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Enzymatic Resolution of 2-Hydroxymethyl-1,4-benzodioxanes

Michael D. Ennis* and David W. Old

Medicinal Chemistry Research
The Upjohn Company
Kalamazoo, Michigan 49001

Abstract: 2-Hydroxymethyl-1,4-benzodioxanes have been shown to be effectively resolved through an enzyme-mediated, stereoselective acylation. Enantiomeric excesses greater than 80% are realized after a single acylation, and enriched material can be upgraded to >96% ee by a second enzymatic resolution.

The 1,4-benzodioxane ring system occupies a traditional place among cardiovascular agents. A number of 2-aminomethyl derivatives of this heterocycle possess antihypertensive properties by virtue of their α-adrenergic antagonism.[1] More recently, some 1,4-benzodioxanes have been found to exhibit strong affinities for other CNS receptors, particularly the serotonergic and dopaminergic sites.[2] The influence of the absolute configuration at the chiral center in 2-substituted-1,4-benzodioxanes in determining receptor binding affinity has been amply documented.[3] The synthesis of optically-pure 2-substituted-1,4-benzodioxanes usually reduces to the preparation of the 2-hydroxymethyl intermediate **1** in chiral form. In this Letter, we will describe the convenient resolution of racemic 2-hydroxymethyl-1,4-benzodioxanes by the Amano P-30 lipase enzyme. In the accompanying Letter, we describe the use of this methodology for the preparation of (+)- and (-)-flesinoxan, an orally-active 5-HT_{1A} agonist.

2-Aminomethyl derivatives of 1,4-benzodioxanes have been resolved by enantioselective crystallization with optically pure binaphthylphosphoric acid.[4] Optically pure **1a** has been prepared previously by coupling a mono-protected catechol with chiral glycidol[5], glycidol tosylate[5], or glycerol derivatives.[3a] Further elaboration provided the benzodioxane system. A Sharpless epoxidation strategy has been utilized on a 1-aryloxy-4-hydroxy-2-butene substrate.[6] We considered that an enzymatic resolution procedure on racemic **1** would be a convenient method of preparing optical isomers of 1,4-benzodioxanes and would allow for the simultaneous generation of both enantiomers. For our own programmatic needs we focused on the 8-methoxy derivative **1b**, but we have demonstrated that this enzymatic methodology is applicable to not only the parent system **1a** (vide infra) but to functionally more complicated substrates as well.[7]

Illustrated in the Scheme is the strategy for application of enzymatic resolution to 1,4-benzodioxanes. We patterned our approach after the work of Bianchi, et. al., who successfully utilized lipase enzymes in organic solvents for the resolution of both primary and secondary alcohols.[8] Resolution is accomplished by a stereoselective acylation reaction in a water-immiscible organic solvent. We had good success using acetic anhydride as the acylating agent and benzene or methylene chloride as the organic solvent. We examined two

Scheme

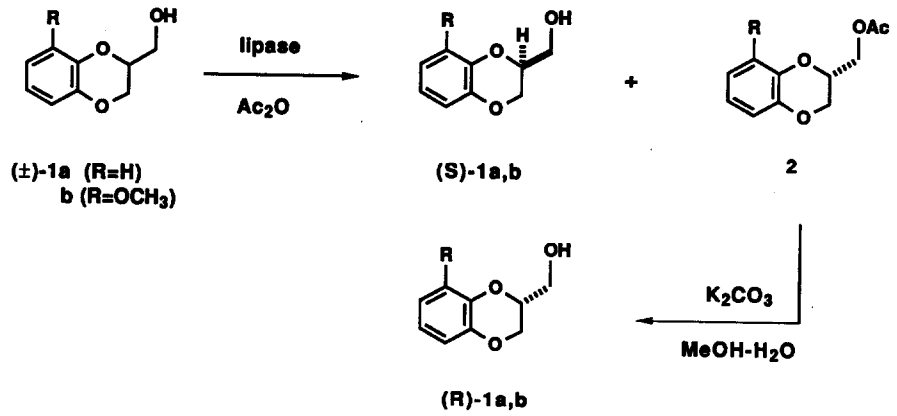

Table: Enzymatic resolutions of 1

run	cmpd (S:R)	enzyme	time, hr	conv, %	(S)-1 % yield	(S)-1 $[\alpha]_D$	(S)-1 % ee[a]	(R)-1 % yield[b]	(R)-1 $[\alpha]_D$	(R)-1 % ee[a]
1	**1b** (50:50)	—[c]	41.5	14	—	—	—	—	—	—
2	**1b** (50:50)	PPL	41.5	52	37	—	0	—	—	—
3	**1b** (50:50)	Amano P-30	2.6	50	44	-28°	83	43	—	81
4	**1a** (50:50)	Amano P-30	1.3	49	48	-23°	62	—	—	—
5	**1b** (91:9)	Amano P-30	<0.5	10	[illegible]			—	—	—
6	**1b** (10:90)	Amano P-30	6.5	84	—	—	—	[illegible]		

[a]Enantiomeric excess determined as described in the text. [b]Yield is for the acetate **2**, but ee reported for the derived alcohol (R)-**1**. [c]Control reaction run with no enzyme present.

different enzymes: porcine pancreatic lipase from Sigma (PPL), and a *Pseudomonas fluorescens* derived lipase from Amano (Amano P-30). Reactions were monitored by HPLC until a 50% conversion was reached, at which point the reaction mixture was simply filtered and chromatographed to give the acetate **2** along with unacylated alcohol **1**.

