Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 6, pp. 1675-1682, 1996

0957-4166/96 $15.00 + 0.00

PII: S0957-4166(96)00201-7

Oxynitrilase-Catalyzed Transformation of Substituted Aldehydes: The case of (±)-2-Phenylpropionaldehyde and of (±)-3-Phenylbutyraldehyde

Bruno DANIELI*[a], Carlo BARRA[a], Giacomo CARREA[b], Sergio RIVA*[b]

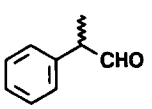
a) Dipartimento di Chimica Organica ed Industriale, Universita' di Milano - Centro C.N.R. di Studio per le Sostanze Organiche Naturali, Via Venezian 21, 20133 Milano, ITALY

b) Istituto di Chimica degli Ormoni, C.N.R., Via Mario Bianco 9, 20131 Milano, ITALY

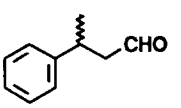
Abstracts: The influence of a stereocenter already present in the molecule on the selectivity displayed by almond oxynitrilase has been addressed, considering the substituted aldehydes (±)-2-phenylpropionaldehyde **1** and (±)-3-phenylbutyraldehyde **2** as model substrates. Only when the stereocenter was adjacent to the aldehyde group (as in **1**) a strong influence on the selectivity of this enzyme was observed, resulting in the formation of the four possible cyanohydrins although in different ratio. On the other hand, the cyanohydrins obtained from **2** had the expected 2*R* configuration.

Stereoselective carbon-carbon bond forming reactions are among the most sought tools of synthetic organic chemistry. Enzymes, due to their ability of catalyzing very selective reactions under mild conditions, are gaining momentum in this important area of research. Aldolases, transketolases and oxynitrilases have been isolated from various natural sources and their catalytic properties studied and exploited with natural and non-natural substrates.[1] Focusing on oxynitrilases, they catalyse the stereoselective synthesis of (*R*)- or (*S*)-cyanohydrines from aldehydes and ketones.[2] These enzymes have showed a great versatility towards different substrates, accepting both aromatic and alyphatic carbonyls. However, despite the great number of compounds considered so far, the influence of a stereocenter already present in the molecule on the selectivity displayed by the oxynitrilase has never been addressed.

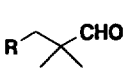
In the frame of our general interest in biocatalysis,[3] we have recently included oxynitrilases among our research topics. Here we will report on the preliminary results that we have obtained considering the substituted aldehydes (±)-2-phenylpropionaldehyde **1** and (±)-3-phenylbutyraldehyde **2** as model substrates for the (*R*)-oxynitrilase from almond.



1



2



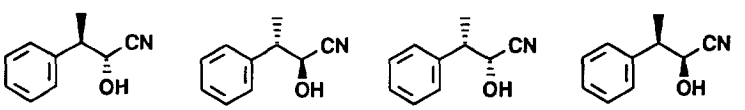
3a R = H

3b-i R = OH; CH_3O; C_2H_5O; $CH_2=CH(CH)_2O$; $C_6H_5CH_2O$; Cl; Br; CH_3COO

RESULTS AND DISCUSSION

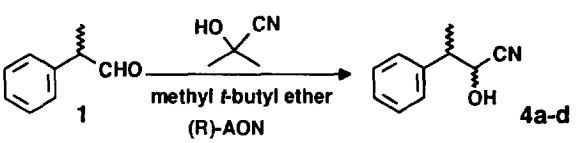
The first asymmetric synthesis of enantiomerically enriched cyanohydrins using the (*R*)-oxynitrilase from almond ((*R*)-AON) was reported by Rosenthaler at the beginning of this century.[4] It is not surprising that this enzyme has been widely investigated later on as it is very abundant in sweet almonds, being not less than 0.4 percent by weight,[5] which is an extraordinary large amount for a single enzyme. A decisive breakthrough for the use of this oxynitrilase came from Efferberger's work,[2] who found that the use of two-phase systems (water/water immiscible organic solvents) greatly reduced the aspecific chemical addition of HCN to the carbonyl group, a very deleterious side-reaction for the enantiomeric purity of the products. Several aromatic and aliphatic aldehydes have been cyanurated by (*R*)-AON.[6] However, only isobutyraldehyde, isovaleraldehyde, pivalaldehyde (**3a**)[7] and some of its derivatives **3b-i**[8] exemplified the use of substituted aldehydes.

