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Rapid Flow-Based Peptide Synthesis

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A flow-based solid-phase peptide synthesis methodology that enables the incorporation of an amino acid residue every 1.8 min under automatic control or every 3 min under manual control is described. This is accomplished by passing a stream of reagent through a heat exchanger into a low volume, low backpressure reaction vessel, and through a UV detector. These features enable continuous delivery of heated solvents and reagents to the solid support at high flow rate, thereby maintaining maximal concentration of reagents in the reaction vessel, quickly exchanging reagents, and eliminating the need to rapidly heat reagents after they have been added to the vessel. The UV detector enables continuous monitoring of the process. To demonstrate the broad applicability and reliability of this method, it was employed in the total synthesis of a small protein, as well as dozens of peptides. The quality of the material obtained with this method is comparable to that for traditional batch methods, and, in all cases, the desired material was readily purifiable by RP-HPLC. The application of this method to the synthesis of the 113-residue *Bacillus amyloliquefaciens* RNase and the 130-residue DARPin pE59 is described in the accompanying manuscript.

Introduction

The total chemical synthesis of peptides has been of great interest for over a century.[1] Chemical synthesis of peptides and proteins enables incorporation of nonproteogenic functionalities, without restriction on their location or number.[2,3] With the introduction of solid-phase peptide synthesis by Merrifield in 1963, the total synthesis of short peptides became routine.[4] Subsequent advances extended the technique to include assembly of long polypeptides,[5–7] and the advent of native chemical ligation (NCL) enabled the preparation of polypeptides of theoretically unlimited length from fragments.[8]

Despite these advances, the time required to assemble polypeptides, either as final targets or as fragments of a larger molecule, imposes a major limitation on studies employing such synthetic material. Most peptides are synthesized with Fmoc protocols, rather than the faster and higher-yielding Boc procedures, because highly toxic hydrogen fluoride is not required and only small amounts of costly trifluoroacetic acid are used.[7] Standard Fmoc solid-phase peptide synthesis methods require 60 to 100 min to incorporate each amino acid residue;[9,10] some specialized procedures use complex microwave systems to reduce this to about 5 min per residue.[11–13] Here, we describe the development of a flow platform that incorporates an amino acid residue every 3 min under manual control or 1.8 min under automatic control, without microwave irradiation.

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Results and Discussion

Apparatus design

To perform such rapid synthesis, we began with an analysis of existing kinetic data. It is known that at room temperature amide bond formation is 99.9% complete in less than 2 min, with 0.5 M amino acids and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) activation,[14] and removal of N-terminal Fmoc protection is effected in 4–6 min with 20% (*v*/*v*) piperidine in DMF.[10,15] Our own model studies corroborated these data: a reaction half-life of (4.6 ± 0.6) s was observed for the formation of an amide bond between 0.3 M activated Fmoc-leucine-COOH and resin-bound phenylalanine at room temperature (see the Supporting Information). Standard procedures allow these steps to proceed for much longer in an effort to improve the quality of difficult sequences, although this strategy is often of marginal benefit.[16]

Assuming the reaction rate for these processes doubles for every 10 °C increase in temperature, at 60 °C amide bond formation should be complete in less than 10 s and Fmoc removal should take less than 20 s. Based on this, we believed that robust Fmoc-based peptide synthesis could be carried out in substantially less than 5 min per residue at 60 °C with conventional heaters.

To study peptide synthesis on these timescales, we sought to build a device capable of delivering preheated reagents and solvents to a synthesis vessel, continuously monitoring the process, and rapidly switching between reagents. The second criterion pushed us to revisit continuous-flow peptide synthesis, as several previous systems were able to effectively track the progress of peptide synthesis by monitoring the UV absorbance of the reaction mixture.[17,18] Furthermore, with a flow-based system, solvents and reagents can be rapidly preheated by pumping them through a high-efficiency heat exchanger with low residence time.[19] This arrangement eliminates the time required to heat reagents after they are delivered to the synthesis vessel and prevents degradation from prolonged storage of reagents at elevated temperature.[20]

Previous flow-based peptide synthesis systems had two major drawbacks, however. First, washes were very slow. All of these systems used a long, packed column containing the solid support (similar to an HPLC column). As with equilibrating an HPLC column, several column volumes of solvent were required to effectively wash them. At low flow rate, this required tens of minutes to hours. As flow rate increased, the solid support collapsed and backpressure rose rapidly. Eventually, the support extruded through the frits used to confine it.[17] Although several solutions to this problem were proposed, none were ultimately successful, and commercial synthesizers of this type are not currently available.[17,18,21,22] Second, these systems recirculated low concentration reagents rather than continuously replenishing high concentration reagents. This conserved activated amino acids, but resulted in slow amide bond formation. To overcome these problems, we designed a low volume, low pressure reaction vessel. This vessel reduced the volume of wash solvent required and enabled delivery of solvent and reagent at high flow rates, thus allowing reagents to be maintained at maximal concentration and to be removed rapidly.

