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Short Research Article

Chemoselective hydrolysis of a radiolabelled nitrile using nitrilases†

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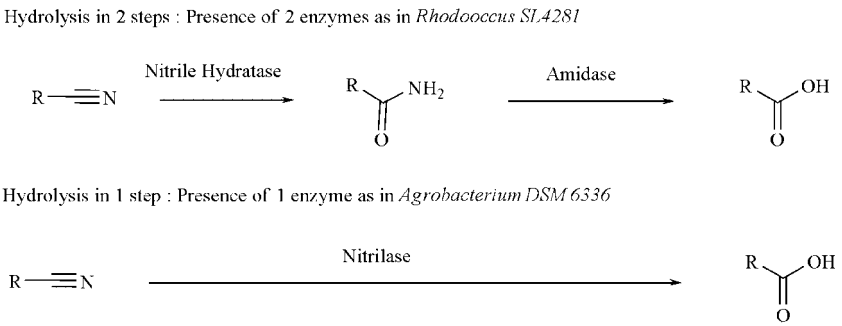
Introduction

One of the most used and readily available radiolabelled starting materials is $K^{14}CN$. In many cases, the synthesis of the radiolabelled compound requires the hydrolysis of ^{14}CN derivative to the corresponding carboxylic acid. The usual harsh conditions of nitrile hydrolysis (strong acid or base) are not compatible with other functions such as esters.[1] According to the literature, microorganisms (e.g. nitrilase, etc.) can hydrolyze nitriles in a chemo[2]- and regio[3]-selective way under mild conditions (pH neutral, 37°C, etc.) with good yields along two possible pathways as described in Scheme 1. To our knowledge, microorganisms have so far never been used in chemoselective hydrolysis of a nitrile in a radiolabelled synthesis.

Results and discussion

^{12}C study of nitrilases: Firstly, we investigated the hydrolysis of cold nitrile derivatives containing an ester function using four different microbial strains. Only *Rhodococcus* sp. SRL4281 and *Agrobacterium* DSM6336 gave the corresponding acids in good yield as shown in Scheme 2. However, as soon as the nitrile function became too sterically crowded, the hydrolysis reaction failed as observed for Entry 4.



Scheme 1

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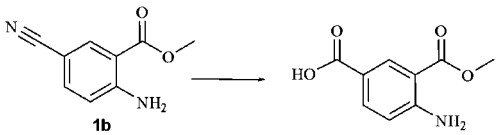
Entry

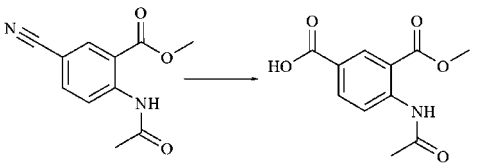
1



1b

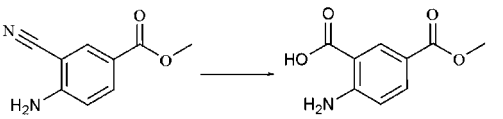
	Agrobacterium DSM 6336	*Rhodococcus* sp SRL4281	*Rhodococcus* sp R312	*Rhodococcus* sp NCIMB 11216
Yield in 1 day	98%	99.5%	22%	15%

2



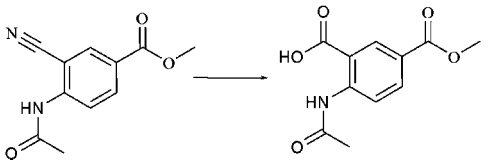
	Agrobacterium DSM 6336	*Rhodococcus* sp SRL4281	*Rhodococcus* sp R312	*Rhodococcus* sp NCIMB 11216
Yield in 2 days	90%	55%	5%	<5%

