Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 2108–2112

Enzyme-promoted desymmetrisation of prochiral bis(cyanomethyl)phenylphosphine oxide

Piotr Kiełbasiński,[a,*] Michał Rachwalski,[a] Małgorzata Kwiatkowska,[a] Marian Mikołajczyk,[a] Wanda M. Wieczorek,[b] Małgorzata Szyrej,[c] Lesław Sieroń[b] and Floris P. J. T. Rutjes[d,*]

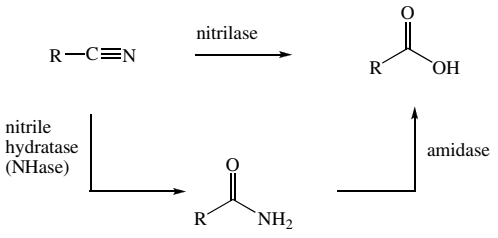
[a]*Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Department of Heteroorganic Chemistry, Sienkiewicza 112, 90-363 Łódź, Poland*
[b]*Institute of General and Ecological Chemistry, Technical University of Łódź, Żeromskiego 116, 90-924 Łódź, Poland*
[c]*Jan Długosz University, Institute of Chemistry and Environmental Protection, al. Armii Krajowej 13/15, 42-201 Częstochowa, Poland*
[d]*Radboud University Nijmegen, Institute for Molecules and Materials, Toernooiveld 1, 6525 Nijmegen, The Netherlands*

Received 27 July 2007; accepted 30 August 2007

Abstract—Prochiral bis(cyanomethyl)phenylphosphine oxide has been successfully transformed into the corresponding optically active monoamide and monoacid with enantiomeric excesses ranging from low (15%) to very high (up to 99%) using a broad spectrum of nitrile-hydrolysing enzymes.

1. Introduction

Nitriles are important intermediates in synthetic chemistry as they are simple to prepare and give access to a wide variety of interesting carboxylic acid derivatives.[1] However, the transformation of nitriles into the corresponding amides or carboxylic acids usually requires rather harsh conditions, for example, concentrated acids or bases, heavy metal salts or elevated temperatures.[2] A solution for these problems seems to be the enzymatic hydrolysis of nitriles. Within the past decade, nitrile-transforming biocatalysts have become very common both in academic laboratories and in industry.[3] These reactions can be conducted under mild conditions (aqueous buffers, neutral pH, ambient temperature) and have an additional advantage of being highly regio- and/or enantioselective.[4] The enzyme-catalyzed hydrolysis of nitriles can follow two different pathways. According to the first one, nitrilases convert nitriles directly into the corresponding acids without the amide as an intermediate. In the second one nitrile hydratases (NHases) transform nitriles into the corresponding amides, which under the action of amidases can be converted into the appropriate acids (Scheme 1).[5,6]



Scheme 1. Nitrile-hydrolysing enzymes.

The nitrilase-mediated selective hydrolysis of dinitriles to cyanocarboxylic acids is of particular synthetic interest, since a selective chemical hydrolysis of dinitriles is virtually impossible.[7]

Our previous work on the enzymatic desymmetrisation of prochiral compounds and on the enzymatic resolution of racemic substrates led to efficient approaches for the synthesis of various classes of optically active compounds.[2,8–11] In particular, our results on the enzymatic desymmetrisation of bis(cyanomethyl)sulfoxide[2] represented the first example of a stereoselective hydrolysis of

* Corresponding authors. Tel.: +48 42 680 3234; fax: +48 42 6847126 (P.K.); fax: +31 24 3653393 (F.P.J.T.R.); e-mail addresses: piokiel@bilbo.cbmm.lodz.pl; f.rutjes@science.ru.nl

0957-4166/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2007.08.027

a dinitrile containing a prostereogenic centre located on a heteroatom, the sulfinyl sulfur. As such, we clearly proved that nitrile-hydrolysing enzymes are capable of recognising and stereoselectively transforming substrates possessing a prochiral heteroatom. We then investigated whether this approach can also be applied to substrates with other prochiral heteroatoms. To this end, we chose a model substrate that contains a prochiral phosphinyl moiety, that is, bis(cyanomethyl)phenylphosphine oxide **2**. To underline the relevance of these investigations, it should be mentioned that P-chiral phosphorus compounds play an important role as ligands in asymmetric catalysis, mechanistic studies and as biologically active species.

