

The assembly and use of continuous flow systems for chemical synthesis

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The adoption of and opportunities in continuous flow synthesis ('flow chemistry') have increased significantly over the past several years. Continuous flow systems provide improved reaction safety and accelerated reaction kinetics, and have synthesised several active pharmaceutical ingredients in automated reconfigurable systems. Although continuous flow platforms are commercially available, systems constructed 'in-lab' provide researchers with a flexible, versatile, and cost-effective alternative. Herein, we describe the assembly and use of a modular continuous flow apparatus from readily available and affordable parts in as little as 30 min. Once assembled, the synthesis of a sulfonamide by reacting 4-chlorobenzenesulfonyl chloride with dibenzylamine in a single reactor coil with an in-line quench is presented. This example reaction offers the opportunity to learn several important skills including reactor construction, charging of a back-pressure regulator, assembly of stainless-steel syringes, assembly of a continuous flow system with multiple junctions, and yield determination. From our extensive experience of single-step and multistep continuous flow synthesis, we also describe solutions to commonly encountered technical problems such as precipitation of solids ('clogging') and reactor failure. Following this protocol, a nonspecialist can assemble a continuous flow system from reactor coils, syringes, pumps, in-line liquid–liquid separators, drying columns, back-pressure regulators, static mixers, and packed-bed reactors.

INTRODUCTION

This century has seen an overwhelming advancement in the understanding and synthesis of organic compounds. By contrast, laboratory technology has remained much the same; reactions are still labor-intensive and are performed in round-bottom flasks housing stir bars and reflux condensers. Although this style of chemistry (termed batch chemistry) is often suitable for chemical transformations, increasing research has demonstrated that continuous flow can provide a range of benefits^{1–9}. Translating reactions into continuous flow can provide levels of control and automation that are not possible in batch reactions. Improving the heat and mass transfer^{10,11}, micromixing¹², radiation penetration^{13–16}, and homogeneity⁶ of reactions has provided practical solutions to improve reaction efficiencies. Moreover, continuous flow allows the safe handling of hazardous species. Generating toxic compounds *in situ* for immediate consumption in sequential reactions avoids stockpiling of risk-prone compounds^{1,4,8}. Harnessing increased reaction efficiencies, environmental sustainability, and improved safety metrics has led to the adoption of continuous flow into both academia and industry^{3,17–21}.

At its core, continuous flow synthesis is achieved by pumping solutions of reactants through reactors in a continuous manner (**Fig. 1a**). For lab-scale purposes, syringes, HPLC systems, or peristaltic pumps drive fluid through reactor coils or microfluidic chips, with the residence time of the fluid dictated by the total flow rate and reactor volume. Although single reactor coils are commonly used, advancements of in-line technology have provided opportunities to explore more complex, multistep transformations^{6,22}. In this context, 'in-line' refers to a manipulation, or analysis, of the continuous flow stream without the solution exiting the continuous flow system. The ability to perform aqueous workups, drying, and other purification procedures has advanced continuous flow notably. Removing impurities and byproducts, or isolating products during multistep reactions has allowed longer, more complex reactions to be translated into continuous flow.

However, advancements in the drying of solutions are required, as this is currently limited by the lifetime of molecular sieves in the system.

Multistep continuous flow systems have provided rapid syntheses of active pharmaceutical ingredients (APIs), natural-product intermediates, and effective functional-group transformations^{3,9}. What started as a niche discipline in academia ~10 years ago has evolved into a rapidly diversifying and expanding research area. Furthermore, materials^{23–26}, biochemical^{27–30}, and biofuel^{31–34} processes have been translated into continuous flow systems with success. In 2005, Lonza stated that ~50% of chemical processes could benefit from translation into continuous flow³⁵. In this respect, it is vital that all researchers wishing to contribute and advance in this area have access to readily available and operationally simple continuous flow equipment.

Although continuous flow chemistry has a plethora of benefits, it has its limitations as well. For example, handling solids in continuous flow is difficult because of reactor clogging. Although advancements in reaction engineering and immobilized reagents have overcome this to a certain extent, further innovation is needed. The most challenging constraints for academics, however, remain the high costs and significant time investment needed to conduct continuous flow research¹³. First, pumping modules are by far the most expensive component of a continuous flow system (\$1,000–20,000 USD). Second, purchasing commercially available reactors and equipment increases the cost of continuous flow synthesis immeasurably. Furthermore, researchers are often burdened by long delivery periods for equipment. Although syringe-pump costs are fixed, the other constraints can be overcome through the assembly of modular continuous flow systems from commercially available and affordable components (**Fig. 1a**). This not only reduces the time and cost associated with continuous flow chemistry but also opens up continuous flow synthesis to all.

