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Substrate Evaluation of *Rhodococcus erythropolis* SET1, a Nitrile Hydrolysing Bacterium, Demonstrating Dual Activity Strongly Dependent on Nitrile Sub-Structure

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Assessment of *Rhodococcus erythropolis* SET1, a novel nitrile hydrolysing bacterial isolate, has been undertaken with 34 nitriles, 33 chiral and 1 prochiral. These substrates consist primarily of β-hydroxy nitriles with varying alkyl and aryl groups at the β position and containing in several compounds different substituents α to the nitrile. In the case of β-hydroxy nitriles without substitution at the α position, acids were the major products obtained, along with recovered nitrile after biotransformation, as a result of suspected nitrilase activity of the isolate. Unexpectedly, amides were found to be the major hydrolysis product when the β-hydroxy nitriles possessed a vinyl group at this position. To probe this behaviour further, additional related substrates were evaluated containing electron-withdrawing groups at the α position, and amide was also observed upon biotransformation in the presence of SET1. Therefore this novel isolate has also demonstrated NHase activity with nitriles that appears to be substrate-dependent.

Introduction

It is generally accepted that nature employs two enzymatic pathways for nitrile hydrolysis, either by direct conversion from a nitrile to a carboxylic acid by nitrilase enzymes[1] or by the hydration of a nitrile followed by hydrolysis of the intermediate amide formed by a combination of nitrile hydratase/amidase.[2] The pharmaceutical industry has considerable interest in the use of nitrile metabolising enzymes for the chemo-, regio- or enantioselective production of carboxylic acids and amides from nitriles under mild conditions.[2,3] In particular, β-hydroxy nitriles, such as 3-hydroxybutyronitrile, 3-hydroxyglutaronitrile and 3-hydroxy-3-phenylpropionitriles, can act as sources of key chiral β-hydroxy carboxylic acids through such hydrolysis reactions. Under classical acid and base conditions, elimination reactions can compete with hydrolysis.[4] Alternatively, nitrile biocatalysis can often selectively facilitate this conversion without affecting other acid- or alkali-labile functional groups present.[5]

Nitrilases, in particular, are found to be inducible enzymes composed of one or two types of subunits of different size and number.[6] Many consist of a single polypeptide with a molecular mass of approximately 40 kDa that aggregates to form the active enzyme. The preferred form of nitrilase enzymes appears to be a large aggregate of 6–26 subunits.[1] The structure of the nitrilase protein is thought to be a novel α-β-β-α sandwich fold with a triad of residues, Glu-Lys-Cys, that is essential for the function of its active site.[7] The substrate scope of a wide range of purified nitrilases has been determined, and it has been found that although most exhibit their highest activity with aromatic nitriles,[8] some nitrilases have a preference for aliphatic nitriles.[9]

The hydrolysis of nitriles under the influence of the nitrilase enzyme does not typically lead to amide as an intermediate, however, this has been detected in some cases, in varying quantities, usually depending on the structure of the nitrile substrate.[10] For example, the nitrilase from *Rhodococcus* sp. ATCC 39484 produced amide during the conversion of phenylacetonitrile,[10g] and the two nitrilases AtNit1 and AtNit4 from *Arabidopsis thaliana* also generated amide as the product with the quantity and enantioselectivity found to be strongly dependent on the nature of the substituent at the α position of the nitrile substrate.[10a] Amides were found to be the major products in ZmNIT2 nitrilase-catalysed hydrolysis of β-hydroxy nitriles.[10e] In addition, during the hydrolysis of mandelonitrile in the presence of PfNLase, the stereochemical configuration of the nitrile was found to exert a major influence on the product profile.[10b] The filamentous fungus *Aspergillus niger* K10 demonstrated broad specificity towards a range of aromatic and aliphatic nitriles, and a high amide/acid ratio or amide only was generated in several cases.[8e,11]

We recently reported a high-throughput bacterial isolate screening strategy that resulted in the identification of a novel nitrile metabolising strain, *Rhodococcus erythropolis* SET1, active towards β-hydroxy nitriles.[12] This organism

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catalysed the hydrolysis of (±)-3-hydroxybutyronitrile with remarkably high enantioselectivity to afford (*S*)-(+)-3-hydroxybutyric acid in 42% yield and >99.9% *ee*. We wished to study the substrate promiscuity of this isolate with a collection of related nitriles with diverse structures and hence examined the effect of varying the alkyl or aryl group β to the nitrile, the substituent present on the hydroxy functionality and the remote proximity of the chiral centre to the cyano group. The results of these studies are reported herein.

Results and Discussion

Biotransformations of Racemic β-Hydroxyalkane and β-Hydroxyaromatic Nitriles (±)-1a–14a

Applying the conditions previously reported in the literature, the (±)-β-hydroxyalkane nitriles **1a**–**4a** were prepared by condensation of acetonitrile with the required aldehyde (Figure 1). In addition, β-aromatic derivatives (±)-**6a**–**10a** were synthesised by $NaBH_4$[3,13] reduction of the corresponding commercially available ketone.[5a] The nitrile hydrolysing activity of SET1 was induced in M9 minimal media containing 3-hydroxybutyronitrile as the sole source of N.[12] By using whole cells of SET1, the biotransformation

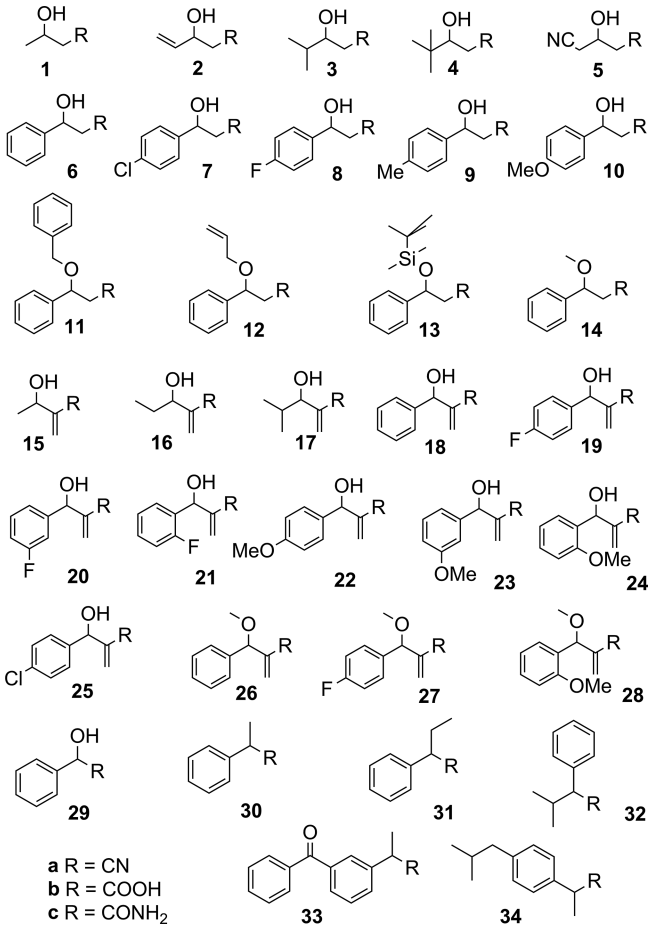


Figure 1. The 34 nitrile substrates evaluated in this study.