3



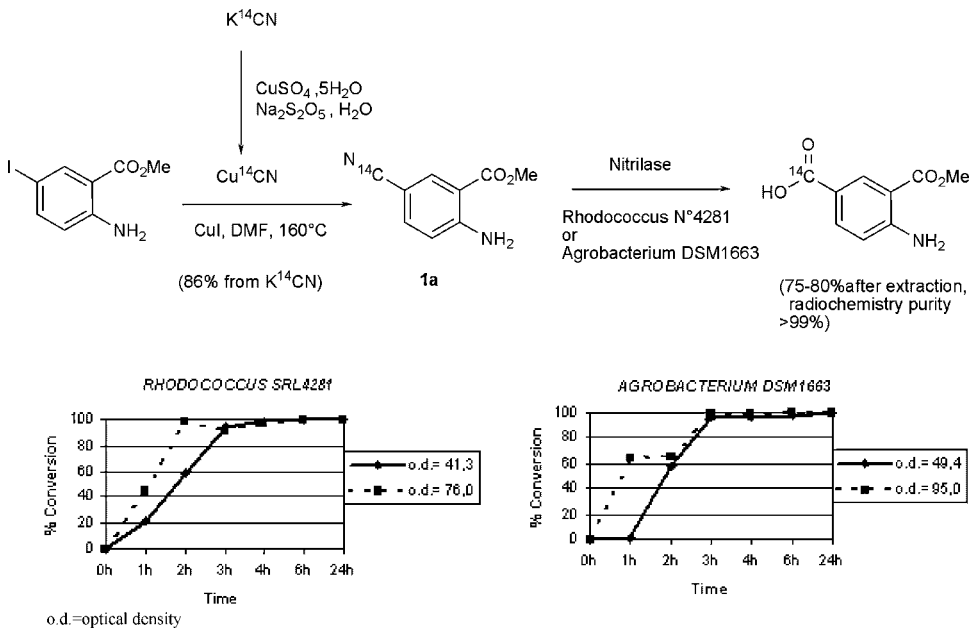
	Agrobacterium DSM 6336	*Rhodococcus* sp SRL4281	*Rhodococcus* sp R312	*Rhodococcus* sp NCIMB 11216
Yield in 4 days	<1%	86%	<1%	<1%

4



	Agrobacterium DSM 6336	*Rhodococcus* sp SRL4281	*Rhodococcus* sp R312	*Rhodococcus* sp NCIMB 11216
Yield	<5%	<5%	<5	<5%

Scheme 2



Scheme 3

^{14}C study of nitrilases: We succeeded in reproducing these results using the ^{14}C-derivate **1a**, which was synthesized in two steps in 86% yield. As for the corresponding unlabelled case **1b**, good results were obtained with the strains of microorganisms *Rhodococcus* sp. SRL4281 and *Agrobacterium* DSM6336 (75–80% yield with radiochemical purity >99%). HPLC analysis showed that the reaction was complete in 4 h (Scheme 3).

Conclusion

We have succeeded in hydrolysing a ^{14}C-radiolabelled nitrile using strains of microorganisms, *Rhodococcus* sp.

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SRL4281 and *Agrobacterium* DSM6336, in good yield and with excellent radiochemical purity. A nitrilase kit (12 purified enzymes) can be purchased from Biocatalytics. This kit was tested and two of the enzymes gave quantitative yield with our model substrate **1b**. However, none was able to hydrolyze sterically crowded substrate (Scheme 2, Case 4). These results need to be confirmed with radiolabelled material, but the use of these purified enzymes is easier than using whole cells, but much more expensive.

REFERENCES

1. To our knowledge, one mild hydrolysis of nitrile to carboxylic acid using tetrafluoropthalic acid was described in moderate to good yield in the literature (Rounds WD, Eaton JT, Urbanowicz JH, Gribble GW. *Tetrahedron Lett* 1988; **29**: 6557–6560), but in our case, these conditions did not work.
2. (a) Cohen MA, Sawden J, Turner NJ. *Tetrahedron Lett* 1990; **31**: 7223–7226; (b) Matoishi K, Sano A, Imai N, Yamaezaki T, Yokoyama M, Sugai T, Ohta H. *Tetrahedron Asym* 1998; **9**: 1097–1102; (c) Cooling FB, Fager SK, Fallon RD, Folsom PW, Gallagher FG, Gavagan JE, Hann EC, Herkes FE, Phillips RL, Sigmund A, Wagner LW, Wu W, DiCosimo R. *J Mol Catal B*: *Enzymatic* 2001; **11**: 295–306.
3. (a) Kakeya H, Sakai N, Sugai T, Ohta H. *Tetrahedron Lett* 1991; **32**: 1343–1346; (b) Yokoyama M, Sugai T, Ohta H. *Tetrahedron Asym* 1993; **4**: 1081–1084; (c) Kerridge A, Parratt JS, Roberts SM, Theil F, Turner NJ, Willetts AJ. *Bioorg Med Chem* 1994; **2**: 447–455.

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