2. Results and discussion

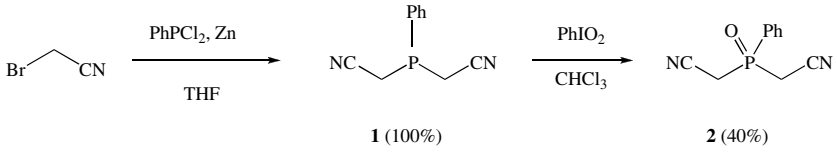
2.1. Synthesis of prochiral bis(cyanomethyl)phosphine oxide 2

The starting material, prochiral bis(cyanomethyl)phosphine oxide **2**, was synthesised via a modification of a method that we described previously.[12] It started from dichlorophenyl phosphine, which upon treatment with bromoacetonitrile in the presence of zinc dust in boiling THF, provided bis(cyanomethyl)phosphine **1** in quantitative yield. The latter compound was (without isolation) oxidised with $PhIO_2$ to give the desired phosphine oxide **2** in 40% yield after purification (Scheme 2). Alternative methods for the synthesis of **2**, as previously described by Dahl et al.,[13,14] appeared to be unsuccessful in our hands.

2.2. Enzymatic hydrolysis of prochiral bis(cyanomethyl)-phenylphosphine oxide 2

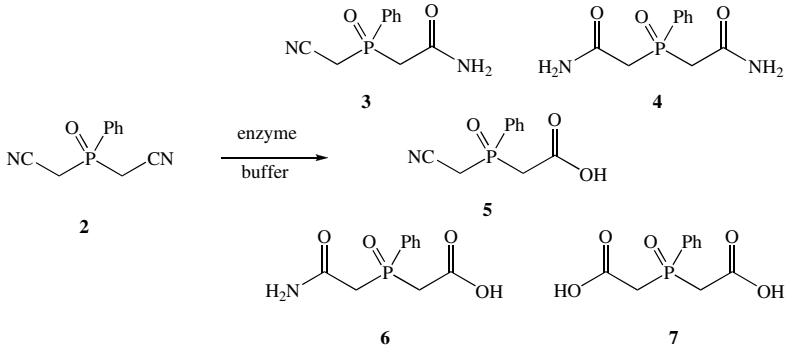
The prochiral substrate **2** was subjected to a general enzymatic procedure, which in principle, can lead to the five possible products **3**–**7** (Scheme 3). Three of them **3**, **5** and **6** may be obtained in each enantiomeric form. The hydrolyses were performed in a potassium phosphate buffer solution of pH 7.2 using a broad spectrum of nitrile-converting enzymes, namely a whole cell preparation from *Rhodococcus erythropolis* NCIMB 11540 (freeze dried cells)[15] and a series of commercially available nitrilases. The reaction times varied from 72 to 144 h. The results are collected in Table 1. Inspection of Table 1 shows that out of the five possible products, only two, cyanomethylphenylphosphinylacetamide **3** and cyanomethylphenylphosphinylacetic acid **5**, were formed in various proportions and enantioselectivities. Both products were isolated by column chromatography and analysed spectroscopically. Their structures were established by ^{1}H NMR, ^{31}P NMR, ^{13}C NMR, MS (CI) and HRMS (CI).

It should be stressed that under the conditions applied, the conversion never reached 100%. In each case, certain amounts of the unreacted substrate were isolated and in three cases (entries 4, 6 and 8) the substrate was the main component of the product mixture. Our investigations showed that the enzyme-promoted hydrolysis of prochiral bis(cyanomethyl)phenylphosphine oxide was not always chemoselective, since in several cases, more than one product was formed. Interestingly, most nitrilases, apart from their normal behaviour, also exhibited nitrile hydratase activity leading both to the monoacid **5** and monoamide **3**. Similar findings have already been reported.[1,2,7] The stereoselectivity of the enzymes towards the heteroatom prostereogenic centre (phosphoryl group) varied from low to very high, being generally lower as compared to bis(cyanomethyl)sulfoxide[2] and C-prochiral hydroxyglutaronitriles.[4] The ee values of monoamide **3** and monoacid **5** were determined by chiral HPLC (Varian Pro Star 210, Chiralpak AS). In two instances, monoamide **3** was obtained in excellent enantiomeric excess (96% and over



1 (100%) **2** (40%)

Scheme 2. Synthesis of bis(cyanomethyl)phenylphosphine oxide.



