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Polymer-Supported Catalase: A Green Approach to the Removal of Hydrogen Peroxide from Reaction Mixtures

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Abstract:

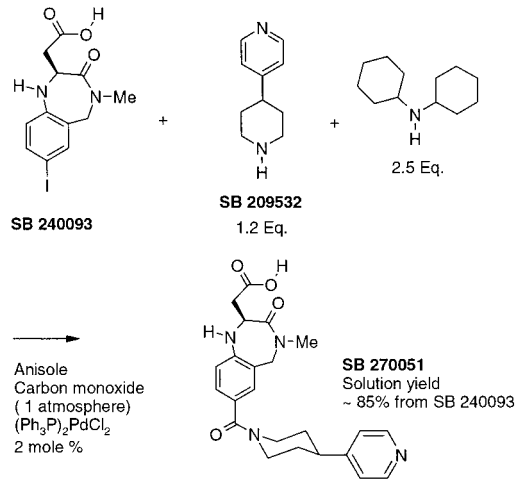
During a programme of work directed at developing a manufacturing route for SB 214857, Lotrafiban, the need arose to find methodology for efficiently destroying hydrogen peroxide in aqueous solutions of the penultimate intermediate, SB 270051. Whilst this was initially achieved using a chemical process, a biotransformation process has now been developed that utilises a polymer-supported catalase enzyme to remove peroxide from reaction mixtures. The biocatalytic approach provides an economic and environmentally friendly solution to peroxide removal when compared to the chemical process.

Introduction

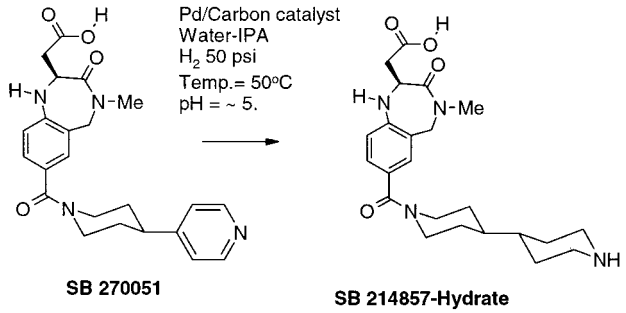
SB 214857-A is a potent GPIIb/IIIa (fibrinogen receptor) antagonist that was being developed by GlaxoSmithKline. Fibrinogen receptor antagonists prevent platelet aggregation; thus, such compounds are designed for the prevention of thrombotic events such as heart attack and stroke.[1] The manufacturing route for SB 214857 had an aminocarbonylation reaction as a key convergent step to assemble to molecule.[2,3] This converts SB 240093 to the penultimate intermediate, SB 270051, Scheme 1.

When this synthetic strategy was being developed, it was envisaged that the product, SB 270051, could be extracted into water from the anisole solvent and directly hydrogenated to give SB 214857, thus avoiding solids isolation and drying (Scheme 2). When the feasibility of this approach was first examined, the reduction of SB 270051 to SB 214845 required large amounts (a weight equivalent!) of platinum oxide catalyst and long reaction times (several days) to go to completion. This could clearly never be a viable industrial process. An extensive investigation into this reduction showed that the presence of iodide anions acting as an efficient catalyst poison was the cause of the long reaction time and requirement for a high catalyst loading.[4,5] In the carbonylation reaction that produces SB 27051, 1 mol of HI per mol of SB 270051 is generated (Scheme 1**)**. The bulk of the HI (∼95%+) is removed during workup as an insoluble salt with dicyclohexylamine. The remaining iodide is present at a level of ∼2000 ppm in the aqueous solution of SB 270051. It was found that if this level could be reduced to less than 100 ppm, then the reduction of the aqueous SB 270051 solution to SB 214857 could be reliably achieved with lower loading of a cheaper catalyst (palladium-on-carbon) in hours rather than days. Thus the reaction became feasible to consider as a manufacturing process (Scheme 2).

Scheme 1. **Aminocarbonylation reaction to produce SB 270051**



Scheme 2. **Hydrogenation of SB 270051 to SB 214857**



Removal of Iodide. Various ways of physically adsorbing iodide were considered but were ruled out for large-scale

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use. The use of metals (e.g., silver nitrate) to form insoluble iodides leads to the precipitation of highly insoluble SB 270051 carboxylate salts. The most efficient process for large-scale use was to oxidise the iodide and then to extract out the resulting iodine. Small-scale studies identified a number of suitable inorganic and organic oxidants although some oxidants were also capable of oxidising SB 270051.

