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Articles

The Discovery and Process Development of a Commercial Route to the Water Soluble Prodrug, Fosfluconazole

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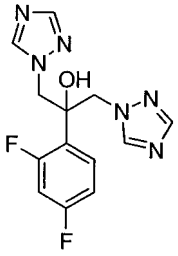
Abstract:

A case history detailing the rationale behind the discovery of 2-(2,4-difluorophenyl)-1,3-bis(1*H*-1,2,4-triazole-1-yl)-2-propyl dihydrogen phosphate, fosfluconazole (2), a water-soluble prodrug of Diflucan, and the subsequent development of a commercial route is presented. Particular items to note are (i) that this compound was discovered in the Chemical Research and Development Department, hence Chemical Research and Development can play a key role in prodrug discovery, (ii) the strategy behind the selection of phosphate ester promoiety, by phosphorylation of a sterically hindered tertiary alcohol, (iii) the development of the initial route to remove thermally hazardous reagents and to improve processing to allow scale-up, and (iv) the identification and development of the proposed commercial process.

Introduction

Fluconazole[1] (Diflucan) **(1)** Pfizer's broad-spectrum antifungal agent was launched in 1988, and its sales have grown strongly, reaching $1 billion per annum in 2000. Its development and subsequent approval during the 1980s came at a time when the number of patients suffering from serious fungal infections had risen sharply amongst cancer, transplantation, and AIDS patients. Diflucan is marketed in both oral and intravenous formulations, the latter being a dilute (2 mg/mL) infusion in saline.



(1)

In the mid 1990s, the identification and development of a highly water soluble prodrug of fluconazole became attractive for Pfizer Global Research and Development since this might offer both clinical and commercial advantages over the Diflucan infusion formulation. A low-volume product would allow for bolus administration, reducing fluid and sodium load, and it could facilitate access to higher doses. A smaller, concentrated product would therefore offer advantages to the patient, as well as being more convenient for storage and in clinical use.

Results and Discussion

Fluconazole has two functional groups from which to attach a promoiety, the tertiary alcohol group and the two triazole rings. At the start of this project, workers at Abbott[2] had recently shown that water-soluble prodrugs could be readily accessed by alkylating a nitrogen-containing heterocycle with bromomethyl acetate to give a quarternary ammonium salt prodrug.

An approach of this type was considered for producing a water-soluble prodrug of fluconazole, but it was deemed to have a number of disadvantages:

(1) The use of an alkylating agent in the final step of a drug synthesis was considered undesirable due to the need for very tight control of the residual alkylating agent in the drug substance.

(2) With two equivalent triazole rings, the alkylation of fluconazole is likely to give mixed products. The plane of symmetry in fluconazole is broken when producing a monoalkylated prodrug, leading to the generation of a chiral centre. The two enantiomers may have different rates of cleavage in vivo, and the development of a racemate is now generally disfavoured in the pharmaceutical industry.

(3) The achiral dialkylated prodrug was more straightforward to access, but as expected for a disalt it proved to be hygroscopic and difficult to handle as a drug substance (Scheme 1).

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(2) Davidsen, S. K.; Summers, J. B.; Albert, D. H.; Holmes, J. H.; Heyman, H. R.; Magoc, T. J.; Conway, R. G.; Rhein, D. A.; Carter, G. W. *J. Med. Chem.* **1994**, *37*, 4423.

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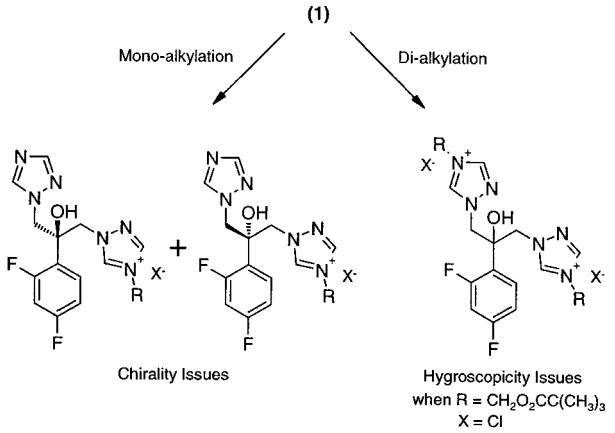
Scheme 1

