Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 342–346.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1388

JLCR

Short Research Article

The use of biocatalysis in the synthesis of labelled compounds†

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Received 25 August 2006; Revised 10 April 2007; Accepted 16 April 2007

Abstract: Since most biotransformations are highly chemo-, regio- and stereoselective, they offer many opportunities for the synthesis of compounds that are not easily attainable by classical organic chemistry, The catalytic properties of the cytochrome P450 (CYP450) enzymes of microorganisms can be exploited to produce suitable N-, S- or O-dealkylated precursors, which can be realkylated with the appropriate labelled reagent. These enzymes are also well reported for hydroxylation of activated or nonactivated carbon centres, to produce drug metabolites. Nevertheless, to use such enzymes, a whole cell system is preferred due to the need for cofactor regeneration. Nitrile hydrolysing enzymes also attract attention, due to their ability to selectively hydrolyse nitrile derivatives under mild conditions. The scope of such biotransformations is discussed. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: biocatalysis; biotransformation; microorganisms; CYP450; nitrilase; nitrile hydratase; isotope labelling

Introduction

Enzymes are well-known catalysts for specific chemical reactions, and their reactions offer excellent regio-, chemo-, stereo- and substrate selectivity. They are now commonly used in organic chemistry, especially as isolated enzymes such as the commercially available hydrolases.

However, biocatalytic systems can operate in two ways: either as isolated enzymes or as whole cell systems, depending on the transformation required. For instance, if cofactor regeneration is necessary or the enzyme involved presents a relative instability, whole cell systems are usually preferred. In this way, cytochrome P450 (CYP450) enzymes can be exploited to catalyse selective N-, O- and S-dealkylation of complex structures. These precursors can then be realkylated with the desired labelled alkyl group.

The CYP450 enzymes of microorganisms are also well described for their metabolizing ability, as 'the microbial model for drug metabolism'.[1] Biotransformations can, therefore, be useful for the preparation of labelled drug metabolites, when the labelled parent compound is available.

Nitrile derivatives are common precursors of ^{14}C-labelled compounds, but their functionalization by hydrolysis often requires harsh conditions incompatible with other functional groups of the molecule. In these cases, hydrolysis under mild conditions is necessary. A family of bacteria called *Rhodoccocus* have long been known for their ability to hydrolyse nitrile-containing compounds at neutral pH under mild, aqueous conditions. This methodology allows the preparation of classes of ^{14}C-labelled molecules, which are otherwise difficult to obtain under classical organic hydrolysis conditions.

Results and discussion

From complex substrates, selectively O-dealkylated compounds can be interesting precursors for the synthesis of labelled compounds. Such dealkylation reactions can be catalysed under mild conditions by CYP450, in a culture broth. SR121960 **1** was deethylated using a culture of the fungus *Cunninghamella echinulata* LCP73.2203 in a fermenter (7 L broth). After extraction of the broth with ethyl acetate and purifica-

