Application of a Practical Biocatalytic Reduction to an Enantioselective Synthesis of the 5*H*-2,3-Benzodiazepine LY300164

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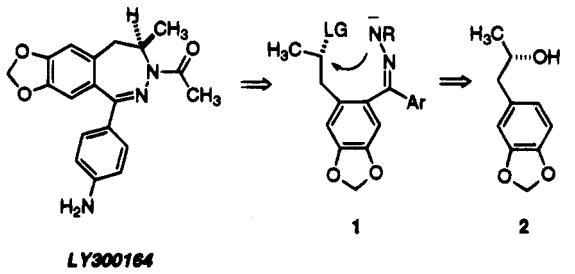
Workers at the Hungarian Institute of Drug Research have recently discovered a novel class of orally active 2,3-benzodiazepines which serve as noncompetitive antagonists of the AMPA subtype of excitatory amino acid receptors.[1] Preclinical studies have demonstrated that these compounds effectively block electroshock and chemically induced seizures in mice as well as provide neuroprotection from ischemic brain damage.[2] One compound of particular interest in this class is the 5H-*N*-acetyl derivative, the more active species of which is the (−) isomer LY300164.[3]

The clinical potential for this new compound has led to interest in developing syntheses that would not be limited by the inherent inefficiency of resolution methods and would be amenable to large-scale execution.[4] We describe here our findings, which detail an unusually effective combination of organic synthesis and biocatalysis. The result is a highly efficient and convergent preparation of the target compound which employs an environmentally benign enantioselective biocatalytic reduction. This step is facilitated on the preparative scale by the application of resin-based technology.[5]

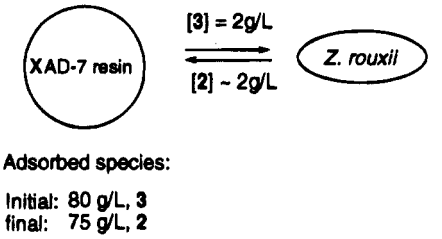
Our retrosynthetic strategy required delivery of the benzodiazepine nucleus by intramolecular cyclization of a suitably functionalized hydrazone (Scheme 1).[6] This disconnection was chosen since it was anticipated that the required stereocenter could be accessed from the relatively simple alcohol **2**. Asymmetric reduction of the corresponding ketone was considered the most desirable option for preparation of the optically active alcohol.[7]

Chemical methods for the enantioselective reduction of methyl benzyl ketones are typically unselective or require the stoichiometric use of costly reagents.[8] Biocatalytic reactions are often more efficient.[9] Unlike closely related examples, however, selectivity and conversion of **3** with Baker's yeast, as well as other common microorganisms, was unsatisfactory.[10]

Scheme 1



Scheme 2



After an extensive screen of organisms, this ketone proved to be an excellent substrate for a whole-cell-mediated process involving an NAD(P)H-dependent oxidoreductase from *Zygosaccharomyces rouxii*.[11,12] High conversion to the desired alcohol was accomplished with excellent stereocontrol (>99.9% ee).

As is common to many oxidoreductase processes, preparative-scale reactions proved problematic due to concentration-dependent substrate and product toxicity to the yeast as well as copious emulsions encountered during product isolation.[13] Sensitivity of the yeast to organic solvents limited the application of standard options which have been developed to circumvent these problems.[14] A successful alternative proved to be use of a solid−liquid biphase reaction system that allowed for efficient production of **2** (Scheme 2).

The ketone substrate was adsorbed to a medium-polarity cross-linked poly(methacrylate ester) resin (Amberlite XAD-7) and introduced into an aqueous suspension of *Z. rouxii*. The adsorptive properties of the resin on both substrate and product enabled a ketone loading of 80 g/L while limiting the effective solution concentration of both **2** and **3** to sublethal concentrations (~2 g/L). Thus, substrate diffused into solution to be reduced by the organism, and product was adsorbed onto the resin

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(7) Copper-catalyzed reaction of (*S*)-propylene oxide with the corresponding aryl Grignard reagent also produces the desired alcohol (91%). Current pricing for the optically pure epoxide is high.

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(9) (a) Csuk, R.; Glänzer, B. I. *Chem. Rev.* **1991**, *91*, 49. (b) Servi, S. *Synthesis* **1990**, 1.

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(11) *Z. rouxii* is readily available from the American Type Culture Collection, Rockville, MD (ATCC 14462). The yeast is used commercially in the production of soy sauce and is classified as a GRAS (generally regarded as safe) organism.

(12) *Z. rouxii*-mediated reduction of β-keto esters was reported during review of this manuscript: Hunt, J. R.; Carter, A. A.; Murrell, J. C.; Dalton, H.; Hallinan, K. O.; Crout, D. H. G.; Holt, R. A.; Crosby, J. *Biocatal. Biotransform.* **1995**, *12*, 159.

(13) Cell death occurred when the yeast was exposed to concentrations of **2** or **3** ≥6 g/L.