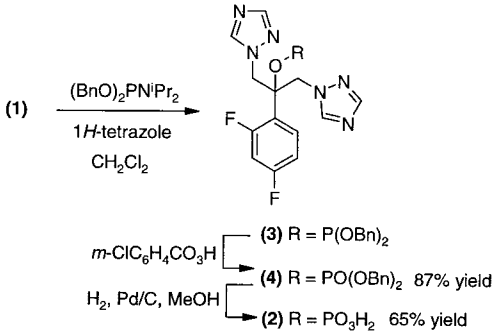


(4) The drugs in this class of prodrugs liberate an equivalent of formaldehyde in vivo when cleaved.[2]

Consequently, we focused our efforts on identifying a suitable polar or polarisable group with which to functionalise the tertiary alcohol group. The potential benefits of such an approach were that all of the issues delineated above could be avoided.

Phosphate esters[3] have frequently been employed as prodrugs since they are readily ionisable and, in a salt form, they significantly increase aqueous solubility. They are also sufficiently stable in the solid state and in aqueous solution to allow their development for intravenous administration. Furthermore, they are readily cleaved in vivo by nonselective alkaline phosphatases, thus releasing the parent drug. However, given the steric hindrance of the tertiary alcohol group of fluconazole, we believed that making such a phosphate ester would be extremely difficult, and indeed if such an ester could be produced, we expected it to be unstable. Nevertheless, we were delighted to be able to access fosfluconazole (**2**)[4] from fluconazole using the phosphorylation procedure developed by Fraser-Reid[5] employing dialkyl phosphoramidites. Hence, when fluconazole was treated with dibenzyl diisopropylphosphoramidite in the presence of 1*H*-tetrazole in dichloromethane, a smooth conversion to the dibenzyl phosphite (**3**) was achieved. This was oxidised in situ with *m*-chloroperoxybenzoic acid to the corresponding phosphate (**4**) in an 87% overall yield. The two benzyl-protecting groups were removed by hydrogenolysis over a palladium on carbon catalyst in methanol to give the free diacid (**2**) in a 65% yield (Scheme 2). Preliminary studies showed that fosfluconazole did indeed have appreciable aqueous solubility (>300 mg/mL), as the disodium salt and that it was readily cleaved in vivo to fluconazole. Additionally, accelerated stability studies showed that it had sufficient stability to allow the preparation of a drug product with a viable commercial shelf life. Consequently, it was nominated for further development.

Scheme 2. **Initial synthesis**



This initial route to fosfluconazole, although relatively high-yielding, had a number of issues that made it inappropriate for further scale-up.

(1) The use of the weakly acidic 1*H*-tetrazole used to promote the initial phosphorylation reaction had serious safety issues, since this reagent is both shock- and heat-sensitive, detonating when heated to its melting point (155 °C).[6]

(2) In addition to this, the use of the *m*-chloroperoxybenzoic acid oxidant also had associated thermal hazards,[7] necessitating the use of an alternative oxidant.

(3) Finally, the hydrogenation conditions proved to be nonrobust, and the isolation of fosfluconazole was complicated by its poor solubility in methanol.

We initially tackled the thermal hazards associated with step 1, and after screening other weak acids, we found that 5-methyl-1*H*-tetrazole, pyridinium hydrobromide with catalytic 4-(dimethylamino)pyridine, and imidazole hydrochloride would each promote the coupling reaction. 1,2,4-1*H*-triazole, which had been successfully used in the phosphorylation at the anomeric position of a sugar,[8] also gave excellent results. We chose 1,2,4-1*H*-triazole for scale-up, and after screening various oxidants we selected 30% aqueous hydrogen peroxide as it yielded a superior product quality. A more comprehensive study of alternative additives has recently been reported by Sanghvi and co-workers in this journal,[9] although we have not evaluated their findings with fluconazole. We next addressed the processing of the final step. Here, the insolubility of the product in methanol meant that the catalyst became poisoned, giving variable product quality together with losses of the product during the catalyst removal. In addition to this, the product formed a tightly bound methanol solvate. Fortunately, these processing issues, together with the concerns of forming dialkyl phosphate esters, could be avoided by changing the solvent medium to aqueous sodium hydroxide in which the product, by design, was soluble and stable. After the removal of the catalyst by filtration and after separation of the toluene by-product, the diacid could be easily crystallised by acidification with sulfuric acid. Consultation with Johnson Matthey led to the