In our approach to the synthetic exploitation of this enzyme we decided to study first the influence of a stereocenter in α to the carbonyl group on the selectivity of (*R*)-AON. Our model substrate **1**, used in racemic form, could, in principle, offer different outcomes. If the stereocenter in α were not effective in the orientation of **1** into the active site of (*R*)-AON, the enzyme would have maintained its R-selectivity and only the two diastereoisomers **4a** (2*R*,3*R*) and **4c** (2*R*,3*S*) would have been obtained. On the other hand, if the enzyme were sensitive to the α-substitution, the formation of the four possible stereoisomers **4a-d** could be expected.



(2R,3R)-4a (2S,3S)-4b (2R,3S)-4c (2S,3R)-4d

Addition of HCN to **1** was performed following the methodology suggested by Kyler[7,9]:



1 4a-d

After acetylation of the reaction mixture, chiral GC analysis clearly indicated that all the four possible diastereoisomers **5a-d** were obtained, even if some sort of selection was operated by (*R*)-AON (**Figure 1, part A**). In order to investigate the partial selectivity displayed by the enzyme, it was necessary to assign the absolute configuration of the four products and to correlate them to their own chiral GC peak.

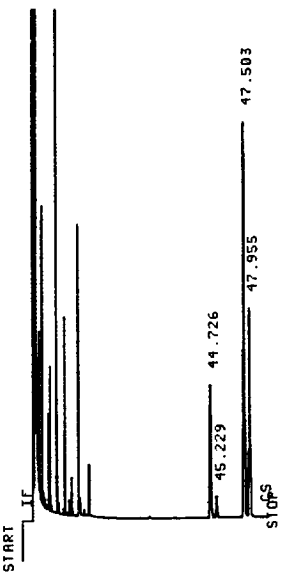
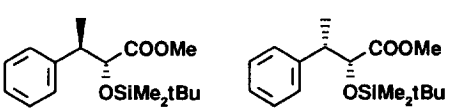


Figure 1. Chiral-GC chromatogram of cyanohydrins acetates 5a-d obtained from (R)-AON.

To this end a preliminary information was obtained by analyzing the reaction mixture using normal phase analytical HPLC. Two peaks were obtained. Samples corresponding to these two separated peaks[10] were collected and injected into the GC. In this way we were able to ascertain that the two less retained GC-peaks corresponded to a pair of enantiomers, and the two most retained ones to the other pair.

The absolute configuration of the products was defined by making recourse to the known selectivity of lipase P in the acylation of racemic cyanohydrins.[11] From the literature data and from what is known on the selectivity of lipase P towards the acylation of secondary alcohols,[12] it was reasonable to expect that the acetylation of an equimolar mixture of **4a-d** (obtained by chemical addition of HCN to **1**) would have occurred preferentially at the (*S*)-2-OH, independently from the configuration at the C-3. Accordingly, the lipase P-catalyzed acetylation of **4a-d** gave an enriched mixture of two diastereoisomers, corresponding to the second (t_R = 45.229 minutes, 91 % e.e.) and to the fourth (t_R = 47.955 minutes, 94 % e.e.) GC peaks in a 38: 62 ratio. This mixture was easily converted, *via* acid methanolysis and silylation, to the corresponding silylated methyl esters, as depicted in **Scheme 1**. The diastereoisomers were then isolated by preparative HPLC and their NMR spectra compared to the ones reported in the literature for the two related compounds **6a** and **6c**.[13]

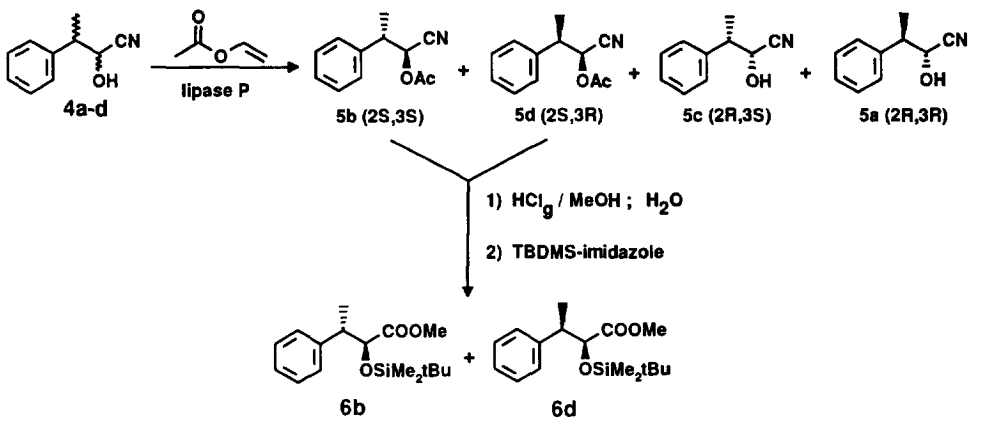


6a (2R,3R) **6c** (2R,3S)