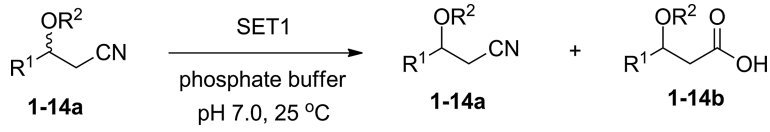
was allowed to occur for 24 to 72 h depending on the substrate under evaluation, periodically monitoring by TLC or HPLC to determine the completion of the reaction. Product *ee* values were determined by HPLC employing a chiral column with analysis in triplicate. The absolute configurations of the products and recovered starting materials were assigned by comparison of our HPLC chromatograms and the specific optical rotations of the purified compounds with those reported in the literature.[3,5a,14–16]

The substrate 3-hydroxybutyronitrile [(±)-**1a**] was hydrolysed with optimum enantioselectivity affording (*S*)-(+)-3-hydroxybutyric acid (**1b**) in 42% yield and >99.9% *ee* (Table 1, entry 1).[12] Despite repeated efforts, unreacted nitrile could not be recovered from the reaction mixture in this case. The preferred temperature for this biotransformation was determined to be 25 °C, above which, although activity was conserved, there was a sharp decrease in enantioselectivity (previously reported results along with additional data provided in the Supporting Information). This temperature was employed for all other biotransformations. In contrast to the results for (±)-**1a**, significantly lower *ee* values were obtained for the acid product of the β-hydroxyalkane nitriles (±)-**2a** (Table 1, entry 2), with degradation of the nitriles (±)-**3a** and -**4a** observed (Table 1, entries 3 and 4). The desymmetrisation of the prochiral 3-hydroxyglutaronitrile (**5a**) was more successful with the acid (*S*)-(+)-**5b** obtained in 67% yield, albeit with a moderate *ee* of 32% (Table 1, entry 5).

It was noted that a longer reaction time of 5–7 d was required for the hydrolysis of β-hydroxyaromatic nitriles (±)-**6a**–**10a** than for their β-hydroxyalkane counterparts (±)-**1a**–**5a**. This can be clearly observed by the difference in the reaction time of 120 h required for the hydrolysis of 3-hydroxy-3-phenylpropionitrile [(±)-**6a**; Table 1, entry 6] compared with 24 h for 3-hydroxybutyronitrile [(±)-**1a**]. Similarly to biotransformations carried out with these -hydroxyaromatic nitriles and other nitrilases, acid was obtained as the product of reaction, it must be noted that in the case of ZmNit2 amide was formed.[10e] In addition, considerably lower yields and *ee* values were obtained with these β-hydroxyaromatic substrates, with the optimum result obtained with the *p*-fluorophenyl β-substituted nitrile (±)-**8a** (Table 1, entry 8), which gave a yield of 42.0% of acid (*S*)-(–)-**8b** with 19.8% *ee* and a recovery of 35.2% of nitrile (*R*)-(+)-**8a** with an *ee* of 23.2%. These results correlate with some previous reports on nitrilase enzymes, which also exhibited low enantioselectivity for such β-hydroxyaromatic nitriles.[17]

The substituent on the benzene ring of the β-hydroxyaromatic nitriles (±)-**6a**–**10a** also appeared to exert some influence on the enantioselectivity and yield. For example, with the electron-withdrawing *p*-fluoro group in (±)-**8a** (Table 1, entry 8), the enantioselectivity of the acid (*S*)-(–)-**8b** produced improved to 19.8% as did the yield to 42% in comparison with the results obtained with the unsubstituted phenyl in (±)-**6a** (Table 1, entry 6). The increase in the *ee* also held for the (*S*)-acid generated from the biotransformation of the *p*-chloro substituted nitrile (±)-**7a** (Table 1, en-

Table 1. Results of the biotransformation of racemic β-hydroxyalkane and β-hydroxyaromatic nitriles **1a**–**10a** and docked analogues **11a**–**14a**.[a]



Entry	Substrate	R^1	R^2	Time [h]	Recovered nitrile **a**		Product acid **b**	
					Yield [%][b]	*ee* [%][c]	Yield %[b]	*ee* [%][c]
1[d]	**1a**	CH_3	H	24	n.d.	n.d.	42.3	(*S*)-(+) > 99.9
2	**2a**	CH_2CH	H	48	50.1	1.4	49.1	1.20
3	**3a**	$CH(CH_3)_2$	H	72	66.4	3.0	n.d.	n.d.
4	**4a**	$C(CH_3)_3$	H	72	72.2	1.8	n.d.	n.d.
5[d]	**5a**	CH_2CN	H	48	22.3	–	67.0	(*S*)-(+)-32.0
6	**6a**	C_6H_5	H	120	80.0	(*S*)-(–)-5.0	19.3	(*R*)-(+)-7.0
7	**7a**	4-ClC_6H_4	H	120	74.1	(*R*)-(+)-8.7	20.1	(*S*)-(–)-15.8
8	**8a**	4-FC_6H_4	H	120	35.2	(*R*)-(+)-23.2	42.0	(*S*)-(–)-19.8
9	**9a**	4-MeC_6H_4	H	120	90.0	(*S*)-(+)-7.4	n.d.	n.d.
10	**10a**	4-$MeOC_6H_4$	H	120	55.1	(*S*)-(–)-21.5	28.2	(*R*)-(+)-7.4
11	**11a**	C_6H_5	Bn	168	91.3	2.1	n.d.	n.d.
12	**12a**	C_6H_5	Allyl	120	97.1	3.4	n.d.	n.d.
13	**13a**	C_6H_5	TBDMS	168	96.2	1.2	n.d.	n.d.
14	**14a**	C_6H_5	Me	120	86.1	(*S*)-(–)-4.2	14.0	(*R*)-(+)-99.9

[a] The biotransformations were carried out by incubating the nitrile (10 mmol/L) in a suspension of *Rhodococcus erythropolis* SET1 (OD_{600nm} = 1) in phosphate buffer (pH 7.0) at 25 °C. [b] Isolated yield. [c] Determined by HPLC analysis using a chiral column (see the Exp. Sect.). The configuration was determined by comparison of HPLC traces and optical rotations with the literature.[5a,21] n.d.: not detected. [d] Chiral HPLC analysis performed on the corresponding benzyl ether.

try 7), but an improvement in yield was not observed. In contrast, this trend was not observed with electron-donating substituents on the phenyl ring of the β-hydroxyaromatic nitrile, as with (±)-**9a** and (±)-**10a** (Table 1, entries 9 and 10), although the recovered nitrile (*S*)-(–)-**10a**, in particular, appeared to be enantioenriched after the biotransformation with an *ee* of 21.5% obtained.

