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# Separation of pyrrolidine allylation products by diastereoselective enzymatic ester hydrolysis

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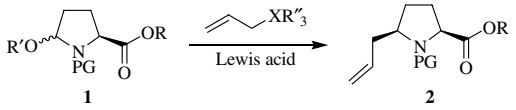
**Abstract**—A multi-parallel enzyme screen has been used to identify potential catalysts for the selective hydrolysis of diastereomeric esters and then subsequently applied in their separation upon scaleup.


Although enzymes have been used extensively for enantioselective synthesis, they have also occasionally been used in diastereoselective synthesis.[1] In the latter scenario, one diastereomer is transformed by the enzyme leaving the other diastereomer untouched, thus allowing facile separation of the two compounds. We recently encountered a very difficult separation of diastereomers and considered the use of enzymes to solve the problem. In this paper we detail a multi-parallel enzyme screen to identify potential catalysts for the selective hydrolysis of diastereomeric esters and its application in their separation on an increased scale.

Treatment of an aminal such as **1** with a Lewis acid creates an iminium intermediate, which can then be trapped by a nucleophile. The reaction of iminium intermediates with various nucleophiles has been widely reported as the key step in the synthesis of numerous nitrogen containing compounds.[2] A particularly useful case is the addition of allyltrimethylsilane (or allyltributylstannane) to various *N*-acyliminium ions bearing an ester side chain.[3] This results in the predominant formation of the *cis* diastereomer **2**[4] (80:20 [5]) (Scheme 1) and the product has been widely used in alkaloid synthesis. Addition of other reagents, such as organo-cuprates, result in high levels of *trans* diastereoselectivity.[3d,6]



1 2

**Scheme 1.** The allylation reaction.

The far from perfect diastereocontrol in the allylation reaction requires separation of the diastereomers. Unfortunately, the *cis*/*trans* diastereomers produced in the allylation reaction are not separable by column chromatography, although they can be separated chromatographically after removal of the nitrogen protecting group, which then requires an additional reprotection step. We considered a less cumbersome alternative strategy: the use of enzymes. This strategy could result in the selective hydrolysis of one ester diastereomer to the carboxylic acid, leaving the other ester diastereomer intact and therefore allowing an easy separation. Indeed, separation of a number of enantiomeric and diastereomeric mixtures of amino acid derivatives bearing an ester group (often a methyl ester) has been achieved by the use of a selective enzymatic ester hydrolysis.[7] Performing an activity screen with a large range of commercially available enzymes in combination with multi-parallel screening equipment to investigate the potential enzymatic separation of the *cis*/*trans* diastereomers could reveal a pragmatic and reproducible method of separation.

In order to investigate the possibility of such a method of separation, preparation of the allylated substrate **8** was required. Treatment of L-pyroglutamic acid **3** with

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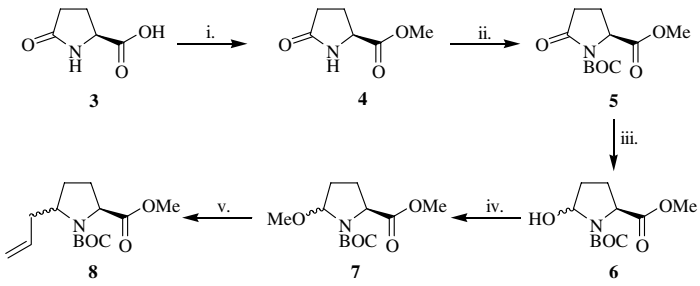



**Scheme 2.** Preparation of allyl-ester **8**. Reagents and conditions: (i) $SOCl_2$ (2 equiv), MeOH, rt, 2 h, 70%; (ii) $BOC_2O$ (1.2 equiv), DMAP (0.1 equiv), MeCN, 3 h, 70%; (iii) $LiEt_3BH$ (1.2 equiv), THF, −78 °C, 2 h, 86%; (iv) *p*-TSA (0.1 equiv), MeOH, 20 h, 75%; (v) $BF_3{\cdot}Et_2O$ (1.03 equiv), allyltrimethylsilane (4.5 equiv), −78 to 0 °C, $Et_2O$, 17 h, 53%, 70:30 *cis*/*trans* (93% isolated yield after BOC-reprotection).

thionyl chloride in methanol gave the methyl ester **4** in 70% yield (Scheme 2). The BOC group was readily introduced to give **5** (70% yield) and subsequent reduction to the hemi-aminal **6** with Super hydride (lithium triethylborohydride) followed by conversion to the methoxy aminal in acidic methanol gave the allylation precursor **7** in good yield. The allylation transformation was then performed, giving **8** in moderate yield (53%) and as a 70:30 mixture of *cis*:*trans* isomers. It was found that the moderate yield was due to *N*-deprotection occurring under the reaction conditions, and the yield could be improved by treating the crude material with $(BOC)_2O$.