The chemical shifts and the coupling constants (see Experimental) indicated that the silyl ether methyl ester less retained in HPLC corresponded to a *syn*-compound, while the other one had an *anti*-configuration. Both of them had a negative specific rotation and therefore they were identified as the methyl *anti*-(2*S*,3*S*)- and *syn*-

(2*S*,3*R*)-2-(*tert*-butyldimethylsilyloxy)-3-phenylbutanoate **6b** and **6d**, enantiomers of the compounds described in the literature.

SCHEME 1



However, at this point it was impossible to correlate the GC peaks of the cyanohydrins acetates to the HPLC peaks of the silyl ether methyl esters. To complete the assignement of the absolute configuration of each product obtained by the cyanuration catalysed by (*R*)-AON, we extensively enzymatically acetylated another chemical sample of **4a-d**. The unreacted cyanohydrin was recovered and acetylated and shown to be a mixture of only two enantiomerically pure stereoisomers, corresponding to the first and the third chiral-GC peaks of **Figure 1**, in a 15 : 85 ratio. This enriched mixture was elaborated to the usual silyl ether methyl ester derivatives and ^{1}H-NMR analysis of these products allowed to identify the major component as a *syn*-isomer. This information, together with the previously described data, allowed to assign unequivocally the 2*R*,3*S*-stereochemistry to the cyanhydrine acetate giving origin to the third chiral GC peak of **Figure 1**, and to fully elucidate the stereochemical outcome of the (*R*)-AON-catalyzed addition of HCN to **1** (**Table 1**).

Therefore we have shown that (*R*)-AON was able to cyanurate both the enantiomers of **1**. However, the absolute configuration of the stereocenter in α to the aldehyde indeed deeply influenced the stereochemical outcome of the reaction. Specifically, when the stereocenter had an *S*-configuration, (*R*)-AON showed the expected preference for the synthesis of *R*-cyanohydrins and the (2*R*,3*S*)-isomer was formed with a diastereomer ratio of 17.3, the ratio between the two possible epimers at C-2 being 51.8 to 3.0. On the other hand, when the stereocenter had an *R*-configuration the selectivity of the enzyme was almost completely lost and the two (2*R*,3*R*)- and (2*S*,3*R*)-cyanohydrins were obtained in nearly equivalent amounts (diasteremer ratio 1.6). Significantly, in this case the selectivity of (R)-AON was inverted as the most abundant diastereoisomer had the (2*S*)-configuration.

Table 1. Chiral-GC peaks and absolute configurations of the cyanohydrins acetates 5a-d.

Compound	Peak (Retention time) [a)]	%	Product
5a	1 (44.726)	17.6	2*R*,3*R*
5b	2 (45.229)	3.0	2*S*,3*S*
5c	3 (47.503)	51.8	2*R*,3*S*
5d	4 (47.955)	27.6	2*S*,3*R*

[a)] **See Figure 1**

We then turned our attention to (±)-2-phenylbutyraldehyde **2** , which contains a β-stereocenter. As shown in the corresponding chiral GC plot (**Figure 2, part A**), in this case (R)-AON gave mainly two of the four possible cyanohydrins, **8a** and **8b** (**Scheme 2, part A**).