The most efficient way to remove iodide, with no loss of SB 270051, was found to be treatment with excess hydrogen peroxide at pH 5 (10 equiv to the amount of iodide known to be present in solution). Under these operating conditions the level of iodide could easily be reduced to 10 ppm or less.

The need to use an excess of hydrogen peroxide was driven by the kinetics of the iodide oxidation. At pH 5 the rate of oxidation is slow, requiring an excess of peroxide to drive the reaction to completion in a reasonable time (1−2 h). The use of stoichiometric hydrogen peroxide requires low pH (∼1) or the use of metal catalysts such as molybdenum or vanadium to get an acceptable reaction rate. SB 270051 is unstable in water below pH 5, and the removal of molybdenum or vanadium salts was not considered an attractive prospect. After removal of iodine, the aqueous solution of SB 270051 had a hydrogen peroxide concentration of ∼2700 mg/L.

Since the aqueous feedstock of SB 270051 was subsequently charged to a hydrogenation reaction, the excess hydrogen peroxide first had to be reduced to less than 1 mg/L for safety reasons.

Removal of Hydrogen Peroxide. Our initial strategy was to add inorganic reducing agents such as sodium metabisulphite or sodium sulphite. These quickly destroyed peroxide, but sulphur residues made the resulting solutions very difficult to hydrogenate.

The use of low-valent metal salts such as stannous chloride was also deemed unattractive. The process that was first scaled-up used palladium-on-carbon as a catalyst to decompose the excess peroxide to oxygen and water. The amount of catalyst and temperature (65−70 °C) were adjusted to give a smooth gas evolution that could be diluted with nitrogen and safely vented.

One peculiar aspect of aqueous SB 270051 is that solutions at ca. neutral pH have the tendency to crystallise when heated. If this occurred during the reaction, the resulting solid coated the catalyst, and the reaction ceased. To ensure that SB 270051 remained in solution during the peroxide destruction, the aqueous solution had to be diluted with 2-propanol (IPA) prior to addition of the Pd catalyst. Whilst this procedure worked and was scaled-up, it was not ideal having an organic solvent mixture heated with an inhomogeneous Pd catalyst in the presence of oxygen. Hence, we decided to look for a safer, more environmentally friendly way to remove excess peroxide.

Enzymic Destruction of Hydrogen Peroxide. Catalase enzymes (EC 1.11.1.6) are used by living systems to break down hydrogen peroxide to water and oxygen.[6] Catalases are widespread and used in many bulk industries and healthcare products, for example, food stuffs, treating peroxide waste after paper bleaching, and contact lens cleaning. In recent years, a number of instances of the use of catalase enzymes to remove hydrogen peroxide (formed as the reduction product of oxygen) from reactions producing pharmaceutical intermediates have been reported.[7−9]

Thus, catalase enzymes presented themselves as potentially useful biocatalysts to remove hydrogen peroxide from aqueous solutions of SB 270051 after iodide removal. A range of commercially available catalases was tested, with mixed results. The use of bovine catalase was ruled out for obvious reasons. In small-scale screening tests, some catalase preparations did destroy peroxide in crude reaction mixtures, but large amounts had to be added. The most promising catalases identified were from the organisms *Aspergillus niger* and *Corynebacterium glutamicum.* These are both available on a large scale, in high purity, and are from nonmammalian sources. The catalase was simply added directly to the aqueous SB 270051 solution and stirred at 25 °C until the residual peroxide level had dropped to below 10 ppm. The solution could then be directly hydrogenated. When this was scaled up in the pilot plant, several deficiencies became apparent. After an initial reaction, gas evolution would cease before all the peroxide had been consumed. The reaction could be restarted by the addition of fresh enzyme, which indicates a problem with the stability or inhibition of the enzyme in this reaction mixture. If too much enzyme is added, however, this led to a retardation of the rate of the subsequent reduction step. This is an inherent problem with the application of a soluble protein that can interfere with the metal catalyst. The aqueous SB 270051 solution would need to be ultrafiltered to remove any protein prior to hydrogenation, but this would add an extra process step to the synthesis. All of these problems arise from the use of soluble enzymes in such a processes.

