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Process Research and Development of a Dihydropyrimidine Dehydrogenase Inactivator: Large-Scale Preparation of Eniluracil Using a Sonogashira Coupling

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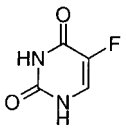
Abstract:

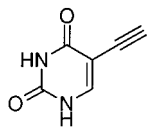
Eniluracil (5-ethynyluracil) is a potent inactivator of the enzyme dihydropyrimidine dehydrogenase, which is the rate-limiting enzyme in the metabolism of 5-fluorouracil, a widely used anticancer drug. The process research and development of a three-stage route to eniluracil is described. A Sonogashira coupling between 5-iodouracil and trimethylsilylacetylene was used to synthesise 5-(2-trimethylsilylethynyl)uracil on a >60 kg scale. Sodium hydroxide deprotection and acidification with acetic acid completed the synthesis of eniluracil in high yield and quality. The optimisation of this process is described with particular attention paid to minimising the input of palladium and copper catalysts and ensuring that the copper catalyst is well suspended in the reaction mixture.

Introduction

5-Fluorouracil (5-FU) is one of the most widely used anticancer drugs for solid tumours. 5-FU is administered by slow intravenous infusion owing to low and variable oral bioavailability due to metabolism by the enzyme dihydropyrimidine dehydrogenase[1] (DPD, also known as uracil reductase), and it is this enzyme that is the target of eniluracil **1** (5-ethynyluracil). Eniluracil is a potent, suicide substrate for DPD and becomes covalently linked to the enzyme,[2] thereby inactivating it. Co-administration of eniluracil with 5-FU leads to higher and more prolonged serum levels[3] of 5-FU and allows oral administration of 5-FU. The synthesis of eniluracil is reported in the literature[4] from the 1970s. Patents were filed[5] by the Wellcome Foundation covering the use of eniluracil as a DPD inactivator to improve therapy with 5-FU.

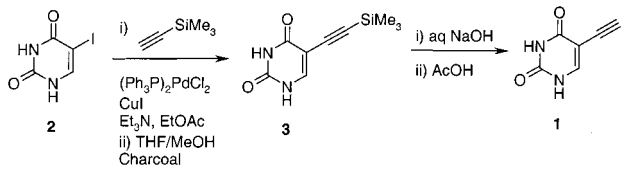


5-Fluorouracil



1
Eniluracil

Scheme 1. **Route to eniluracil**



The Sonogashira reaction[6] is well-known in the literature and has achieved widespread use as a mild method for introducing an acetylene into a molecule via coupling with a vinyl or aryl halide. The use of trimethylsilylacetylene[7] (TMSA) was introduced in 1980, and this provides a mild, safe and high-yielding method for the introduction of an unsubstituted ethyne fragment. Later papers describe Sonogashira-type coupling of acetylenes with 5-iodouracil[8] and close analogues.[9] It is the development and scale-up of a Sonogashira coupling as the foundation of a route to eniluracil that forms the subject of this paper.

Issues with the Initial Process

When the project was transferred to Stevenage, a three-stage pilot-plant process[10] had already been devised and operated (Scheme 1). Stage 1 consisted of a Sonogashira coupling of TMSA with 5-iodouracil **2** (5-IU) catalyzed by 1.5 mol % $PdCl_2$ and 10 mol % CuI. This was followed by two charcoal treatments (2 × 0.9 weights of charcoal) and recrystallisations, which were required to control the heavy metal contamination of 5-(2-trimethylsilylethynyl)uracil **3** (stage 2). Finally, stage 3, a deprotection with aqueous sodium hydroxide, was used to remove the trimethylsilyl group.

The route was considered suitable for the routine long-term manufacture of eniluracil; however, a number of key

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(10) See the Supporting Information for details of this process.

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issues with the process were identified: high levels of copper and palladium in the drug substance, the colour of the drug substance, less than optimal yields, and complex work-up and isolation procedures. The factors which were selected as most relevant in addressing these issues were: the quantities of palladium and copper catalysts used, the reaction temperature, the formation of the palladium catalyst[11] ($(Ph_3P)_2PdCl_2$) in situ, and the work-up procedure. It was apparent that at the original reaction temperature of refluxing ethyl acetate (ca. 75 °C) the reaction was complete very quickly (<10min) and that a significant amount of catalyst decomposition occurred as indicated by precipitation of a black solid. Furthermore, the boiling point of the acetylene component is only 53 °C, and considerable losses could be expected at 75 °C.