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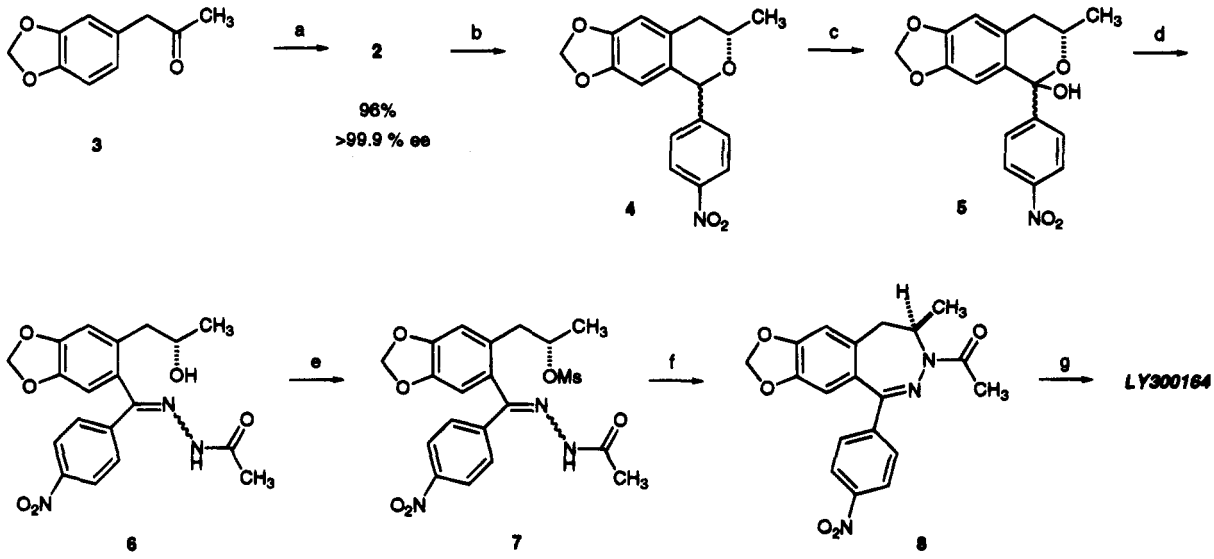
Scheme 3[a]



[a] Reaction conditions: (a) *Z. rouxii*, XAD-7 resin (96%); (b) *p*-NO_2PhCHO, HCl, toluene (90%); (c) 50% NaOH, air, DMSO/DMF (>95%); (d) H_2NNHAc, EtOH, catalytic HCl (91%); (e) CH_3SO_2Cl, Et_3N, CH_2Cl_2 (87%); (f) *t*-BuOLi, THF (91%, >99.9% ee); (g) Pd/C, H_2, EtOH (87%).

following its formation. The operability of the reaction was further enhanced by the size differential of the resin (~500 μm) and the yeast cells (~5 μm), which enabled the product-containing resin to be collected on a 150 μm filter screen; the yeast slurry could be disposed without environmental concerns. A simple rinse of the resin with acetone liberated the product cleanly. Although the use of resins for *in situ* extractive bioconversions has been described, application in biocatalytic processing is rare and, to our knowledge, has not been previously employed in yeast-mediated reductions.[5]

Acid-catalyzed reaction of **2** with 4-nitrobenzaldehyde led to convergent introduction of the remaining carbon constituents and generation of an isomeric mixture of optically pure **4** (Scheme 3). Conversion of **4** to the desired ring system required oxidation at C-1. Although the oxidation chemistry of 1-aryl isochromans has been developed as a method for the production of dicarbonyl compounds, reactions which allow selective C-1 functionalization are unknown.[15,16]

The propensity for isochromans to undergo autoxidation to yield the corresponding peroxide and peroxide dimer led to our evaluation of a similar process.[16] This approach was pursued in spite of the surprising observation that unlike 1-H and 1-alkyl derivatives, 1-arylisochromans are known to resist photochemically induced autoxidation.[17] In the event, reaction of the carbanion generated by NaOH at 5−10 °C in DMSO/DMF with air led to clean conversion to the desired hemiketal.[18] The intermediate peroxide was not detected and was presumably reduced immediately upon its formation by DMSO.[19] Reaction of **5** with acetic hydrazide in refluxing ethanol gave a 1:1 *E/Z* mixture of hydrazones **6**.

Cyclization of the *E/Z* mixture of **6** directly to **8** was accomplished by employing standard Mitsunobu conditions (THF, DEAD, PPh_3). High conversion was realized (90%), but product isolation was complicated, and **8** was isolated in only 73%. A stepwise procedure proved more desirable. Treatment of **6** with methanesulfonyl chloride and triethylamine cleanly provided the desired mesylates **7** in high yield without competing *N*-mesylation. Cyclization was readily accomplished by treatment of **7** in THF with lithium *tert*-butoxide. Both *E* and *Z* mesylate isomers converged to the desired product **8** in 91% isolated yield. The seven-membered ring was isolated exclusively without production of products from competing reactions of the other nucleophilic positions. The only observable byproduct was a trace of the corresponding elimination product (<0.15%).

Both the Mitsunobu procedure and the mesylate displacement reaction proceded with complete inversion of the stereocenter and provided optically pure penultimate intermediate **8** (>99.9% ee). The synthesis was completed by reduction of the nitro group under standard conditions to deliver LY300164 in 87%.

In summary, we have developed an efficient and environmentally benign synthesis of the 5*H*-2,3-benzodiazepine LY300164 that provides the optically pure compound in 51% overall yield. Intramolecular hydrazone alkylation led to a remarkably facile and selective formation of the benzodiazepine. Furthermore, the application of resins to whole-cell-based biotransformations should find general utility for similar reactions that are complicated by component inhibition and product isolation.

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Supporting Information Available: Experimental details and characterization data for **2**, **4**−**8**, and LY300164 (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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