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The Synthesis of (+)- and (-)-Flesinoxan: Application of Enzymatic Resolution Methodology

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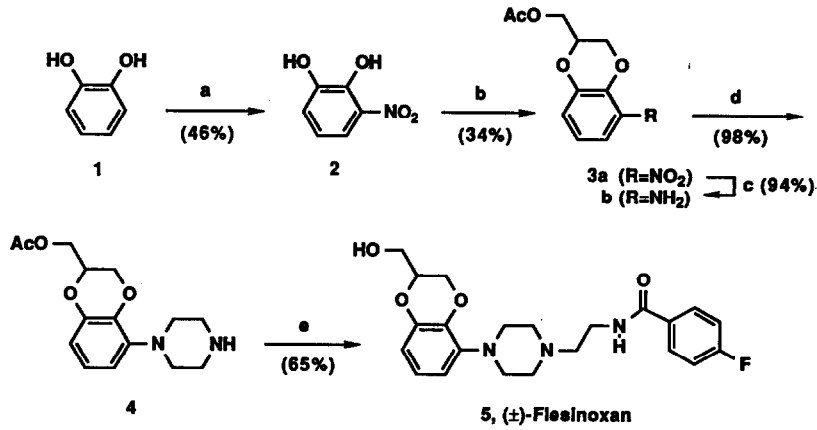
Abstract: The synthesis of (±)-flesinoxan was carried out in seven steps starting from catechol. An enzymatic resolution was utilized to prepare each enantiomer. Based upon the known preferences of the enzyme system used, we have assigned the (R)-configuration to the (+)-flesinoxan isomer.

(+)-Flesinoxan is an orally-active 5-HT_{1A} agonist discovered by the Duphar pharmaceutical company.[1] This potent compound has the distinction of being the first such agent to be evaluated in clinical trials as an antihypertensive. Although the synthesis of racemic flesinoxan has been described in patent literature,[2] the preparation and assignment of the optical isomers has never been disclosed. This Letter describes our preparation of both (+)- and (-)-flesinoxan. Highlighting this synthesis is a final stage resolution mediated by a lipase enzyme.[3]

Illustrated in the Scheme is the synthesis we carried out to produce racemic flesinoxan **5**. The Duphar patent starts with the aniline **3b**. Surprisingly, we were unable to find a description of either **3b** or the precursor nitro-compound **3a** in the chemical literature. We prepared the nitro-alcohol **3a** by the two-step sequence shown. Two-phase nitration of catechol[4] generated a separable mixture of the desired 3-nitro isomer **2** and the unwanted 4-nitro isomer.[5] Reacting **2** with epichlorohydrin in basic ethanol produced a mixture of regioisomeric benzodioxanes, from which was obtained the desired 5-nitro isomer by chromatography (30%).[6] This isomer was converted to the acetate **3a** in 87% by standard methodology and reduced by catalytic hydrogenation to provide the aniline **3b** (94%). Treatment of **3b** with bis-(2-chloroethylamine) hydrochloride in refluxing chlorobenzene effectively bis-alkylates the aniline nitrogen to generate the aryl piperazine **4** in 98% yield. Alkylation of the secondary amine in **4** with 4-fluorobenzoylaziridine[7] provides flesinoxan acetate (65%), which upon hydrolysis under standard conditions gives racemic flesinoxan **5**.

In the previous Letter, we described our studies using Amano P-30 lipase to kinetically resolve 2-hydroxymethyl-1,4-benzodioxanes. In these studies, we were able to selectively acylate the (R)-benzodioxane isomer. Two applications of this enzyme-mediated acetylation resulted in optically pure material (>96% ee). It was our intent to apply this methodology to resolve racemic flesinoxan into its optical isomers. The results of the Amano P-30 acylations with (±)-flesinoxan are summarized in the Table. In contrast to our earlier work, the lipase mediated acylations involving flesinoxan were significantly slower than those with simplified, non-nitrogenous 1,4-benzodioxans. This necessitated using larger amounts of both the enzyme preparation and