Process Improvements

It was soon established that the process used for the in situ formation of the catalyst by mixing $PdCl_2$ and Ph_3P was very inefficient (as judged by the almost complete insolubility of $PdCl_2$ in EtOAc) compared to using preformed $(Ph_3P)_2PdCl_2$, and hence the preformed catalyst was used in all further experiments. The Pd catalyst loading was reduced from 1.5 mol % to 0.5 mol % which maintained an acceptable reaction rate, but further reduction to 0.1 mol % gave a very slow reaction. For the sake of robustness and reaction rate a level of 0.5 mol % was adopted. Similarly, the loading of CuI was reduced from 10 mol % to 0.5 mol % but further reduction or omission gave an unacceptable conversion. These modifications had a number of clear benefits: reduced catalyst purchase cost, reduced heavy metal contamination of drug substance, intermediate, and waste streams. These experiments were carried out at a reaction temperature of 25 °C which gave ca. 95% conversion in 6 h. More importantly, the colour of the reaction mixture was maintained as pale yellow throughout, with no precipitation of dark solids, which would indicate catalyst decomposition and lead to high levels of heavy metal contamination in the isolated intermediate **3**. It was found that effective de-oxygenation of the reaction mixture was necessary to minimise heavy metal contamination; hence, the reaction mixture was degassed by application of three vacuum−nitrogen purge cycles prior to introduction of the TMSA.

A screen of various solvents for the Sonogashira reaction (ethyl acetate, DMF, THF, MeOH, EtOH, and *n*-PrOH) was carried out. This confirmed ethyl acetate as the solvent of choice despite the very low solubility of both **2** and **3**. The low solubility of **3** allowed direct isolation from the reaction mixture (10 vol of ethyl acetate were required to achieve a mobile slurry) by filtration with minimal losses in the filtrate. The previous process had required a slurry with aqueous EDTA to reduce the levels of copper, but with the massively reduced input of CuI this became unnecessary. Instead, a water slurry was used to remove the triethylammonium iodide formed in the reaction. This was best carried out in a mechanical filter-dryer which allowed effective mixing of water with the initial filter cake followed by in situ vacuum-drying to give the intermediate grade (IG) **3**. This material was off-white and typically had metal levels of: Pd ca. 600 ppm and Cu ca. 100 ppm. The earlier process provided dark grey-coloured material with metal levels of: Pd >5000 ppm and Cu >13000 ppm.

The much reduced heavy metal contamination of the IG **3** allowed a single, much reduced charge of charcoal to be used in stage 2; typically 0.4−0.6 wt. This led to improved yields and alleviated the issues of filtration of charcoal on scale-up. Stage 2 consisted of treating a solution of **3** with charcoal (0.4 wt) in a solvent mixture of boiling THF/MeOH 1:1 (this gave better solubility than either solvent alone) and filtration of the hot mixture. The charcoal was collected in a pressure filter jacketed with hot water to avoid crystallisation in the filter. A vacuum distillation of solvent from the filtrate was found to be beneficial to minimise the formation of impurities. Addition of water as an anti-solvent led to isolation of purified **3** as a white crystalline solid with very low levels of palladium (typically <2 ppm) and copper (typically <1 ppm).

Stage 3 was relatively unaltered except for the omission of all charcoal and filter aid. The purified **3** was dissolved in 1 M sodium hydroxide and when the reaction was complete, the hexamethyldisiloxane (HMDSO) by-product was separated and discarded before a line-filtration of the disodium salt solution into the crystallisation vessel. The product **1** was formed by acidifying to pH 5 with acetic acid, collecting the fine solid by filtration and washing with water followed by acetone. This allowed isolation of very high quality material, in a virtually quantitative yield. The HPLC purity was typically >99.9%, and the largest impurity in all batches was acetone (typically 0.3% w/w determined by NMR) which could not be reduced even by extended drying at 50° under vacuum. Levels of palladium were typically 2 ppm, and copper, <1 ppm.

Issues during Development

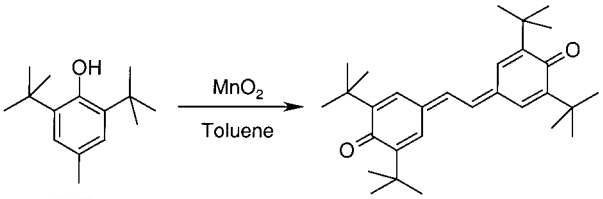
It was found in some laboratory-scale reactions that the copper iodide was not properly suspended but formed a solid mass at the bottom of the flask and resulted in a failed reaction. Copper iodide is a very dense solid (*d* 5.62 g/mL), and even finely divided material requires vigorous agitation to remain suspended. We were concerned about this issue in large conical-bottomed vessels in the pilot plant, and thus calculations were carried out to determine a minimum stirring rate for the particular plant we were planning to use. These calculations indicated that a stirring speed of 120 rpm would effectively suspend copper iodide with a particle size of less than 150 μm. By adding the copper iodide powder to a reaction mixture stirred at 120 rpm we avoided any failed reactions on plant.

