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Aminotransferase Catalysis Applied to the Synthesis of a PAF Antagonist

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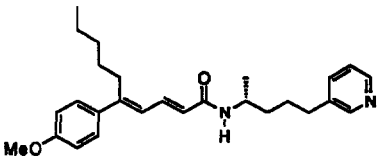
Abstract—(*R*)-α-Methyl-3-pyridinebutanamine (**5**) was required as a key intermediate for production of the PAF antagonist [(*R*)-(*E*,*E*)-5-(4-methoxyphenyl)-*N*-1-methyl-4-(3-pyridinyl)butyl-2,4-decadienamide (**1**). A method meeting the requirements of technical simplicity and economic viability was developed using an aminotransferase ex. *B. megaterium* to catalyze a kinetic resolution of racemic α-methyl-3-pyridinebutanamine (**3**). 5-(3-Pyridinyl)-2-pentanone (**4**) produced from the (*S*)-enantiomer, with co-generation of alanine, is recycled by catalytic reductive amination.

While, as a class, they have yet to yield an approved therapeutic agent, pharmaceutical research continues apace on various types of platelet activating factor (PAF) receptor antagonists.[1] Compound **1** represents a group of PAF antagonists characterized by the presence of a pentadienamide unit and Kierstead and coworkers, who discovered this type, reported both oral activity and high enantiospecificity in the expression of pharmacological effects.[2] A decision to advance compound **1** to clinical evaluation made a process yielding the pure (*R*)-enantiomer highly desirable, as it was known to be at least four times more active than the (*S*)-enantiomer in blocking PAF-induced bronchoconstriction in guinea pigs, with a substantially longer duration of action.



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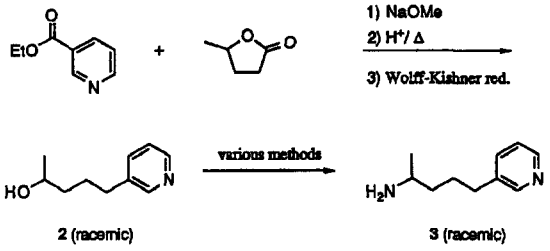
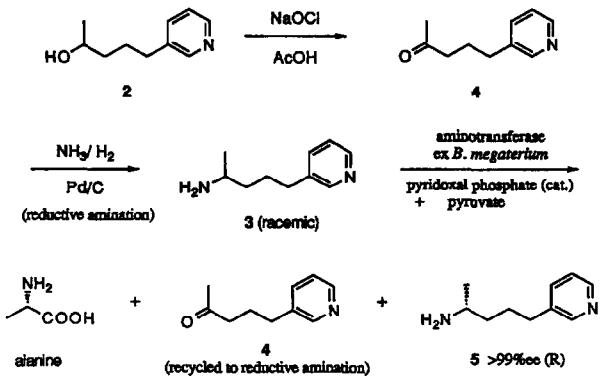
An earlier publication from these laboratories delineated the realization of an efficient synthesis for compound **1**.[3] The pentadienoic acid portion of the structure was effectively addressed with a vinylogous Reformatsky strategy and construction of the aminopentylpyridine moiety proved to be equally facile using a reaction sequence keyed on the Claisen ester condensation of ethyl nicotinate and γ-valerolactone (Scheme I).[3]

However, the various methods explored for obtaining **3** in enantiomerically pure form, either via enantioselective routes from alcohol **2** or by resolution of racemic **3**, entailed varying degrees of difficulty, success, and overall reliability—particularly when viewed in the context of large scale production. The potential for applying biological catalysis was recognized at an early stage and a procedure predicated on lipase-catalyzed kinetic resolution of alcohol **2** afforded material with 98–99 % *e.e.* in lab scale runs.[3] This did not constitute an adequate basis for technical scale production of compound **1** because of the number of steps required (5 steps) and because the level of enantiomeric excess achieved required double processing through the enzymatic hydrolysis.



Scheme I.

The emerging technology of enzymatic transamination is uniquely suited to the preparation of chiral primary amines in enantiomerically pure form[4] and looked particularly attractive for the problem in hand. Our expectations were quickly and fully met with the solution depicted in Scheme II.



Scheme II.

Dedicated to Professor J. Bryan Jones on the occasion of his 60th birthday.

Sodium hypochlorite in acetic acid, an oxidation method popularized by Stevens and co-workers,[5] proved to be highly practical for conversion of alcohol **2** to ketone **4** and catalytic reductive amination of this material afforded the racemic amine **3** in high yield.