Scheme



a) HNO_3, ether, 0°→RT. b) NaOH(aq), EtOH, epichlorohydrin, ↑↓; then AcCl, pyr. c) H_2 (40 psi), 10% Pd/C, EtOH. d) $(ClCH_2CH_2)_2NH_2^+Cl^-$, chorobenzene, ↑↓. e) 4-fluorobenzoylaziridine, Et_3N, acetone, ↑↓; then K_2CO_3, MeOH.

acetic anhydride. Unfortunately, the large excess of acylating agent helped promote the non-enzymatic (and non-selective) acetylation of our substrate as evidenced by a control reaction (entry 1). In order to minimize this detrimental chemical reaction, we employed a reaction protocol in which the acetic anhydride was added slowly (several hours via syringe pump) to the reaction flask. In one run (entry 3) we saturated the organic phase with water prior to initiating the reaction, hoping to minimize the non-enzymatic acylation by destroying acetic anhydride which is not intimately incorporated into the enzyme preparation. Although this particular reaction proceeded slowly and required the addition of 3 equiv. of acetic anhydride, it nevertheless demonstrated very high stereoselectivity. These resolutions are extremely easy to carry out. Briefly, a mixture of flesinoxan, acetic anhydride, and the Amano P-30 enzyme preparation are gently stirred in a water-immiscible solvent (eg, benzene, dichloromethane, or both). The reaction is monitored by HPLC and terminated when the desired conversion is reached (50% when starting with racemic substrates). The resulting acetate and remaining alcohol are easily separated by chromatography. Enantiomeric excess is assayed by optical rotation and through the preparation and examination of Mosher esters.

As can be seen from the Table, the stereoselectivity for Amano P-30 mediated acylations of flesinoxan using our "standard" conditions[3] was approximately 90:10, which is nearly identical to that seen in our earlier studies with simpler benzodioxane substrates (entry 2). In the run employing water-saturated solvent (entry 3), we were able to isolate unreacted (S)-flesinoxan which assayed for 99:1 optical purity. In order to obtain the (R)-isomer, it was necessary to hydrolyze the enantiomerically-enriched acetate **6** and to subject the resulting alcohol to another lipase-mediated acylation reaction. In this way, we were able to prepare multi-gram quantities of (R)-flesinoxan which exhibited an optical rotation of +26° and assayed via Mosher esters for 98% ee (entry 4).

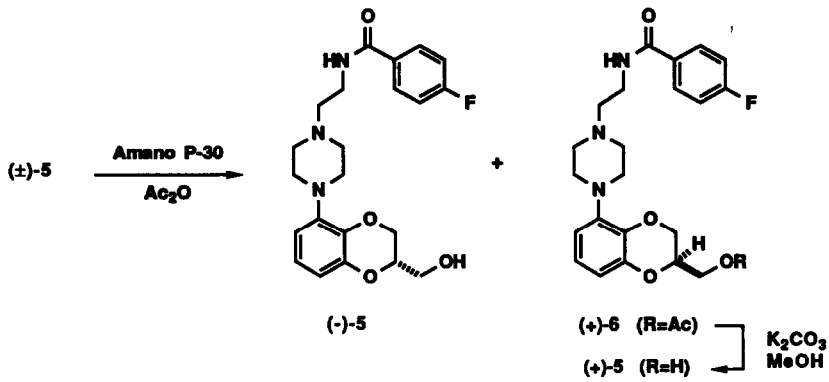


Table: Enzymatic resolution of flesinoxan

entry	initial R:S ratio	time, hr	conv, %	(-)-5			(+)-5		
				% yield	$[\alpha]_D$	% ee[a]	% yield[b]	$[\alpha]_D$	% ee[a]
1	50:50	4.5	21[c]	—	—	—	—	—	—
2	50:50	4.5	49	43	-23°	84	52	+20°	76
3	50:50	144[d]	50	[illegible]			48	+22°	78
4	76:24	72	68	25	-20°	70	[illegible]		

[a]Enantiomeric excess as determined by ^{19}F NMR of derived Mosher esters. [b]Represents overall yield for acylation and hydrolysis. [c]Control reaction without enzyme. [d]Reaction solvent (CH_2Cl_2) saturated with H_2O.