PROTOCOL

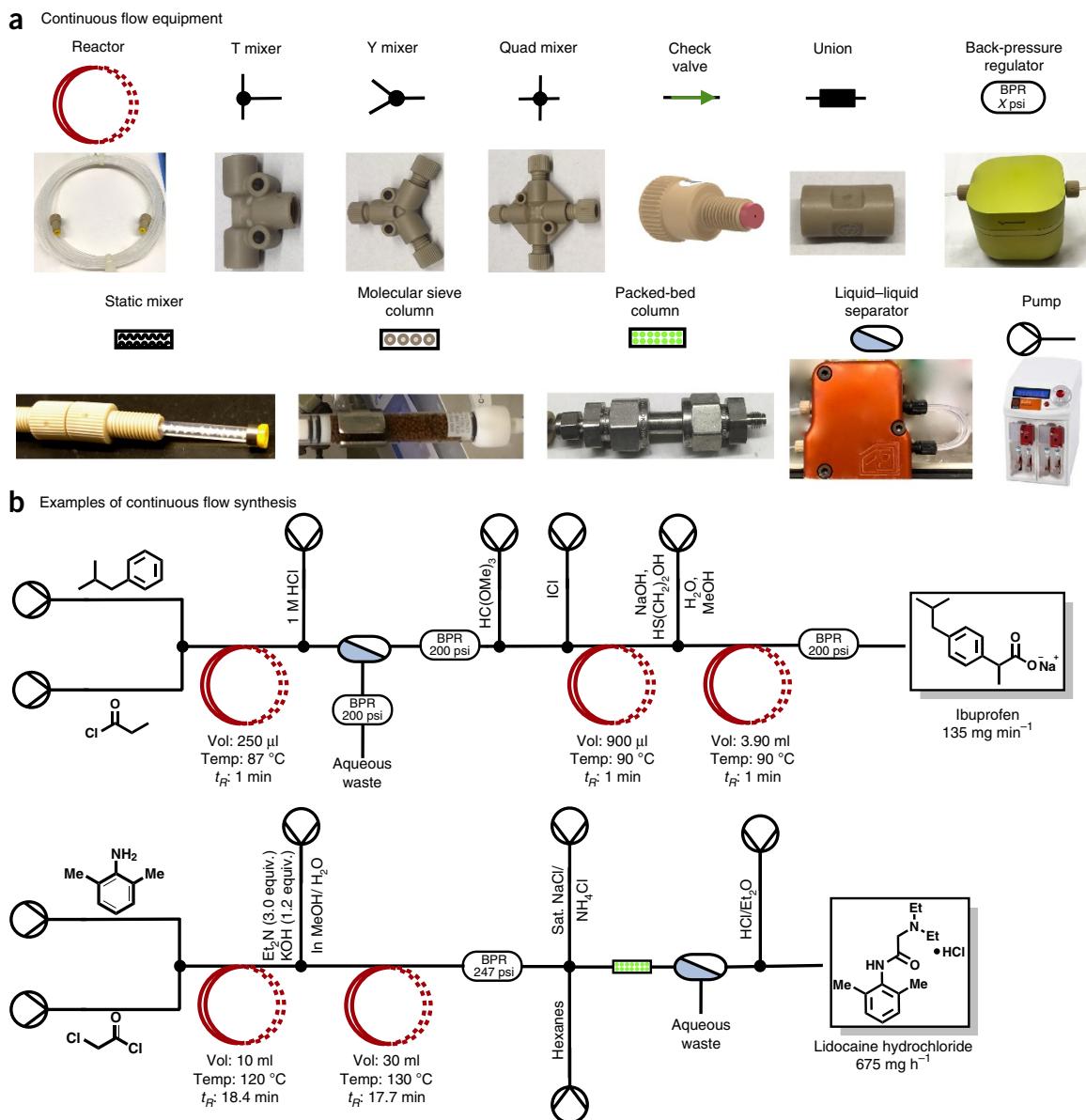


Figure 1 | Continuous flow equipment and its use in the multistep continuous flow synthesis of active pharmaceutical ingredients. **(a)** Typical equipment used in the assembly of the continuous flow systems that are discussed in this protocol. **(b)** Multistep continuous flow syntheses of ibuprofen and lidocaine hydrochloride, as published by our laboratory^{37,36}. BPR, back pressure regulator; sat., saturation; t_R , residence time of the fluid in the system.

Development of the protocol

We maintain that a continuous flow apparatus should be (i) modular, so that it can be easily replaced to create a variety of systems, (ii) quick to assemble from readily available and cost-effective parts, and (iii) easy to operate, allowing both new and experienced researchers to harness the benefits of continuous flow chemistry. For example, the synthesis of lidocaine hydrochloride requires assembly of multiple syringes, pumps, reactor coils, a back-pressure regulator, and an in-line liquid–liquid separator (Fig. 1b). To then synthesize ibuprofen, users need only re-arrange the syringe pumps, add a new reactor coil, and then exchange a static mixer for a back-pressure regulator. This whole process takes ~30 min and allows the synthesis of another API.