Enhanced chiral recognition and improved enantioselectivity were observed previously for the NHase/amidase biotransformation employing AJ270 upon the addition of a removable docking group onto the free hydroxy moiety of 3-hydroxy-3-phenylpropionitrile [(±)-**6a**].[18] Although this strategy was employed for NHase/amidase-catalysed hydrolysis, the effect of such a docking group in the substrate during hydrolysis in the presence of nitrilase enzyme was investigated. Thus, to improve the *ee* of the acid (*R*)-(+)-**6b** obtained upon biotransformation of the corresponding nitrile and possibly the related analogues discussed above, the protected benzyloxy [(±)-**11a**], allyloxy [(±)-**12a**], trimethylsilyloxy [(±)-**13a**] and methoxy [(±)-**14a**] derivatives were prepared from (±)-**6a** by using previously reported procedures.[19]

Introduction of a methyl group, in particular, onto the free hydroxy of the nitrile in (±)-**14a** resulted in a dramatic increase in the enantioselectivity upon biotransformation. Although 7% *ee* of the acid (*R*)-(+)-**6b** was obtained from the biotransformation of the unprotected substrate (±)-**6a** in 19% yield (Table 1, entry 6), <99.9% *ee* of the acid (*R*)-(–)-**14b** was produced, albeit in the slightly lower yield of 14% (Table 1, entry 14). This 14-fold increase in *ee* may be due to the improved fit of the β-aromatic substrate, with the β-methoxy docking moiety enhancing the chiral recognition of this substrate by the enzyme, leading to a more enantioselective biotransformation.[20] It appears that the size of the docking group also has an impact on the *ee* values. For example, when a more sterically demanding group was present, as in the case of protected substrates (±)-**11a**–**13a**, no acid was detected and racemic starting nitrile was recovered in almost quantitative yield.

In summary, for this series of substrates (±)-**1a**–**14a**, the presence of an alkyl or aryl substituent at the β-position as well as a free or protected β-hydroxy moiety significantly influenced the outcome of the biotransformation reaction. It is also of note that for all the nitriles (±)-**1a**–**14a**, the acid was produced as the reaction product along with remaining nitrile with no amide detected or recovered. This suggests catalysis of the hydrolysis reaction by a nitrilase enzyme within the whole cell of SET1.

Biotransformations of Racemic β-Hydroxyalkane and β-Hydroxyaromatic α-Methylene Nitriles 15a–28a

To further demonstrate the application of *R. erythropolis* SET1 in nitrile biotransformations, we examined the biotransformation of a variety of α-methylene β-hydroxy-functionalised nitriles. Such synthetically useful Baylis–Hillman adducts often demonstrate anti-bacterial, anti-tumour and antifungal activity.[16b]

The starting nitriles (±)-**15a**–**28a** were prepared by the DABCO-catalysed reaction of acrylonitrile with substituted

aliphatic or aromatic aldehydes.[22] The induction, biotransformation and analytical conditions were as reported in the previous section.

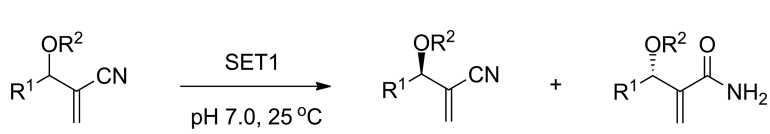
Interestingly, SET1 influenced the hydrolysis of this series of nitriles, and in all cases under the biotransformation conditions amides were produced with good-to-excellent *ee* values and yields in some cases (Table 2, entries 1, 5 and 10). This is in sharp contrast to the previous behaviour observed with this isolate when acids were the products obtained from the reaction along with recovered nitrile. This can be exemplified by comparing the results obtained from the biotransformation of substrates 3-hydroxy-2-methylenebutanenitrile [(±)-**15a**] and 3-hydroxybutyronitrile [(±)-**1a**]. In the latter case, the acid (*S*)-(+)-**1b** was formed with >99.9% *ee* in 42% yield from **1a** (Table 1, entry 1), whereas upon the introduction of the vinyl functionality at the α position in (±)-**15a**, the amide (*S**)-**15c** was obtained in 43.2% yield and with 87.8% *ee* (Table 2, entry 1). Unreacted nitrile (*R**)-**15a** was also recovered after biotransformation in 42% yield and with 76.5% *ee*.

In addition to amide formation, some of the β-hydroxyalkane α-methylene substrates, for example, (±)-**17a**, proved more stable/tolerant of the reaction media than their α-unsubstituted analogues. In the case of (±)-**17a** compared with (±)-**3a** (Table 2, entry 3 vs. Table 1, entry 3), a significant yield of 44% of the amide product was isolated with a moderate *ee* of 16.1%, whereas no acid product was obtained in the biotransformation of (±)-**3a** with reduced recovery of the starting nitrile, potentially due to degradation by the microbial whole cell.

As was the case for β-hydroxyalkane nitriles (±)-**1a**–**5a** when compared with their aromatic counterparts, the biotransformation reactions were observed to be complete in shorter times for β-hydroxyalkane α-methylene nitriles (±)-**15a**–**17a** than for their β-hydroxyaromatic α-methylene analogues (±)-**18a**–**25a** (48–72 h vs. 120 h). In addition, a lower *ee* of 42.3% of the amide product (*S*)-(+)-**18c** along with an almost 50% reduction in its yield was observed for the biotransformation of the β-hydroxyaromatic α-methylene nitrile (±)-**18a** (Table 2, entry 4) when compared with its β-hydroxyalkane α-methylene analogue (±)-**15a** from which amide (*S**)-**15c** was obtained with 87.8% *ee* (Table 2, entry 1) and in 43.2% yield. Similar reductions in yield and *ee* were observed for the acid products isolated after the biotransformation reactions when comparing within the series the results of α-unsubstituted β-hydroxyalkane and β-hydroxyaromatic nitriles in Table 1.

It was also observed that higher *ee* values of the amides were obtained when the aromatic ring in the β-hydroxyaromatic α-methylene nitrile contained the electron-withdrawing F group at the *para* position of the β-aromatic ring, as in (±)-**19a** (Table 2, entry 5), and the electron-donating OMe group at the *ortho* position of the aromatic ring, as in (±)-**24a** (Table 2, entry 10), rather than at alternative positions around the ring. In particular, when such groups were present at the *meta* position of the ring, amides were produced with significantly lower *ee* values [a 10-fold reduction in the case of the amide produced from *m*-fluoro-substituted nitrile (±)-**20a** vs. *p*-fluoro-substituted nitrile (±)-**19a**]. Somewhat surprisingly, the recovered nitrile appeared to be more enantioenriched when it was *meta*-substituted, with nitriles **20a** and **23a** recovered with 46.0 and 38.9% *ee*, respectively (Table 2, entries 6 and 9). The *p*-chloro analogue (±)-**25a** did not undergo the biotransformation to

Table 2. Results of the biotransformation of racemic β-hydroxyalkane α-methylene and β-hydroxyaromatic α-methylene nitriles **15a**–**25a** and docked analogues **26a**–**28a**.[a]