Scheme 3. Possible products of enzymatic hydrolysis of **2**.

Table 1. Enzymatic hydrolysis of prochiral **2**

Entry	Enzyme	Reaction time [h]	Product, yield [%]	$[\alpha]_D$[a]	ee [%]	Abs. conf.
1	Nitrilase 101	144	**2**, 10.4			
			3, 4.6	−4.0	56[c]	(*R*)
			5, 73.1	−1.5	53[c]	(*S*)
2	Nitrilase 102	144	**2**, 8.2			
			3, 9.6	−3.5	49[c]	(*R*)
			5, 82.2	−0.45	16[c]	(*S*)
3	Nitrilase 103	144	**2**, 4.0			
			3, 8.6	−3.8	55[c]	(*R*)
			5, 85.3	−0.42	15[c]	(*S*)
4	Nitrilase 105	144	**2**, 80.0			
			3, 7.4	−2.6	36[c]	(*R*)
5	Nitrilase 106	144	**2**, 17.3			
			3, 10.8	+7.2	>99[b]	(*S*)
			5, 51.0	−2.0	70[b]	(*S*)
6	Nitrilase 107	144	**2**, 78.0			
			3, 3.7	−3.0	42[c]	(*R*)
7	Nitrilase 108	72	**2**, 16.0			
			3, 18.7	−6.9	96[c]	(*R*)
			5, 70.7	−1.7	60[c]	(*S*)
8	Whole cells[d]	144	**2**, 75.0			
			3, 11.1	−2.8	39[c]	(*R*)

[a] In $D_2O + CD_3COCD_3$ (*c* 1).
[b] Determined by chiral HPLC.
[c] Enantiomeric excess, determined by comparison of $[\alpha]_D$ values.
[d] *Rhodococcus erythropolis* NCIMB 11540 (freeze dried for the cases, in which ee's were determined by chiral HPLC cells).

99%) albeit in yields of 18.7% and 10.8%, respectively (entries 5 and 7). Since compound **3** proved to be crystalline, we were able to perform X-ray analysis and determine its absolute configuration as (+)-(*S*) (Fig. 2). The highest ee value of monoacid **5** was 70% (entry 5). Since **5** appeared to be an oil and, moreover, was never obtained in a fully enantiopure form, it was more difficult to determine its absolute configuration. Therefore, we decided to measure the CD spectra of amides (+)-(*S*)-**3** and (−)-(*R*)-**3** and of acid (−)-**5**, assuming that comparison of the Cotton effects exhibited by each sample would allow us to determine the absolute configuration of the acid. This assumption seemed justifiable because the stereogenic phosphorus atom is in both products bound to three identical substituents, the fourth one being only slightly different at a distant position (CH_2CO–N vs CH_2CO–O). The CD spectra are shown in Figure 1.

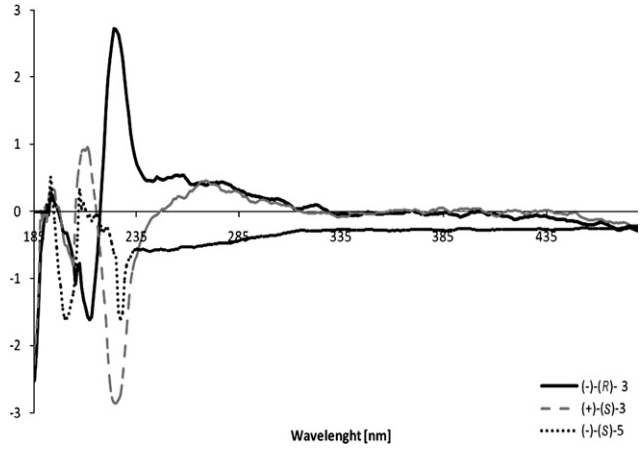


Figure 1. The CD spectra of compounds **3** and **5**.