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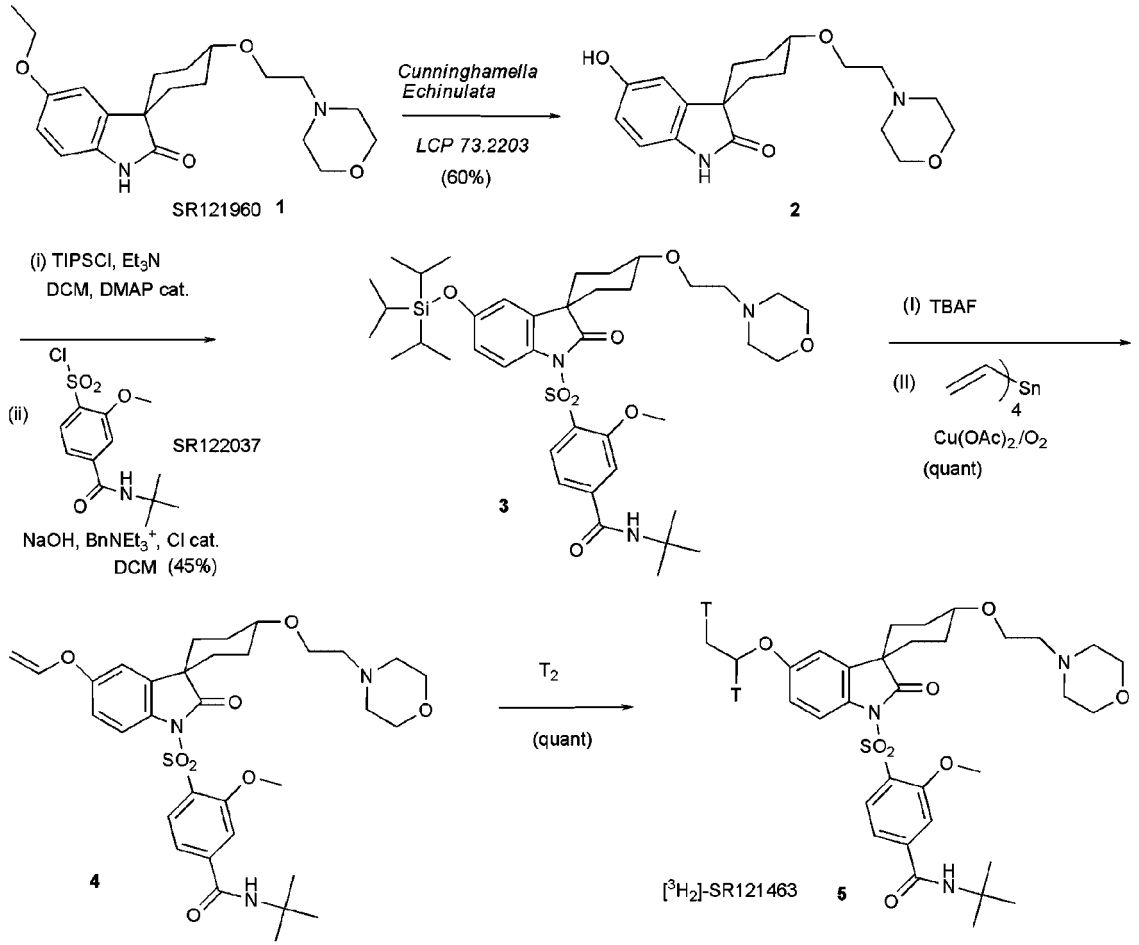
†Proceedings of the Ninth International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds, Edinburgh, 16–20 July 2006.



Scheme 1

tion by flash chromatography on silica gel, 1.2 g of the desired phenol derivative **2** was produced. The yield was 60% at 0.3 g/L as the initial concentration of starting material **1**. The phenol function was protected with a TIPS group before coupling the benzene sulphonyl chloride derivative SR122037 to afford compound **3** with 45% yield in two steps. After the removal of the TIPS protecting group with TBAF, tetravinyltin was used to vinylate the phenolic group and to obtain the unsaturated tritiation precursor **4**. The double bond of precursor **4** was successfully tritiated using tritium gas to give [$^3H_2$]-SR121463 **5** with a specific activity of 56 Ci/mmol. It should be noticed that attempts to directly dealkylate SR121463 failed (Scheme 1).

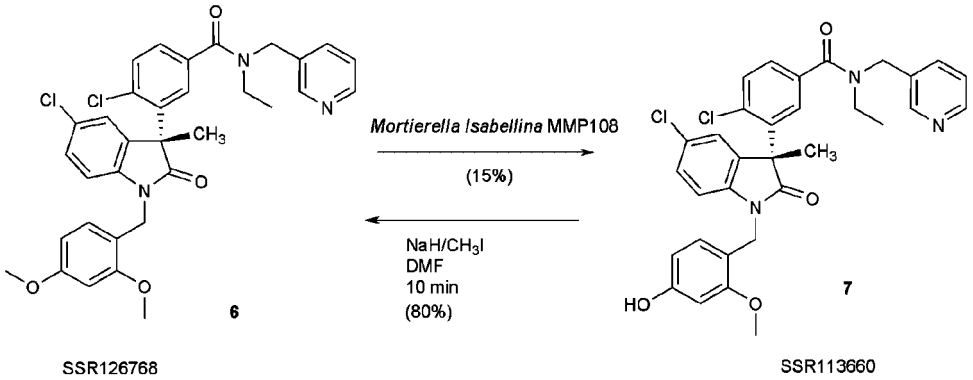
This dealkylation–realkylation strategy can also be used to prepare interesting precursors for PET studies, as illustrated in Scheme 2.