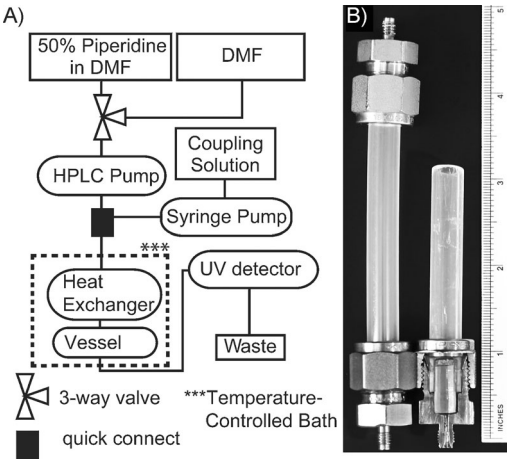

Figure 1. Flow platform for Fmoc SPPS. A) Schematic of the synthesizer. The reaction vessel can be placed in a temperature-controlled bath. B) The assembled reaction vessel (left) and a cutaway showing the down-stream components (right).

To deliver the reagents required for peptide synthesis without a complex fluidic manifold, the apparatus shown in Figure 1A was developed. An HPLC pump was used to deliver either DMF or 50% (*v*/*v*) piperidine in DMF for the common washing and deblocking steps, and a syringe pump was used to deliver coupling reagents. The HPLC pump solvent was selected by a manually actuated three-way valve, and the HPLC pump outlet was attached to the reaction vessel through a luer-lock quick connect. For the coupling step, the quick connect was manually disconnected from the HPLC pump and connected to a syringe pump that delivered a solution of activated amino acid. The effluent was passed through a UV detector to continuously monitor the absorbance at 304 nm, a wavelength at which Fmoc amino acids and the dibenzofulvene-piperidine deprotection adduct absorb strongly.[18]

The first-generation reaction vessel (Figure 1B) was designed to be simple and easy to construct, while giving low backpressure. A 6.4 mm ($^1/_4$") inner diameter × 76 mm (30") long perfluoroalkoxy tube was used with Swagelok reducing unions at the inlet and outlet. A frit was positioned in the outlet by using a short piece of tubing with a 6.4 mm outer diameter. Installation of the outlet fitting and concurrent compression of the ferrule and tube sealed the frit in place. The total volume of the vessel was ~2.5 mL. This vessel was assembled without machine- or glass-shop support, and allowed us to conduct model studies with up to 100 mg of resin.

To verify the feasibility of Fmoc SPPS with this system, we synthesized the model peptide Fmoc-ALFALFA-$CONH_2$ on a 0.1 mmol scale (100 mg of resin). Based on an initial estimate, we chose to start with a 2 min DMF wash at 10 mL min^{-1}, 2 min Fmoc deprotection at 6 mL min^{-1}, another 2 min DMF wash, and a 6 min room temperature coupling with 2 mmol of activated amino acid at 1 mL min^{-1}. This sequence yielded highly pure material, thus enabling peptide synthesis at 12 min per residue. To achieve maximal concentration of activated amino acid and rate of amide bond formation, coupling solutions were prepared by dissolving amino acids in one equivalent of 0.4 M 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphonate (HBTU) in DMF. The activating base was added immediately before use to give a final concentration of activated amino acid of about 0.3 M. This concentration of coupling reagent was used for all experiments, including those with HATU activation (Figure 3A, below).

Based on our initial investigations and prior reports, we decided to carry out all subsequent studies at 60 °C to minimize the cycle time without significantly increasing formation of side products.[23,24] To consistently and quickly bring reagents to 60 °C, a heat exchanger was placed between the synthesis vessel and the luer-lock quick connect. A 1.6 m coil of tubing (1.6 mm outer diameter, 0.76 mm inner diameter) was used. This preheat loop was immersed with the reaction vessel in a water bath maintained at 60 °C, and effectively increased the temperature of the solvent from 18 °C (RT) to 59 °C, as measured by a thermocouple in the outlet of the loop. PFA tubing was effective at flow rates up to 20 mL min^{-1}; stainless steel was used for all experiments at higher flow rates.

Determination of minimum cycle time and model studies

To determine minimal cycle times, each step of peptide synthesis was studied. First, the time required to wash amino acid solution out of the reaction vessel was investigated as a function of flow rate by monitoring the UV absorbance of the effluent. At 1 mL min^{-1}, about 16 min (16 mL) was required to remove 99% of the amino acid precursor. At higher flow rates, however, the amount of required solvent decreased: only 1 min (10 mL) was required at 10 mL min^{-1}. To guarantee an effective wash, 20 mL of DMF were used. This was delivered over two minutes at 10 mL min^{-1} because the first-generation reaction vessel could not reliably tolerate higher flow rates. Analysis of the crude peptides did not show double incorporation of amino acids, and increasing the wash volume did not improve crude peptide quality.

