Application Note 59:

Automated photochemical library synthesis

Produced by Vapourtec

Abstract

A synthesis of a library of compounds enables the chemist to rapidly screen possible chemistries and produce small quantities to be used for other screening processes.

This application note describes:

- Rapid synthesis of a library of 12 [2+2]photo-cycloadducts in 50 mg quantities
- Rapid screening of a range of photosensitizers
- Optimization and scale-up of two products to several grams scale

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Background

Photochemistry offers a vast array of opportunities for the development of interesting arrays of novel small molecules, which are often of special interest to medicinal chemists.[1] The [2+2] photocycloaddition is undisputedly the most important and most frequently used photochemical reaction and can be particularly useful for the synthesis of highly functionalized cyclobutanes of interest in drug discovery and natural product synthesis.[2,3]

The utilization of such chemistry has often been limited by the need for specialist equipment, labor intensive optimization procedures and very low mass throughputs. Noting the practical difficulties associated with this chemistry in batch photochemical reactors, not least of which is maintaining a suitably high concentration of ethene, the scarcity of photochemical reactors capable of working at pressure and the very modest batch throughput, we hereby seek to show the advantages of the Vapourtec UV-150 photochemical flow reactor, and the steps that can be taken to avoid some common pitfalls of photochemical reactions using the examples from our library synthesis.

Herein we describe the synthesis of a library of representative small, medicinally interesting molecules using the Vapourtec UV-150 continuous flow photochemical reactor, and an R-Series equipped with an automated liquid handler. Many commercially available batch photochemical reactors are only capable of producing small, milligram quantities of product. We have successfully synthesized a library in approximately 50 mg quantities with the aim of producing enough material for characterization, and other stages of compound screening. The examples selected were chosen to illustrate a range of molecular polarities and solubilities from small lipophilic, highly soluble examples to highly polar, low solubility examples see Figure 1 and Table 1. With the library completed, two examples were selected, optimized, and produced at larger scale.

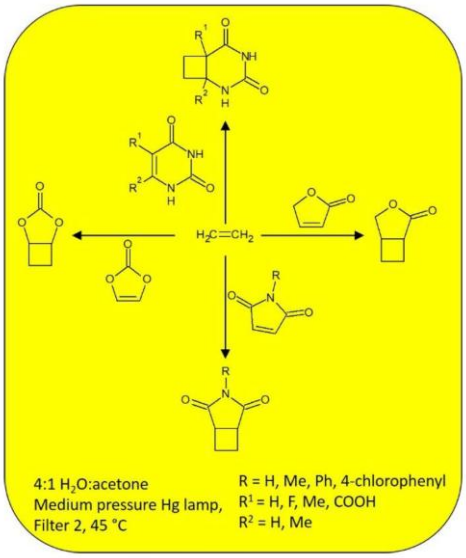


Figure 1: the synthesis of a library of representative small, medicinally interesting molecules using the Vapourtec UV-150 continuous flow photochemical reactor

Setup

All reactions were performed using a Vapourtec R-Series equipped with an R2S+ pump module with 5 ml sample loops, and a 10 ml UV-150 continuous flow photochemical reactor, as shown in Figure 2. An automated liquid handler was connected to the system, controlled by flow control software, and used to load one 5 ml sample loop and to collect product. Ethene 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 substrates were prepared in a 4:1 v/v H_2O:acetone solution in concentrations of 100 mM for maleimide and its derivatives, and 75 mM for all other substrates due to challenging solubility.

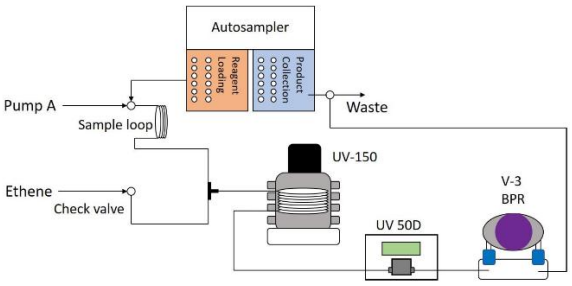


Figure 2: A schematic of the continuous flow reactor used to the [2+2] photocycloaddition library synthesis. Reagents are loaded by the autosampler into the sample loop and then combined with ethene before passing into the UV-150 photochemical reactor. Inline UV analysis is used to monitor the progress of the reaction, and an SF-10 (stand-alone V-3 pump) in pressure regulation mode maintains reaction pressure. The product is collected into vials by the autosampler.