15-28a **15-28a** **15-28c**

Entry	Substrate	R^1	R^2	Time [h]	Recovered nitrile **a**		Product amide **c**	
					Yield [%][b]	*ee* [%][c]	Yield [%][b]	*ee* [%][c]
1	**15a**	CH_3	H	48	42.0	(*R**)-76.5	43.2	(*S**)-87.8
2	**16a**	CH_3CH_2	H	72	69.3	4.2	22.0	(*S*)-(+)-10.8
3	**17a**	$CH(CH_3)_2$	H	72	42.2	(*R**)-12.0	44.1	(*S*)-(+)-16.1
4	**18a**	C_6H_5	H	120	72.4	(*R**)-18.7	22.2	(*S*)-(+)-42.3
5	**19a**	4-F-C_6H_5	H	120	61.3	(*R**)-22.5	30.2	(*S**)-50.4
6	**20a**	3-F-C_6H_5	H	120	43.2	(*R**)-46.0	30.1	(*S**)-4.9
7	**21a**	2-F-C_6H_5	H	120	69.5	(*R**)-14.3	26.4	(*S**)-38.4
8	**22a**	4-MeO-C_6H_5	H	120	64.6	(*R**)-10.8	28.5	(*S*)-(+)-32.1
9	**23a**	3-MeO-C_6H_5	H	120	45.2	(*R**)-38.9	27.3	(*S*)-(+)-11.6
10	**24a**	2-MeO-C_6H_5	H	120	82.3	3.8	12.8	(*S*)-(+)-60.5
11	**25a**	4-Cl-C_6H_5	H	120	97.0	2.8	n.d.	n.d.
12	**26a**	C_6H_5	CH_3	120	58.6	8.2	27.1	(*S*)-(+)-33.0
13	**27a**	4-F-C_6H_5	CH_3	120	60.8	(*R*)-10.3	22.0	(*S**)-26.4
14	**28a**	2-MeO-C_6H_5	CH_3	120	76.3	1.5	12.2	(*S**)-31.3

[a] The biotransformations were carried out by incubating the substrate (10 mmol/L) in a suspension of *Rhodococcus erythropolis* SET1 (OD = 1) in phosphate buffer (pH 7.0) at 25 °C. [b] Isolated yield determined by using flash chromatography. [c] Determined by HPLC analysis using a chiral column. The configurations were determined by comparison of HPLC traces and optical rotations with the literature when available. Tentative assignments are denoted by the use of *; n.d.: not detected.

any great extent (Table 2, entry 11), with the starting material recovered in quantitative yield.

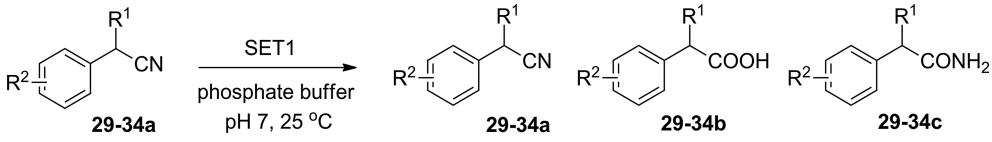
Comparison of the reactions of β-hydroxyaromatic α-methylene nitriles (±)-**19a**–**24a** with their α-unsubstituted aromatic analogues (±)-**6a**–**10a** revealed no significant increase in yield of the hydrolysed product when the vinyl group was introduced. For example, the yield of amide (*S*)-(+)-**18c** obtained after purification was 22.2% (Table 2, entry 4), whereas that of the acid (*R*)-(+)-**6b** was 19.3% (Table 1, entry 6). However, more significantly, a six-fold increase in *ee* was observed for the amide (*S*)-(+)-**18c** when compared with the *ee* of the acid product (*R*)-(+)-**6b** produced in the absence of the vinyl group at the α position. Such increases in *ee* were also observed for nitriles with *p*-fluoro- and *p*-methoxy-substituted phenyl rings at the β-position along with vinyl groups at the α position in comparison with their α-unsubstituted counterparts [nitriles (±)-**19a** vs. (±)-**8a**, Table 2 entry 5 vs. Table 1, entry 8 as an example].

To assess the role of the hydroxy group in the biotransformation of the β-hydroxy α-methylene nitrile substrates, it was protected by methylation as described previously. Here, however, the *ee* of the amide product appeared to decrease upon protection. The *ee* of the (*S*)-amide product obtained decreased from 42.3% (Table 2, entry 4) for the reaction of the unprotected nitrile (±)-**18a** to 33.0% for the transformation of the *O*-methylated nitrile (±)-**26a** (Table 2, entry 12). A reduction in the *ee* of the amide was also observed from 50.4% after the biotransformation of nitrile (±)-**19a** to 26.4% in the case of the *O*-methylated (±)-**27a** (Table 2, entry 13). Alhough the configurations of the biotransformation products were assigned as noted previously by comparison of HPLC and optical rotation data with literature values, the absolute configurations of several of the products were assigned tentatively in this series.

The unexpected quantities of the amides formed during the biotransformations of (±)-**15a**–**28a** in the presence of SET1 led us to investigate mandelonitrile (±)-**29a** and related α-alkyl-substituted aryl nitriles (±)-**30a**–**34a** to assess the effect of alternative groups at the α position on the type and amount of biotransformation product obtained. To circumvent the degradation of mandelonitrile in aqueous solution at pH 7, the biotransformation was carried out at the lower pH of 4.[10f] A slight decrease in the activity of SET1 was observed at this lower pH, which correlates with the results of pH versus activity studies carried out for 3-hydroxybutryonitrile [(±)-**1a**] in the presence of SET1 (the results of these studies are presented in the Supporting Information). In addition, rather than the exclusive formation of amide or acid, both amide (*R*)-(–)-**29c** and acid (*S*)-(+)-**29b** were isolated after the hydrolysis along with recovered nitrile (Table 3, entry 1). It is apparent from the time course of the reaction (data not shown) that both acid and amide are formed concurrently and that temporary accumulation of the amide and subsequent hydrolysis does not occur. The *S* enantiomer of mandelic acid [(*S*)-(+)-**29b**] was produced in an extremely high *ee* of >99.9%, albeit in a low yield of 8.2%.