In order to perform a selectivity screen, it was necessary to isolate the individual *cis*/*trans* ester diastereomers. This was achieved by removal of the protecting group, followed by separation by column chromatography and reprotection.

With these pure diastereomers in hand, an activity assay was performed in parallel. One hundred and twenty commercially available hydrolases (proteases, esterases and lipases) were combined with 1 mg of ester in the presence of a red coloured, buffered pH indicator solution in 96-well plates. In the wells containing an active enzyme, that is, when ester hydrolysis occurred giving the corresponding acid, the concurrent pH change resulted in a colour change of red to yellow, indicating a positive hit (wells E3 and F3, Fig. 1. The control wells G3 and H3 contain the same enzyme but not the ester as occasionally the enzyme alone can trigger a colour change). If the same enzyme produced a negative hit

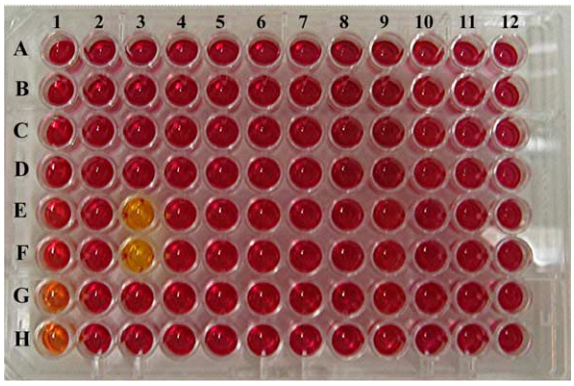
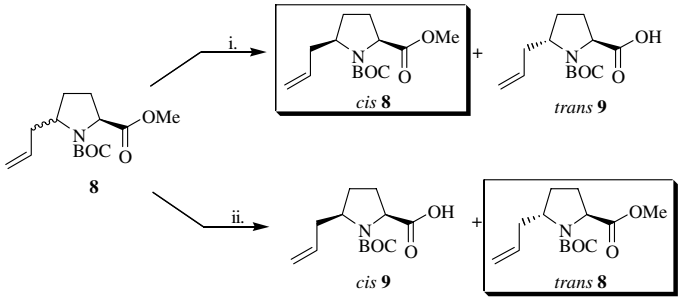



**Figure 1.**

for the other diastereomer then this indicates a useful enzyme for the selective hydrolysis of one diastereomer in the presence of the other. There were several enzymes which showed high activity but low selectivity, but gratifyingly two selective enzymes were found. An esterase from *Candida lipolytica* (Fluka), selectively hydrolysed the *trans* diastereomer but not the *cis*, and a lipase from *Rhizomucor miehei* (Novozymes), selectively hydrolysed the *cis* diastereomer but not the *trans*.

The separation of a mixture of *cis*/*trans* diastereomers was then investigated on synthetic scale (2.92 g). Treating the 7:3 *cis*/*trans* mixture of diastereomers of **8**, formed in the allylation reaction, with *Candida lipolytica* esterase, resulted in a highly selective hydrolysis




**Scheme 3.** Diastereoselective enzymatic ester hydrolysis. Reagents and conditions: (i) $NaCl_{(aq)}$ (0.1 M), sodium phosphate buffer (pH 7, 0.04 M), *Candida lipolytica* esterase (Fluka: 46056), rt, 70 h, *cis* **8**, 86% of theoretical; (ii) $NaCl_{(aq)}$ (0.1 M), sodium phosphate buffer (pH 7, 0.04 M), *Rhizomucor miehei* lipase (Novozymes), 43 h, *trans* **8**, 52% of theoretical.

of the *trans* diastereomer allowing the *trans* carboxylic acid **9** to be washed out in the aqueous phase leaving highly pure *cis* **8** in excellent yield (86% of theoretical) (Scheme 3). Treating the same mixture of diastereomers with *R. miehei* lipase resulted in a less selective ester hydrolysis, with 52% of the *trans* ester being recovered, after the *cis* diastereomer had been completely hydrolysed.

In conclusion, the use of a selective enzymatic ester hydrolysis complements the allylation reaction perfectly. The ester moiety, initially responsible for providing moderately high diastereoselectivities during the allylation reaction, subsequently provides a handle with which to remove the unwanted *trans* diastereomer, allowing rapid access to enantiomerically and diastereomerically pure *cis* intermediates.

## Acknowledgements

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.12.062. Experimental procedures and full characterisation for all compounds. This material is available free of charge via the internet.

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