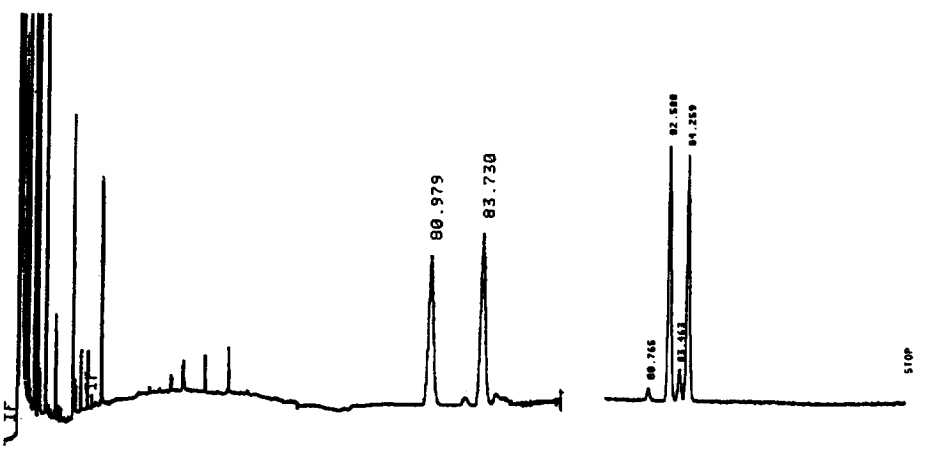
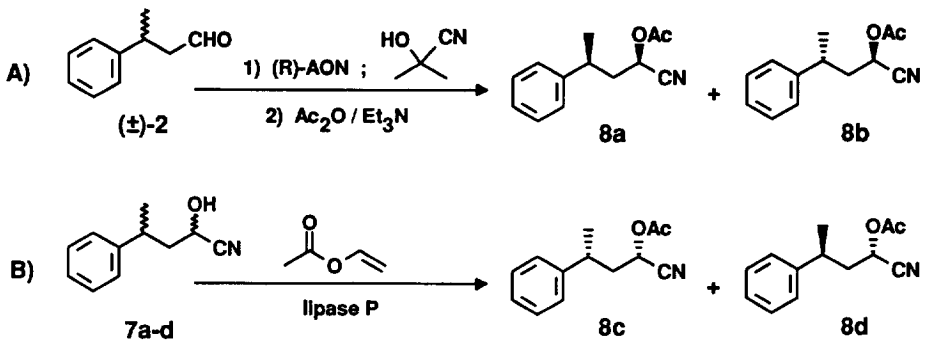


Figure 2. Chiral-GC chromatograms of cyanohydrins acetates 8a-d obtained from (R)-AON (part A) and from lipase P (part B).

Even though we did not perform any correlation study, a strong indication of the stereochemistry of these products was again given by the lipase P-catalyzed-acetylation of the racemic cyanohydrins (**7a-d**). Two main acetates were obtained (**8c** and **8d**), which corresponded to the previous minor peaks (**Figure 2, part B** and **Scheme 2, part B**). Taking for granted the (*S*)-selectivity of lipase P towards racemic cyanohydrins, these data strongly supported the conclusion that the cyanohydrins obtained by catalysis of (*R*)-AON had the expected 2*R*-configuration, as depicted in the part A of **Scheme 2.**

SCHEME 2



In conclusion, we have shown that only the substituents adjacent to the reactive center of the aldehyde have a strong influence on the selectivity of this oxynitrilase. It seems reasonable to hypothesise that this is the result of the interaction of α-sustituents with the aminoacids located in the proximity of the active site of (*R*)-AON. Unfortunately, the X-ray structure of almond oxynitrilase has not been resolved yet[14] and therefore it is not possible to rationalise our results by molecular modelling. Studies are in progress to extend these preliminary observations to other α-substituted aldehydes and the results will be reported in due course.

EXPERIMENTAL

Materials and Methods. Oxynitrilase from almonds was purchased from Sigma. Lipase P (from *Pseudomonas cepacia*) was from Amano. (±)-2-Phenylpropionaldehyde and (±)-3-phenylbutyraldehyde were from Aldrich. ^{1}H-NMR spectra were recorded on a Bruker AC-300. HPLC analyses and separations were obtained using analytical and semi-preparative silica gel columns (250 x 4.6 mm and 500 x 9.4 mm, Whatman) respectively. Enantiomeric excess of cyanohydrins acetates was determined by chiral GC with a CP-Cyclodextrin -β-2,3,6-M-19 column (50 m, 0.25 mm ID, Chrompack) using H_2 as the carrier gas under the following conditions: **5a-d**, oven temperature from 110°C (initial time 3 min) to 170°C with a heating rate of 1°C/min; **8a-d**, oven temperature from 110°C (initial time 3 min) to 135°C with a heating rate of 0.5°C/min. TLC were obtained on precoated silica gel 60 F_{254} plates (Merck) and compounds were detected using $KMnO_4$ (0.5 g) dissolved in 1 N NaOH (100 mL).