In contrast, the absence of the α-hydroxy or another electron-withdrawing functionality in nitrile (±)-**30a** generated only the corresponding carboxylic acid product (*S*)-(+)-**30b** with an excellent *ee* of >99.9% (Table 3, entry 2) in addition to recovered (*R*)-nitrile in 40.1% yield and 63.0% *ee*. The substrate (±)-**31a**, related to (±)-**30a** but differing in the size of the alkyl group at the α-position, also provided only the acid product along with recovered nitrile; however, a dramatic decrease in the *ee* of the acid was observed with the increase in size of the alkyl substituent (Table 3, entry 3 vs. entry 2, 49.5% vs. 99.9% *ee*) with only a minor reduction in product yield (19.3 vs. 17.3%). The amide was formed in one case in this series of substrates, (±)-**33a**, which afforded after the biotransformation, along with the acid (*S*)-(+)-**33b** with an excellent *ee* of 96.7%, an equivalent amount of the amide (*R*)-(–)-**33c** with a lower *ee* of 45.7% as well as recovered nitrile. The production of amide in this case may possibly be due to inductive electron-with-

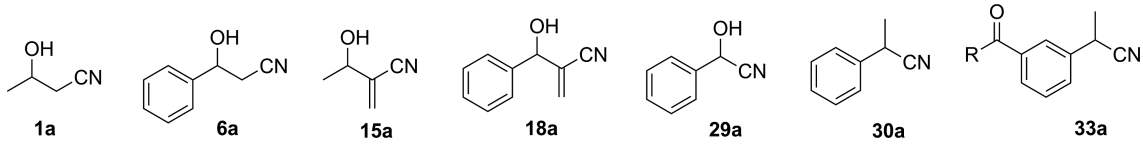
Table 3. Biotransformations of α-alkyl-substituted aryl nitriles.[a]



Entry	Substrate	R^1	R^2	Recovered nitrile **a** Yield [%],[b] *ee* [%][c]	Product acid **b** Yield [%],[b] *ee* [%][c]	Product amide **c** Yield [%],[b] *ee* [%][c]
1	**29a**[d]	OH	H	43.2, (*S*)-(–)-46.0	8.2, (*S*)-(+)-99.9	44.1, (*R*)-(–)-3.7
2	**30a**	CH_3	H	40.1, (*R*)-(+)-63.0	19.3, (*S*)-(+)-99.9	n.d.
3	**31a**	CH_3CH_2	H	45.0, (*R*)-(+)-55.5	17.0, (*S*)-(+)-49.5	n.d.
4	**32a**	*i*Pr	H	73.1, 1.2	n.d.	n.d.
5	**33a**	CH_3	3-COC_6H_5	54.1, (*R*)-(+)-41.7	20.0, (*S*)-(+)-96.7	20.2, (*R*)-(–)-45.7
6	**34a**	CH_3	4-*i*Pr	89.2, 1.2	n.d.	n.d.

[a] The biotransformations were carried out by incubating the substrate (10 mmol/L) in a suspension of *Rhodococcus erythropolis* SET1 (OD_{600nm} = 1) in phosphate buffer (pH 7.0) at 25 °C. [b] Isolated yield determined by using flash chromatography. [c] Determined by HPLC analysis using a chiral column. The configurations were determined by comparison of HPLC traces and optical rotations with the literature and authentic standards when available; n.d.: not detected. [d] The hydrolysis of mandelonitrile was performed in phosphate buffer at pH 4.0.

Table 4. Variation and yields of different substrate types as well as *ee* values obtained from relevant nitriles (n.d.: not detected).



Substrate	1a	6a	15a	18a	29a	30a	33a
Acid [%]	42.2	19.3	n.d.	n.d.	8.2	19.3	20.0
ee [%]	>99.9 (*S*)	7.0 (*R*)	n.d.	n.d.	>99.9 (*S*)	>99.9 (*S*)	96.7 (*S*)
Amide [%]	n.d.	n.d.	43.2	22.2	44.1	n.d.	20.2
ee [%]	n.d.	n.d.	87.8 (*S*)	42.3 (*S*)	3.7 (*R*)	n.d.	45.7 (*R*)

drawal at the α position by a *m*-oxo-substituted aryl ring or steric effects. A comparison of the acid and amide quantity, structural variation and *ee* value is provided for relevant substrates of the series (±)-**1**–**34a** in Table 4. It can be observed that when there is no substitution or a sterically non-demanding alkyl group is present at the α position (as in nitriles **1a**, **6a** and **30a**), the acid is formed with high *ee* values in several cases, whereas in the presence of an electron-withdrawing or conjugating group at this position in the starting nitrile, amide is formed or a combination of amide and acid in varying ratios (**15a**, **18a**, **29a** and **33a**).

Mechanistic Insights into Nitrilase/NHase Behaviour

We suggest that a nitrilase enzyme system is responsible for the generation of the acid and amide products. It is thought that a conserved catalytic triad of Glu-Lys-Cys is present at the active site of nitrilases[23] and that hydrolysis reactions may take place via the formation of a thioimidate intermediate produced by attack of the thiol group of the cysteine residue on the carbon atom of the nitrile, followed by the addition of a H_2O molecule.[8d] To determine whether the catalytic cysteine residue is present in SET1, the biotransformation of 3-hydroxybutyronitrile [(±)-**1a**] was examined in the presence of a range of inorganic salts that have previously been shown to interact with nitrilase enzymes (Figure 2).[8f,24]

It can be seen from the data in Figure 2 that the thiol-complexing reagents of Zn^{2+}, Ag^{+} and Hg^{2+} in particular, at a concentration of 1 mM, significantly if not completely inhibit the activity of SET1. The most pronounced effect was observed with Hg^{2+} ions, and this indicates the possible involvement of a thiol-containing conserved cysteine residue in the catalytic mechanism and points to the presence of a nitrilase enzyme.

When amides have been produced as the products of biotransformations employing nitrilase, NHase activity has been postulated to be part of the biocatalytic mechanism.[10] It has been proposed that upon formation of the thioimidate intermediate, if the N atom of the tetrahedral intermediate contains a positive charge stabilised by the triad Glu residue (**Ia**, Figure 3), ammonia may be eliminated to form acid as the product.[10f] In contrast, if this positive charge lies on the amino Lys residue rather than on the N atom,

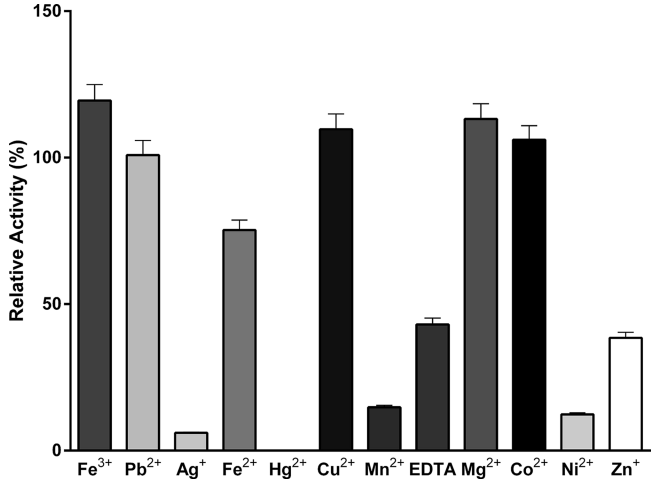


Figure 2. Effect of metal ions on the nitrilase activity of *R. erythropolis* SET1. Reactions were performed for 24 h in potassium phosphate (100 mM, pH 7.0) containing 1 mM of inorganic metal ions. The activities were determined in triplicate by using Nesslers microscale colorimetric assay and are reported relative to that observed in the absence of metal ions.

alternative thiol elimination may occur to form the amide (via intermediate **Ib**). If an electron-withdrawing group is present at the α position it may destabilise **Ia** leading to more of the amide-forming aryl intermediate **Ib**. The relative quantity of the amide could be linked to the extent of destabilisation of **Ia** through inductive effects at the α position. In addition, in the case of α-methylene β-hydroxy nitriles, intramolecular hydrogen bonding of the β-hydroxy with the amino moiety via a six-membered ring may lead to increased formation of the more favourable intermediate **Ib**.[10e] It is also thought that steric interactions could play a significant role by weakening potential interactions between the charged N atom and Glu residue in intermediate **I**, and again this could be a factor in the case of the biotransformations of nitriles such as **15a**, **18a**, **29a** and **33a**.