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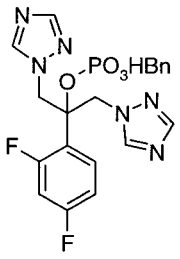
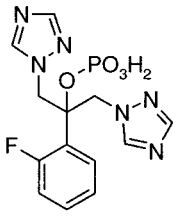
selection of the optimum palladium-on-carbon catalyst, which minimised both under-reaction, leading to the monobenzyl ester (**5**), and over-reduction, leading to the defluorinated product (**6**). This route was successfully scaled up in large-scale laboratory equipment to prepare two 2-kg batches to fund toxicology and early clinical studies.



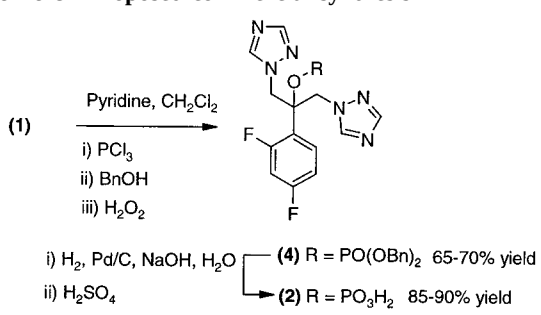
(5)



(6)

Although the initial route had been quickly developed into one that was more appropriate for scale-up, we did not consider this route to be commercially viable. This was because there was only a limited bulk availability of the dibenzyl diisopropylphosphoramidite reagent. This in turn was due to difficulties in preparing and purifying this liquid reagent to a sufficiently high quality on a bulk scale, and as a result, both the high costs and poor availability led us to seek an alternative set of phosphorylation conditions. We were initially attracted to forming the phosphorus oxygen bond with the phosphorus already at the correct oxidation state, but we were unable to identify any conditions that would allow us to achieve this. Instead we were forced to establish conditions that coupled the phosphorus at the more reactive phosphite oxidation state, with subsequent oxidation in situ. Fortunately, we managed to successfully establish a procedure employing cheap and readily available materials that was suitable for scale-up into both the pilot plant and our production facilities. In this procedure, fluconazole was combined with pyridine in a suitable solvent (initially ethyl acetate, later dichloromethane) with sequential additions of phosphorus trichloride, benzyl alcohol, and hydrogen peroxide to provide the desired protected phosphate ester (**4**) in 65−70% (Scheme 3). We found that the process produced marginally purer products when the reaction was run in ethyl acetate, and the processing was more straightforward in this solvent during work-up. Nevertheless, we had concerns about the potential formation of ethyl chloride during the initial phases of the reaction and the potential engineering measures that would be required to manage the safety issue of adding

Scheme 3. **Proposed commercial synthesis**



hydrogen peroxide to a highly flammable solvent. We subsequently changed the solvent to dichloromethane prior to scale-up. We were keen to avoid the use of pyridine and we have screened many different bases (e.g., trialkylamines, *N*-methylimidazole, and DBU) to achieve this conversion without success. We have now scaled up this process to produce approximately 1 tonne of the dibenzyl phosphate ester (**4**) in our manufacturing facilities in Sandwich.

We have experienced challenges scaling up the hydrogenation step in our pilot plant and manufacturing facilities during the fosfluconazole development program. For example, we found that as we scaled up the process, drying times increased, and under these conditions, hydrolysis of the product back to fluconazole was observed. This was minimised by giving the filter cake a thorough wash with acetone to displace the water required to hydrolyse the phosphate ester, which in turn, reduced the drying times required. Other challenges have pertained to the control of various impurities that may be present in the drug substance at parts per million levels. The use of a palladium-on-carbon catalyst in the final step of a drug substance synthesis has led to the introduction of an additional operation to remove trace levels of this metal. This has been successfully achieved by incorporating a carbon treatment of an aqueous solution of the disodium salt of the prodrug after the catalyst filtration, and prior to the crystallisation of the diacid.

