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A Practical and Robust Process to Produce SB-214857, Lotrafiban, ((2*S*)-7-(4,4′-Bipiperidinylcarbonyl)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1, 4-Benzodiazepine-2-acetic Acid) Utilising an Enzymic Resolution as the Final Step

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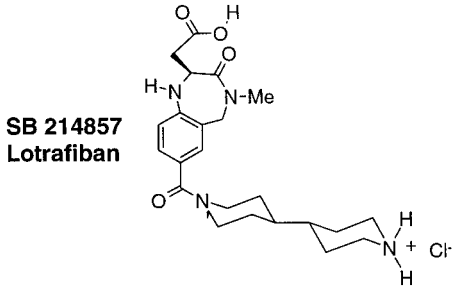
Abstract:

During the scale-up of a chemical process to produce phase II supplies of the chiral compound Lotrafiban, partial racemisation occurred to produce drug substance of unacceptable chiral purity. A new route capable of producing several hundred kilograms of Lotrafiban of high chiral purity had to be rapidly identified and scaled up. The strategy adopted was to employ an enzymic resolution as the final step, thus introducing the chirality under very mild conditions to prevent any racemisation. This was achieved using an immobilised form of the *Candida antarctica B* lipase in water at 30 °C. The biotransformation was demonstrated to be a robust, reliable, and an economic way to introduce the chirality into the Lotrafiban molecule.

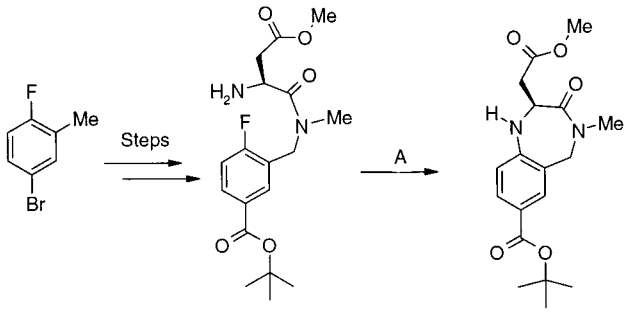
Lotrafiban, **SB-214857**, is an orally active GPIIb/IIIa fibrinogen receptor antagonist designed for the prevention of thrombotic events.[1] The route of synthesis developed to



make the initial clinical supplies was derived from the original Medicinal Chemistry synthesis[2] using (*S*)-aspartic acid as the source of chirality.

A key step in this route involved forming the 1,4-benzodiazepine ring via an intramolecular displacement of fluoride from a monocyclic intermediate, incorporating a synthon derived from aspartic acid (Scheme 1). Whilst the reaction was low-yielding (~40%), the 1,4-benzodiazepine product, SB-218093, was always isolated in 100% ee when the reaction was run in the laboratory.

Scheme 1. **Benzodiazepine synthesis via fluoride displacement**



However, on scaling up the process in the pilot plant, the chiral purity of the SB-218093 product fell to ~96% ee. This translated into drug substance of an unacceptable ee of 91%. Despite intensive investigations, the ee of the drug substance could not be satisfactorily upgraded by crystallisation or the problem of partial racemisation of SB-218093 solved. The most likely cause of the drop in chiral purity was felt to be base-catalysed racemisation via fluoride ion. It was felt that the 4A sieves in the plant reactors were less efficiently scavenging the HF liberated in the displacement reaction.

Thus, to meet clinical supplies requirements for Lotrafiban of high chiral purity a new synthetic route had to be quickly developed.

Whilst the fluoride displacement chemistry was being scaled up, a number of other routes to SB-214857 were being evaluated.[3,4] At this time, an efficient route that could produce the racemic methyl ester of SB-214857, (SB-215346, Scheme 2) was coming on-stream.[4] It was decided that to minimise the possibility of racemisation, the chirality would be introduced in the final step via a resolution using the mildest conditions that could be identified. A biocatalytic resolution seemed an ideal solution.