Results

The library of cyclobutyl adducts was prepared using the R-Series equipped with an autosampler, which is able to automatically load reagents and collect products. The reaction substrates outlined in Table 1 were each prepared in an appropriate solvent, with acetone as a photosensitizer. 20 ml aliquots were put into 20 ml scintillation vials and equipped with a vial cap that is fitted with a septum. Critically, this septum vial enables the use of volatile reagents (acetone in this instance) that would otherwise evaporate, especially in the air-flow experienced in a fume hood. When the vials are loaded into the autosampler, the needle is able to pierce the septum and extract the required volume of substrate. Using this approach, it is also possible to blanket the substrate solution with an inert gas if needed.

The library was planned so that each substrate, pre-mixed with the photosensitizer, was loaded into one 5 ml sample loop and then pumped to a T-piece where it mixed with ethene delivered from the second pump. The combined reaction mixture passed into the UV-150 where the [2+2] photocycloaddition took place, then to an inline UV detector, and SF-10 in pressure-regulation mode. Pressure is critical to a gas-liquid reaction, as the heated gas will otherwise expand and force the reaction mixture out of the reactor, reducing the residence time and adversely affecting the reaction performance. After depressurization the flow passes to the liquid-handler which dispenses the product into individual collection vials. The residence time within the reactor varied depending on the solubility of ethene in the solution, so inclusion of inline UV/Vis was critical to the preliminary experiments to ensure the product was not missed.

Table 1 summarizes the results of the library, and Figure 3 shows an excerpt of the data collected. The library took 350 minutes (approx. 6 hours) and was set running at the end of the working day to run overnight under the control of flow control software.

Table 1: Result of [2+2] photocycloaddition library. [a] determined by ^{1}H NMR. [b] undiscernible due to large amount of aliphatic polymer present. [c] starting material fully consumed but hydrolysis product obtained in > 99% without any [2+2] cyclobutyl adduct. [d] highly insoluble product, NMR not obtained.

Substrate	Conversion[a] / %
Maleimide	> 99
n-methyl maleimide	> 99
n-phenyl maleimide	> 99
n-(4-chlorophenyl)maleimide	N/A[b]
2-(5H)-furanone	70
Vinylene carbonate	65
Uracil	>99
5-methyl uracil	54
6-methyl uracil	62
5-hydroxymethyl uracil	52
5-fluorouracil	0[c]
5-carboxy uracil	N/A[d]

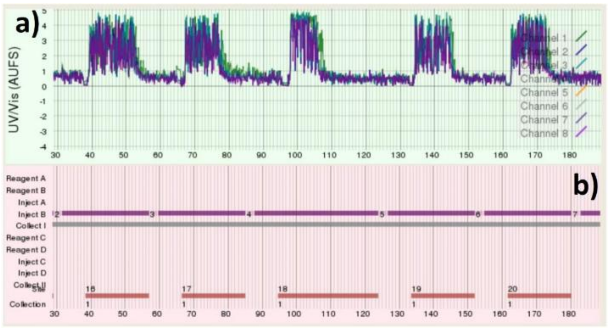


Figure 3: a) elution of product from the reactor as measured by inline UV/Vis spectroscopy. The noise that can be seen in the UV/Vis signal is

caused by the Taylor flow of the liquid reagent and ethene B) Position of valves during the reaction. Notice the purple indicating that reagent was being delivered, with the reagent vial numerically displayed. Also notice the orange bar indicating collection, and to which vial. Reagent 4/collection 18 was permitted a longer collection window as discussed in the text

What became clear from NMR analysis was the presence of a highly aliphatic, polymeric material. Given the use of ethene gas we surmised that this was polyethene, which is known to form photochemically in the presence of oxygen and with sufficiently energetic wavelengths.