However, in the hydrolysis of α-methylene β-hydroxy nitriles, in particular, enzyme linkage to the unsaturated nitrile by conjugate addition of a thiol residue to a doubly activated Michael-type acceptor may be an alternative albeit less likely mechanism. If this was the case, upon the addition of the first molecule of water, the relative rates of elimination of the thiol-containing cysteine residue from

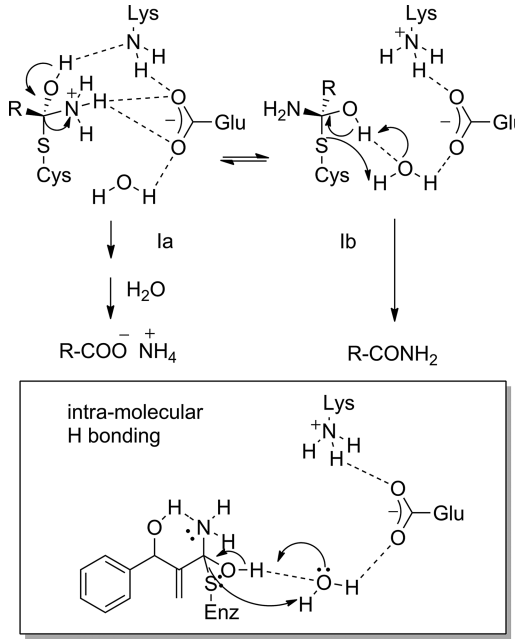


Figure 3. Proposed nitrilase mechanism for amide formation adapted from[10f] and including additional stabilisation by proposed intermolecular hydrogen bonding.

tetrahedral intermediates obtained restoring unsaturation could influence the ratio of acid/amide formation. Reversible thiol conjugate addition has been demonstrated to be possible in doubly activated acrylonitrile substrates.[25] As the enzyme has not been isolated and the crystal structure not yet determined, further studies are required to determine the precise mechanism.

Conclusions

We have shown that *R. erythropolis* SET1 catalyses the hydrolysis of a variety of structural analogues of 3-hydroxybutyronitrile to afford both acids and amides in high yields and *ee* values along with recovered enantioenriched nitrile in several cases. This work has also identified key substrate structural attributes that may influence the activity and enantioselectivity of the isolate. The isolate appears to transform β-hydroxyalkane and β-hydroxyaromatic nitriles with significantly improved yields in the case of β-alkyl substrates. The enantioselectivity may also be improved dramatically by incorporating a docking group at the hydroxy position in β-hydroxyaromatic nitriles. This effect has previously been reported for the NHase/amidase-catalysed hydrolysis of β-hydroxy nitriles rather than nitrilase-catalysed hydrolysis. Protecting the β-hydroxy moiety with a methyl group, in particular, led to a significant increase in the *ee* in one case and may be a viable strategy in the future for related substrates and SET1.

We also consistently observed that when an electron-withdrawing group was present in the nitrile at the α position the product bias shifts towards the amide rather than exclusively the acid. We have proposed a dual nitrilase/NHase activity within the microbial whole cell that is highly dependent on the structure of the nitrile. The possible involvement of a cysteine residue in the catalytic cycle has been demonstrated by the almost complete suppression of the activity of SET1 in the presence of thiol-complexing metal agents. Further studies on *R. erythropolis* SET1 are underway in our laboratory to gain an understanding of its enantioselectivity and NHase activity.

Experimental Section

Materials and Methods: All reagents were purchased from commercial sources and used without additional purification. 3-Hydroxybutyronitrile (**1a**), mandelonitrile (**29a**), 2-phenylpropionitrile (**30a**), 2-phenylbutyronitrile (**31a**) and 3-methyl-2-phenylbutanenitrile (**32a**) were purchased from Sigma–Aldrich. 3-Hydroxypentane-1,5-dinitrile (**5a**), 2-(3-benzoylphenyl)propanenitrile (**33a**) and 2-(4-isobutylphenyl)propanenitrile (**34a**), were purchased from TCI Chemicals Europe. NMR spectra were recorded with a JEOL ECX 400 MHz spectrometer. The chemical shifts (expressed in ppm) of the ^{1}H and ^{13}C NMR spectra are referenced to the solvent peaks. TLC was performed on aluminium-backed sheets with silica gel 60 F_{254} (Merck). Column chromatography was performed with silica gel 60 (0.04–0.063 mm, Merck). HPLC analysis was performed with a Hewlett-Packard 1050 series instrument, LCMS was carried out with an Agilent technologies 1200 series instrument with an LC/MSD Trap XCT detector and GC–MS with a Varian 450 GC coupled with a 220 MS.

Determination of Substrate Specificity

Preparation of the Starting Nitriles and Their Spectroscopic Data: The following racemic β-hydroxy nitriles were synthesised by reduction ($NaBH_4$, EtOH) of the corresponding ketones, and the products **6–10a** were identified by comparison with NMR data reported in the literature.[5a] The Baylis–Hillman nitriles **15a**–**26a** were prepared by DABCO-promoted Morita–Baylis–Hillman reaction of acrylonitrile with various aldehydes to produce the corresponding α-methylene β-hydroxy nitriles as previously reported,[22] and the products were identified by comparison with the corresponding reported NMR data.[22] Substrates **11a**, **12a** and **14a** were prepared from 3-hydroxy-3-phenylpropionitrile as previously reported by Ma et al.[20] The synthetic procedures and characterisation data for all such previously prepared compounds are provided in the Supporting Information.