During a routine user test of a new batch of 5-IU it was noticed that the reaction mixture turned a dark brown colour (normally yellow) and achieved only 11.7% conversion with the normal charge of catalyst. By increasing the catalyst loading to 1.5 mol % a 93.1% conversion could be achieved, but this would not be acceptable for processing because of the much higher heavy metal contamination in the IG **3**. We discussed the possible contaminants with the supplier of the

(11) More precisely $(Ph_3P)_2PdCl_2$ is a precursor to the true catalyst: $(Ph_3P)_2Pd$.

batch of 5-IU and began to suspect that elemental sulfur may be present from sodium thiosulfate used to quench excess iodine in the iodination of uracil. A re-work procedure was devised, consisting of dissolving the rather insoluble 5-IU in 2 M sodium hydroxide, clarifying the hazy solution through a 1 μm line filter and precipitating the 5-IU by acidifying with concentrated hydrochloric acid. This procedure gave a 94.2% recovery on a 200 g scale, and the resultant material gave a satisfactory user test. The re-work was rapidly scaled-up to process >200 kg of 5-IU and to allow us to continue with our campaign with minimal loss of time. It is interesting to note that in all respects, other than the user test, this batch of 5-IU passed our specification, and it was shown that the sulfur impurity was present at only 0.2% w/w. The specification was subsequently revised to incorporate a clarity test of a caustic solution of 5-IU.

It seemed prudent to try to integrate stages 1 and 2 by processing the initial filter cake (including the triethylammonium iodide) directly in stage 2. This would have the benefits of fewer washes and less waste, as well as one less drying step, and would therefore save time and energy. To this end the initial filter cake from stage 1 was washed with ethyl acetate, dissolved in boiling THF/MeOH, and treated with charcoal (0.4 wt). **3** was isolated in the normal way, and it was shown that the triethylammonium iodide had been successfully removed but the material was bright yellow rather than white. The yellow-coloured impurity was isolated, and inspection of its NMR spectrum showed that it was not drug-related but indicated that it was derived from butylated hydroxytoluene **4** (BHT) the standard stabiliser found in THF. An authentic sample of a known[12] BHT dimer **5** was prepared by treatment of BHT with manganese dioxide followed by chromatography to give a pure sample of **5** as a deep orange solid, which was identical to that isolated from the yellow **3**. This indicated that the stabiliser in the THF was being oxidised by the palladium and copper contaminants in the IG **3** but only in the presence of triethylammonium iodide. Although it was possible to remove the BHT dimer **5** at stage 3, we elected not to implement this integrated stage for the pilot-plant campaigns.



BHT, **4** **5**

Conclusions

A Sonogashira coupling of 5-IU with TMSA has been optimized and scaled up to >60 kg batch size in 1500 L plant. Particular attention was paid to minimising input of palladium and copper catalysts, which allowed the preparation of good quality material with a minimum amount of charcoal to remove heavy metal contaminants. The process has been successfully operated on numerous occasions in the pilot plant at Stevenage and subsequently validated at a manufacturing site. At the manufacturing site stages 1 and 2 were combined into a single process with the IG **3** being dissolved in the filter-dryer. This led to further improvements in the environmental impact of the process and the occupational health benefit of reduced solids handling.

Experimental Section

Reactions were carried out in 1500 L glass-lined carbon steel reactors and the intermediate isolated in a Hastelloy (C22) Schenk mechanical filter-dryer with a poroplate filter of 1.2 m^2. The drug substance was isolated in a stainless steel enclosed pan filter (internal diameter 890 mm) and dried in a vacuum oven. All temperatures are in degrees Celsius. Levels of palladium and copper were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES) and are quoted in parts per million. HPLC was carried out using the following method: eluent A: 970:30:1 water:methanol:acetic acid, eluent B: 100:900:1 water: methanol:acetic acid. An isocratic elution of 100% A for 10 min and then a gradient to 100% B over 20 min; column 150 mm × 4.6 mm YMC-AQS-3 120A, 3 μm; flow rate 1.0 mL/min; temperature 40 °C; UV detection at 281 nm.