A strain of fungus *Mortierella isabellina* MMP108 was selected for its ability to selectively monodemethylate the compound SSR126768 **6**. Two hundred mg of the desired phenol **7** was produced using a 7-day long fed batch culture of the fungus in a fermenter (7 L broth). A large amount of the parent drug **6** was recovered and a desethyl amide compound was also produced during the fermentation. Compound **7** was thus purified by preparative HPLC after extraction of the broth with ethyl acetate. Nevertheless the yield remained low (15%, at 0.3 g/L as the initial concentration of the starting material **6**), but we were able to prepare this interesting precursor **7** in one step.

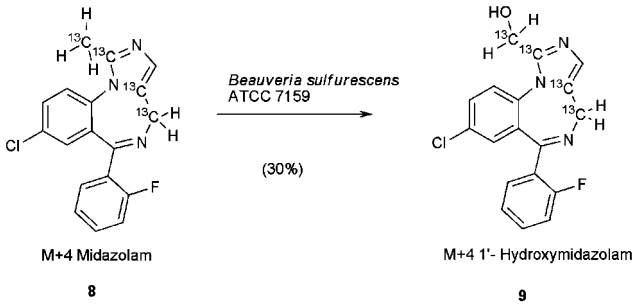
Model compound **7** was successfully remethylated using methyl iodide and sodium hydride in DMF for 10 min, in order to confirm that compound **7** could be used as a PET precursor.

In 1974, Smith and Rosazza[1] first introduced the concept of using microorganisms as 'microbial models for mammalian metabolism.' While conducting microbial hydroxylation of aromatic substrates, they noticed a similarity between microbial metabolites and those obtained from mammalian systems. In pharmacokinetic and metabolism laboratories, Midazolam is used to phenotype hepatic CYP3A activity. Formerly Midazolam clearance was used as a marker, but now, the monitoring of the formation of the major metabolite of

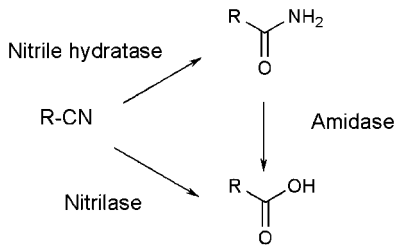
DOI: 10.1002.jlcr



Scheme 2



Scheme 3



Scheme 4

Midazolam is the preferred method. So, for this mass spectrometry-based bioanalytical assay, stable-isotope labelled 1′-hydroxymidazolam is required (Scheme 3).

Since the parent [$^{13}C_4$]-labelled drug **8** was available, it was directly converted to its [$^{13}C_4$]-labelled metabolite **9** using a culture of *Beauveria sulfurescens* ATCC7159 in 30% yield.

This example demonstrates that bioconversion can be an interesting alternative route to synthesize labelled metabolites.

Nitrile hydrolysing enzymes

K^{14}CN is the most commonly used radiolabelled starting material for organic synthesis. In most cases, the synthesis of a radiolabelled compound requires the hydrolysis of a CN derivative. Classical hydrolysis often requires harsh conditions in terms of pH and temperature. Some other functions in the molecule are often affected by this reaction, leading to degradation or undesirable side products. A nitrile hydrolysis system operating under mild conditions would hence be very useful.

Enzyme-catalysed hydrolysis of nitriles has been shown to proceed by either direct transformation to carboxylic acids with a nitrilase[2] or via the corresponding amide mediated by a nitrile hydratase/amidase system[3] (Scheme 4). A range of nitrile hydrolysing microorganisms has been isolated and is available from several collection of microorganisms (ATCC, DSM, NBRC, NCIMB, etc.). Furthermore, purified enzymes are now commercially available (Biocatalytics).