Chemical synthesis of 4a-d. Racemic **1** (2 g, 15 mmol) was dissolved in 7.5 mL of AcOH. A solution of NaCN (2.2 g, 45 mmol) in H_2O was dropped into the flask and the reaction mixture was left at r.t. for 1 h. After this time, the solution was diluted with 25 mL of H_2O and extracted with diethyl ether. Following anhydrification and evaporation of the solvent, the crude residue was purified by flash chromatography (eluent

hexane/AcOEt 85:15) to give 1.88 g of **4a-d** (78 % yield). ^{1}H-NMR ($CDCl_3$) δ : 7.30 (m, 5H); 4.49 and 4.48 (d, 1H, H-2); 3.15 (m, 1H, H-3); 1.46 and 1.44 (d, 3H, CH_3).

Enzymatic synthesis of 4a-d. Racemic **1** (0.5 g, 3.77 mmol) was dissolved in 23 mL of di-isopropyl ether containing 0.45 mL (4.88 mmol) of acetone cyanohydrin. (R)-AON (1500 units) was dissolved in 0.6 mL of 0.1 M citrate buffer pH 5.5 and added to the organic solution. This final biphasic mixture was shaken at r.t. and the reaction outcome was followed by TLC (eluent CH_2Cl_2/toluene/AcOEt 50:30:3). After seven days the two phases were separated and the water layer extracted with di-isopropyl ether. Following anhydrification with Na_2SO_4 and evaporation of the solvent, the crude residue was purified by flash chromatography to give 365 mg (60 % yield) of **4a-d**. Acetylation of this mixture with Ac_2O/Et_3N gave **5a-d** in a 17.6 : 3.0 : 51.8 : 27.6 ratio (see **Figure 1**).

Lipase P-catalyzed acetylation of 4a-d. Chemically prepared **4a-d** (0.5 g) was dissolved in 20 mL of a 1:1 mixture of methyl-*t*-butyl-ether and vinyl acetate. Lipase P adsorbed on celite[15] (5 g) was added and the suspension was shaken at room temperature for 48 h, monitoring the reaction by TLC (eluent hexane/AcOEt 85:15). Usual work-up and purification by flash chromatography allowed the isolation of 167 mg of **5a-d**. The ratio among the four different cyanohydrin acetates was 1.6 : 37.1 : 1.8 : 59.5 as determined by chiral-GC.

Methyl 2-(*t*-butyldimetylsilyloxy)-3-phenylbutanoate 6b and 6d . The above described cyanohydrin acetates (150 mg) were dissolved in 3 mL of diethyl ether. Anhydrous MeOH (170 μL) was added and then HCl was bubbled into the solution till saturation. The reaction was left overnight at 5°C and then 30 min at r.t.. The solvent was evaporated, the oily residue suspended in 5 mL of H_2O and extracted with CH_2Cl_2. Following anhydrification, the solvent was evaporated and the residue silylated using 1-(*t*-butyldimethylsilyl)imidazole (325 μL) in DMF (6 mL). Following usual work-up, the crude material was purified by preparative HPLC (eluent hexane/AcOEt 100:0.5; flow rate 2.5 mL/min; λ_{max}= 254 nm) to give two products which were identified as **6b** and **6d** by comparing their ^{1}H-NMR with the literature data. ^{1}H-NMR ($CDCl_3$) **6b** δ: 7.30 (m, 5H); 4.21 (d, 1H, H-2); 3.63 (s, 3H, $COOCH_3$); 3.12 (dt, 1H, H-3); 0.79 (s, 9H); -0.11 (s, 3H); -0.19 (s, 3H). **6d** δ: 7.30 (m, 5H); 4.22 (d, 1H, H-2); 3.61 (s, 3H, $COOCH_3$); 3.21 (dt, 1H, H-3); 0.83 (s, 9H); -0.11 (s, 3H); -0.24 (s, 3H).

Enzymatic synthesis of 8a-d. Racemic **2** (100 mg, 0.67 mmol) was dissolved in 4 mL of di-isopropyl ether containing 80 μL (0.88 mmol) of acetone cyanohydrin. (R)-AON (150 units) was dissolved in 0.5 mL of 0.1 M citrate buffer pH 5.5 and added to the organic solution. After seven days of shaking at r.t., the two phases were separated and the products isolated according to the usual procedure. Flash chromatography (eluent hexane-AcOEt 15: 85) gave 95 mg of **8a-d** (see **Figure 2, part A**). ^{1}H-NMR ($CDCl_3$) δ: 7.30 (m, 5H); 4.17 (t, 1H, H-2); 3.12-2.92 (m, 1H, H-4); 2.20-2.02 (m, 2H, H-3); 1.31 (d) and 1.33 (d) (3H, CH_3).