The monoamide (+)-(*S*)-**3** exhibits two strong Cotton effects—a positive one at 218 nm and a negative one at 223 nm. Obviously, the enantiomeric monoamide (−)-(*R*)-**3** exhibits the Cotton effects that are mirror images of the above. In turn, the monoacid (−)-**5** exhibits two Cotton effects which are weaker than those of the amides. Since the shapes of the curves and the signs of the Cotton effects of (+)-(*S*)-**3** and (−)-**5** are similar, it is reasonable to assume that the absolute configuration of the monoacid is (−)-(*S*).

As far as the mechanism of the above desymmetrisation is concerned, two courses of the reaction must be considered: in the first one, monoamide **3** and monoacid **5** are formed concurrently; in the second one the amide is formed first and then subsequently hydrolysed to the acid. Identical absolute configurations of both products obtained in the same reaction (Table 1, entry 5) undoubtedly speak in favour of the first assumption. The cases in which the amide and the acid formed in the same reaction have opposite absolute configurations (Table 1, remaining entries) may suggest that the real stereorecognition is a result of a kinetic resolution at the stage of the hydrolysis of the initially formed racemic amide. However, in the latter case the concurrent formation of both products cannot be excluded, as has been shown by Sheldon et al.,[1] who proposed a bidirectional mechanism of action of nitrilases leading to both types of products. Taking into account the fact that amides are hardly accepted as substrates by nitrilases, the bidirectional mechanism seems more reasonable.

3. X-ray analysis

The crystal structure of (+)-**3** contains two crystallographically independent molecules (A and B) in the asymmetry unit (Fig. 2).

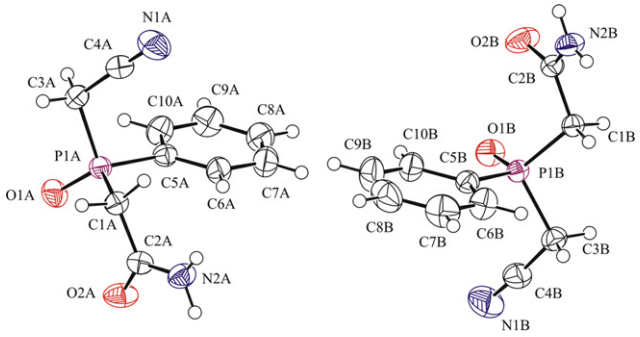


Figure 2. The molecular structure of two independent molecules A and B of (+)-**3**, showing 50% probability displacement ellipsoids and the atom-numbering scheme.

The angles about the P atom range from 103.06(8)° to 103.27(8)° (C1–P1–C3) and from 115.92(8)° to 115.92(8)

(O1–P1–C1) in molecules A and B, respectively, indicating a distorted tetrahedral environment. The absolute configuration at the atom P is (*S*) in both crystallographically independent molecules. In both molecules a significant shortening of the P1–C1 bond lengths is observed: 1.7877(17) Å for molecule A and 1.7939(16) Å for molecule B; a typical P–C single bond length is 1.816(2) Å.

4. Conclusions

Enzymatic hydrolysis of prochiral bis(cyanomethyl)phenylphosphine oxide was achieved for the first time using a broad spectrum of nitrile-converting enzymes under mild conditions (buffer solution of pH 7.2, 30 °C). Two products were formed, cyanomethylphenyl-phosphinylacetamide and cyanomethylphenyl-phosphinylacetic acid, in different proportions and various enantioselectivity ranging from 15% to 99% ee. The absolute configurations of the products have also determined.

5. Experimental

5.1. General

The enzymes were purchased from BioCatalytics Europe GmbH, Grambach, Austria. NMR spectra were recorded on Bruker instruments at 200 MHz with D_2O and CD_3COCD_3 as solvents. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter ($c = 1$). Column chromatography was carried out using Merck 60 silica gel. TLC was performed on Merck 60 F_{254} silica gel plates. The enantiomeric excess (ee) values were determined by chiral HPLC (Varian Pro Star 210, Chiralpak AS).