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(1) Samanen, J. M.; Ali, F. A.; Barton, L. S.; Bondinell, W. E.; Burgree, J. L.; Callahan, J. F.; Calvo, P. R.; Chen, W.; Chen, L.; Erhard, K.; Feuerstein, G.; Heys, R.; Hwang, S.-M.; Jakas, D. R.; Keenan, R. M.; Ku, T. W.′ Kwon, C.; Lee, C.-P.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M.; Peishoff, C. E.; Rhodes, G.; Ross, S.; Shu, A.; Simpson, R.; Takata, D.; Yellin, T.-O.; Uzsinskas, I.; Veslavsky, J. W.; Yuan, C.-K.; Huffman, W. F. *J. Med. Chem*. **1996**, *39*, 4867.

(2) Ku, T. W.; Ali, F. E.; Bondinell, W. E.; Erhard, K. F.; Huffman, J. W.; Venslavsky, J. W.; Yuan, C. C.-K. *Tetrahedron Lett*. **1997**, *38*, 3131.

(3) Hayes, J. *Synlett* **1999**, 865.

(4) Etridge, S. K.; Hayes, J. F.; Walsgrove, T. C.; Wells, A. S. *Org. Process Res. Dev*. **1999**, *3*, 60.

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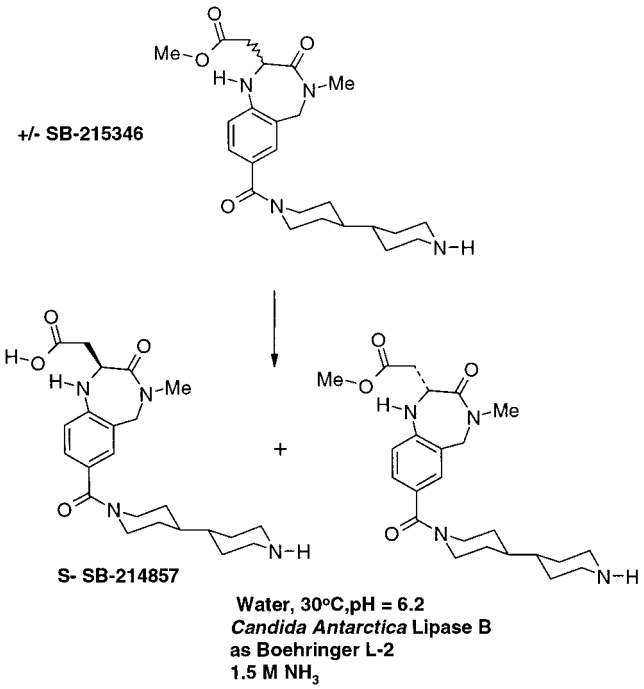
Scheme 2. **Bioresolution of ±SB 215346**



The goal was to identify an enzyme that would hydrolyse the racemic ester in SB-215346 to give the acid with the (*S*) stereochemistry required for Lotrafiban. A range of commercially available esterases, lipases, and proteases was screened for activity against SB-215346 in water.[5] Some esterases and proteases showed activity in the hydrolysis of the unwanted (*R*)-enantiomer, but poor ee's were obtained (~70%). Most of the commercially available lipases were inactive or showed very weak activity. The exception to this being *Candida antarctica* B (CAB) lipase.[6] This lipase quickly hydrolysed the (*S*)-enantiomer of SB-215346 in aqueous solution and was found to be totally stereoselective. Once all the (*S*)-isomer had been converted, the reaction ceased, and no hydrolysis of the (*R*)-enantiomer could be detected, even after prolonged reaction times (Scheme 2).