We then investigated the rate of Fmoc removal by monitoring formation of the UV-active dibenzofulvene-piperidine adduct. We chose to use 50% (*v*/*v*) piperidine in DMF over the more common 20% (*v*/*v*), because our preliminary work indicated this removes Fmoc more rapidly. To decouple the time spent removing the Fmoc protecting group and the time required to wash the byproduct from the resin, deprotection reagent was delivered briefly, the resin was washed, and more deprotection reagent was delivered. A second UV absorbance peak indicated formation of additional dibenzofulvene-piperidine, and incomplete initial deprotection. The initial deprotection was performed at 10 mL min^{-1} for 60, 30, 15, or 6 s. A second peak was observed after a 6 s deprotection, but not after a 15, 30, or 60 s deprotection. Fmoc removal is reported to be sequence-dependent,[8] so, to ensure robust deblocking, the deprotection reagent was delivered for 20 s at 10 mL min^{-1}.

With wash and deblocking conditions established, the time required for robust amide bond formation was determined by synthesizing two model peptides: LYRAG-$CONH_2$ and Fmoc-ALF-$CONH_2$. Each peptide was synthesized five times, and, for each synthesis, 0.3 M amino acid solutions were coupled for a nominal time of 90, 45, 30, 15, or 7 s (Figure 2). For syntheses with 90, 45, and 30 s couplings, 2 mmol of each amino acid was used. As the syringe pump could not infuse 6 mL (2 mmol, 0.3 M) of amino acid solution in less than 30 s, for the synthesis with 15 s couplings, 3 mL (1 mmol) was used, and 1.2 mL (0.4 mmol) was used for the synthesis with 7 s couplings. For Fmoc-ALF-$CONH_2$, we found no significant difference in the quality of the crude product as a function of coupling time. For LYRAG-$CONH_2$ we observed a significant increase in the Arg deletion peptide when all residues were coupled for 7 s. Based on these results, a 30 s coupling time was selected. The timeline of the final synthesis used with the first-generation reaction vessel is shown in Figure 2C.

To explore our approach under a variety of synthetic conditions, we studied the synthesis of residues 65–74 of the acyl carrier protein (ACP). This peptide serves as a model to validate new peptide-synthesis protocols, as it is considered difficult to prepare.[14,25–27] The main synthetic impurity is a chromatographically resolved Val deletion. The LCMS data for the synthesis of ACP(65–74) with our methodology, as well as two controls, is shown in Figure 3. When using our protocol and the HATU coupling agent, a minor Val deletion product was observed. When using HBTU, more Val deletion was observed; this is consistent with prior reports.[14,25] ACP(65–74) synthesized with our flow system and synthesis timeline, but at room temperature, showed large Val and Gln deletions, thus confirming that reaction temperature is important. No major differences in the crude product from this flow-based room-temperature synthesis and an analogous room-temperature batch synthesis that followed the same synthetic timeline were observed.

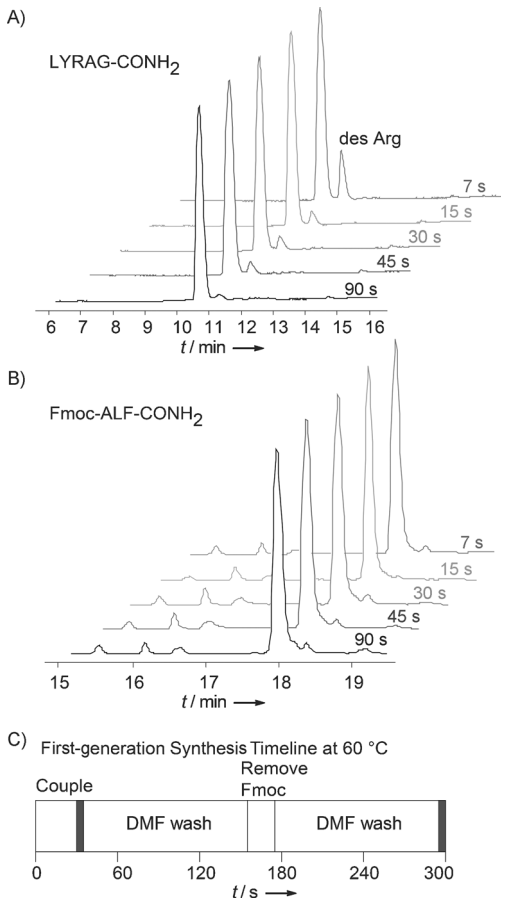

Figure 2. Systematic investigation of coupling time. A) LC data for crude LYRAG-$CONH_2$ produced by coupling every amino acid for a nominal 90, 45, 30, 15, or 7 s. The amount of Arg deletion peptide was greatest for 7 s coupling. B) LC data for the synthesis of Fmoc-ALF-$CONH_2$ under the same conditions: there was no change in peptide quality with reduction in coupling time. C) The final synthetic timeline used with the first-generation reaction vessel; gray bars indicate time required to move the quick connect. An amino acid residue is incorporated every 300 s. Total ion current is displayed in each chromatogram.