To advance this concept, modular continuous flow equipment has been developed that is readily assembled from affordable and

commercially available components, allowing researchers to explore continuous flow synthesis without purchasing expensive equipment. In addition, the synthesis of APIs and commodity chemicals such as ibuprofen³⁶, lidocaine hydrochloride³⁷, diphenhydramine hydrochloride^{37,38}, diazepam³⁷, fluoxetine hydrochloride³⁷, atropine³⁹, rufinamide⁴⁰, aliskiren^{41,42}, and AS-136A (ref. 43) has been achieved (Fig. 1b). Furthermore, fluorination using SF₆ (ref. 44), biocatalysis⁴⁵, controlled polymer growth⁴⁶, and a range of synthetic transformations has been mediated by ‘in-lab’-constructed systems^{16,47–57}.

As with traditional organic synthesis, a range of accessories is required for continuous flow synthesis. Typically, systems are operated with a back-pressure regulator to ensure fluid homogeneity as it proceeds through the reactor; if there is no back pressure, then the liquid passes through the reactor too quickly

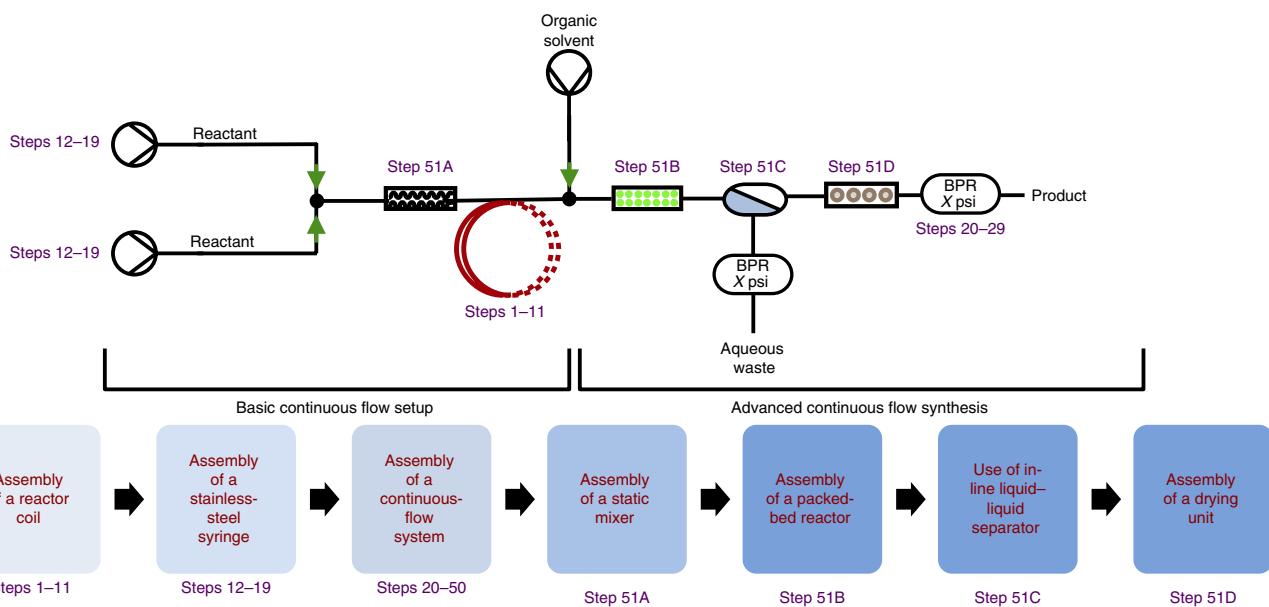


Figure 2 | Outline of the equipment constructed in this protocol. First, the assembly of a basic continuous flow setup is detailed in Steps 1–50; then the assembly of advanced continuous flow equipment is detailed in Step 51A–D.

because of lack of resistance, resulting in variable fluid residence times (Fig. 1a). Operating under a back pressure also allows solvents to be heated above their atmospheric boiling points, affording opportunities for improved reaction kinetics. Analogous to stir bars in round-bottom flasks, static mixers and packed-bed reactors micromix solutions in a continuous manner (Fig. 1a). Packed-bed reactors can also house solid or immobilized reagents in such a way that solids can participate in flow chemistry. Moreover, in-line liquid–liquid separators housing a semipermeable membrane can isolate immiscible fluids for intermediate purification. Finally, drying units containing molecular sieves can remove water from solutions before subsequent moisture-sensitive transformations. Combining these modules into specific orders allows researchers to translate a large number of synthetic protocols into continuous flow.