A final note must be made regarding absolute stereochemical assignments. We have depicted the absolute stereochemistry of (+)-flesinoxan as (R) based upon the known preference for the Amano P-30 lipase to selectively acylate the (R)-isomer.[8] To our knowledge this preference is without exception. Furthermore, we have demonstrated that the Amano P-30 lipase system used for these resolutions exhibits the same (R)-isomer preference in related 2-hydroxymethyl-1,4-benzodioxane substrates.[3] The observation that both flesinoxan and simple benzodioxanes are resolved to nearly identical levels of optical purity also suggests that these systems interact with the Amano P-30 lipase in a similar manner. As stated earlier, we are not aware of any published reports describing the preparation of optically-active flesinoxan. Furthermore, we have never seen a structure of this agent published in any of the papers describing the biology of (+)-flesinoxan. Admittedly, our assignment is based solely upon the known preference of the Amano P-30 enzyme to acylate the (R)-enantiomer and as such does not stand as a proof. However, we feel this precedent is very well-documented and are confident in our assignment.

Experimental Procedures

(-)-Flesinoxan, (-)-5: Racemic flesinoxan (1.00 g) was dissolved in 20 mL of H_2O-saturated CH_2Cl_2. To this solution was added the enzyme preparation[8] (270 mg) followed by a solution of acetic anhydride (0.12 mL, 0.51 equiv) in CH_2Cl_2 (9 mL) slowly over 10 hours. The reaction was monitored by HPLC (Ultrasphere ODS 5μm column, 50% CH_3CN/pH 3 phosphate buffer, 4 mL/min; R_t **5** = 3.51 min, R_t **6** = 6.16 min). Additional amounts of acetic anhydride (1 equiv) were periodically added as solutions (0.2 M in benzene) over 4-5 hours. After 144 hours and a total of 3 equiv. of acetic anhydride had been added, the reaction reached 50% conversion. The reaction mixture was filtered and the filter cake washed thoroughly with CH_2Cl_2. The combined organics phases were stirred vigorously for 3 hrs with 125 mL saturated aqueous Na_2CO_3, separated, and dried over $MgSO_4$. Purification by silica gel chromatography (10% MeOH/EtOAc) gave (+)-**6** (536 mg, $[\alpha]_D$ +34°) and (-)-flesinoxan (407 mg, 41%); $[\alpha]_D$ -27° (c 1, MeOH). The HCl salt of (-)-flesinoxan was prepared and recrystallized from EtOH/Et_2O; mp 183.0-184.5°C, $[\alpha]_D$ -24° (c 1, MeOH).

(+)-Flesinoxan, (+)-5: (+)-Flesinoxan was obtained from two applications of the enzymatic esterification procedure. Combining several lots of (+)-**6** and hydrolysing them gave 2.34 g of (+)-flesinoxan which was enriched in the (R)-isomer (R:S 76:24). This material was dissolved in CH_2Cl_2 (120 mL) and treated with the Amano P-30/Celite enzyme preparation (635 mg). As above, solutions of acetic anhydride in benzene were periodically added over 4-5 hours until HPLC analysis indicated the reaction had gone to 68% completion, at which point it was worked up as described above. Chromatography provided (+)-**6** (1.83 g, 70%, $[\alpha]_D$ +41°, c 1, MeOH), which was hydrolyzed to give (+)-flesinoxan (1.70 g, 99%); [a]$_D$ +26 (c 1, MeOH). The HCl salt was prepared and recrystallized from EtOH/Et_2O; mp 183.0-184.0°C $[\alpha]_D$ +25° (c 1, MeOH).

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5. The desired 3-nitro isomer was separated from the 4-nitro isomer by exploiting its preferential solubility in hot heptane.
6. Structural assignment to these regioisomers was non-trivial. Absolute proof was obtained from an X-ray crystal structure of the derived piperazine **4**. We thank Steve Mizsak and Connie Chidester (Physical and Analytical Chemistry, The Upjohn Co.) for determining this structure.
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