Application Note 60:

Photochemical hydration and scale-up of an anti-cancer candidate

Produced by Vapourtec

Abstract

In our application note 59 we found an unexpected photohydration taking place, instead of a cycloaddition during a photochemical library synthesis of [2+2] cycloadducts. Investigating further, we learned that it has not been reported in continuous flow in the literature before. We decided to explore this reaction further in this application note, which describes:

- Efficient and clean functionalization of a candidate for an anti-cancer pharmaceutical
- **New reaction** for continuous flow
- Rapid investigation of reaction conditions, enabling double the throughput
- Optimization of a photochemical reaction
- Throughput of **0.67 g/hr** from a photochemical reaction
- **Isolated yield of 80%** with 99% purity

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Background

During a recent photochemical [2+2] library synthesis project (Application note number 59) using a series of uracils and maleimides, we noticed that one example, 5-fluorouracil, gave the hydrated product 6-hydroxy-5-fluoro-5,6-dihydro uracil as the principle product in preference to the expected [2+2] cycloadduct, Figure 1. [1] A directed search using the SciFinder database revealed **no reports** of this photochemical hydration being carried out in flow before, so here we demonstrate the easy optimization of this reaction using the highly versatile Vapourtec UV-150 photochemical reactor for what we believe is the first time in continuous flow.

Uracil is one of the four principle nucleotides in the nucleic acid of RNA and it and its derivatives have enormous potential as pharmaceutical compounds; indeed, 6-hydroxy-5-fluoro-5,6-dihydro uracil and other close analogues have shown anti-tumor activity.[2] Any fast, clean and efficient method of functionalizing these compounds to enhance their activity or solubility could present a very useful pathway to some very important compounds.

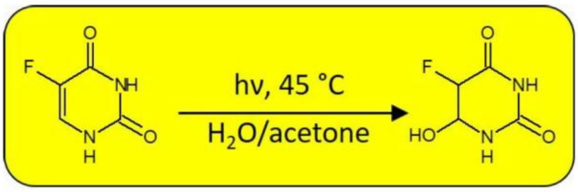


Figure 1: Photochemical hydration of 5-fluorouracil using the UV-150 photochemical reactor

Setup

All reactions were performed using a Vapourtec R-Series equipped with an R2S+ pump module, and a 10 ml UV-150 continuous flow photochemical reactor, as depicted in Figure 2. An automated liquid handler was connected to the system, controlled by flow control software, and used to collect product. Ethene gas was required to replicate the conditions used during the library synthesis application note in which this product was first observed. The gas was delivered from an ethene cylinder to a V-3 pump, which was used to pump the ethene in gas delivery mode. The reactions were irradiated using a Vapourtec medium-pressure mercury lamp with specific wavelengths transmitted by means of Vapourtec filter number 2.

All reagents were used as supplied by Sigma-Aldrich or Fisher-Scientific. The reaction substrate was prepared in a 4:1 v/v H2O:acetone solution at 75 mM, and degassed by bubbling nitrogen for 30 mins.

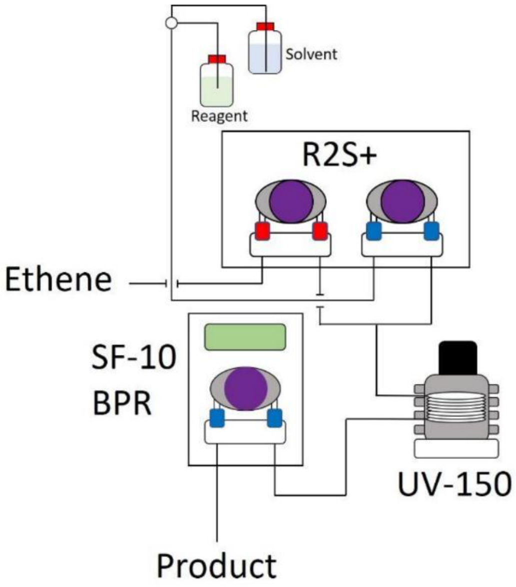


Figure 2: Schematic of the R-Series used. 5-fluorouracil and ethene are pumped using an R2S+ and combine in a t-piece before entering the UV-150 photochemical reactor. Pressure is controlled by an SF-10 in pressure-regulation mode and the product is collected automatically under the control of flow control software.

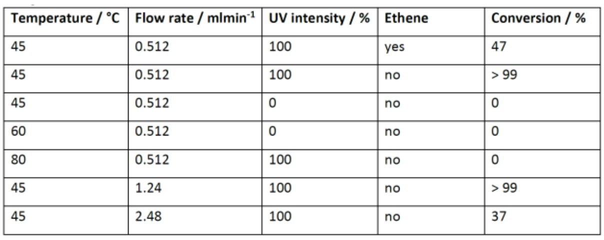
Results

Following the recent success of our application note 59: Photochemical library synthesis, (available here), we were keen to further explore the photochemical hydration of 5-fluorouracil that we observed. As a transformation that has not been reported in flow before, we felt this was an exciting opportunity to demonstrate an optimization and scale-up using the UV-150 photochemical reactor.