Overview of the procedure

In this protocol, the assembly, use, and troubleshooting of modular continuous flow systems are discussed. For the reader's convenience, the PROCEDURE (Fig. 2) first describes the assembly of basic continuous flow equipment, then describes the incorporation of advanced in-line technologies for more complex syntheses, and finally describes the use of the earlier PROCEDURE steps in a real-life example: the synthesis of a sulfonamide.

For basic continuous flow systems, the assembly of reactor coils from PFA tubing and the assembly of stainless-steel syringes are discussed. Finally, the assembly of a continuous flow system housing a back-pressure regulator, two syringes, and a single reactor coil is demonstrated. For those new to continuous flow, this is the most basic setup. The skills learned in this section, however, can be applied to the assembly of more complex systems in future syntheses.

In the advanced section, the assembly, inclusion, and use of in-line technology will be described to allow more complex, multistep continuous flow transformations (Fig. 1b). First, the

assembly of a static mixer and a packed-bed reactor for increased mixing of solutions is discussed. Second, the use of a liquid–liquid separator for intermediate purification is detailed. Finally, how to assemble a drying unit housing molecular sieves is demonstrated. This protocol allows an undergraduate researcher to quickly assemble reactor coils, static mixers, stainless-steel syringes, packed-bed reactors, back-pressure regulators, in-line liquid–liquid separators, and drying units. Compiling this equipment into a simple modular continuous flow system allows those new to the field to explore continuous flow synthesis.

Controls

Control reactions are essential to the optimization and understanding of continuous flow systems. To effectively determine the yield, or conversion, of a transformation occurring within the continuous flow system, the reaction must be immediately quenched upon exit. There are several types of quenches, including (i) chemical, (ii) thermal, and (iii) isolation. Chemical quenches destroy at least one of the reagents to avoid further reactivity. Thermal quenches slow the rate of reaction significantly, so that the reaction mixture can be isolated or analyzed. Isolation quenches remove a reactant from the reaction mixture through a physical process such as evaporation, separation, or precipitation. To test whether your quench is suitable, first analyze the solution immediately after quenching it. Second, perform the same analysis on a quenched solution after 30 min. If a change in the yield results after 30 min, then the quench is ineffective. This consideration is especially important for those performing 'flash chemistry'^{58,59}. Here, synthetic transformations are mediated on very short time scales (seconds). Furthermore, it is important to separate reactive reagents into separate syringes to avoid reactivity before they enter the continuous flow system. Note that several continuous flow systems allow in-line reaction monitoring that removes the need for reaction quenching^{60–62}. However, this is beyond the scope of this work.

PROTOCOL

Experimental design

Reactor coils form the basis of continuous flow systems. In a continuous flow synthesis, pumps (i.e., syringe pumps, HPLC pumps, piston pumps, or peristaltic pumps) drive fluid through reactors coils that can be heated, cooled, and/or irradiated. The length and volume of the reactor coil determines the length of time (i.e., the residence time) it takes for a fluid to pass through the reactor. Changing the flow rate of fluid through a reactor, or the length and volume of a reactor, can change the residence time. The equations to calculate the required reactor-coil length for a specific residence time are provided in the **Supplementary Equations**. Unlike most batch reactions, continuous flow systems often operate under a back pressure, controlled by a back-pressure regulator. Importantly, this pressure must be considered when carrying out the appropriate safety assessment. For example, operating a back pressure of 200 psi means that if the reactor tubing breaks, 200 psi of pressure will be immediately released from the reactor coil, along with its contents. Although continuous flow synthesis is ideal for performing high-temperature and high-pressure reactions, if users believe there could be a problem with pressure and temperature, we suggest using stainless-steel tubing. Note that we have used 0.02-inch PFA tubing at 200 °C with a back pressure of 200 psi, and we believe that

this could be near the limits of this tubing. In addition, although Harvard pumps are suitable for a wide variety of chemicals, they have their own limitations; users should refer to usage guides for chemical compatibility details.

This protocol describes the assembly and operation of a simple continuous flow system harnessing two syringe pumps at an elevated pressure. Steps 20–30 describe how to set a back pressure using a Zaiput variable back-pressure regulator. A variable back-pressure regulator offers reaction flexibility and, in our experience, lasts longer than other back-pressure regulators, but other options are available, such as the fixed-back-pressure regulator cartridges from IDEX Health and Science (e.g., cat. nos. P-795 and P-764). Operating under a back pressure allows solvents to be heated above their atmospheric boiling points while providing a smooth flow of fluid through the system. After charging a back-pressure regulator, Steps 31–50 describe the assembly of a continuous flow system housing two syringe pumps, a T mixer with check valves, a single reactor coil, and a back-pressure regulator. This setup allows two solutions to be mixed and reacted, the product to be collected for analysis, and then the system to be cleaned. Although a simple system, the skills in this section are invaluable for the assembly of more complex systems.

