Data-rich automated liquid-phase dipeptide synthesis in transient flow

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Abstract Automated transient flow synthesis with online analytics is a powerful tool capable of efficiently collecting high quality data for process development. We showcase this efficiency using our automated reactor platforms on a dipeptide synthesis example. The campaign required only three reaction runs, the first an initial scoping reaction to get an overview of the chemical space and identify variables that most influenced the reaction outcome. The remaining two runs focused on the chemical space of interest and revealed minimum additive loadings required to achieve high yields. In total, only 7.5 hours of reactor time was required to collect 122 high-quality quantitative HPLC data points.

Introduction Efficient and high-quality data collection underpins process development campaigns. This is often achieved through coupling modern high-throughput experimentation (HTE) technologies with automation. This marriage enables reliable and robust data collection practices by standardising experimental protocols, while simultaneously increasing throughput with minimal manual input.[1] HTE methods in batch are commonplace in the pharmaceutical industry, and increasingly so in academic labs too, providing a platform for rapid data collection.[2–4] However, due to the small scale of these reactions and limitations in sampling methods, the outputs are often only single time-point data – applicable to hit identification and screening efforts, but limited in the knowledge that can be extracted from each individual reaction. When using modern batch screening platforms, important process development variables such as temperature, reaction time, concentration and solvent must be treated as discrete variables, and therefore require increased experimentation to explore adequately. These systems can be further hindered by poor mass and heat transfer too, meaning processes developed on these platforms are often not suitable for scale-up.[5]

In contrast, transient flow with online analytics allows for data-rich experimentation. This differs from steady-state continuous flow synthesis, which is increasingly commonplace in the pharmaceutical industry,[6,7] by creating dynamic reaction environments through variation of reaction inputs during the course of an experiment. Precise reactor characterisation and input controls allow reaction outputs to be correlated to these dynamic inputs, enabling sampling across diverse chemical space within a single experimental run.[8,9] In practice, this allows temperature, reaction time, concentrations and solvents to be varied over the course of a reaction and therefore become continuous variables. Coupling this approach with automation and online analytics results in a highly efficient and reliable data collection tool.[10]

With this in mind, we set out to probe the utility of our automated transient flow reactors for developing reaction understanding in a pharmaceutically relevant reaction. We selected the coupling of two amino acids to make a dipeptide as liquid phase peptide synthesis (LPPS) is an ever important area of research for developing and manufacturing therapeutics.[11]

Results and Discussion Our investigation began by examining the coupling of Fmoc-Ile-OH **1** and H-Phe-OtBu.HCl **2** using HATU and DIPEA as coupling agent and base respectively to produce dipeptide **3**, Figure 1A. We opted for a 3-reservoir reactor connected to an online HPLC, Figure 1B, for this initial scoping reaction. Limiting reagent **1**, DIPEA (in a ratio of 1:3) and internal standard (biphenyl) were added to the first reservoir. The second contained **2** and HATU in a ratio of 1:1.5. The third reservoir contained only solvent DMF and its flow rate was varied to keep the concentration of limiting reagent **1** constant at 0.02 M in the reactor. The multivariant design space included temperature ramps between 40 and 80 °C, reservoir 2 equivalents range between 1-2 and residence times of 2-16 minutes. This was accomplished in a single experimental run, requiring only 3 hours of automated system run time.

The initial results indicated the formation of **3** was dependent on the equivalents of reservoir 2 in the reactor, Figure 1C. Temperature and residence time did not appear to influence the reaction outcome. Postulating that this effect was due to HATU loading, we therefore elected to examine coupling agent and base loading independently.

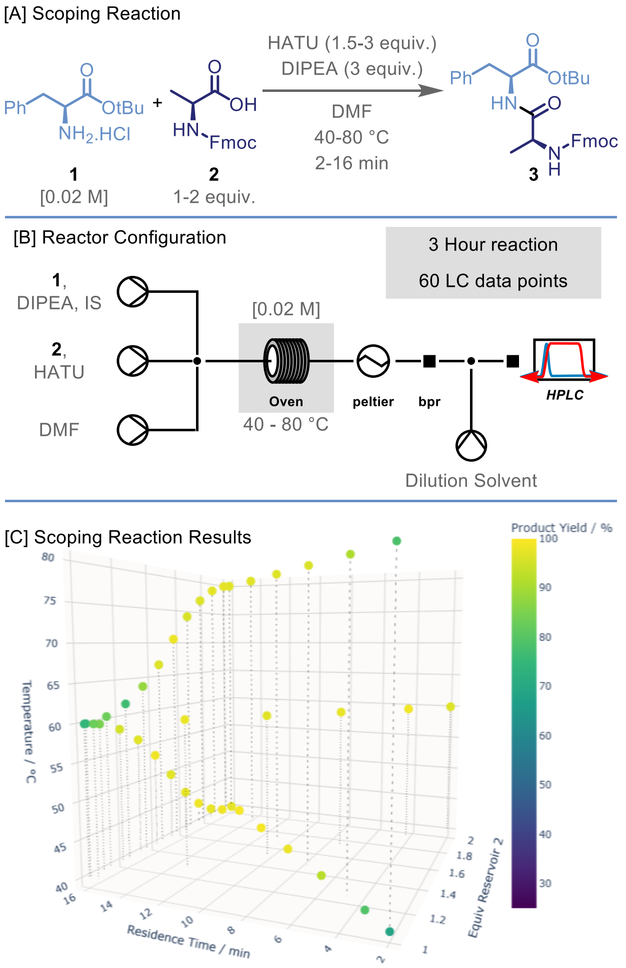
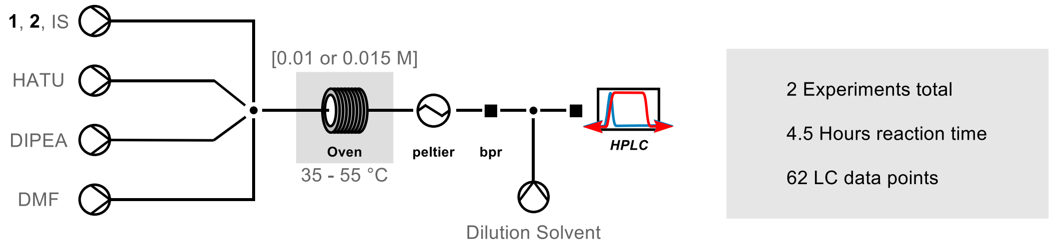


