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Nitrilase-catalysed hydrolysis of cyanomethyl *p*-tolyl sulfoxide: stereochemistry and mechanism

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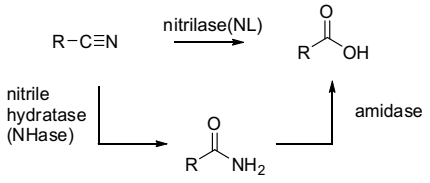
Abstract—Several commercially available nitrilases have been used for the enantioselective hydrolysis of cyanomethyl *p*-tolyl sulfoxide into the corresponding amide and acid, which are formed in different proportions and with varying stereoselectivities, depending on the nitrilase involved. It was shown that the externally added amide is not transformed into the acid, which can be explained by assuming that both products must be produced in concurrent reactions. It was also demonstrated that the absolute configuration of the substrate exerts substantial influence on the product ratio. Two alternative explanations of the stereochemical course are presented.

1. Introduction

Nitriles constitute as an important and versatile class of intermediates for synthetic chemistry.[1] They are synthetically particularly useful and, hence, widely used for the preparation of various carboxylic acids and amine derivatives. However, their chemical transformation into the corresponding carboxamides and carboxylic acids or esters usually requires the use of strong bases, acids or heavy metal salts. Therefore, an alternative approach to achieve this, which rests upon the use of nitrile hydrolysing enzymes, has been a fast developing area of research in recent years.[2–7]

It is generally accepted that the enzyme-catalysed hydrolysis of nitriles follows one of the two pathways (Scheme 1).[8] In the first pathway, a nitrilase (NL) converts the nitrile directly into the carboxylic acid, via addition of two molecules of water. In the second one, a nitrile hydratase (NHase) catalyses single hydration of the nitrile to give the corresponding amide, which is followed by amidase-catalysed hydrolysis to the appropriate acid. Thus, according to this scheme, the latter pathway requires the simultaneous presence of two different enzymes, which can be achieved using microorganisms or whole cell techniques.



Scheme 1. Pathways of enzyme-catalysed nitrile hydrolysis.

This simple picture has recently turned out to be insufficient for explaining new findings. For example, it was reported that certain nitrilases, besides their anticipated behaviour, exhibited NHase activity, which resulted in the formation of both the corresponding amides and acids.[9–15] In principle, such results could be explained by invoking the second pathway. However, on the basis of common observations that nitrilases do not hydrolyse carboxamides,[13] the assumption was made that both products of the hydrolysis were formed in parallel reactions. A plausible mechanism, which could account for such results, was proposed by Sheldon et al.[13]

Our previous work on the deracemisation of racemic and desymmetrisation of prochiral heteroorganic compounds led to efficient approaches for the synthesis of a variety

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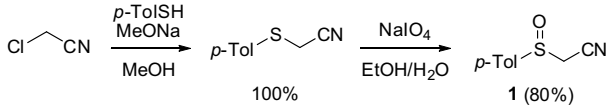
of optically active heteroatom derivatives.[14–18] In particular, our results on the enzymatic desymmetrisation of bis(cyanomethyl) sulfoxide[14] and bis(cyanomethyl)phenylphosphine oxide[15] represented the first examples of a nitrilase-catalysed stereoselective hydrolysis of dinitriles containing prostereogenic centres located on a heteroatom—the sulfinyl sulfur and phosphinyl phosphorus, respectively. In these cases, the hydrolysis also led to two out of five possible products, namely the corresponding monoamide and monoacid, formed in different proportions and with varying enantiomeric excesses, depending on the kind of the nitrilase. Interestingly, in certain cases the absolute configurations of the amide and acid produced in the same reaction were identical, while in others they were opposite. The former would substantiate the assumption that both products were formed in a concurrent reaction (as proposed by Sheldon et al.),[13] while the latter would suggest that the amide was first formed non-stereoselectively and then stereoselectively hydrolysed to the acid under kinetically controlled resolution conditions.