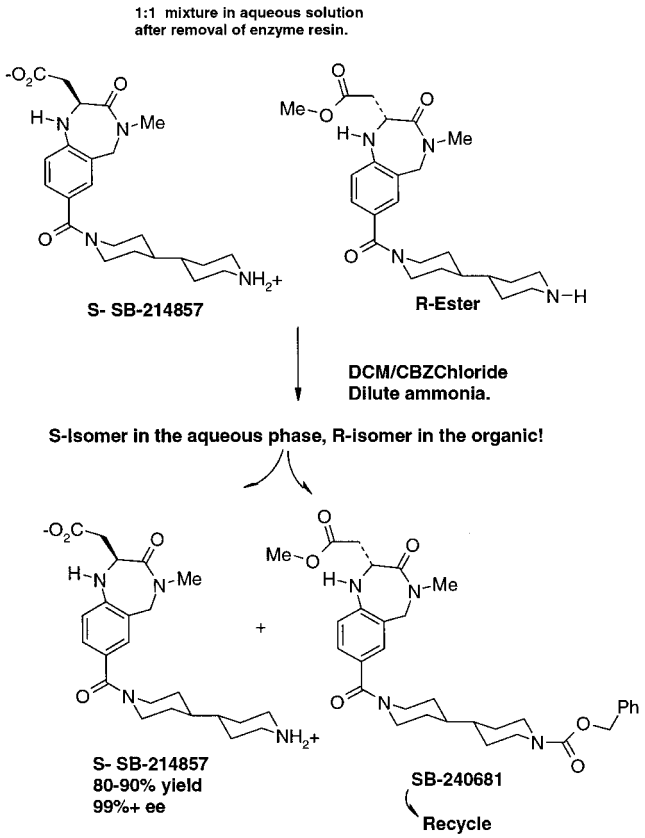
Having identified the desired enzyme for the biotransformation, the reaction had to be developed into a workable plant process. Initially, the enzyme was charged as a solution in water or added as a freeze-dried solid. This was deemed undesirable for large-scale work since protein would have to be removed from the aqueous solution of SB-214857. The solution to this problem was to use the CAB lipase immobilised on an inert support. We first used Novozym 435, a commercial preparation of the CAB lipase supplied by NovoNordisk. This biocatalyst worked well in initial reactions, but the activity quickly dropped off in subsequent repeat reactions. This was identified as being due to protein loss from the resin. Novozym 435 was designed as a catalyst for use in anhydrous systems (the lipase is only absorbed onto the resin).[7] The aqueous environment used in the resolution of SB-215346 was simply dissolving the lipase away from the solid support. A much better form of CAB lipase catalyst was the L-2 resin supplied by Boehringer Mannheim. This preparation uses a macroporous cross-linked resin to support the enzyme, but the protein is covalently bonded to the polymer, making it ideal for use in aqueous environments. The resin was charged at 10 wt % (this was never optimised) to SB-215346. This gave a reaction time of ~2 h at 25 °C in 10−15 vols of water as the solvent. Attempts to run the process at a more concentrated level led to the premature crystallisation of SB-214857. Provided the temperature was maintained below 30 °C, repeat laboratory experiments showed that at least 100 reuses of the resin should be possible. Analysis showed negligible protein loss from the resin into the aqueous environment. Thus, a few kilograms of resin were sufficient to produce hundreds of kilograms of SB-214857.

When scaled-up, the L-2 resin also proved to be very robust in the pilot plant, and multiple reuses were possible. After use, some resin was stored for over a year in 3.5 M ammonium sulphate and showed no loss of activity or chiral selectivity on reuse.