Aminotransferases able to convert prochiral ketones stereospecifically to single enantiomer α-methyl arylalkyamines have been described by Stirling *et al.*[7] To access a target (*R*)-amine, an (*R*)-specific enzyme presented with the corresponding ketone and suitable amino donor, can be used in a synthesis mode. Alternatively, presented with the racemic amine mixture and an amino acceptor, an (*S*)-specific enzyme will enantioselectively convert the (*S*)-enantiomer back to the ketone leaving the target (*R*)-enantiomer in high enantiomeric purity. In this case, the readily available racemic amine proved to be an excellent kinetic and stereospecific fit for an available (*S*)-aminotransferase and the latter resolution mode was adopted.

The racemic amine **3** is thus combined with excess pyruvate in the presence of catalytic amounts of an (*S*)-aminotransferase from *Bacillus megaterium* and pyridoxal phosphate in aqueous buffer. The (*S*)-enantiomer undergoes deamination coupled to the conversion of pyruvate to alanine and leaves a mixture of desired unreacted (*R*)-amine **5** and deamination product, ketone **4**. The amine **5** and ketone **4** are then separated from the reaction mixture, and each other, by selective extraction at controlled pH, exploiting the difference in pK_a of the pyridine (5.5) and amine groups (10.5), and purified by distillation.

Particularly in cases where the 'wrong' enantiomer is associated with potentially undesirable side effects, the production of enantiomerically pure drug substances must meet two important criteria: (a) a very high level (≥ 99 %) of enantiomeric purity must be consistently achievable and (b) accurate and reliable analytical methods must be available to measure the enantiomeric purity. Having met point (a), attention was now focused on *e.e.* analysis and excellent methods based on chromatography with chiral columns were developed. As shown in Figure 1, the acetamides of the racemic amine **3** give good separation on a permethylated β-cyclodextrin lined capillary column. By this method, batches of (*R*)-amine **5** produced via the aminotransferase catalyzed process were shown to be consistently close to 100 % *e.e.* An equally effective HPLC method was developed using a Daicel Crownpack CR (+) chiral column (Figure 2).

When integrated with the conventional chemical steps of the process described in Reference 3, the aminotransferase technology provided a practical method for the production of PAF antagonist **1** and this was demonstrated on a multi-kilogram scale.

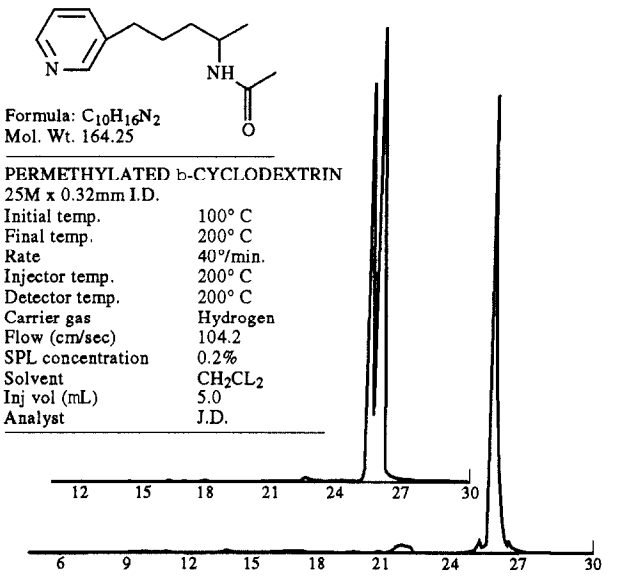


Figure 1. Chiral gc analyses of racemic and resolved amines

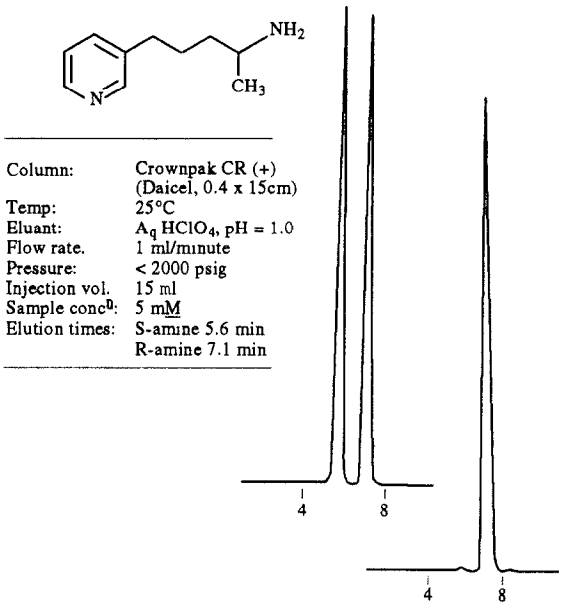


Figure 2. Chiral HPLC analyses of racemic and resolved amines.