A number of immobilised catalase preparations have been reported, but most could be ruled out for various reasons. The most attractive carrier for the protein that would be compatible with large-scale batch processing was a Eupergit type (macroporous acryate beads).[10] The catalase enzymes of choice could be easily attached, by covalent bonding, to Eupergit C250L, a support containing oxirane functionalities that react with various groups on the protein.[11] Two other supports, Eupergit C and cyanogen bromide-activated Sepharose were also used to support the catalase but were not progressed on the basis of cost, activity, and stability comparisons. Mixing a solution of catalase with the support at ambient temperature gave at least a 98% attachment of protein. Although binding was effective in a few hours, the protein was mixed with the resin for 72 h, resulting in a

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more stable form of the immobilised enzyme; this presumably arises from multiple points of attachment of the protein to the support. A range of protein loadings from 10 to 200 mg/g did not appear to have a significant affect on protein activity (data not shown).

The supported catalase enzymes proved to be much more reliable. A 0.5-g portion of dry resin containing 0.7 mg of enzyme could treat a 200-mL portion of SB 270051 solution, containing around 20 g of SB 270051, in less than 1 h at 25 °C. The resin could be filtered off at the end of the reaction, and the SB 270051 in aqueous solution could be hydrogenated directly to give the same yield and quality of product as obtained in the palladium-treated route. Typically, a 20 wt % charge (based on SB 270051) of 10% Pd on carbon catalyst and 50 psi hydrogen pressure would reduce solutions of SB 270051 obtained from either peroxide destruction process in ~4 h. The yields of SB 214857 were typically 70% from SB 240093 and the products indistinguishable by analysis (weight-based assay, impurity profile, chiral assay).

The recovered enzyme resin could be reused in subsequent, sequential reactions with identical results. Nine sequential recycles of the supported enzyme were followed by soaking the resin in the reaction mixture solution for 24 h with no loss of activity. Similarly, sequential challenge of the enzyme with hydrogen peroxide solution did not cause any reduction in the activity level or dissociation of the protein from the support.

Thus, the catalase resins provided an effective, economical, safe, and environmentally friendly solution to the problem of removing peroxide in the processing of SB 270051 solutions.

(1) No palladium-on-carbon is used for peroxide destruction.

(2) The reactions are run in water alone—no IPA cosolvent is required. This removes the need to dispose of IPA waste and also makes the reaction more volume-efficient.

(3) No heating or cooling is required, thus saving energy. The chemical process runs at 70 °C—the enzyme reaction proceeds at ambient temperature, releasing oxygen in a safe and controlled manner.

(4) The catalase resin can be recovered and reused many times. The actual number of possible reuses has yet to be determined, but the results from the initial small-scale tests suggest the catalase on the resin is very stable, for example, at least 10 cycles possible with no inhibition by the reaction mixture and no detectable protein leaching. Eupergit supports also seem to be very robust under processing conditions. It should be noted that Pen G acylase on Eupergit resin can be reused ~700 times.[11]

On a manufacturing scale, if 30 t of SB 214857 were to be produced per year this would require ~3 t of palladium-on-carbon catalyst just to remove peroxide. If the catalase resin could be reused just 50 times, only 0.015 t would be required! Apart from the green aspects, at this level the cost of enzyme and resin become negligible.

Unfortunately, the project was terminated before the supported catalase resin step could be scaled-up and tested. The laboratory work would indicate however that this technology would have produced a robust and "green" manufacturing process. It also provides a good example of a "nonchiral" use of a biotransformation in process chemistry, an area in which we feel more thought could be directed.

Experimental Section

Corynebacterium glutamicum catalase was obtained from Boehringer Mannheim as a freeze-dried preparation and stored at 4 °C. Eupergit C 250L was obtained from Röhm GMBH as macroporous, spherical "beads" and stored at <−15 °C.