Having identified robust conditions for the resolution, a simple workup procedure to separate the chiral products was sought. Traditionally, when a racemic ester is resolved into a chiral acid and a chiral ester, the products are easily separated by basification, extraction of the ester into a solvent, and then acidification followed by extraction or crystallisation of the acid. In the case of SB-215346, the (*R*)-ester proved to be very water-soluble and could not be efficiently extracted away from the required (*S*)-acid unless the pH was very high (12−14). In practice this meant that fairly rapid chemical hydrolysis of some (*R*)-SB-215346 always occurred, lowering the ee of the isolated (*S*)-acid. On a small scale, chromatography on ion-exchange resins or XAD 2 could separate the products; however, a more elegant separation was sought for large-scale production. Due to the basicity of the piperidine nitrogen in SB-214857, at pH 7 this compound exists in the zwitterionic form, with the piperidine nitrogen protonated. It was reasoned that treatment of a mixture of the zwitterionic SB-214857 and the (*R*)-ester free base at pH 7.0 with a limited quantity of a mild acylating agent would result in the (*R*)-ester only reacting, having an unprotonated, hence nucleophilic, piperidine nitrogen. Indeed, this proved to be the case. On treating the 1:1 aqueous solution of (*S*)-acid and (*R*)-ester with a dichloromethane solution of BOC anhydride or CBZ chloride (the latter was preferred for large-scale use), with the pH maintained at 7.0, the (*R*)-ester partitioned into the organic solvent as the carbamate, SB-240681, whilst the required (*S*)-acid remained unreacted in the aqueous phase. The desired product was then isolated simply by concentration and cooling. The SB-240681 could be recovered in quantitative yield from the dichloromethane layer and racemised[8] and deprotected to regenerate SB-215346 to be recycled back into the resolution process (Scheme 3).

(5) Enzyme screening kits from Altus Biologics, Roche Molecular Biochemicals, and Ensynthase Engineering.
(6) Anderson, E. M.; Larrson, K. M.; Kirk, O. *Biocatal. Biotransform.* **1998**, *16*, 181.
(7) Michiyo, I. WO/88/02775.
(8) Andrews, I.; Atkins, R.; Bellingham, R. F.; Breen, G.; Carey, J. S.; Etridge, S. K.; Hayes, J.; Hussain, N.; Morgan, D. O.; Share, A.; Smith, S.; Walsgrove, T. C.; Wells, A. S. *Tetrahedron Lett.* **2001**, *42*, 4915.

Scheme 3. **Downstream enantiomer separation**



The only byproducts identified from the treatment with CBZ chloride were very minor amounts of amide dimers (1−2%) arising via the formation of mixed anhydrides.

Apart from resolving the racemate, some SB-215346 of ∼93% ee was prepared from the plant batches of 96% ee SB-218093. This SB-215346 was then converted to 100% ee SB-214857 by running the enzyme hydrolysis to 90% conversion and then separating the (*S*)-acid and the small amount of residual (*R*)-ester as before.

In conclusion, the enzymic resolution route provided a robust and efficient way to prepare large quantities of drug substance of high chiral purity. The simple and mild process minimised the chance of any racemisation and gave excellent enzyme life. Allowing for multiple reuses, the calculated cost of enzyme resin per kg of drug substance was between £5 and £10.

Whilst a more efficient manufacturing route was eventually developed that employed an earlier resolution, this biotransformation provided a pathway to keep the project alive and supplied with crucial clinical and tox supplies (several hundred kilograms of Lotrafiban were prepared by this route).

Experimental Section

Reagents and solvents were used as supplied by standard commercial bulk suppliers. SB-215346 was produced by published procedures.[4] The *Candida antarctica B* lipase resin, L-2 (now designated Chirazyme L-2 C-1) was supplied by Boehringer Mannhiem (now Roche Molecular Biochemicals).

The resolution was carried out in a standard 2400-L glass-lined vessel equipped with a pH probe inserted via a port into the reaction mixture (rather than a circulating loop). The titrant could be added manually via a head tank or more effectively controlled via an autotitrator system. The variable speed agitator was set to gently suspend the resin in the reaction mixture. Once used in processing, L-2 resin was stored wet with 3.5 M ammonium sulphate at 5 °C when not in use.

Chiral analysis of SB 214857 was performed by capillary electrophoresis. The capillary was 75 μm polyimide-coated fused silica (50/57), capillary cartridge 100 μm × 200 μm. Running buffer was 0.1 M, pH 3, lithium phosphate containing 1.5 mM methyl-β-cyclodextrin and 0.05% w/v hydroxyethyl cellulose. The prerinse solution was 0.1 M sodium hydroxide. The applied voltage was 30 kV, ramped from 0 to 30 kV over 0.2 min. The capillary temperature was 20 °C, UV detection at 200 nM, and the analysis time was 25 min.