(*R*,*S*)-2-[(4-Fluorophenyl)(methoxy)methyl]acrylonitrile (27a): Solid Ag_2O (0.646 g, 2.82 mmol, 1 equiv.) and iodomethane (1.59 g, 11.3 mmol, 4 equiv.) were added to 2-[(4-fluorophenyl)(hydroxy)-methyl]acrylonitrile (0.5 g, 2.82 mmol, 1 equiv.) successively and the resulting mixture was stirred in the dark overnight. The reaction mixture was diluted with acetone (5 mL) and filtered by gravity. The solvent was removed in vacuo to afford the crude product. The crude product was subjected to silica gel column chromatography eluting with a mixture of hexane/ethyl acetate (80:20). The title product was obtained as a colourless oil (0.22 g, 41%). The enantiomers were separated by HPLC analysis using a Chiralcel OJ-H column with hexane/propan-2-ol (90:10) containing 0.1% TFA as the mobile phase at a flow rate of 0.8 mL/min to give to peaks: t_1 = 11.0 min, t_2 = 11.5 min. IR (KBr disc): $\tilde{\nu}_{max}$ = 2227, 1604, 1224, 953, 840 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.51 (td, J = 7.3, 1.8 Hz, 1 H, Ar-H), 7.35–7.31 (m, 1 H, Ar-H), 7.22–7.20 (m, 1 H, Ar-H), 7.09–7.05 (m, 1 H, Ar-H), 6.05 (s, 1 H, CH_2=C), 5.99 (s, 1 H, CH_2=C), 5.13 (s, 1 H, 3-H), 3.42 (s, 3 H, CH_3-O) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.0 (C-1′), 134.1 (C-4′), 128.1 (CH_2=C), 128.0 (C-2′, C-6′), 116.9 (C-2), 116.0

(C-1), 115.1 (C-3′, C-5′), 78.1 (C-3), 57.0 (CH_3-O) ppm. MS: *m*/*z* = 192.2 $[MH]^+$. HRMS (ESI): calcd. for $C_{11}H_{11}NOF$ 192.0825 $[MH]^+$; found 192.0817,.

(*R*,*S*)-2-[Methoxy(2-methoxyphenyl)methyl]acrylonitrile (28a): Solid Ag_2O (0.619 g, 2.6 mmol, 1 equiv.) and iodomethane (1.45 mL, 10.4 mmol, 4 equiv.) were added to 2-[hydroxy(2-methoxyphenyl)-methyl]acrylonitrile **24a** (0.5 g, 2.6 mmol, 1 equiv.) successively and the resulting mixture was stirred in the dark overnight. The reaction mixture was diluted with acetone (5 mL) and filtered by gravity. The solvent was removed in vacuo to afford the crude product. The product was subjected to silica gel column chromatography eluting with a mixture of hexane/ethyl acetate (60:40). The title product was obtained as a colourless oil (0.20 g, 39% yield). The enantiomers were separated by HPLC analysis using a Chiralcel OJ-H column with hexane/propan-2-ol (90:10) containing 0.1% TFA as the mobile phase at a flow rate of 0.8 mL/min to give two peaks: t_1 = 11.2 min, t_2 = 12.2 min. IR (KBr disc): $\tilde{\nu}_{max}$ = 2225, 1601, 1246, 1090, 747, 668 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.45 (d, *J* = 7.5 Hz, 1 H, Ar-H), 7.29 (t, *J* = 7.3 Hz, 1 H, Ar-H), 7.01 (t, *J* = 6.4 Hz, 1 H, Ar-H), 6.88 (d, *J* = 8.2 Hz, 1 H, Ar-H), 5.96 (d, *J* = 6.8 Hz, 2 H, CH_2=C), 5.20 (s, 1 H, 3-H), 3.82 (s, 3 H, O-CH_3), 3.37 (s, 3 H, CH_3-O) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.1 (C-1′), 147.8 (*C*H_2=C), 145.5 (C-5′), 143.6 (C-3′), 128.3 (C-2′), 127.4 (C-2), 127.1 (C-4′), 117.8 (C-1), 71.4 (C-3), 57.6, (*C*H_3-O), 56.1 (*C*H_3-O) ppm. MS: *m*/*z* = 204.1 $[MH]^+$. HRMS (ESI): calcd. for $C_{12}H_{13}NO_3$ 203.0946 $[M]^+$; found 203.0955.

General Procedure for the Biotransformation of Racemic Nitriles: *Rhodococcus erythropolis* SET1 was grown in M9 minimal media by using 3-hydroxybutyronitrile (**1a**) as the sole nitrogen source. The culture was then re-suspended in potassium phosphate buffer (0.1 M, pH 7.0) to give an OD_{600nm} of 1.0. The resting cells were activated at 25 °C for 30 min through orbital shaking. Racemic nitrile was added in one portion to the flask, and the mixture was incubated at 25 °C by using an orbital shaker (250 rpm). The reaction, monitored by TLC or LCMS, was quenched after a specified period of time (see Tables 1 and 2 and the Supporting Information) by removing the biomass through centrifugation. The resulting aqueous solution was adjusted to pH 12 with aqueous NaOH (2 M) and extracted with ethyl acetate (3× 120 mL). After drying ($MgSO_4$) and removal of the solvent under vacuum, the residue was subjected to silica gel column chromatography using a mixture of hexane and ethyl acetate as the mobile phase to give amide product and the recovered nitrile in some cases. The aqueous phase was then adjusted to pH 4 with hydrochloric acid (2 M) and extracted with ethyl acetate (3× 120 mL). After drying ($MgSO_4$) and removal of the solvent under vacuum, the residue was purified by silica gel column chromatography by employing a mixture of hexane and ethyl acetate as eluent to give the pure acid product. The structures of all the products were fully characterised by spectroscopic analyses. The *ee* values were determined by HPLC analysis using a chiral stationary phase (see the Supporting Information). These and specific optical rotation values were compared with literature data to assign the stereochemistry. The spectroscopic data associated with new products are presented in this section. All data associated with previously reported products are provided in the Supporting Information.

(*S*)-2-[(4-Fluorophenyl)(hydroxy)methyl]acrylamide (19c): The title product was obtained as a white solid (26 mg, 30.2% yield), m.p. 85.1–85.6 °C. Chiral HPLC analysis using a Chiralcel OJ-H column with hexane/propan-2-ol (90:10) containing 0.1% TFA as the mobile phase at a flow rate of 0.8 mL/min gave two peaks: t_{minor} = 16.09 min, t_{major} = 17.20 min. IR (KBr disc): $\tilde{\nu}_{max}$ = 3378, 3195, 1657, 1625, 862, 816 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.31–7.27 (m, 1 H, Ar-H), 7.10 (t, *J* = 7.8 Hz, 2 H, Ar-H), 6.95 (m, 1 H, Ar-H), 6.25 (br, 1 H), 5.92 (s, 1 H, CH_2=C), 5.86 (s, 1 H, CH_2=C), 5.62 (br, 1 H), 5.51 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.5 (C-1), 161.7 (C-1′), 143.9 (C-2), 143.4 (C-4′), 130.1 (*C*H_2=C), 122.6 (C-5′), 121.7 (C-3′), 114.7 (C-6′), 113.2 (C-2′), 73.9 (C-3) ppm. MS: *m*/*z* = 196.1 $[MH]^+$. HRMS (ESI): calcd. for $C_{10}H_{11}NO_2F$ 196.0774 $[MH]^+$; found 196.0773.