Method for the Production of Eupergit C 250L-Immobilised Catalase. The catalase was reconstituted in KPO_4 buffer (1 M, pH 7.5) at a concentration of 1.67 g of powder/L of buffer, and mixed gently. After approximately 30 min the enzyme was dissolved, and to the solution was added Eupergit C 250L at 167 g/L with mixing. The mixture was allowed to stand at ambient temperature for 72−96 h, after which the immobilised enzyme preparation was washed with 3 vols of KPO_4 buffer (1 M, pH 7.5) followed by 3 vols of deionised water.

Chemistry. Details of the large-scale aminocarbonylation of SB240093 to SB 270051 are to be published elsewhere.[3]

Aqueous solutions of SB 270051. To avoid isolation and solids handling, SB 270051 was not isolated, but formed from SB 240093 and carried through in solution.

The aminocarbonylation reaction was run in 8 vols of anisole. When complete the reaction was quenched with toluene (2 vols) and water (8 vols). The solids (dicyclohexylamine HI salt and some palladium black) were removed by filtration and washed with water (2 vols). The aqueous phase was basified with 2.0 M sodium hydroxide to pH 12 and washed with ethyl acetate to remove residual dicyclohexylamine. This gave an aqueous solution of SB 270051 at ~10 wt %/v containing ~2000 ppm iodide (as determined by ion chromatography analysis). The pH was then adjusted to 5.0 with 30 wt %/wt phosphoric acid solution.

Typical treatments for 200 mL of this solution are as follows.

Palladium-on-Carbon. Celite (1.5 g) was added. During the peroxide treatment some tars are precipitated. Adsorption onto Celite was found to be the most effective way to remove the tar without encountering downstream filtration problems. Hydrogen peroxide solution (36 mL, 3 wt %/wt) was added. The mixture was stirred for 30 min and then treated with dichloromethane (40 mL).

The mixture was stirred for 3 min and then filtered; the organic layer was discarded. The aqueous layer was re-extracted with dichloromethane (2 × 40 mL).

The pH was adjusted to 7 with 2 M sodium hydroxide and IPA added (60 mL) followed by palladium-on-carbon catalyst (2 g wet catalyst, 10% dry weight Pd, Johnson Matthey type 87L). The mixture was heated to 60° C for 2−3 h after which the peroxide level was less than 1 mg/L as judged by analysis with Quantofix peroxide test strips, ensuring appropriate dilutions were made at higher peroxide concentrations to keep the assay with the linear range.

The mixture was cooled and filtered. The IPA−water solution was concentrated under reduced pressure to adjust

the concentration SB 270051 to ∼10 wt %/v. The pH was adjusted to 5 with phosphoric acid solution and the solution was then hydrogenated using the following conditions, 20wt %/wt based on SB 270051 10% Pd on carbon catalyst/50 °C/50 psi H_2 pressure. After 4 h, HPLC analysis showed less than 1% residual SB 270051. SB 214857 could then be isolated in ∼70% overall yield from SB 240093.

Catalase Resin (*Corynebacterium glutamicum* on Eupergit C250-L). After dichloromethane extraction of the liberated iodine, the pH was adjusted to 7 with 2 M sodium hydroxide solution. A portion of the Eupergit C250-L catalase resin was added (0.95 g wet resin,∼0.46 g dry weight containing ∼0.7 mg catalase). The reaction was stirred at 25° C.

After 1 h all the peroxide had been destroyed (less than 1 mg/L). The resin was simply filtered off and the aqueous solution of SB 270051 hydrogenated after adjustment to pH 5.The recovered resin was reused in a second reaction with identical results. Both hydrogenations proceeded at rates identical to those in palladium-on-carbon treated solutions (4 h to reduce SB 270051 to less than 1%) and the product, SB 214857, was isolated in identical yield and quality.

Multiple Reuse of Catalase Resins. A 20 mL sample of SB 270051/peroxide reaction mixture was treated with 0.45 g of wet resin (∼0.2 g dry weight). All the peroxide was destroyed after 3 min at 25 °C. The resin was removed by filtration, washed with water, and reused. In nine consecutive reactions the same batch of catalase resin destroyed the peroxide in less than 3 min. The catalase resin was then soaked in reaction mixture for 24 h and reused. On the 10th reuse no loss of activity at all was detected, confirming that the solid-supported catalase was not inhibited by the reaction mixture. Identical results were found for both the Eupergit C-250L and cyanogen bromide-activated Sepharose resins.

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