MATERIALS

REAGENTS

- Dibenzylamine, 97% (Sigma-Aldrich, cat. no. D34108-100G)
! CAUTION This chemical is corrosive and toxic. It should always be handled in a fume hood with the appropriate personal protective equipment.
- 4-Chlorobenesulfonyl chloride, 97% (Sigma-Aldrich, cat. no. 133698-100G) **! CAUTION** This chemical is corrosive and toxic. It should always be handled in a fume hood with the appropriate personal protective equipment.
- Triethylamine, >99.5% (Sigma-Aldrich, cat. no. 471283-100ML)
! CAUTION This chemical is corrosive, toxic, and flammable. It should always be handled in a fume hood with the appropriate personal protective equipment.
- Anhydrous dichloromethane (DCM), >99.8% (Sigma-Aldrich, cat. no. 270997-100ML) **! CAUTION** This chemical is toxic. It should always be handled in a fume hood with the appropriate personal protective equipment.
- Anhydrous DMSO, >99.9% (Sigma-Aldrich, cat. no. 276855-100ML)
- Hexanes, >97% (Sigma-Aldrich, cat. no. 34859-1L) **! CAUTION** This chemical is toxic, flammable, and hazardous to the aquatic environment. It should always be handled in a fume hood with the appropriate personal protective equipment.
- Ethyl acetate, 99.7% (Sigma-Aldrich, cat. no. 34858-1L) **! CAUTION** This chemical is toxic and flammable and should always be handled in a fume hood with the appropriate personal protective equipment.
- Chloroform-*d* (CDCl_3), 100%, 99.96 atom% *D* (Sigma-Aldrich, cat. no. 151858-10G) **! CAUTION** This chemical is toxic. It should always be handled in a fume hood with the appropriate personal protective equipment.
- Silica column (Biotage SNAP, 25-g KP column; Biotage, cat. no. FSK0-1107-0025)
- Magnesium sulfate, anhydrous, >99.5% (Sigma-Aldrich, cat. no. M7506-500G)
- 1 M hydrochloric acid (Sigma-Aldrich, cat. no. H9892-100ML)
- Acetone for HPLC analysis, ≥99.9% (Sigma-Aldrich, cat. no. 270725)
! CAUTION Acetone is highly flammable and volatile.
- Absolute ethanol, ≥99.8% (Sigma-Aldrich, cat. no. 24102)
! CAUTION Ethanol is highly flammable and volatile.
- Toluene for HPLC, 99.9% (Sigma-Aldrich, cat. no. 34866)
! CAUTION Toluene is highly flammable.

EQUIPMENT

- ▲ **Critical** The equipment required for assembly of modular continuous flow systems is grouped below. This protocol uses 1/16-inch-outer-diameter PFA tubing, as this provides the largest range of operational flexibility. If larger tubing is required, the complementary ferrules, super flangeless nuts, and low-pressure PEEK unions should be used. For example, if you are using 1/8-inch-outer-diameter PFA tubing, ensure that the ferrules and super flangeless nuts are compatible with 1/8-inch-diameter tubing.
- DuPont 0.02-inch-inner-diameter, 1/16-inch-outer-diameter PFA tubing (IDEX Health & Science, cat. no. 1512L)
- Polymer-tube cutters for 1/16-inch- and 1/8-inch-outer-diameter tubing (IDEX Health & Science, cat. no. A-327)
- Ferrule sets for 1/16-inch-outer-diameter tubing (IDEX Health & Science, cat. no. P-250)
- Super flangeless nuts for ≤1/16-inch-outer-diameter tubing (IDEX Health & Science, cat. no. P-141)
- Low-pressure PEEK unions for 1/16-inch-outer-diameter tubing (IDEX Health & Science, cat. no. P-702)
- Extender tool for standard head nuts (IDEX Health & Science, cat. no. P-299)
- Sharp hooked tweezers (Sigma-Aldrich, cat. no. T4912)
- Pliers
- Cable ties (Staples, cat. no. 192314)
- 8-ml stainless-steel syringe kit with a 1/16-inch nut (Harvard Apparatus, cat. no. 702267)
- 5/16-inch wrench
- Bench vise
- Standard 1/4-28 in-line check valves (IDEX Health & Science, cat. no. CV-3301)
- Nonmetallic 1/4-28 in-line check valves (IDEX Health & Science, cat. no. CV-3320)
- PEEK T-junction mixer for 1/16-inch-outer-diameter tubing, 0.20-inch through hole (IDEX Health & Science, cat. no. P-714)
- Syringe pumps (PhD syringe pump; Harvard Apparatus, cat. no. 70-3005, or Asia syringe pump; Syrris, cat. no. 2200292)
- Compressed gas tank and regulator
- Krytox LVP grease (Sigma-Aldrich, cat. no. Z273546)
- Type HT Teflon static mixer, with 10 mixing-elements (StaMixCo, <http://www.stamixco-usa.com>)
- DuPont 0.062-inch-inner-diameter, 1/8-inch-outer-diameter PFA tubing (IDEX Health & Science, cat. no. 1521L)