(*S)-2-[(3-Fluorophenyl)(hydroxy)methyl]acrylamide (20c):** The title product was obtained as a white solid (30.1% yield), m.p. 92.3–93.0 °C. Chiral HPLC analysis using a Chiralcel OJ-H column with hexane/propan-2-ol (90:10) containing 0.1% TFA as the mobile phase at a flow rate of 0.8 mL/min gave two peaks: t_{minor} = 17.27 min, t_{major} = 18.10 min. IR (KBr disc): $\tilde{\nu}_{max}$ = 3382, 3187, 1658, 1625, 1044, 947 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.31–7.27 (m, 1 H, Ar-H), 7.10 (t, *J* = 7.8 Hz, 2 H, Ar-H), 6.95 (td, *J* = 5.0, 3.2 Hz, 1 H, Ar-H), 6.31 (br, 1 H), 5.92 (s, 1 H, CH_2=C), 5.71 (br, 1 H), 5.51 (s, 1 H, CH_2=C), 5.50 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.45 (C-1), 161.67 (C-1′), 143.9 (C-2), 143.4 (C-3′), 130.1 (C-5′), 122.63 (*C*H_2=C), 121.7 (C-4′), 114.7 (C-2′), 113.2 (C-6′), 73.92 (C-3) ppm. MS: *m*/*z* = 196.1 $[MH]^+$. HRMS (ESI): calcd. for $C_{10}H_{11}NO_2F$ 196.0774 $[MH]^+$; found 196.0777.

(*S)-2-[(2-Fluorophenyl)(hydroxy)methyl]acrylamide (21c):** The title product was obtained as a white solid (29 mg, 26.4% yield), m.p. 108.3–109.1 °C. Chiral HPLC analysis using a Chiralcel OJ-H column with hexane/propan-2-ol (90:10) containing 0.1% TFA as the mobile phase at a flow rate of 0.8 mL/min gave two peaks: t_{minor} = 18.7 min, t_{major} = 20.10 min. IR (KBr disc): $\tilde{\nu}_{max}$ = 3369, 3200, 1664, 1592, 804, 759 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.53 (td, *J* = 7.6, 1.8, 1.4 Hz, 1 H, Ar-H), 7.24–7.16 (m, 1 H, Ar-H), 7.16 (t, *J* = 7.6 Hz, 1 H, Ar-H), 6.22 (br, 1 H), 5.86 (s, 1 H, CH_2=C), 5.79 (s, 1 H, CH_2=C), 5.54 (br, 1 H), 5.46 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.5 (C-1), 142.9 (C-F), 139.2 (C-2), 129.5 (C-2′), 127.9 (C-3′), 124.3 (*C*H_2=C), 122.0 (C-5′), 115.3 (C-4′), 115.1 (C-6′), 68.9 (C-3) ppm. MS: *m*/*z* = 196.1 $[MH]^+$. HRMS (ESI): calcd. for $C_{10}H_{11}NO_2F$ 196.0774 $[MH]^+$; found 196.0774.

(*S)-2-[(4-Fluorophenyl)(methoxy)methyl]acrylamide (27c):** The title product was obtained as a white solid (19 mg, 22.0% yield), m.p. 101.2–102 °C. Chiral HPLC analysis using a Chiralcel AD-H column with hexane/propan-2-ol (90:10) containing 0.1% TFA as the mobile phase at a flow rate of 0.8 mL/min gave two peaks: t_{minor} = 11.60 min, t_{major} = 11.11 min. IR (KBr disc): $\tilde{\nu}_{max}$ = 3197, 1657, 1629, 1101, 861, 822 cm^{-1} ^1H NMR (400 MHz, MeOD): δ = 7.38–7.29 (m, 2 H, Ar-H), 7.16 (t, *J* = 7.7 Hz, 1 H, Ar-H), 7.06 (t, *J* = 10.1 Hz, 1 H, Ar-H), 5.92 (s, 1 H, 3-H), 5.51 (d, *J* = 10.1 Hz, 2 H, CH_2=C), 3.28 (s, 3 H, CH_3O-CH) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 171.0 (C-1), 143.6 (C-1′), 129.7 (C-2), 129.6 (*C*H_2=C), 128.4 (C-4′), 124.0 (C-5′, C-3′), 119.6 (C-6′, C-2′), 75.1 (C-3), 56.2 (*C*H_3-O) ppm. GC–MS (EI): retention time = 11.48 min; *m*/*z* (%) = 209 (26.3), 194 (16.1), 178 (46.1), 161 (12.4), 139 (100), 123, 44. HRMS (ESI): calcd. for $C_{11}H_{13}NO_2F$ requires 210.0930 MH^+; found 210.0929.

(*S)-2-[Methoxy(2-methoxyphenyl)methyl]acrylamide (28c):** The title product was obtained as a white solid (15 mg, 12.2% yield), m.p. 103.0–104.2 °C. Chiral HPLC analysis using a Chiralcel OJ-H column with hexane/propan-2-ol (90:10) containing 0.1% TFA as the mobile phase at a flow rate of 0.8 mL/min gave two peaks: t_{minor} = 17.04 min, t_{major} = 20.4 min. IR (KBr disc): $\tilde{\nu}_{max}$ = 3187, 1662, 1629, 1090, 1064, 884, 852 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.36 (dd, *J* = 7.7, 1.8 Hz, 1 H, 3′-H), 7.28 (dd, *J* = 7.3, 1.8 Hz, 1 H, 5′-H), 6.97 (t, *J* = 7.3 Hz, 1 H, 4′-H), 6.91–6.86

(d, J = 8.2 Hz, 1 H, 6′-H), 6.82 (br, 1 H), 6.18 (s, 1 H, 3-H), 5.51 (s, 1 H, CH_2=C), 5.43 (s, 1 H, CH_2=C), 3.82 (s, 3 H, CH_3-O-Ar), 3.38 (s, 3 H, CH_3-O) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.3 (C-1), 157.2 (C-1′), 141.3 (C-2), 130.3 (C-3′), 130.1 (C-5′), 129.3 (C-2′), 127.2 (*C*H_2=C), 124.8 (C-4′), 120.8 (C-6′), 57.2 (Ar-O*C*H_3), 55.5 (*C*H_3O-C) ppm. LRMS (ES): m/z = 222.1 $[MH]^+$. HRMS (ESI): calcd. for $C_{12}H_{16}NO_3$ 222.1130 $[MH]^+$; found 222.1133.

Acknowledgments

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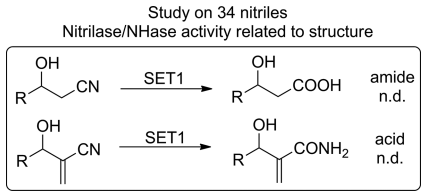
Enzyme Dual Activity

The nitrile hydrolysing bacterial isolate *Rhodococcus erythropolis* SET1 has demonstrated dual nitrilase/NHase activity depending on the nitrile structure. β-Hydroxy nitriles unsubstituted α to the nitrile group gave acids along with recovered nitrile as a result of nitrilase activity of the isolate, whereas amides were the major product from α-vinyl β-hydroxy nitriles due to NHase activity.



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Substrate Evaluation of *Rhodococcus erythropolis* SET1, a Nitrile Hydrolysing Bacterium, Demonstrating Dual Activity Strongly Dependent on Nitrile Sub-Structure

Keywords: Biotransformations / Enzyme catalysis / Reaction mechanisms / Nitriles