Initially the library conditions that had first yielded this product were re-visited and a repeat experiment performed with 5 ml of 75 mM starting material. As had been the case during the original library, ethene was delivered into the reaction prior to the reactor and the ethene rich solution was irradiated using a medium-pressure mercury lamp equipped with Vapourtec filter 2, to prevent the generation of un-wanted radicals that could initiate side reactions (see application note 59 for more details). A comparable result to the library was obtained so the investigation was expanded. Under the same conditions, the reaction was carried out without ethene, and a significantly cleaner NMR was obtained, that also indicated full conversion of the starting material. Neither of these findings is unexpected; the absence of ethene removes the possibility for the formation of [2+2] adducts or polymeric materials, and also increases the residence time inside the reactor.

To prove this was indeed a photochemical process, the experiment was repeated without ethene and without the lamp, at 45, 60 and 80 °C. In these experiments, none of the 5-fluorouracil was consumed, and analysis by NMR shows a clean spectrum of the starting material, %, as reported in Table 1.

Table 1: Several conditions explored during the investigation into the photohydration of 5-fluorouracil



Temperature / °C	Flow rate / mlmin^{-1}	UV intensity / %	Ethene	Conversion / %
45	0.512	100	yes	47
45	0.512	100	no	> 99
45	0.512	0	no	0
60	0.512	0	no	0
80	0.512	100	no	0
45	1.24	100	no	> 99
45	2.48	100	no	37

With conditions giving clean product identified, the flow rate was explored to increase throughput for a scaled-up synthesis of the product. The flow rate was doubled from 0.512 ml/min to 1.24 ml/min, and doubled again to 2.48 ml/min at 45 °C. The product at these conditions was collected under automatic control by flow control software. Analysis by NMR revealed that doubling the flowrate, and therefore doubling the throughput, had little effect on the conversion of 5-fluorouracil (>99%) but when the flowrate was doubled again, the conversion fell to approx. 37%.

Using the conditions found that doubled the reaction throughput and retained high conversion, the reaction was run for 2.5 hours, resulting in an isolated yield of 79.9% (1.68 g of product) in 99.0% purity following a simple filtration. Interestingly, the collected product solution was colored green, as had been observed in the small-scale experiments, but after running for the scale synthesis a small quantity (7% by mass of overall material obtained) of insoluble material could be seen at the bottom of the collection vessel. Small quantities of insoluble particulates like this are quite common in any synthesis, and present a challenge in flow because of their potential to cause blockages. Typically, blockages from small particulates occur where there is a restriction in the flow path, most commonly the device being used to control pressure. Any BPR that uses a needle and seat-type arrangement is at risk of being blocked by particles such as this, which would have caused the synthesis to stop while the BPR was cleaned or replaced. In this work an SF-10 peristaltic pump, which has the function to operate as a back-pressure regulator, was used to control the system pressure. Using the peristaltic BPR there is no restriction to the flow path and solids are kept moving, preventing blockages and enabling long experiments.

Conclusion

Here we have described a simple and efficient photochemical functionalization of a pharmaceutically important compound. We have also shown how, in the previous application note, 59, the Vapourtec R-Series can be used for reaction screening, which revealed a synthetic pathway that has not been reported in continuous flow in the literature before. Now, using the R-Series and UV-150 photochemical reactor, we have shown how the synthesis can be more thoroughly investigated and scaled up to gram quantities quickly and in high purity.

Analysis

Immediate analysis was performed in-house by HPLC to give a qualitative indication of how the reaction had proceeded. Samples of the crude reaction mixture were dried using a V10 evaporator and sent for external NMR analysis by Tocris Bioscience.

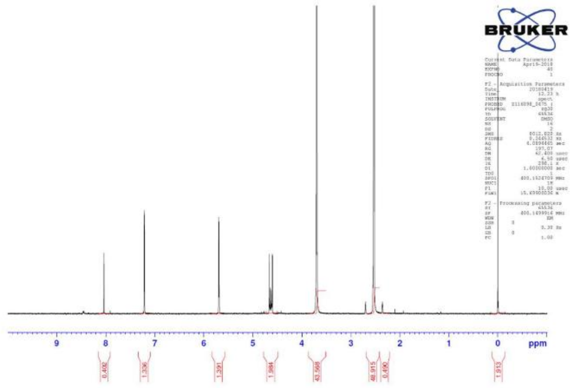


Figure 3: NMR of the isolated product following a simple filtration. Peak at δ 4.6 corresponds to 5-H of the hydrated 6-hydroxy-5-fluoro-5,6-dihydrouracil. Trace amounts of the starting material can be seen at δ 7.8 (6-H).

References

1. Zupancic, N., Zupan, M., Sket, B., Collection of Czechoslovak Chemical Communications, **1984**, 49(7), 1592-9.
2. van der Wilt, C. L., Visser, G. W. M., Braakhuis, B. J. M., Wedzinga, R., Noordhuis, P., Smid, K., Peters, G. J. British Journal of Cancer, **1993**, 68(4), 702-7.