- Ferrule sets for 1/8-inch-outer-diameter tubing (IDEX Health & Science, cat. no. P-359X)
- Super flangeless nuts for 1/8-inch-outer-diameter tubing (IDEX Health & Science, cat. no. P-331)
- Low-pressure PEEK unions for 1/8-inch-outer-diameter tubing (IDEX Health & Science, cat. no. P-703)
- Stainless-steel 10-μm frits (IDEX Health & Science, cat. no. A-107)
- Swagelok stainless zero-volume reducing union, 1/4-inch outer diameter (Swagelok, cat. no. SS-400-6-1ZV)
- Swagelok stainless ferrule set (one front and one back ferrule; Swagelok, cat. no. SS-100-SET)
- Metal-tube cutter (3–30 mm, 1/8–1 1/8-inch)
- Seamless stainless-steel tubing with a 0.180-inch inner diameter, 1/4-inch external diameter, 0.035-inch wall thickness, and maximum pressure of 4,375 psi at 72 °F (Grainger, cat. no. 3ACH4)
- 3-ml Disposable syringe
- Sand, 50- to 70-mesh particle size (Sigma-Aldrich, cat. no. 274739)
- Spatula
- 9/16-inch-, 1/2-inch-, and 5/16-inch-sized wrenches
- Metal file
- Deburring tool
- Small drill bit
- Liquid–liquid membrane-separator kit (Zaiput Flow Technologies, cat. no. SEP-10)
- Variable pore-size PTFE hydrophobic replacement membranes (Zaiput Flow Technologies, 0.5- and 1.0-μm pore sizes)
- 2× Variable back-pressure regulator kit containing a back-pressure regulator and gas adaptor (Zaiput Flow Technologies, cat. no. BPR-10)
- 3/32-inch Allen key
- 5/64-inch Allen key
- Omnifit EZ chromatography column kit with 1× adjustable and 1× fixed end piece, 150-mm length (Omnifit, cat. no. 006EZ-05-15-FF)
- 4-Å molecular sieves, beads, 4–8 mesh (Sigma-Aldrich, cat. no. 208590)

Equipment for the example reaction:

- Cable ties (Staples, cat. no. 192314)
- Variable back-pressure regulator kit containing a back-pressure regulator and a gas adaptor (Zaiput Flow Technologies, cat. no. BPR-10)
- PEEK Y-Assembly PEEK mixer for 1/16-inch-outer-diameter tubing, 0.60-inch through hole (IDEX Health & Science, cat. no. P-514)
- 3× 8 ml Stainless-steel syringe kit with a 1/16-inch nut (Harvard Apparatus, cat. no. 702267)
- 2× Syringe pumps (PhD syringe pump; Harvard Apparatus, cat. no. 70-3005)
- Krytox LVP grease (Sigma-Aldrich, cat. no. Z273546)
- 2× Hot plate (Heindolph, MR Series Magnetic Stirrer Hotplate)
- Oil bath (30-cm diameter)
- Silicon oil with a temperature range from –50 °C to +200 °C (Sigma-Aldrich, cat. no. 85409-1L)
- 3× 25 ml Glass beakers (Sigma-Aldrich, cat. no. BR91214)
- 2× 10 ml Volumetric flasks (Sigma-Aldrich, cat. no. Z326178)
- 2× 250 ml Volumetric flasks (Sigma-Aldrich, cat. no. Z326798)
- 125-ml Separatory funnel (Sigma-Aldrich, cat. no. Z550582)
- Powder funnel with an ST/SN joint (Sigma-Aldrich, cat. no. Z517828)
- 250-ml Round-bottom flask (Sigma-Aldrich, cat. no. Z414506)
- Whatman qualitative filter paper, Grade 1 (Sigma-Aldrich, cat. no. Z274844)
- Whatman weighing paper (Sigma-Aldrich, cat. no. WHA10347670)
- Ohaus New Explorer weighing balance (Sigma-Aldrich, cat. no. Z760420)
- 12-inch Spatula (Sigma-Aldrich, cat. no. Z283274)
- 1/2-inch PTFE stir bar (Sigma-Aldrich, cat. no. Z126942)
- 20- to 200-μl Pipette and disposable tips (Sigma-Aldrich, cat. no. Z710180)
- 1,000-μl Pipette and disposable tips (Sigma-Aldrich, cat. no. Z710199)
- Glass Pasteur pipette (Sigma-Aldrich, cat. no. CLS709B9)
- Pasteur pipette rubber bulb (Sigma-Aldrich, cat. no. Z111589)
- 2× Binder clips (Staples, cat. no. 378813)
- 2× Clamp
- 10-ml Disposable plastic syringe
- Hypodermic needles
- Rotary evaporator

PROCEDURE

Section 1—Basic continuous flow equipment: assembly of a reactor coil ● TIMING ~10 min

▲ CRITICAL Refer also to **Supplementary Video 1**.