Conclusions

A highly water-soluble prodrug of Diflucan (**1**) has been rationally designed by functionalising the tertiary alcohol as the phosphate ester to give fosfluconazole (**2**). The development of the initial phosphoramidite route to remove the thermal hazards and to improve the processing in the final step that are described in this contribution, allowed the support of exploratory development. The subsequent identification and development of a viable manufacturing route has allowed several hundred kilograms of fosfluconazole to be prepared in both pilot-plant and production facilities.

Experimental Section

Proton NMR data were recorded on a Varian Unity 300 spectrometer operating at 300 MHz. Microanalytical data were obtained from Exeter Analytical UK Ltd. Mass spectra were obtained on a Micromass Autospec-Q. Melting points were determined on a Buchi melting point apparatus.

Dibenzyl 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazole-1-yl)-2-propyl phosphate (4). Method 1. A solution of 2-(2,4-difluorophenyl)-1,3-bis(1*H*-1,2,4-triazole-1-yl)-2-propan-2-ol (10 g, 32.6 mmol), 1*H*-tetrazole (6.85 g, 97.8 mmol), dibenzyl diisopropyl phosphoramidite (22.55 g, 65.2 mmol) in dichloromethane (100 mL) was stirred at ambient temperature under nitrogen for 2 h. The mixture was then cooled to 0 °C, and a solution of *m*-chloroperoxybenzoic acid (13.5 g, 50−55% w/w, 39 mmol) in dichloromethane (50 mL) was added, maintaining the temperature at 0 °C. The resulting mixture was allowed to warm to ambient temperature for 1 h before washing with aqueous sodium metabisulfite and sodium bicarbonate. After drying ($MgSO_4$)

the solvent was removed and replaced with methyl isobutyl ketone (37 mL) and *tert*-butyl methyl ether (74 mL). After granulating at −10 °C for 1 h the product was filtered and washed with ice cold methyl isobutyl ketone and *tert*-butyl methyl ether (1:3, 15 mL) and dried at 50 °C under vacuum for 18 h to afford the title compound (16.05 g, 87%) as a white solid: mp 92−93 °C. ^{1}H NMR ($CDCl_3$) δ 4.90 (2H, d), 4.95 (2H, d), 5.05 (2H, d), 5.19 (2H, d), 6.58−6.73 (2H, m), 6.88−6.95 (1H, m), 7.20−7.30 (4H, m), 7.32−7.38 (6H, m), 7.80 (2H, s), 8.36 (2H, s). *m*/*z* 567 $[M + H]^+$. Found: C, 57.12; H, 4.46; N, 14.85. $C_{27}H_{25}F_2N_6O_4P$ requires: C, 57.24; H, 4.46; N, 14.84.

Method 2. A solution of 2-(2,4-difluorophenyl)-1,3-bis-(1*H*-1,2,4-triazole-1-yl)-2-propan-2-ol (1.5 kg, 4.9 mol), 1*H*-1,2,4-triazole (507 g, 7.35 mol), dibenzyl diisopropyl phosphoramidite (2.53 kg, 7.35 mol) in dichloromethane (7.5 L) was refluxed under nitrogen for 20 h. The mixture was then cooled to 10 °C, and 30% hydrogen peroxide (1 L, 8.8 mol) was added, maintaining the temperature between 10 and 25 °C. The resulting mixture was allowed to warm to ambient temperature for 1 h before separating the phases. The organic phase was washed with aqueous sodium metabisulfite, hydrochloric acid, and water. The solvent was removed and replaced with methyl isobutyl ketone (5 L) and *tert*-butyl methyl ether (10 L). After granulating overnight at ambient temperature and at 0 °C for 1 h, the product was filtered and washed with methyl isobutyl ketone and *tert*-butyl methyl ether (1:3, 2 L). The product was finally dried at 60 °C under vacuum for 18 h to afford the title compound (2.26 kg, 81%) as a white solid.