Next, two additional "difficult" peptides were prepared: a conotoxin variant and a fragment of HIV-1 protease.[14] The initial syntheses of these peptides yielded several products of equal molecular mass; these were determined to be diastereomers arising from racemization of cysteine during activation. We therefore carried out model studies with the peptide GCF, and found several conditions that produced less than 1% dia-

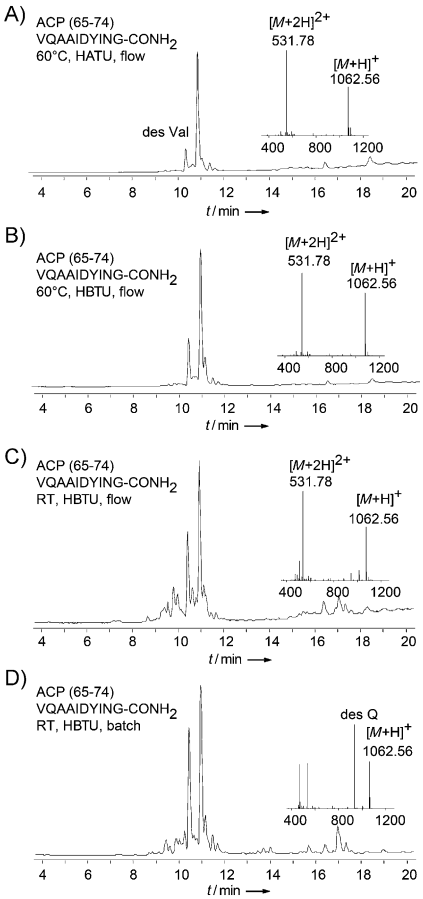

Figure 3. LCMS data for ACP(65–74) model studies. The synthetic timeline in Figure 2C was used. Crude LCMS chromatograms for ACP(65–74) synthesized at A) 60 °C with HATU as activator, B) 60 °C with HBTU as activator, C) RT with HBTU as activator, and D) RT with a manual batch method following the same timeline. Total ion current is displayed in each chromatogram.

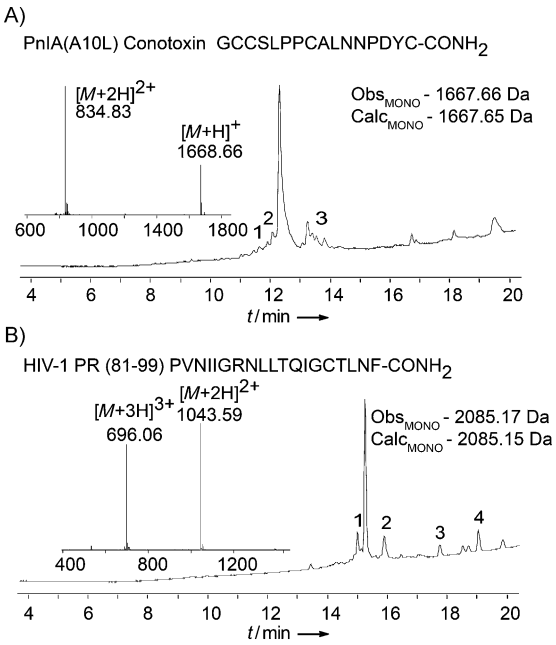

Figure 4. Synthesis of difficult peptides under flow. LCMS data for the crude peptides: A) PnIA(A10L);1 = Cys deletion, 2 = Cys deletion, 3 = incomplete side-chain protecting group removal; B) HIV-1 PR (81–99); 1 = peptide truncation at Arg; 2, 3, 4 = incomplete side-chain protecting group removal. Total ion current is displayed.

stereomer while maintaining the same cycle time (Figure S5 in the Supporting Information). This level of racemization is consistent with previous reports for Fmoc protocols,[28] and the identified conditions were also used to couple racemization-prone histidine and tryptophan when used in the syntheses of Barnase (RNAse from *Bacillus amyloliquefaciens*) and a DARPin (see the accompanying manuscript: 10.1002/cbic.201300797). The peptides shown in Figure 4 were prepared on a 0.1 mmol scale by using modified activation conditions for Cys with the first-generation synthesis vessel and cycle.

To demonstrate the suitability of this flow-based synthesis method for ligation-based protein synthesis, it was applied to the synthesis of a 58-residue trihelical protein based on the Z domain of protein A ("affibody"). The synthesis strategy used peptide-hydrazides as thioester precursors. Peptide hydrazides can be oxidized with $NaNO_2$ to form a C-terminal acyl-azide, which reacts with a thiol to form a peptide thioester suitable for use in native chemical ligation.[8,29] The LCMS data for the crude synthetic peptides are shown in Figure 5. We purified each peptide, synthesized the affibody according to the strategy in Figure 5A, and isolated highly pure, full-length affibody after purification (Figure 5E). The fragments were produced and cleaved from the resin in one day. In contrast, production of similar fragments with optimized Boc in situ neutralization protocols required more than three days, and yielded crude peptides of similar quality (Figure S7).