Lipase P-catalyzed acetylation of 7a-d. Chemically prepared **7a-d** (25 mg) was dissolved in 1 mL of a 1:1 mixture of methyl-*t*-butyl-ether and vinyl acetate. Lipase P adsorbed on celite[15] (50 mg) was added and the

suspension was shaken at 45°C, monitoring the reaction by TLC (eluent hexane/AcOEt 75:25) and chiral GC. After 24 hours conversion was about 50 % (as judged by TLC) and chiral GC analysis showed that the cyanohydrins acetates **8a-d** were present in a 1.1 : 48.4 : 1.7 : 48.6 ratio (see **Figure 2, part B**).

REFERENCES AND NOTES

1. For recent reviews see: a) Wong, C.H.; Whitesides, G.M. '*Enzymes in Synthetic Orgainc Chemistry*', Pergamon Publishers: Trowbridge (U.K.). 1994; pp 195-251. b) Drauz, K.; Waldmann, H. '*Enzyme Catalysis in Organic Synthesis*', VCH Publishers: Weinheim (Germany). 1995; pp. 547-593.
2. For a recent review see: Effenberger, F. *Angew.Chem.Int.Ed.Engl.*, **1994**, *33*, 1555.
3. a) Danieli, B.; Luisetti, M.; Riva, S.; Bertinotti, A.; Ragg, E.; Scaglioni, L.; Bombardelli, E. *J.Org.Chem.*, **1995**, *60*, 3637. b) Carrea, G.; Ottolina, G.; Riva, S. *TIBTECH*, **1995**, *13*, 63. c) Danieli, B.; Riva, S. *Pure & Applied Chem.* **1994**, *66*, 2215.
4. Rosenthaler, R. *Biochem. Z.*, **1908**, *14*. 238.
5. Smitskamp-Wilms, E.; Brussee, J.; Van der Gentile, A.; Van der Schanemburg, G.J.M., Sloothaok, J.B. *Recl.Trav.Chim. Pays-Bas* **1991**, *110*, 209.
6. a) Effenberger, F.; Ziegler, T.; Forster, S. *Angew Chem.Int.Ed.Eng.* **1987**, *26*, 458. b) Brussee, J.; Loos, W.T.; Kruse, C.G.; van der Gentile, A. *Tetrahedron* **1990**, *46*, 979. c) Huuhtanen, T.T.; Kanerva, L.T. *Tetrahedron:Asymmetry* **1992**, *3*, 1223. d) Menendez, E.; Brieva, R.; Rebolledo, F.; Gotor, V. *J.Chem.Soc.Chem.Commun.* **1995**, 989.
7. Ognyanov, F.; Datcheva, V.K.; Kyler, K.S. *J.Am.Chem.Soc.* **1991**, *113*, 6992.
8. Effenberger, F.; Eichhorn, J.; Roos, J. *Tetrahedron:Asymmetry* **1995**, *6*, 271.
9. This methodology was chosen among the different protocols found in the literature because of the minimal spontaneous chemical reaction of the *in situ* generated HCN with our substrate.
10. Separation was achieved only on analytical scale. Attempts to reproduce this purification procedure with preparative HPLC were unsuccessfull due to solubility problems.
11. a) Inagaki, M.; Hatanaka, A.; Mimura, M.; Hiratake, J.; Nishioka, T.; Oda, *J. Bull.Chem.Soc.Jpn.* **1992**, *65*, 111. b) Effenberger, F.; Gutterer, B.; Ziegler, T.; Aichholz, R. *Liebigs Ann.Chem.* **1991**, 47.
12. a) Weissfloch, A.N.E.; Kazlauskas, R.J. *J.Org.Chem.*, **1995**, *60*, 6959. b) Kazlauskas, K.J.; Weissfloch, A.N.E.; Rappaport, A.T.; Cuccia, L.A. *J.Org.Chem.*, **1991**, *56*, 2656.
13. Akita, H.; Fumichi, A.; Koshiji, H.; Horitoshi, K.; Oishi, T. *Chem.Pharm.Bull.*, **1984**, *32*, 1324.
14. Lauble, H.; Muller, K.; Schindelin, H.; Forster, S.; Effenberger, F. *Proteins: Struct.Funct.Genet*, **1994**, *19*, 343.
15. Secundo, F.; Riva, S.; Carrea, G. *Tetrahedron:Asymmetry*, **1992**, *3*, 267.

(*Received in UK* 27 *February* 1996; *accepted* 10 *April* 1996)