Prevention of Undesired Radicals

When preparing for continuous photochemical reactions it is important to consider any side reactions that may also be initiated photochemically. Of particular concern is exposure to high energy, short-wavelength (<250 nm) outputs from the medium-pressure mercury lamp which could, especially in the presence of oxygen, generate highly reactive radical species that interfere with the desired reaction. In the example of the [2+2] photocycloaddition of ethene to maleimide and uracil derivatives, there is a risk of these short wavelengths generating oxygen radicals that in turn could initiate the polymerization of ethene in the reactor.

To prevent this radical formation, one example, n-methylmaleimide, in which there was a high concentration of polythene, was re-run with the reagent and solvent both de-gassed with nitrogen (anhydrous solvents were used and nitrogen was bubbled through the solution for 30 min before use. A nitrogen atmosphere was maintained over the solvent throughout the experiment). Vapourtec filter 2 was already being used to exclude the highest energy wavelengths emitted from the medium-pressure mercury lamp, and this was retained. The resulting NMR showed that the formation of polythene had been prevented by the de-gassing procedure, and as such, the degassing was applied to all subsequent syntheses. When selecting an appropriate filter, it is critical to consider not just the wavelengths transmitted, but also the quantity of photons transmitted at that wavelength; in a photochemical reaction, the photon flux (number of photons per second, per unit area) is a significant factor to consider in reaction design and is often the limiting factor. Acetone is known to absorb strongly between 240 and 290 nm and so in principle any filter that allows greater transmission at these wavelengths, and therefore has a higher photon flux, ought to cause a greater number of excitations of the acetone. Thus, although Vapourtec filters 3 and 7 also transmit in the region absorbed by acetone, filter 2 was selected because the photon flux at the required wavelengths is significantly higher (visit here for a detailed comparison of Vapourtec filters)

Reaction Optimization

Following the success of the library synthesis, two compounds were chosen for optimization and scale-up; the cyclobutyl-adducts of maleimide and uracil.

Uracil proved challenging to dissolve in the water/acetone mixture required for the reaction, and so the concentration had been reduced to 75 mM in a 4:1 v/v solution of water and acetone. To try and increase the throughput of the reaction for scale up, a screen of photosensitizers was devised, again using the autosampler to automatically load the required solution of uracil and photosensitizer

(these solutions were prepared to 75 mM in 3:1 H_2O:ethanol v/v with 10 mol% loading of the relevant photosensitizer), and collect the resulting crude product solutions into separate vials. Each solution was degassed and used with Filter 2 to prevent polymer formation.

Table 2 outlines the photosensitizers tested in the attempt to identify a highly active sensitizer that would permit shorter residence times and increase throughput. The photosensitizers were selected based on their availability, and their maximum absorption wavelength.

Table 2: Photosensitizers trialled for uracil. Used at 10 mol% of a 75 mM uracil solution

Photosensitisers for Uracil
4-aminoacetophenone
3-nitroacetophenone
Acetophenone
3-aminoacetophenone
4-methylacetophenone
Benzil
Benzophenone
3-hydroxyacetophenone

The photosensitizer screening took 4 hours to complete under automated control by flow control software and the collected product samples were dried using a V10 evaporator and sent for analysis. Interestingly, despite each sensitizer absorbing strongly in the regions transmitted by filter 2, only acetophenone caused any conversion of uracil (approximately 10 % converted). As such, it was decided that acetone would be used for the scale-up as it had given the best performance of the photosensitizers so far and its removal from the product is trivial. To further explore the potential increase in throughput of the uracil cyclobutyl adduct, an investigation into temperature was carried out, at a four-fold and eight-fold excess of ethene as outlined in Table 3. For this investigation, the conditions used for the experiments outlined in Table 1 were not suitable because any increase in reaction rate would be difficult to measure. Therefore, the investigation outlined in Table 3 was performed at half of the residence time (which by HPLC resulted in poor conversion). Thus, any conditions that retained a high conversion could have the potential for improving the throughput of the synthesis.