Acceleration and scale up

After these model studies, we sought to increase the synthesis scale and to decrease the cycle time of this system. All attempts to increase the flow rate or add more resin to the first-generation reaction vessel were thwarted by rapidly increasing backpressure. A high-pressure stainless steel reaction vessel was constructed to study the effect of simply providing more pressure to maintain a high flow rate. This resulted in extrusion of the resin through the frit, as has been previously observed.[17]

Therefore, the second-generation synthesis vessel shown in Figure 6 was constructed. The diameter is twice that of the first-generation vessel, and volume-limiting inserts restrict the volume to 2 mL (about the same as the first-generation). Increasing the diameter of the vessel drastically reduced the backpressure, and maintaining a comparable volume allowed the same volumes of solvents and reagents to be used. This vessel accommodated up to 200 mg of resin, and flow rates up to 60 mL min^{-1}. More resin should not be used, because the resin swells as the peptide elongates, and the volume-limiting inserts restrict the swollen volume to 2 mL. Long peptides might result in reactor failure if more than 200 mg of resin is

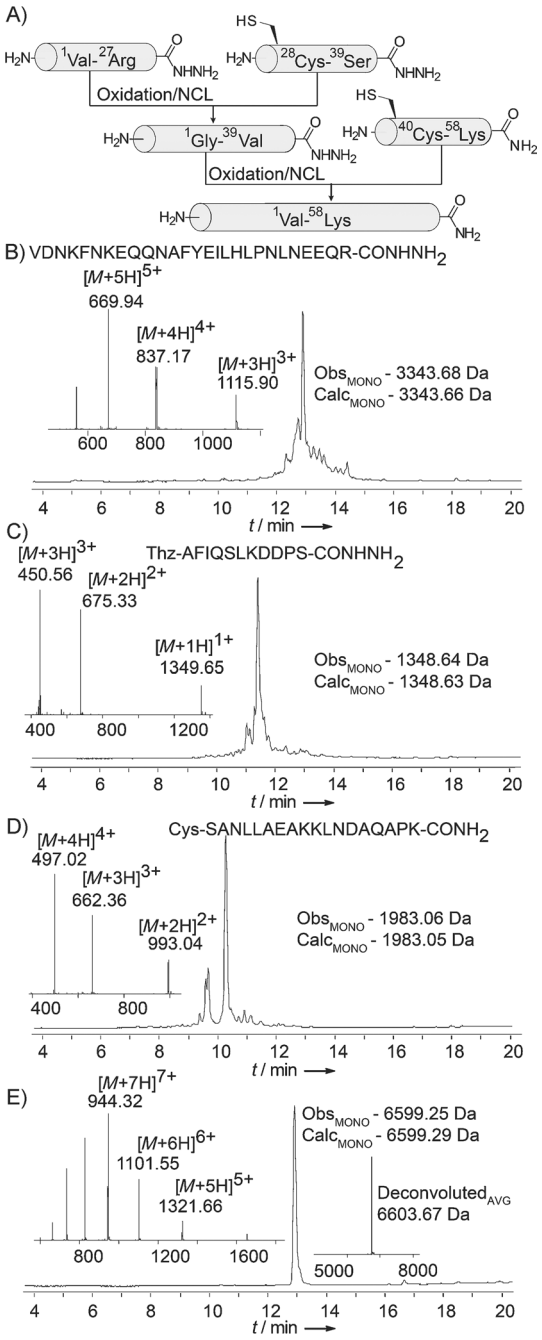

Figure 5. Synthesis of the affibody. A) Synthesis scheme used to produce the affibody. B) Crude fragment [1–27]-$CONHNH_2$. C) Crude fragment Thz-[28–39]-$CONHNH_2$. D) Crude fragment Cys-[40–58]-$CONH_2$. E) Purified full-length affibody. Observed and calculated masses are monoisotopic. Total ion current is displayed in each chromatogram.