5.2. Synthesis of prochiral 2

THF (30 mL) and zinc dust (1.31 g, 0.02 mol) were placed in a 100 mL three-neck round-bottom flask equipped with a mechanical stirrer, dropping funnel and reflux condenser with a $CaCl_2$ tube. A mixture of bromoacetonitrile (2.40 g, 0.02 mol) and dichlorophenyl phosphine (1.79 g, 0.01 mol) in THF (5 mL) was slowly added to the mixture under gentle reflux. During the addition, a crystal of I_2 was added to initiate the reaction (the brown colour of iodine disappeared almost immediately). After the addition, the mixture was very vigorously stirred and refluxed for 1 h. The reaction was monitored by ^{31}P NMR and by TLC. After 1 h, the ^{31}P NMR spectrum showed one signal ($\delta = -29.4$) and the TLC plate revealed only one spot. After cooling to room temperature, water was added and the mixture was extracted with chloroform (3×20 mL). The combined organic layers were dried over anhydrous $MgSO_4$. After evaporating off $CHCl_3$ phosphine **1** was obtained in quantitative yield. This compound was dissolved in chloroform and $PhIO_2$ (1.18 g, 0.005 mol) was added to the solution. The reaction was followed by TLC and after its completion $CHCl_3$ was evaporated and the residue was separated via column chromatography (CH_2Cl_2/acetone in gradient) to obtain the desired phosphine oxide **2** (8.16 g, 40%) as a white powder, mp 136–137 °C; ^1H NMR (CD_3COCD_3): $\delta = 3.83$ (d, $J = 14.2$ Hz, 4H), 7.96–8.03 (m, 5H); ^{31}P NMR (CD_3COCD_3): $\delta = 24.4$; ^{13}C NMR (CD_3COCD_3): 20.8, 22.1, 114.41 (d), 130.8 (d), 132.6 (d), 135.3. MS (CI): *m*/*z* 205 (M+H). HRMS (CI) calcd for $C_{10}H_9N_2OP$: 204.0454; found 204.0458.

5.3. Enzymatic hydrolysis of 2—general procedure

Compound **2** (0.050 g, 0.245 mmol) was dissolved in a buffer solution and the enzyme (5 mg) was added. The reaction was shaken at 30 °C for 72–144 h (see Table 1) and monitored by TLC. Water was then evaporated and the residue was separated using column chromatography (chloroform/methanol in gradient) to yield the corresponding products **3** and **5**. The yields and specific rotations are shown in Table 1.

5.3.1. Cyanomethylphenylphosphinylacetamide 3. White crystals (MeCN), mp = 183–185 °C; ^1H NMR ($D_2O + CD_3COCD_3$): $\delta = 3.63$ (AB, 2H), 3.86 (AB, 2H), 7.75–7.84 (m, 5H); ^{31}P NMR ($D_2O + CD_3COCD_3$): $\delta = 33.9$; ^{13}C NMR ($D_2O + CD_3COCD_3$): 18.03, 38.80, 117, 133.4, 169.06. MS (CI): *m*/*z* 223 (M+H). HRMS (CI) calcd for $C_{10}H_{11}N_2O_2P$: 222.0556; found 222.0553.

5.3.2. Cyanomethylphenylphosphinylacetic acid 5. Yellow oil; ^1H NMR ($D_2O + CD_3COCD_3$): $\delta = 2.93$ (AB, 2H), 3.06 (AB, 2H), 7.47–7.82 (m, 5H); ^{31}P NMR ($D_2O + CD_3COCD_3$): $\delta = 17.8$; ^{13}C NMR ($D_2O + CD_3COCD_3$): 21.46, 31.80, 118.45, 129.3, 133.8, 179.4. MS (CI): *m*/*z* 224 (M+H), 180 (M−CO_2).