According to the literature, these enzymes can operate in a chemo[4]-, regio[5]- and stereo[6]-selective way. The chemoselective hydrolysis was successfully applied to several substrates (**10**, **12**, **14**) containing a methyl ester group. In the case of the hindered nitrile **16** no strain or enzyme was able to perform the reaction (Scheme 5).

When using the whole cell system, the strain was grown on a specific medium containing an inducer of the activities. The cells were then harvested by centrifugation and re-suspended in a phosphate buffer at pH 7. The substrate, solubilized in a minimum amount of ethanol, was added to the mixture and the reaction was monitored by LC-UV. After complete conversion, the cells were harvested by centrifugation and the supernatant was acidified to pH 2 and extracted with ethyl acetate. No further purification was necessary.

This methodology was successfully applied to ^{14}C-labelled molecule **18** (Scheme 6).

Conclusion

We have demonstrated that biocatalysis is an efficient tool for the preparation of a range of valuable precursors of labelled molecules. This methodology allows an access in one step to intermediates, which could have been difficult or impossible to obtain in a

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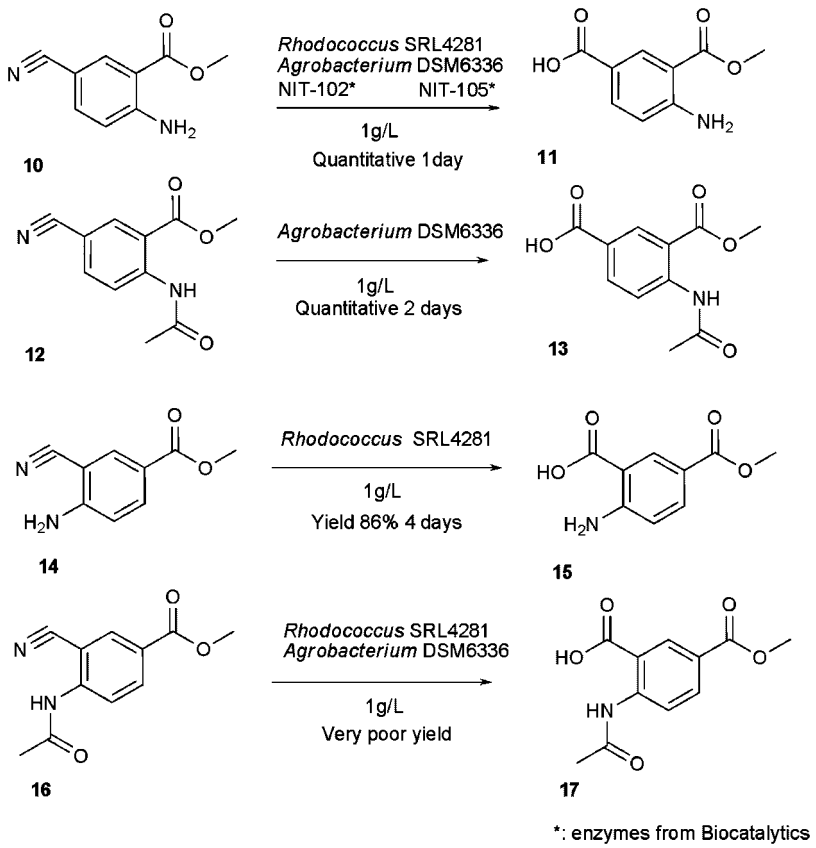
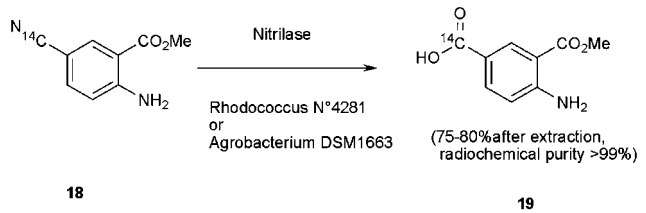


Scheme 5



Scheme 6

classical chemical approach. It is especially useful at the small scales often required in labelled compound synthesis.

Acknowledgement

We are grateful to Elisabeth Kauffmann for analytical support.

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DOI: 10.1002.jlcr