Figure 1. [A] Summary of the scoping reaction. [B] Reactor configuration for the scoping reaction. [C] Calibrated results from the scoping reaction given as percentages.

[A] Reactor Configuration



[B] 3D Representation of Results

[C] Equiv. HATU vs Equiv. DIPEA

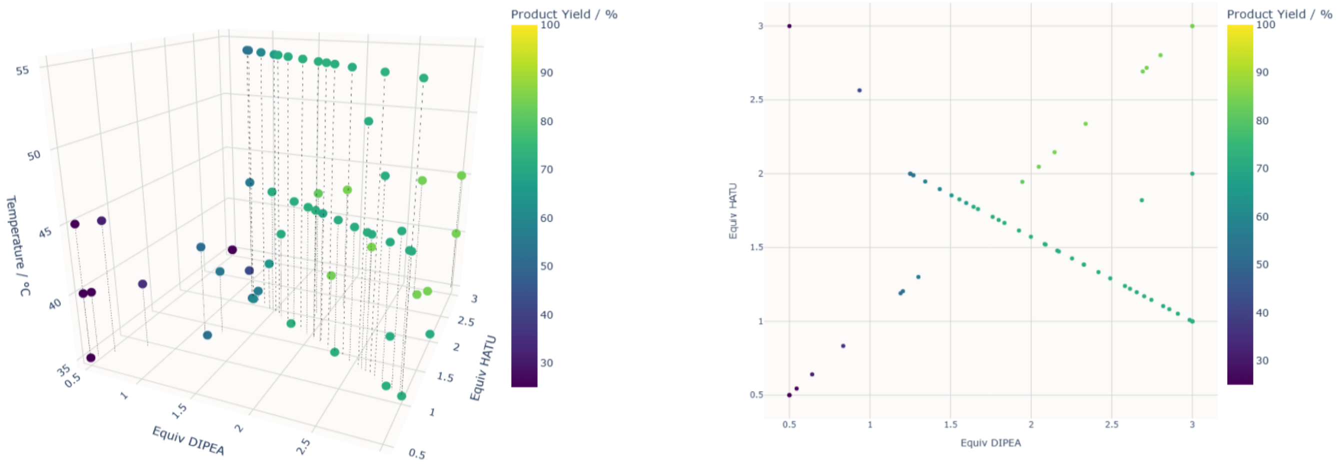


Figure 2. [A] Reactor configuration for the 4-reservoir reactions. [B] 3D representation of results showing product yield for equiv. DIPEA vs equiv. HATU vs Temperature. [C] 2D representation of results highlighting product yield for equiv. HATU vs equiv. DIPEA.

To probe the effect of increased equivalents of HATU and DIPEA independently we switched to a 4-reservoir system. This enabled experiments to be run with 4 variables; HATU equiv., DIPEA equiv., Temperature, and Residence Time, Figure 1A. Control experiments confirmed that **1** and **2** were stable in a single reservoir for the duration of the reaction. Just 2 reaction runs and 4.5 hours of reactor time were required to complete this second data collection campaign, which resulted in 62 HPLC data points. Once again, temperature and residence time did not prove to be the main factors promoting formation of **3**, with high yields being observed at both ends of the temperature and residence time ranges, Figure 2B. Separating HATU and DIPEA into individual reservoirs highlighted the sensitivity to each component in the formation of **3**. The greatest yields were observed when both HATU and DIPEA loading were above 1.7 equivalents. This rapid data collection could then be used to inform trade-offs in yield vs material costs or sustainability for the process space and identified conditions. Data collection across various dynamic reaction conditions, including residence time, allows this data to be used for kinetic modelling purposes too.

Conclusion Herein we demonstrated that automated transient flow is capable of performing rapid multiparameter exploration of an amino acid coupling. An initial scoping reaction identified that the reaction could be run at lower temperatures with shorter residence times, and that the reaction was sensitive to HATU and DIPEA equivalents. Two further reactions collected sufficient data to identify minimal loadings of these components required for high reaction yields. The entire campaign required only 7.5 hours of reactor time and a single operator. The full interactive data set is available in the SOLVE Chemistry webapp with other case studies.

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