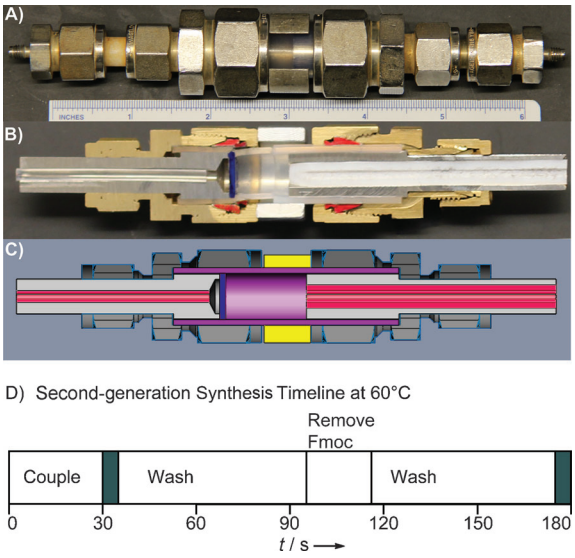

Figure 6. Second-generation reaction vessel. A) Assembled unit. B) Cutaway showing fittings (brass), frit (blue), and large ferrules (red). The image has been color enhanced, and background objects were removed. C) False-color drawing of the cutaway showing fittings (dark gray) and the frit (blue). D) The final synthetic timeline used with the second-generation reaction vessel; gray bars indicate time required to move the quick connect. An amino acid residue is incorporated every 180 s.

used. Higher flow rates were not tested, but the observed backpressure indicates they are possible.

With this new reaction vessel, cycle time was reduced by washing at a higher flow rate. As expected, the required wash time continued to decrease with increasing flow rate: 99% amino acid removal was effected in 36 s at 20 mL min^{-1} and in 20 s at 40 mL min^{-1}. To allow operators adequate time to prepare each successive amino acid, one minute washes at 20 mL min^{-1} were used. When the instrument was made available for general laboratory use, users occasionally successfully ran the system with 30 s washes at 40 mL min^{-1}.

To verify that the performance of the second-generation synthetic protocol and vessel (using faster cycles and fewer equivalents of amino acids) was comparable to that of the first-generation synthetic procedure, we synthesized ACP(65–74), the PnIA(A10L) conotoxin, the HIV-1 protease fragment, and the affibody fragments on 200 mg of resin with the timeline shown in Figure 6D. The crude peptides were of comparable quality (Figures S9–S11), and this reactor was made available for general use.

Dozens of unique peptides were made for diverse applications, and almost all were of high crude purity. Several representative case studies are included here and in the Supporting Information (Figures 7 and S12–S14). This method is sufficiently robust that all of these peptides were synthesized without UV monitoring of the reactor effluent. Figure 7 shows a library of model cysteine-containing peptides, including some of low crude purity. Additional, longer analogues of the cysteine contain peptides (Figure S13), a library of ten model glutathione-like peptides prepared in a single day (Figure S12), and two biotinylated peptides (Figure S14) are presented in the Supporting Information; all were of high crude purity. In all cases, peptides were produced on a 0.2 mmol scale, the major peak was the desired product, and crude material was successfully purified in one preparative RP-HPLC step.

This method was also used to produce peptide fragments of two additional proteins, the 113-residue Barnase and the 130-residue peptide pE59 DARPin. Following rapid optimization of the synthetic protocol, full-length proteins were obtained. For

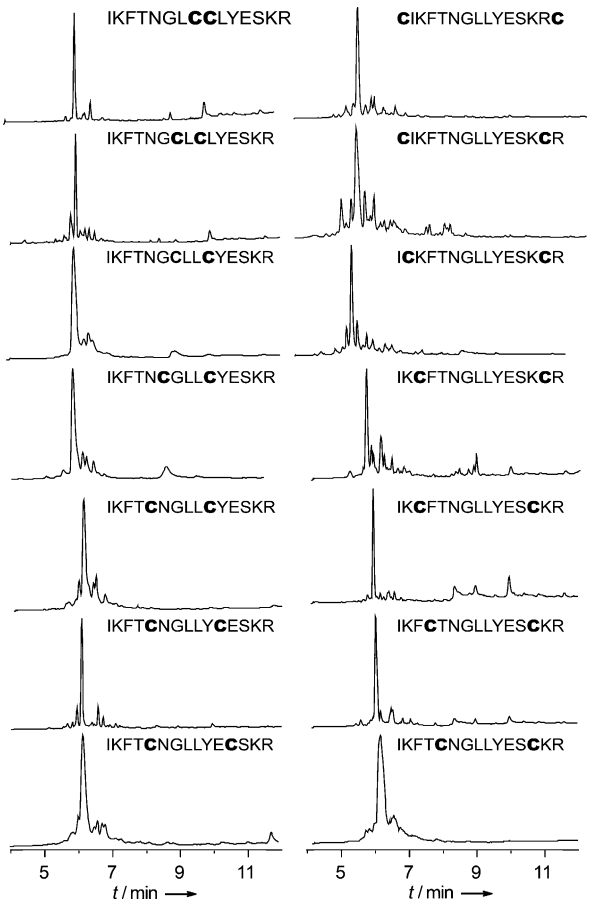

Figure 7. LCMS traces of model 15-mer peptides for cysteine macrocyclization research synthesized in 45 min each. Cysteine is shown in bold type, and total ion chromatograms are shown. All peptides were produced as C-terminal amides, and, in all cases, the main peak was the desired mass (1772.9 Da found and calculated, monoisotopic).

a detailed account of the synthesis of these peptides (including effective and ineffective methods for suppressing aspartamide formation, deletions, methionine oxidation, and premature termination of the peptide chain), see the accompanying manuscript.

