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Enzymatic reduction of α-substituted ketones with concomitant dynamic kinetic resolution

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ABSTRACT

Racemic α-substituted ketones were converted to the corresponding chiral alcohols with high diastereo- and enantioselectivities using enzymatic reduction with concomitant dynamic kinetic resolution. Reductions of N-protected α-amino ketones by microorganisms and commercial enzymes provided N-protected α-amino alcohols. Choice of buffer was found to be a crucial factor for the successful reduction and simultaneous dynamic resolution of an α-methyl ketone to the corresponding chiral alcohol.

1. Introduction

Ketoreductases are frequently used to reduce ketones to chiral alcohols [1]. With an enolizable group at the α-position the stereochemistry at the adjacent carbon can often be established due to keto-enol tautomerism and selection of only one of the ketone enantiomers for reduction by the enzyme leading to reduction with concomitant dynamic kinetic resolution (DKR) [2–5]. Although there are many examples of such ketone reduction with DKR, there are only a few reports on synthesis of α-alkyl or N-protected α-amino hydroxy esters from the corresponding ketones using enzymatic reduction of ketone and DKR [6–8].

Compound **1** (Fig. 1) is a positive allosteric modulator for the metabotropic glutamate receptor (mGluR5) considered for development as a possible treatment of neurological disorders [9]. The initial synthetic route to **1** used an intermediate *syn*-(1*R*,2*R*) N-protected amino alcohol **2** prepared by an aminohydroxylation catalyzed by OsO_4 which was further converted to a key intermediate **3** for the synthesis of **1** [10]. The aminohydroxylation reaction had only moderate regioselectivity and enantioselectivity, and required careful attention to downstream removal of osmium. Using alternative chemistry, both the *syn*-**2**, *anti*-**4** protected amino alcohols were further converted to **3** the penultimate for the initial synthetic route.

The alcohol **8** (Fig. 2) is a key chiral intermediate for a GPR40 modulator drug candidate **10** for the treatment of diabetes [11,12]. Initial synthesis of **8** was carried out in seven steps with three isolations involving Evan's auxiliary to create chiral centers, and finally hydrogenation of benzyl amine **9** to **8** with an overall yield of 12% [13].

This report describes the preparation of both *syn*- and *anti* N-protected amino alcohols **2** and **4** from ketone **5** (Fig. 1), and *anti* alcohols **9** and **12** from ketone **11** (Fig. 2). In both cases, an α-substituted racemic ketone was enzymatically reduced to a chiral alcohol with the stereochemistry at the second chiral center (α-carbon) set via a dynamic resolution. Summaries of our work on enzymatic reductions and the detailed synthesis of drug candidates have been reported by us in separate publications [13,14].

2. Experimental

Starting materials and product markers were prepared as described [9–14]. Enzymes were purchased from commercial sources. Microorganisms were purchased from ATCC (ATCC#) or obtained from our own culture collection (SC#). Reaction conversion and purity of isolated product were determined from achiral HPLC area percentage (AP). Diastereromeic excess (de) and enantiomeric excess (ee) were determined from chiral HPLC.

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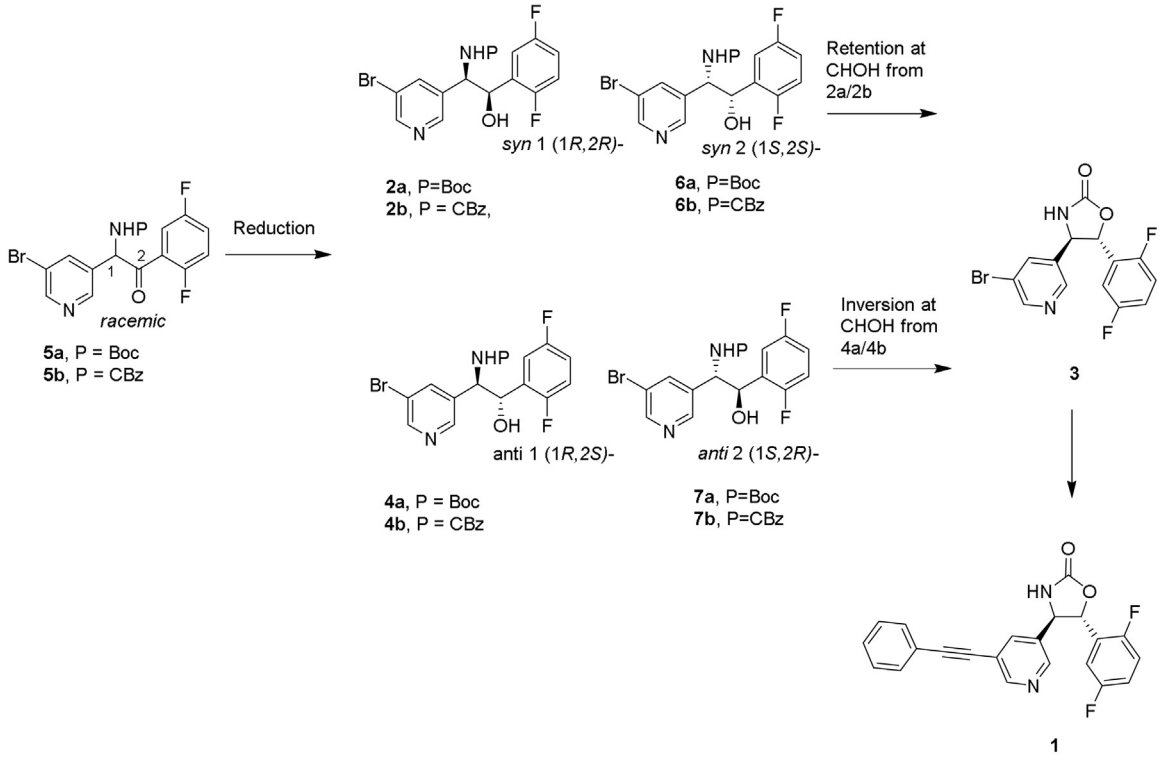