1| Cut a DuPont 0.02-inch-inner-diameter PFA tubing to the required length using polymer-tube cutters. Use the equations found in the **Supplementary Equations** to calculate the reactor volume required for a specific residence time at a desired flow rate.

▲ CRITICAL STEP Use polymer-tube cutters to cut the PFA tubing to reduce the chance of the reactor leaking. Polymer-tube cutters provide a flush cut, ensuring optimal sealing of the fitting.

2| Insert one end of the PFA tubing through a super flangeless nut, ensuring that the thread of the nut is pointing toward the open end of the PFA tubing.

3| Slide the stainless-steel ring of the ferrule set for the 1/16-inch-outer-diameter tubing onto the PFA tubing using tweezers. Ensure that the slanted part of the stainless-steel ring is facing toward the open end of the PFA tubing and that the thicker portion is facing toward the nut (**Fig. 3a,d**).

▲ CRITICAL STEP If the stainless-steel ring is installed backwards, the reactor will not seal properly, and, as a consequence, it can leak.

4| Slide the yellow plastic component of the ferrule set for the 1/16-inch-outer-diameter tubing onto the PFA tubing using tweezers. Ensure that the cone portion of the yellow plastic component is facing the super flangeless nut and the stainless-steel ring (**Fig. 3b,d**).

▲ CRITICAL STEP If the yellow plastic component is installed backwards, the reactor will not seal properly, and, as a consequence, it can leak.

5| Insert the end of the PFA tubing into a low-pressure PEEK union for the 1/16-inch-outer-diameter tubing. Ensure that the PFA tubing is constantly pushed into the low-pressure union while finger-tightening the super flangeless nut (**Fig. 3c**).

PROTOCOL

▲ **Critical Step** If the PFA tubing is not constantly pressed into the union during tightening, the tubing may not be aligned with the ferrule during compression. This can lead to reactor failure and leaking.

- 6| Further tighten the super flangeless nut into the low-pressure union using the extender tool for standard head nuts. ▲ **Critical Step** Do not overtighten the ferrule, as this will lead to deformation and/or cracking of the nut. This can lead to reactor failure and leaking.

- 7| Unscrew the flangeless nut from the union using the extender tool for standard head nuts. Ensure that the ferrule set is flush with the PFA tubing.

▲ **Critical Step** If this is not perfect, cut off the ferrule and the super flangeless nut and repeat the process. Be mindful that cutting the tubing will decrease the residence time of the fluid in the reactor.

TROUBLESHOOTING

- 8| Repeat Steps 2–7 on the other end of the PFA tubing.

- 9| Coil the PFA tubing into a circle with a diameter less than that of the bath used to mediate the temperature of the reactor coil (**Fig. 3e**). This can be achieved by wrapping the tubing around a circular object such as a paper cup.

- 10| Use two cable ties to fasten the opposite sides of the reactor coil into shape (**Fig. 3f**).

- 11| Finish the reactor coil by cutting the cable ties to a workable length (**Fig. 3g**).

Assembly of a stainless-steel syringe ● TIMING ~10 min

! **Caution** Although stainless-steel syringes are ideal for those who are new to the field of continuous flow chemistry, some reagents are not compatible with stainless steel. Furthermore, the grease required for stainless-steel syringes can pose contamination concerns with nonpolar compounds. In this case, users should use glass or plastic syringes. It is worth noting that plastic and glass syringes are more affordable, but using stainless-steel syringes allows fluid delivery at higher pressures (>25 bar). Furthermore, glass syringes can shatter, and plastic syringes can crack if the system clogs, posing a safety concern. Although stainless-steel syringes require greater financial investment, their long-term use and wide operational applicability is highly advantageous.

▲ **Critical** This part of the protocol describes the assembly of stainless-steel syringes for use in a continuous flow system. Here, 8-ml stainless-steel syringes are used because of their ability to handle a wide variety of organic solvents as compared with 20-ml stainless-steel syringes; this relates to O-ring degradation. Furthermore, 8-ml syringes are capable of dealing with higher-pressure systems, and, as a consequence, they fail less frequently. If a 20-ml stainless-steel syringe is required, ensure that perfluoroelastomer barrel O-rings are installed (Harvard Apparatus, cat. no. PY2 5013-089). **Supplementary Video 2** shows the assembly of the stainless-steel syringe.

- 12| Unpack the contents of the stainless-steel syringe kit (**Fig. 4a**). The kit should contain a stainless-steel syringe, its plunger, a metal ferrule set (components 2a and 2b), a metal nut (component 1), and a syringe connector (component 3).