6. Crystallographic data

Molecular formula: $C_{10}H_{11}N_2O_2P$. Formula weight: $M_r = 222.18$. Crystallographic system: monoclinic. Space group: $P2_1$; $a = 13.457(3)$ Å, $b = 5.7298(11)$ Å, $c = 13.966(3)$ Å, $\alpha = 90.00°$, $\beta = 91.49(3)°$, $V = 1076.5(4)$ Å^3, $Z = 4$, $D_x = 1.371$ Mg m^{-3}. Mo Kα radiation. Cell parameters from 5646 reflections: $\theta = 2.9$–$31.4°$, $\mu = 0.24$ mm^{-1}, $T = 295(2)$ K.

Crystallographic data for (+)-(*S*)-**3** have been deposited with the Cambridge Crystallographic Data Center No. CCDC 654477 and can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: (internat.) +44-1223/336-033; e-mail: mailto: deposit@ccdc.cam.ac.uk].

Acknowledgements

The work was carried out under the Polish–Dutch Joint Research Project: 'Dynamic kinetic resolution and desymmetrisation of sulfur and phosphorus compounds using enzymes'. Financial support from the Ministry of Science and Information Society Technologies (Poland), Grant No. 3 T09A 166 27 for P.K., is gratefully acknowledged.

References

1. Fernandes, B. C. M.; Mateo, C.; Kiziak, C.; Chmura, A.; Wacker, J.; van Rantwijk, F.; Stolz, A.; Sheldon, R. A. *Adv. Synth. Catal.* **2006**, *348*, 2597–2603.
2. Kiełbasiński, P.; Rachwalski, M.; Mikołajczyk, M.; Szyrej, M.; Wieczorek, M. W.; Wijtmans, R.; Rutjes, F. P. J. T. *Adv. Synth. Catal.* **2007**, *349*, 1387–1392.
3. Kubac, D.; Cejkova, A.; Masak, J.; Jirku, V.; Lemaire, M.; Gallienne, E.; Bolte, J.; Stloukal, R.; Martinkova, L. *J. Mol. Catal. B: Enzym.* **2006**, *39*, 59–61.
4. Wang, M.-X.; Liu, C.-S.; Li, J.-S. *Tetrahedron: Asymmetry* **2001**, *12*, 3367–3373.
5. *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 2, pp 699–716.
6. Faber, K. *Biotransformations in Organic Chemistry*, 4th ed.; Springer: Berlin, 2004, pp 149–159.
7. (a) Effenberger, F.; Osswald, S. *Synthesis* **2001**, 1866–1872; (b) Zhu, D.; Mukherjee, C.; Biehl, E. R.; Huaa, L. *Adv. Synth. Catal.* **2007**, *349*, 1667–1670.
8. Kiełbasiński, P.; Żurawiński, R.; Albrycht, M.; Mikołajczyk, M. *Tetrahedron: Asymmetry* **2003**, *14*, 3379–3384.
9. Kiełbasiński, P.; Mikołajczyk, M. In *Enzymes in Action: Green Solutions for Chemical Problems*; Zwanenburg, B., Mikołajczyk, M., Kiełbasiński, P., Eds.; Kluwer: Dordrecht, 2000; pp 161–191.
10. Mikołajczyk, M.; Kiełbasiński, P.; Żurawiński, R.; Wieczorek, M. W.; Błaszczyk, J. *Synlett* **1994**, 127–129.
11. Kiełbasiński, P.; Rachwalski, M.; Mikołajczyk, M.; Moelands, M. A. H.; Zwanenburg, B.; Rutjes, F. P. J. T. *Tetrahedron: Asymmetry* **2005**, *16*, 2157–2160.
12. Kiełbasiński, P.; Mikołajczyk, M. *Synthesis* **1995**, 144–146.
13. Dahl, O. *Acta Chem. Scand. B* **1976**, *30*, 799–800.
14. Dahl, O.; Henriksen, U.; Trebbien, C. *Acta Chem. Scand. B* **1983**, *37*, 639–642.
15. Vink, M. K. S.; Wijtmans, R.; Reisinger, C.; van den Berg, R. J. F.; Schortinghuis, C. A.; Schwab, H. A.; Schoemaker, H. E.; Rutjes, F. P. J. T. *Biotechnol. J.* **2006**, *1*, 569–573.