Fig. 1. Reduction of ketone **5.**

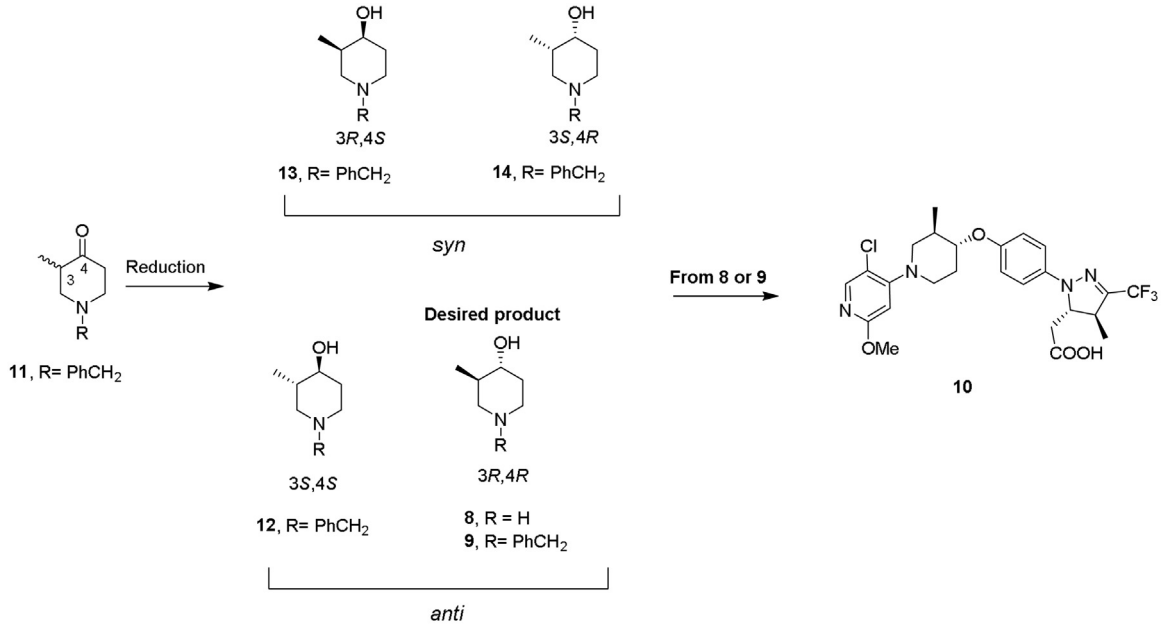

Fig. 2. Reduction of ketone **11.**

2.1. Screening for reduction with commercial enzymes

Ketoreductases (1 mg) were screened in 1-mL reactions in 24-well plates containing 1 mg ketone dissolved in 0.02 mL DMSO, 1 mM NADP or NAD, 5 mg glucose, 0.05 mg glucose dehydrogenase (3.2 U, Amano), 1 mM dithiothreitol and 0.98 mL 0.1 M potassium phosphate buffer pH 7. After 20–87 h at 30 °C and 400 rpm, reactions were quenched with 1 mL methanol and analyzed by HPLC. For the Codexis screening kit and KRED panels the manufacturer's protocol was followed.

2.2. Screening for reduction with microorganisms

Microbial strains were screened in 24-well plates containing inoculum and 2 mL F7 medium (1% malt extract, 1% yeast extract, 0.1% peptone and 2% dextrose adjusted to pH 7) per well. Plates were incubated for 1 day at 28 °C, 600 rpm then 2 mg ketone in 0.04 mL DMSO was added and the incubation was continued for 2 days. The incubations were quenched by addition of 2 mL methanol per well and analyzed by HPLC.