The optical purity of the unacylated alcohol **1** was determined in three ways. Derivation of **1** as both the Mosher ester and the O-methylmandelate provided compounds which could be assayed for diastereomer ratios by HPLC. The ^{19}F-NMR of the Mosher derivatives provided the third direct measure of optical purity.[9] To determine the optical purity of the acylated product **2**, we simply hydrolyzed the ester and analyzed the resulting alcohol as just described. The results of these experiments are shown above.

As can be seen from the Table, both enzymes studied displayed catalytic activity for the acylation of racemic **1b** (entries 2 and 3). The control experiment (entry 1) verified that the non-enzymatic acylation reaction proceeds at a very slow rate (only 14% conversion after nearly 42 hours). The PPL enzyme was not only significantly less active than Amano P-30, but was also completely non-stereospecific in catalyzing the acylation of **1b**. On the other hand, the Amano P-30 reaction was both extremely fast (50% conversion in 160 min) and highly stereospecific. The unacylated alcohol **1b** recovered from this reaction assayed to a 91:9 ratio of Mosher and O-methylmandelate diastereomers (entry 3). This ratio was mirrored when acetate **2b** was hydrolyzed and analyzed. This comparison between the PPL and Amano P-30 enzymes is consistent with that reported by Bianchi, et al.[8]

We have represented the absolute stereochemistry of the unreacted alcohol as (S) based upon the known preference of Amano P-30 the acylate the (R)-isomer in other systems.[8] Further support for this assignment is provided by entry 4, where the parent 2-hydroxymethyl-1,4-benzodioxane **1a** was subjected to the Amano P-30 acylation. This reaction proceeded quickly and provided the (S)-**1a** in excellent yield and 62% ee. The material thus obtained had a optical rotation of -23°, which is opposite to that of the known (R)-isomer[3a] and is therefore consistent with established (R)-isomer preference for this enzyme.

Although we were encouraged by the relatively high levels of stereoselectivity displayed by the Amano P-30 acylations, the 83% ee nevertheless falls short of today's standards for optical purity. We therefore resubjected the now enantiomerically enriched alcohols to a second treatment with the enzyme. By taking material which was 91:9 (S:R) and attempting to selectively acylate the 9% unwanted (R)-isomer, one could anticipate the recovery of even further enriched (S)-**1b**. Similarly, one could take **1b** which is enriched to a 10:90 (S:R) level and run the acylation reaction to nearly 90% conversion. The alcohol derived from the acetate formed in this reaction should be even more stereochemically enriched. These ideas were successfully reduced to practice as illustrated by entries 5 and 6. The reaction of (S)-enriched **1b** very quickly reached 10% conversion and provided an 82% yield of recovered (S)-**1b**. We determined that this material was greater than 96% enantiomerically enriched as judged by the three assays described above. For the (R)-enriched isomer, the reaction took longer and was eventually stopped at 84% conversion. The (R)-**2** acetate, obtained in 75% isolated yield, was hydrolyzed to give the (R)-**1b** alcohol. Again, the optical purity of this material was determined to be greater than 96% ee.

We have demonstrated the utility of the Amano P-30 lipase enzyme in the resolution of 2-hydroxymethyl-1,4-benzodioxanes. With two-passes of the enzyme system, high levels of enantiomer enrichment can be realized. This procedure is rapid and extremely simple to carry out. In the accompanying Letter, we demonstrate the application of this methodology in the synthesis of (+)- and (-)-flesinoxan.

Lipase catalyzed acylation of 1b.

A solution of **1b** (3.29 g, 16.8 mmol) in benzene (42 mL) was treated at room temperature with the Amano P-30 enzyme (640 mg) prepared as described in reference 8. The progress of the reaction was followed by HPLC, and after 160 min was found to be 50% complete. The reaction mixture was gravity filtered and the filter cake was washed several times with ether. The combined filtrates were washed with 5% aqueous Na_2CO_3, dried over Na_2SO_4, filtered, and concentrated to give a mixture of the acetate **2b** and unreacted **1b** (3.8 g). This mixture was chromatographed on silica gel to obtain 1.44 g of (S)-**1b** (44%) and 1.73 g of **2b** (43%).

HPLC analysis of the Mosher ester of 1b.

The Mosher ester of **1b** was prepared by coupling with (S)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetic acid using DCC/DMAP. Diastereomers were analyzed on a Beckman Ultrasphere ODS Reverse Phase HPLC column (5 μ) using 64% MeOH/H_2O flowing at 5 mL/min. R_t for (S)-**1b** derivative: 40.13 min. R_t for (R)-**1b** derivative: 37.75 min.

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