Automation

We believed our rapid synthesis method might prove difficult to automate. To demonstrate automation, the proof-of-principle instrument shown in Figure 8A was constructed. Two HPLC pumps delivered reagents; one delivered piperidine or solutions of amino acids and HBTU in DMF, and the other delivered *N*,*N*-diisopropylethylamine (DIEA) to activate the amino acids. A static mixer was used to ensure effective mixing of DIEA and the amino acid solution, and the valve positions and pump flow rates were controlled by an Arduino microcontroller. Amino acids were stored as 0.4 M solutions with equimolar HBTU in DMF; these were stable for weeks without the activating base when stored in sealed vessels. The system used the heat exchanger, the second-generation synthesis vessel, and the UV detection described above without modification (details of construction in the Supporting Information).

The automated cycle time was not limited by the rate at which a user could complete manual tasks or at which a syringe pump could infuse, so the timeline was substantially accelerated (Figure 8B). Two 45 s washes at 50 mL min^{-1} (maximum available), a 7 s coupling, and a 10 s deprotection resulted in incorporation of an amino acid residue every 107 s (1.8 min). The coupling time and deprotection time constitute small fractions of the total time, so were not optimized. With these cycles, the heptapeptide ALFALFA-CONHNH$_2$ was produced in 12.5 min, ACP(65–74) was produced in 17.8 min, and (ALF)$_7$, a model 21-residue peptide prepared to demonstrate that the system was robust, was produced in 37.5 min. The crude quality of (ALF)$_7$ was nearly identical to that from a synthesis using the manual second-generation protocol (Figure S15).

Conclusions

The reported methods generate high quality peptides extremely rapidly, on scales relevant for research. Based on the published syntheses of ACP(65–74), the conotoxin, and the HIV protease fragment,[14] together with our experiments with manual Boc syntheses of affibody fragments and the synthesis of ACP(65–74) at room temperature in batch and under flow, we believe that crude material obtained by our methods is nearly identical to that obtained by traditional batch processes.

Extensive work has shown that SPPS chemistry can be fast and efficient at elevated temperatures.[12] The reported system further accelerates SPPS chemistry—far beyond what is currently possible with microwave-assisted or other rapid peptide synthesizers—by leveraging a flow-based approach. In addition to continuously supplying high concentration reagents, the flow-based platform overcomes a number of significant obstacles that hinder standard and microwave-assisted approaches. First, the completely sealed reaction vessel and heat exchanger can be immersed in a temperature-controlled bath; this allows solvents and reagents to be heated in a consistent and controlled manner immediately before reaching the resin bed. Rapid preheating is crucial to avoid thermal degradation of reagents[20] while quickly reaching the desired temperature, but this is extremely difficult in a batch system. Second, the flow platform can be scaled up without increasing cycle time. As demonstrated in the transition from the first- to second-generation reaction vessels, increasing the diameter and flow rate effectively increases the maximum scale without slowing synthesis. Third, stirring is not required to effect adequate mass transfer, thus eliminating failure-prone moving parts and facilitating scale-up. Fourth, high quality peptides can be obtained quickly without double coupling, double deprotection, or colorimetric tests of coupling efficiency. During our studies with ACP(65–74), we observed no decrease in the Val deletion peptide after double coupling Val and double deprotecting the preceding Gln, and these results are consistent with our experience optimizing the fragments of Barnase and a DARPin. Such addition-