Method 3. A mixture of 2-(2,4-difluorophenyl)-1,3-bis-(1*H*-1,2,4-triazole-1-yl)-2-propan-2-ol (79.8 kg, 261 mol) and pyridine (68.0 kg, 861 mol) in dichloromethane (640 L) was stirred at −13 °C under nitrogen. Phosphorus trichloride (38.0 kg, 276 mol) was added to the mixture over 5 min followed by a dichloromethane (30 L) wash. The mixture was then reacted at 13 °C for 2 h before benzyl alcohol (65.0 kg, 602 mol) was added over 2 h, maintaining the temperature between 14 and 16 °C. A dichloromethane (30 L) line wash was added, and the mixture was stirred between 10 and 15 °C for 2 h. The mixture was then cooled to 0 °C, and 30% hydrogen peroxide (106 kg, 935 mol) was added over 3 h, maintaining the temperature below 20 °C. After reacting for 1 h, the mixture was allowed to settle. After separating, the organic phase was washed with sodium metabisulfite (12 kg in 160 L of water), dilute hydrochloric acid (6 L of concentrated acid in 160 L of water), and water (320 L). The product solution was then concentrated under vacuum and the dichloromethane replaced with methyl isobutyl ketone (229 L) and *tert*-butyl methyl ether (296 L). After granulating at 2 °C for 1 h the product was filtered and washed with *tert*-butyl methyl ether (2 × 70 L) and dried at 40 °C under vacuum for 8 h to afford the title compound (97.7 kg, 66%) as a white solid.

2-(2,4-Difluorophenyl)-1,3-bis(1H-1,2,4-triazole-1-yl)-2-propyl dihydrogen phosphate (2). A slurry of dibenzyl 2-(2,4-difluorophenyl)-1,3-bis(1*H*-1,2,4-triazole-1-yl)-2-propyl phosphate (30.1 kg, 53.13 mol), 5% palladium-on-carbon catalyst (50% wet, type 5R39, 1.5 kg), and sodium hydroxide (4.36 kg, 108.9 mol) in low-endotoxin water (75.7 L) was hydrogenated at ambient temperature and 414 kPa (60 psi) for 12 h. The slurry was filtered, and the catalyst was washed with low-endotoxin water (9.8 L). After separating the toluene by-product, the aqueous phase was slurried with carbon (3.1 kg) for 30 min. After the carbon was removed by filtration, the aqueous phase was acidified to pH 1.45 by that addition of sulfuric acid (6.69 kg) in low-endotoxin water (25 L) over 2 h. The resulting slurry was granulated at ambient temperature for 1 h and then filtered. The product was sequentially washed with filtered low-endotoxin water (103 L) and filtered acetone (103 L). The product was dried under vacuum at 50 °C for 12 h to give the title compound (18.1 kg, 88%) as a white powder: mp 223−224 °C. ^{1}H NMR (DMSO) δ 5.07 (2H, d), 5.24 (2H, d), 6.77−6.83 (1H, m), 7.00−7.18 (2H, m), 7.75 (2H, s), 8.53 (2H, s). Found: C, 40.28; H, 3.39; N, 21.63; $[MH]^+$ 387.0786. $C_{13}H_{13}F_2N_6O_4P$ requires: C, 40.43; H, 3.39; N, 21.78; $[MH]^+$ 387.0782.

Benzyl 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazole-1-yl)-2-propyl hydrogen phosphate (5) characterisation data: ^{1}H NMR (DMSO) δ 4.99 (2H, d), 5.11 (2H, d), 5.26 (2H, d), 6.77−6.83 (1H, m), 7.00−7.18 (2H, m), 7.30−7.42 (5H, m), 7.81 (2H, s), 8.59 (2H, s). Found: $[MH]^+$ 477.1255. $C_{20}H_{19}F_2N_6O_4P$ requires: $[MH]^+$ 477.1246.

2-(2-Fluorophenyl)-1,3-bis(1H-1,2,4-triazole-1-yl)-2-propyl dihydrogen phosphate (6) characterisation data: ^{1}H NMR (DMSO) δ 5.13 (2H, d), 5.28 (2H, d), 6.88−6.94 (1H, m), 7.03−7.11 (2H, m), 7.21−7.26 (1H, m), 7.72 (2H, s), 8.52 (2H, s). Found: $[MH]^+$ 369.0868. $C_{13}H_{14}FN_6O_4P$ requires: $[MH]^+$ 369.0871.

Acknowledgment

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