The use of prochiral substrates did not allow us to distinguish the two possible pathways employed by the enzymes. First of all, it was not possible to determine whether the first step leading to the amide was stereoselective, since the unreacted substrate was not chiral. Additionally, it appeared rather difficult to synthesise a racemic monoamide for subjection to hydrolysis by a nitrilase. In order to gain better insight into the hydrolysis mechanism, we decided to investigate the nitrilase-catalysed kinetic resolution of a racemic cyanomethyl sulfoxide since this would provide more information about the stereochemistry of the reaction. As a model substrate, the easily available racemic cyanomethyl *p*-tolyl sulfoxide **1** was chosen.

2. Results and discussion

2.1. Synthesis of substrates

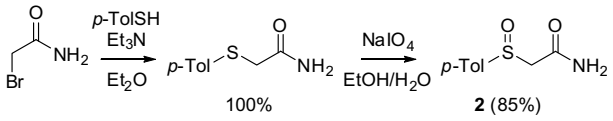
2.1.1. Synthesis of racemic and enantiomeric cyanomethyl *p*-tolyl sulfoxides. The starting material, racemic cyanomethyl *p*-tolyl sulfoxide, **1**, was synthesised in a two-step procedure starting from chloroacetonitrile, which on subjection to *p*-toluenethiol in the presence of sodium methoxide in methanol provided cyanomethyl *p*-tolyl sulfide in quantitative yield. The latter was oxidised with sodium periodate in a 1:1 mixture of water and ethanol to give the desired sulfoxide **1** in 80% yield after purification (Scheme 2).



Scheme 2. Synthesis of racemic sulfoxide **1**.

The enantiopure compounds (+)-(*R*)-**1** and (−)-(*S*)-**1** were synthesised using a method described by Hiroi and Umemura.[19]

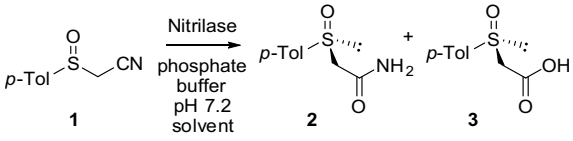
2.1.2. Synthesis of racemic *p*-toluenesulfinylacetamide 2. Racemic **2** was synthesised via a two-step method from bromoacetamide, which upon treatment with *p*-toluenethiol in the presence of triethylamine in Et_2O, provided the corresponding sulfide in quantitative yield. The latter was oxidised with sodium periodate in a 1:1 mixture of water and ethanol to give the desired racemic amide **2** in 85% yield after purification (Scheme 3).



Scheme 3. Synthesis of racemic amide **2**.

2.2. Kinetic resolution of racemic cyanomethyl *p*-tolyl sulfoxide 1

The racemic sulfoxide **1** was subjected to hydrolysis in a phosphate buffer and a co-solvent to dissolve the substrate, by a variety of nitrilases under kinetic resolution conditions. The hydrolysis should in principle give rise to two products: *p*-toluenesulfinylacetamide **2** and *p*-toluenesulfinylacetic acid **3**, together with (probably) enantiomerically enriched starting material **1** (Scheme 4, absolute configurations of **2** and **3** are arbitrarily chosen). The products were purified by column chromatography and analysed spectroscopically. The results are shown in Table 1.



Scheme 4. Enzymatic hydrolysis of sulfoxide **1**.

The enantiomeric excesses of acid **3** were calculated by comparing the $[\alpha]_D$ value of a given sample with that reported in the chemical literature,[20] while the enantiomeric excesses of unreacted nitrile **1** and amide **2** were determined by chiral HPLC. The absolute configurations of **1** and **3** are known from the literature to be (+)-(*R*).[19,20] To determine the absolute configuration of amide **2**, CD spectra of (+)-(*R*)-**1**, (+)-(*R*)-**3** and of (+)- and (−)-**2** were measured as shown in Figure 1.

It is justifiable to assume that the comparison of the Cotton effects exhibited by each sample allows to ascribe an absolute configuration to amide **2**, since the stereogenic sulfur atom in all the compounds is bound to three identical substituents, and the fourth is only slightly different at a distant position. As can be seen in Figure 1, the Cotton effects exhibited by the laevorotatory amide (−)-**2** and the laevorotatory acid (−)-(*S*)-**3** are almost identical. Furthermore, the Cotton effects exhibited by the dextrorotatory amide (+)-**2** and the dextrorotatory nitrile (+)-(*R*)-**1** are identical as well. Hence, it seems reasonable to assign the absolute configuration of amide **2** as being (+)-(*R*).