Typical Pilot-Plant Biotransformation. Demineralised water (1100 kg) and SB-215346 (racemic methyl ester, 76.3 kg, 161 mol at 93% purity) were charged to a reactor at 26 to 28 °C. Whilst the solid was being charged, ∼1.0 N HCl was added (concentrated HCl, 79 kg, in deionised water, 630 kg) to maintain the pH between 6 and 7. When the solid addition was finished, the mixture was stirred for 30 min, maintaining the pH between 6.3 and 6.8. At the end of the 30-min stir, all the SB 215346 had dissolved. A total of 105 L of titrant had been added.

The titrant was changed to ∼1.5 N ammonia solution (94.5 kg, 0.88 M ammonia plus 1260 kg of deionised water) and Boehringer L-2 enzyme resin (6 kg) was added. The reaction was stirred at 29 to 30 °C, maintaining the pH between 6 and 6.8 by the addition of ∼1.5 N ammonia solution. After 2.5 h demand for titrant ceased, and the end of the reaction was confirmed by HPLC analysis (1:1 mixture of acid and residual ester). The L-2 resin was filtered off through a single bag Gaff filter, and the vessel, lines, and filter were washed with deionised water (290 kg). The resin was then reused in another resolution or stored. If the resin was to be stored, the Gaff filter was filled with 3.5 M ammonium sulphate solution and the resin allowed to soak for 2−3 h, drained, and stored at 5 °C, damp with the ammonium sulphate solution.

The aqueous filtrate was then stirred whilst simultaneously adding a solution of benzyl chloroformate (15.8 kg, 83.6 mol at 90% purity, in dichloromethane, 396 kg) and ∼1.5 N ammonia solution (146 kg) at such a rate as to maintain the pH between 6.8 and 7.2. The head tank and lines were then washed through with dichloromethane (20 kg). After the addition, the reaction was allowed to stir for 30 min at 24 °C. The phases were then allowed to separate for 15 min, and the organic phase was removed. The aqueous phase containing the (*S*)-acid, SB-214857, was washed with dichloromethane (390 kg) and then heated under vacuum at ∼50 °C to remove 1260 kg of distillate.

The pH was maintained between 6.3 and 7.4 by periodically adding small amounts of ∼1.5 N ammonia solution.

At the end of the distillation the resulting slurry was cooled and stirred at −1−3 °C for 16 h and isolated via centrifugation, washed with deionised water (105 kg) and dried at 25 °C under vacuum for 60 h to give the required product as a hydrate. The yield was 35.2 kg of white solid containing 17.6% water. This equates to a 42% overall yield from the racemate SB-215346 (or 84% of available (*S*)-isomer). The product was 99% ee (*S*)-isomer as determined by capillary electrophoresis analysis. This material was greater than 99% pure by HPLC analysis (corrected for water) and had an impurity profile of greater than 99.5% by HPLC analysis.

The dichloromethane extract containing the (*R*)-N-CBZ isomer (SB-240681) was retained and bulked together with extracts from other runs (typically 3 or 4 would be combined). The dichloromethane was stripped off and replaced with 60/80 petrol (∼5 vols) and cooled to 5 °C to crystallise the product that was isolated in a centrifuge, washed with 60/80 petrol and dried under vacuum. Typical recovery was 50% from racemic SB-215346 (∼100% of available (*R*)-isomer).

The SB-240681 was typically better than 95% pure by HPLC assay and 98% ee by chiral HPLC assay.

The recovered SB-240681 could be racemised and then deprotected via transfer hydrogenation to regenerate SB-215346. This material could be reused in the resolution to generate further supplies of SB-214857 hydrate.

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