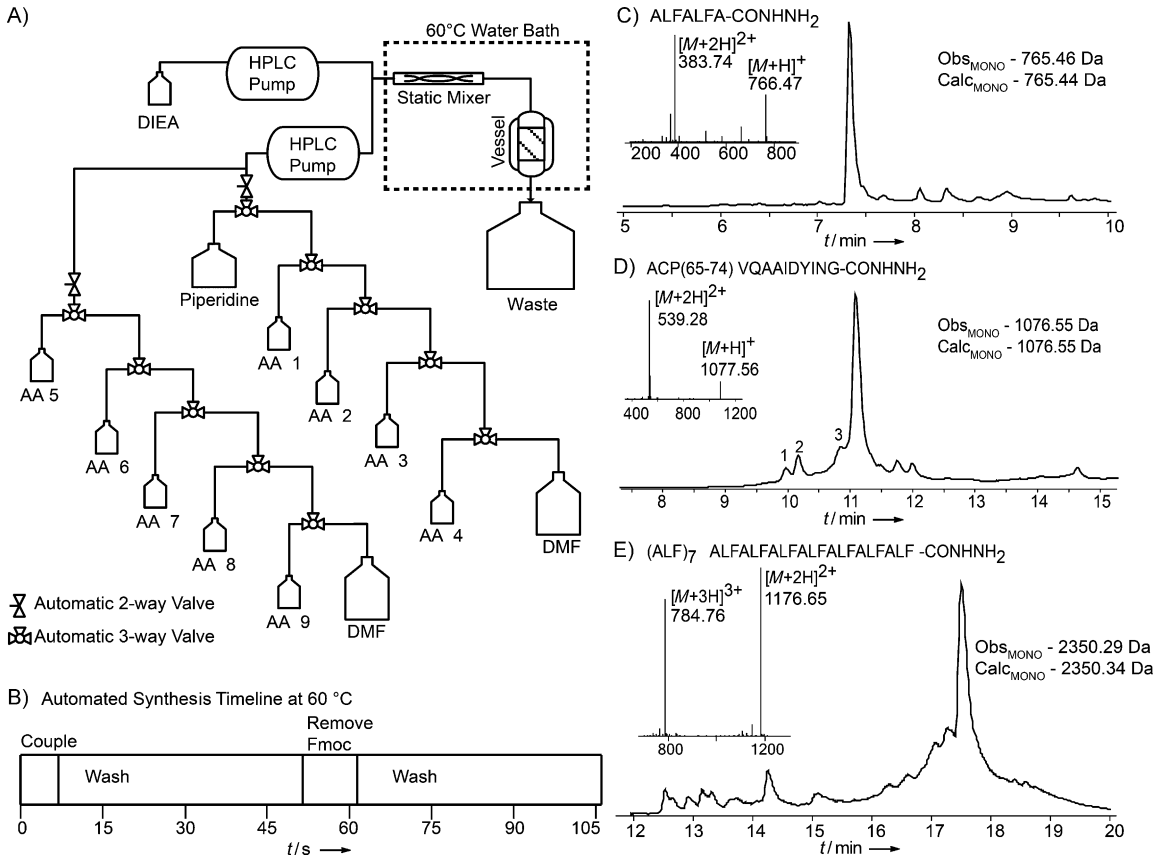

Figure 8. Automated peptide synthesis platform. A) Schematic representation. B) Synthesis timeline used to incorporate an amino acid residue every 107 s. C) ALFALFA-CONHNH$_2$ assembled in 12 min. D) ACP(65–74) assembled in 18 min (1, 2 = Ile deletion, 3 = hydrolysis of the C terminus). E) (ALF)$_7$ assembled in 37 min. Total ion chromatograms are shown.

al steps are often employed in batch mode synthesis, thus hindering the progress of the synthesis. Finally, automation of this system enables faster cycle times, in contrast to the often slow progress of automated batch synthesis.[14]

For the first time, the reported platform enables efficient, rapid Fmoc synthesis of polypeptides, and provides a reproducible and systematic study of flow-based Fmoc SPPS chemistry at elevated temperature. To prove the utility of this system, we produced dozens of peptides suitable for various applications, including three fragments of an affibody; these were successfully ligated to produce a full-length protein.

By reanalyzing flow-based SPPS[17,18,21,22] and carefully designing a new system, we were able to overcome two long-standing challenges that have constrained rapid peptide synthesis. First, we were able to reduce the wash time from several minutes to one minute (or less) by minimizing the volume of the system. Although rarely discussed, washing the resin requires significant time and solvent in most SPPS systems. With the reported system, wash times and solvent usage are significantly reduced, but washing remains a key challenge in further accelerating the cycle.

Second, we eliminated the extremely high capital and maintenance costs of microwave heating by employing a simple, effective heat exchanger in a water bath. As a UV detector is not essential, our system can be assembled for about $1000 with used pumps. Furthermore, no component of the system requires servicing by an expert, thus drastically reducing instrument downtime. We believe that lowering the cost and complexity of rapid peptide synthesis is a major step towards its general adoption by chemists. Similarly, the reported system uses the cheapest, most-common peptide synthesis chemistry. All reagents are commercially available, and no unusual hazards are associated with chain assembly or cleavage.

This platform was used to produce dozens of peptides, and, in an accompanying manuscript, we report the total synthesis of Barnase (113 residues) and a DARPin (130 residues). Importantly, during the synthesis of the fragments of these proteins, most of the major side reactions in peptide synthesis were encountered. The accompanying manuscript provides an extensive description of procedures to overcome these side reactions, and constitutes a detailed tutorial for sequence-specific optimization of long and complex peptides with our flow-based synthesizer.

In conclusion, we have developed a rapid, highly robust peptide-synthesis platform. The system can be easily and cheaply assembled, then leveraged to generate high quality peptides. This and our subsequent work provide a guide for chemists inexpert in peptide and protein synthesis to quickly

and independently carry out total synthesis of these complex biomolecules without the need for sophisticated tools, reagents, or equipment.

Experimental Section

For syntheses and apparatus, see the Supporting Information.

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Keywords: flow chemistry · native chemical ligation · peptides · rapid synthesis · solid-phase synthesis

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