52–8.00 (6 H, superimposed m and br s, Ph, NH_2); δ_C (75 MHz; [$^2H_6$]-DMSO) 170.06 (CO), 164.67 (CO), 139.64 (CH-Ph), 129.28 (C-Ph), 129.24 (CH-Ph), 128.79 (CH-Ph), 117.54 (CN), 66.87 (CH), 38.50 (CH_2) and 22.33 (CH_2); m/z 232 (M^+, 1 %), 167 (28), 149 (84), 127 (26), 122 (21) and 105 (100).

(RS)-3-t-Butyldimethylsilyloxy-4-cyanobutanoic acid dimethylamide 21

A solution of the acid **18** (0.729 g, 3 mmol) in CH_2Cl_2 (10 cm^3) was treated with pyridine (5 drops) and then with a 2 M solution of oxalyl chloride in CH_2Cl_2 (2.1 cm^3, 4.2 mmol). The solution was stirred for 45 min at room temperature, then for 30 min at 30–40 °C, and finally for a further 15 min at room temperature. This solution was dropped into an ice-cold 40 % aqueous solution of dimethylamine (15 cm^3). The reaction mixture was stirred for 10 min at room temperature and then diluted with $CHCl_3$ (30 cm^3). The organic extract was washed with water (3 x 20 cm^3) and dried ($MgSO_4$). After evaporation of the solvent under reduced pressure the residue was purified by flash chromatography over silica gel (40 g) using EtOAc–petrol ether (40–60 °) (4 : 1) as eluant to give the amide **21** (0.739 g, 91 %); bp 200 °C/0.5 mmHg (bath temp.; Kugelrohr); (found: C, 57.4; H, 10.0; N, 10.05. $C_{13}H_{26}N_2O_2Si$ requires C, 57.7; H, 9.7; N, 10.35 %); ν_{max} (film)/cm^{-1} 2256 (CO), 1743 (CO) and 1657 (CO); δ_H (300 MHz, $CDCl_3$) 0.07 (3 H, s, $SiMe_2$), 0.13 (3 H, s, $SiMe_2$), 0.90 (9 H, s, Bu^t), 2.62 (4 H, m, CH_2), 2.93 (3 H, s, NMe), 3.03 (3 H, s, NMe) and 4.44 (1 H, m, CH); δ_H (75 MHz, $CDCl_3$), 169.56 (CO), 117.42 (CN), 65.78 (CH), 39.89 (CH_2), 37.35 (NMe), 35.27 (NMe), 26.52 (CH_2), 25.62 (Me), 17.83 (C), –4.88 (SiMe) and –5.01 (SiMe); m/z 270 (M^+, <1 %), 255 (29), 213 (100) and 172 (58).

(RS)-4-Cyano-3-hydroxybutanoic acid dimethylamide 22

A solution of the amide **21** (0.675 g, 2.5 mmol) in tetrahydrofuran (7 cm^3) was treated with a 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (3 cm^3, 3 mmol) and stirred for 20 min at room temperature. The solvent was removed under reduced pressure. The residue was purified by flash chromatography over silica gel (30 g) using EtOAc–EtOH (4 : 1) as eluant to give the hydroxy amide **22** as an oil (0.375 g, 96 %); ν_{max} (film)/cm^{-1} 3388 (OH), 2250 (CN), 1743 (CO) and 1631 (CO); δ_H (300 MHz; $CDCl_3$) 2.55 (4 H, m, CH_2), 2.92 (3 H, s, NMe), 2.98 (3 H, s, NMe), 4.28 (1 H, m, CH) and 4.75 (1 H, br s, OH); δ_C (75 MHz; $CDCl_3$) 171.06 (CO), 117.36 (CN), 64.47 (CH), 37.85 (CH_2), 37.10 (NMe), 35.21 (NMe) and 24.80 (CH_2); m/z 156 (M^+, 8 %), 141 (3), 129 (37), 116 (62), 87 (31) and 72 (100); (found: M^+ 156.09008. $C_7H_{12}N_2O_3$ requires 156.08988).

(RS)-3-Benzoyloxy-4-cyanobutanoic acid dimethylamide 23

An ice-cold solution of the hydroxy amide **22** (0.325 g, 2.08 mmol) in pyridine (5 cm^3) was treated with benzoyl chloride (0.422 g, 3 mmol). After 10 min the cooling bath was removed and stirring was continued at room temperature for 30 min. The reaction mixture was diluted with EtOAc (40 cm^3) and the organic extract was washed with an aqueous solution of $NaHCO_3$ (10 cm^3) and water (2 x 10 cm^3). After drying ($MgSO_4$) the solvent was

removed under reduced pressure. The residue was purified by flash chromatography over silica gel (30 g) using petrol ether (40–60 °)–EtOAc (1 : 4) as eluant to give the benzoate **23** (0.560 g, 100 %); mp 70–71.5 °C (from diethyl ether–hexane); (found: C, 64.5; H, 6.2; N, 10.95. $C_{14}H_{16}N_2O_3$ requires C, 64.6; H, 6.2; N, 10.8 %); ν_{max} (KBr)/cm^{-1} 2250 (CN), 1719 (CO), 1646 (CO) and 1602 (CO); δ_H (300 MHz, $CDCl_3$) 2.96 (3 H, s, NMe), 3.01 (4 H, m, CH_2), 3.04 (3 H, s, NMe), 5.60 (1 H, m, CH) and 7.50–8.10 (5 H, m, Ph); δ_C (75 MHz; $CDCl_3$) 168.19 (CO), 165.29 (CO), 133.53 (CH-Ph), 129.79 (CH-Ph), 129.30 (C-Ph), 128.51 (CH-Ph), 116.62 (CN), 66.98 (CH), 37.14 (NMe), 35.95 (CH_2), 35.28 (NMe) and 22.93 (CH_2); m/z 260 (M^+, 8 %), 155 (28), 139 (12), 105 (100) and 83 (88).