Table 3: Effect of temperature and ethene excess on conversion of uracil. Conversion determined by NMR

Temperature/°C	Ethene Excess	Conversion/ %
45	8	14
45	4	18
60	8	8
60	4	19
30	8	14
20	8	21

From Table 3 it can be seen that an improved conversion was observed at 20 °C, however at this lower temperature the solubility of uracil became problematic and began to cause blockages. Therefore, it was decided to continue using 45 °C as had been used in the library synthesis.

Key to this investigation was the ease with which the range of conditions could be examined. Using the automated control features of flow control software combined with the use of an auto-sampler, enabled fast experiment times without

committing a researcher to anything other than preparation of the reagent solutions. As can be seen from Table 3, none of the conditions tested at the reduced residence time permitted much conversion. Therefore, the scale-up of the uracil cyclobutyl adduct was performed under the same conditions used for Table 1.

Scale-up and purification

Having further studied the effects of several reaction parameters, the synthesis of the maleimide and uracil cycloadducts were scaled up. Our investigations to this point had identified conditions that achieved 86% conversion, cleanly to the maleimide cycloadduct product. These conditions were selected for scale-up of the maleimide adduct because, although there were conditions that resulted in higher conversion, they required double the residence time, greatly reducing the throughput. Sacrificing the highest conversion enabled a greater quantity of the product to be synthesized per hour. Under these conditions, 1.73 g of crude product was produced in a little over 2.5 hours (0.64 g/hr) with a conversion of 80% to the maleimide cycloadduct, demonstrating an almost 35-fold scale-up. The scale-up of the cycloaddition to uracil was also completed successfully, giving 1.80 g of crude product in approximately 6.5 hours, with 85% conversion to the cycloadduct. This synthesis incorporated several cycles of washing, in which water was pumped at 5 ml/min for two system volumes after every 30 ml of reagent had been added, to prevent any build-up of the cyclobutyl adduct, which was less soluble in the reaction solvent.

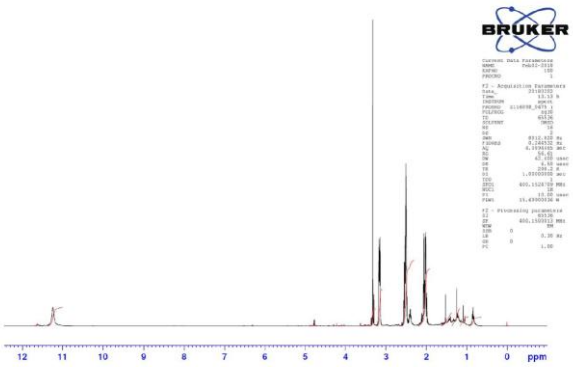
Conclusion

Library synthesis is a critical tool for medicinal and pharmaceutical chemists, however when applied to batch photochemical reactions, has always been limited to very low mass throughputs due to limitations as a result of the Beer-Lambert law.

In this application, we have demonstrated how the powerful combination of an R-Series, UV-150 photochemical reactor and autosampler can be used to perform a photochemical library synthesis quickly and with mass throughputs that are typically superior to commercially available lab-scale batch photochemical reactors. The automated control capability of flow control software permits safe operation out-of-hours, and remote access where necessary, freeing the user for other tasks while the traditionally labor-intensive library synthesis is carried out. Inline UV/Vis spectroscopy was used to ensure product collection was accurate, and two products were successfully scaled up to several gram quantities with good conversion to the desired product.

Analysis

All NMR were performed by Tocris Bioscience. An example NMR following the synthesis of the maleimide cyclobutyl adduct is below.



References

1. See for example: A. B. Beeler, *Chem. Rev.* **2016**, 116, 17, 9629-9630
2. Sergeiko, A., Poroikov, V. V., Hanus, O., Dembitsky, V.M., *Open Med Chem J*. **2008,** 2, 26–37
3. Poplata, S., Tröster, A., Zou, Y-Q., Bach, T., *Chem Rev*, **2016**, 116(17), 9748–9815