Stage 1: Intermediate grade 5-(2-trimethylsilylethynyl)uracil 3. $(Ph_3P)_2PdCl_2$ (0.95 kg, 1.35 mol, 0.005 equiv) and finely ground CuI (0.26 kg, 1.37 mol, 0.005 equiv) were added to a well-stirred suspension of **2** (64.20 kg, 269.8 mol) in ethyl acetate (514 L) at 15−25°. The mixture was then deoxygenated by evacuating and flushing with nitrogen three times. TMSA (31.3 kg, 318.7 mol, 1.15 equiv) followed by triethylamine (31.7 kg, 313.3 mol, 1.15 equiv) was added and the addition flask rinsed with ethyl acetate (128 L) into the reactor. The suspension was stirred under nitrogen at 25° for 19 h. The solids were isolated by filtration in a mechanical filter-dryer under nitrogen and washed sequentially with ethyl acetate (2 × 128 L), water (3 × 128 L) and finally ethyl acetate (2 × 128 L). The product was dried under vacuum in the filter-dryer at 50° overnight to give IG **3** as an off-white powder 52.42 kg, 251.7 mol, 93.3% theory yield, 81.7% w/w yield. HPLC 98.5% purity. ^{1}H NMR (400 MHz; d_6-DMSO) δ: 11.35 (br s, 2H), 7.79 (s, 1H), 0.18 (s, 9H). ICP-OES: Pd 550 ppm, Cu 120 ppm.

Stage 2: 5-(2-Trimethylsilylethynyl)uracil 3. A mixture of IG **3** (50.44 kg, 242.2 mol) and decolourising charcoal (Norit SX+, 21.50 kg) in a mixture of THF (504 L) and MeOH (504 L) was heated at reflux (61−62°) for 1 h. The suspension was filtered hot (ca. 60°) by pumping through a filter train consisting of a pressure filter, a GAF filter (5 μm bag), and a 1 μm line filter in series. The charcoal filter cake was washed with hot (ca. 60°) THF/MeOH (1:1, 252 L). The wash was added to the distillation vessel and the mixture concentrated to 252 L under reduced pressure (batch temperature ca. 20−30°) to give a slurry. The temperature of the slurry was adjusted to 25−30°. The slurry was diluted with water (252 L) over 30 min at 25−30°, cooled to 20°, and aged at 15−20° for 30 min. The product was isolated by filtration in a mechanical filter-dryer, washed with a mixture of water and MeOH (2:1, 2 × 100 L), and dried in the filter-dryer at 50° overnight to give **3** as a white

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powder: 47.92 kg, 230.1 mol, 95.0% yield. HPLC 99.9% purity. ^{1}H NMR (400 MHz; d_6-DMSO) δ: 11.35 (br s, 2H), 7.79 (s, 1H), 0.18 (s, 9H). ICP-OES: Pd 1.6 ppm, Cu <0.7 ppm.

Stage 3: 5-Ethynyluracil 1 (Eniluracil). A 1 M aqueous solution of NaOH (1162 L) was prepared by adding 50% w/w aqueous NaOH (93.0 kg, 1162 mol, 5.2 equiv) to purified water (744 L) and diluting with further purified water (372 L). **3** (46.48 kg, 223.2 mol) was added at 15−20°, and the mixture stirred vigorously at 20° for 2 h. The agitator was stopped and the HMDSO allowed to separate. The lower aqueous layer was clarified by filtration through a GAF filter (5 μm bag) and a 1 μm line filter, in series. The HMDSO was extracted with purified water (93 L), the phases were allowed to separate, and the lower aqueous phase was transferred into main batch via the filter train. The upper HMDSO layer was discarded. The pH of the combined filtrate and wash was adjusted to 5.0 by adding filtered acetic acid (98.6 kg, 1642 mol, 7.36 equiv) at 20−25°. The resultant suspension was cooled to 20° and aged at 15−20° for at least 30 min. The product was isolated by filtration, washed with filtered, purified water (2 × 116 L), washed with filtered acetone (2 × 116 L), and dried in a vacuum oven at 50° overnight to give **1** as an off-white powder: 30.06 kg, 220.9 mol, 99.0% theory yield, 64.7% w/w. HPLC 99.95% purity. ^{1}H NMR (400 MHz; d_6-DMSO) δ: 11.36 (br s, 2H), 7.82 (s, 1H), 4.03 (s, 1H). ICP-OES: Pd 2 ppm, Cu <1 ppm.

Acknowledgment

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Supporting Information Available

Additional detail of the original process at the point of transfer to Stevenage.. This material is available free of charge via the Internet at http://pubs.acs.org.

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