Methyl (RS)-3-benzyloxy-4-cyanobutanoate ***24***

An ice-cold solution of benzyl 2,2,2-trichloroacetimidate (2.1 g, 8.7 mmol) in CH_2Cl_2 (70 cm^3) was treated with trimethylsilyl trifluoromethanesulfonate (0.150 cm^3, 0.8 mmol) and then with a solution of the hydroxy nitrile **13** (0.605 g, 4.23 mmol) in CH_2Cl_2 (10 cm^3). The reaction mixture was stirred for 20 h at room temperature and then concentrated under reduced pressure to a volume of *ca* 20 cm^3. The remaining solution was treated with hexane (20 cm^3) and cooled to *ca* –20 °C for 15 min. The precipitated 2,2,2-trichloroacetamide was removed by filtration. The crystals were washed with CH_2Cl_2–hexane (1 : 1, 2 x 10 cm^3). The filtrate was washed with a saturated aqueous solution of $NaHCO_3$ (10 cm^3) and water (2 x 10 cm^3), dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography over silica gel (50 g) using $CHCl_3$–acetonitrile (99 : 1) as eluant to give the crude benzyloxy ester **24** as an oil (0.562 g, 57 %); δ_H (300 MHz, $CDCl_3$) 2.70 (4 H, m, CH_2), 3.71 (3 H, s, OMe), 4.15 (1 H, quintet, *J* 6, CH), 4.64 (2 H, s, OCH_2), 7.30 (5 H, m, Ph).

(RS)-3-Benzyloxy-4-cyanobutanoic acid ***25***

A solution of crude **24** (0.445 g, 1.91 mmol) in MeOH (5 cm^3) was treated with NaOH (0.080 g, 2 mmol) and water (0.2 cm^3). After standing for 72 h at room temperature the solvent was removed under reduced pressure and the residue was dissolved in water (20 cm^3). The solution was extracted with EtOAc (2 x 10 cm^3) and subsequently acidified with acetic acid. The acidic aqueous solution was extracted with EtOAc (3 x 10 cm^3). The combined acidic extracts were dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash chromatography over silica gel (10 g) using petrol ether (40–60 °)–EtOAc–glacial acetic acid (10 : 10 : 0.2) as eluant to give the benzyloxy acid **25** as an oil (0.254 g, 61 %); ν_{max} (film)/cm^{-1} 3600–2400 (COOH), 2252 (CN) and 1715 (CO); δ_H (300 MHz, $CDCl_3$) 2.74 (4 H, m, CH_2, 4.14 (1 H, quintet, *J* 6, CH), 4.65 (2 H, s, CH_2O), 7.35 (5 H, m, Ph) and 8.80 (1 H, br s, COOH); δ_C (75 MHz, $CDCl_3$) 175.59 (CO), 136.91 (C-Ph), 128.60 (CH-Ph), 128.23 (CH-Ph), 127.93 (CH-Ph), 116.79 (CN), 72.41 (CH_2O), 70.97 (CH), 38.72 (CH_2) and 23.01 (CH_2); m/z 219 (M^+, 16), 107 (100), 91 (72), 79 (24); (found: M^+ 219.09005. $C_{12}H_{13}NO_3$ requires M 219.08954).

(RS)-3-Benzyloxy-4-cyanobutanoic acid amide ***8***

A solution of the benzyloxy acid **25** (0.250 g, 1.14 mmol) in CH_2Cl_2 (5 cm^3) was treated with five drops of pyridine and subsequently with a 2 M solution of oxalyl chloride in CH_2Cl_2 (0.75 cm^3, 1.5 mmol). The reaction mixture was stirred for 45 min at room temperature, then for 30 min at 30–40 °C, and finally for a further 15 min at room temperature. This solution was added dropwise to an ice-cold 33 % aqueous solution of ammonia (3 cm^3) and stirred for 10 min at room temperature. The reaction mixture was diluted with $CHCl_3$ (20 cm^3) and washed with water (3 x 10 cm^3). The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography over silica gel (30 g) using EtOAc as eluant to give the benzyloxy amide **8** as colourless crystals (0.200 g, 80 %); mp 91-92 °C (from EtOAc–hexane); (found: C, 65.7; H, 6.5; N, 12.4. $C_{12}H_{14}N_2O_2$ requires C, 66.0; H, 6.5; N, 12.8 %); ν_{max} (KBr)/cm^{-1} 3401 (NH), 3177 (NH), 1690 (CO) and 1637 (CO); δ_H (300 MHz, $CDCl_3$) 2.45–2.77 (4 H, m, CH_2), 4.15 (1 H, m, CH), 4.62 (2 H, AB-system, *J* 11.5, OCH_2) and 7.30 (5 H, m, Ph); δ_C (75 MHz, $CDCl_3$) 171.68 (CO), 137.05 (C-Ph), 128.62 (CH-Ph), 128.22 (CH-Ph), 127.96 (CH-Ph), 117.05 (CN), 72.40 (OCH_2), 71.59 (CH), 40.30 (CH_2) and 22.96 (CH_2); m/z 191 (M^+ – HCN, 1 %), 149 (3.5), 127 (1), 112 (97) and 91 (100).

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