Experimental Section

Preparation of 5-(3-pyridinyl)-2-pentanone (4)

To a solution of 5-(3-pyridinyl)-2-pentanol (**2**) (165 g, 1.0 mol) in acetic acid (1L) was added 1.23 M sodium hypochlorite solution (1L) dropwise over 70 min, keeping the temperature between 20–25 °C. After stirring for 2.5 h, the reaction mixture was quenched with isopropanol (50 mL). After stirring overnight, the residual oxidant was quenched by the addition of saturated $NaHSO_3$ solution (5 mL) (potassium iodide-starch test showed negative). The mixture was concentrated at 40–50 °C under reduced pressure (aspirator), and the residue was dissolved in CH_2Cl_2 (500 mL) and water (250 mL). Then the pH of the aqueous layer was adjusted to 11 by the addition of 4N NaOH (*ca* 375 mL). The organic layer was separated, and the aqueous layer was back-extracted with CH_2Cl_2 (2 x 200 mL). The combined organic layers were washed with saturated $NaHCO_3$ (200 mL), dried, and concentrated to dryness to give 159 g (97.3 %) of **4** as a dark amber oil. This material was used in the next step without any further purification. The spectroscopic and analytical

characteristics of **4** were reported previously.[6] The identity of material prepared by this method was verified by NMR.

^{1}H NMR ($CDCl_3$) δ 1.87 (2H, quintet, J = 8 Hz), 2.10 (3H, s), 2.42 (2H, t, J = 8 Hz), 2.59 (2H, t, J = 8 Hz), 7.19 (1H, dd, J = 8 and 6), 7.47 (1H, d, J = 8 Hz), 8.40 (2H, m).

Preparation of (±)-α-methyl-3-pyridinebutanamine (3)

A 500 mL autoclave was charged with **4** (10 g, 61.3 mmol), 10 % Pd on carbon (1 g), and methanol (50 mL). To this mixture was added a methanol solution of ammonia, which was prepared by dissolving 50 mL of liquid ammonia in 150 mL of methanol at –70 °C. The mixture was stirred at room temperature under 300 psi of hydrogen for 66 h (*ca* 50 % of reaction after 18 h). After the catalyst was removed by filtration, the filtrate was concentrated. The residue was then distilled under high vacuum to give 8.12 g (80.7 %) of **3** (bp 85–90 °C/0.5 mm Hg) as a colorless oil. The spectroscopic and analytical characteristics of **3** were reported previously.[6] The identity of material prepared by this method was verified by NMR.

^{1}H NMR ($CDCl_3$) δ 1.03 (3H, d, J = 7 Hz), 1.38 (2H, quintet, J = 8 Hz), 1.39 (2H, br s), 1.63 (2H, m), 2.60 (2H, t, J = 8 Hz), 2.89 (1H, sextet, J = 7 Hz), 7.20 (1H, dd, J = 8 and 6), 7.48 (1H, d, J = 8 Hz), 8.41 (2H, m).

Preparation of (R)-α-methyl-3-pyridinebutanamine (5)

(±) α-Methyl-3-pyridinebutanamine (**3**) (29 g, 176.8 mmol), pyruvic acid (11 g, 125 mmol), orthophosphoric acid (2 g, 20 mmol) and pyridoxal 5-phosphate (5 mg, 0.02 mmol) were dissolved in 1 L of deionized water and brought to 33 °C. The pH of the solution was verified at 7.3. Celgene S-AT biocatalyst[7] (4 g) was added to start the reaction and the mixture was maintained at 33 °C with gentle agitation. After 16 h, the enantiomeric excess was measured by direct analysis of the reaction mixture via HPLC to be > 99 %, and 35 % HCl (33 mL) was added to adjust the pH to < 4.

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The volume was then reduced to 130 mL by distillation (100 °C) to remove water. After cooling to room temperature, the pH was adjusted to 7.5 using 50 % NaOH solution, and the 5-(3-pyridinyl)-2-pentanone (**4**) removed by extraction (2 x) with 130 mL portions of CH_2Cl_2. These extracts were combined and concentrated to a yellow oil (13.3 g) which was distilled under high vacuum to give 11 g of **4** (bp 102–103°C/0.4 mm Hg).

The pH of the aqueous phase was then adjusted to > 13 using 50 % NaOH and the amine removed by extraction (2 x) with 130 mL portions of CH_2Cl_2. These extracts were combined and concentrated to a yellow oil (13.6 g) which was distilled under high vacuum to give 12.5 g of 99.6 % *e.e.* (*R*)-α-methyl-3-pyridinebutanamine (**5**) (bp 105–106 °C/0.6 mm Hg) as a clear colorless liquid. The identity of this material was verified by NMR.

Acknowledgement

The expert assistance of Pat Riggio in developing the analytical chiral GC method is greatly appreciated.

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