- 13| Connect the stainless-steel syringe to the syringe connector and finger-tighten it. Use a bench vise to hold the syringe in place while performing this step (**Fig. 4b,c**).

▲ **Critical Step** Overtightening can deform the O-ring in the syringe connector, leading to failure.

- 14| Cut a 30-cm long piece of DuPont 0.02-inch-inner-diameter PFA tubing and ensure that both ends have been cut flat with a polymer-tube cutter.

▲ **Critical Step** Use polymer-tube cutters to cut the PFA tubing to reduce the chance of the reactor leaking. Polymer-tube cutters provide a flush cut, ensuring optimal sealing of the fitting.

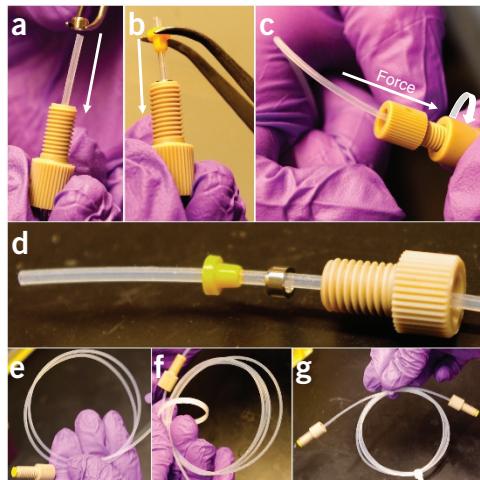


Figure 3 | Reactor coil assembly. (a) Slide the stainless-steel ring of the ferrule set onto the PFA tubing using tweezers. Ensure that the smaller-diameter face is pointing toward the end of the PFA tubing. (b) Slide the yellow-plastic component of the ferrule set onto the PFA tubing with the neck positioned closest to the super flangeless nut and stainless-steel ring. (c) Force the PFA tubing into the low-pressure union and finger-tighten the super flangeless nut into the low-pressure union. (d) The correct order and orientation for the ferrule set on the PFA tubing. (e) Coil the reactor into a circular shape. (f) Use two cable ties to fasten the reactor coil. (g) Cut the cable ties to size to reveal the finished reactor coil.

15 Pass the PFA tubing through the metal nut (component 1), ensuring that the threaded side of the nut is facing the end of the PFA tubing (**Fig. 4d**).

16 Slide the stainless-steel ring (component 2a) onto the PFA tubing using tweezers. Ensure that the thicker side of the stainless-steel ring is facing toward the nut and that the thinner side is facing the end of the PFA tubing. Next, slide the stainless-steel cone (component 2b) onto the PFA tubing using tweezers. Ensure that the larger-diameter side of the stainless-steel cone is facing the metal nut and the stainless-steel ring, and that the smaller-diameter face is pointing toward the end of the PFA tubing (**Fig. 4d**). Note that the Swagelok ferrules do not sit flush with the tubing, as described for the super flangeless nut and ferrule in the previous section. Swagelok ferrules sit a few millimeters onto the PFA tubing.

▲ **Critical Step** If the Swagelok ferrules are not correctly connected, the syringe can leak.

17 Insert the open end of the PFA tubing into the syringe connector (component 3). Ensure that the PFA tubing is constantly forced into the syringe connector, and tighten the nut by hand (**Fig. 4e**).

▲ **Critical Step** If the PFA tubing is not constantly pushed into the syringe connector while tightening, the depth of the Swagelok ferrules may be incorrect and reactor leaking can occur.

18 Use two 5/16-inch wrenches to further tighten the metal nut onto the syringe connector.

▲ **Critical Step** Do not overtighten the metal nut, as this can lead to constriction of the PFA tubing, and, in extreme cases, can break the PFA tubing. A properly tightened ferrule will remain in position when the nut has been disconnected from the syringe connector but does not pinch the PFA tubing.

TROUBLESHOOTING

19 Attach a super flangeless nut and a ferrule set to the other end of the PFA tubing, as described in Steps 2–7 in the reactor-coil assembly (**Fig. 4f**).

TROUBLESHOOTING

Assembly of a basic continuous flow system ● TIMING ~30 min

20 *Pressurization of the Zaiput variable back-pressure regulator* (Steps 20–29, 10 min; **Supplementary Video 3**). Unpack the contents of the variable back-pressure regulator kit to reveal a back-pressure regulator and a gas-delivery tube.

21 Attach the gas-delivery tube to a tank of compressed gas housing an adjustable regulator with a needle valve (**Fig. 5a**). Note that air is typically used, but N₂ or argon can also be used.

22 Set the compressed gas regulator pressure to the desired pressure to be programmed into the variable back-pressure regulator. For example, 100 pounds per square inch, gauge (psig) of compressed air equals a back pressure of 100 psig in the variable back-pressure regulator (**Fig. 5b**).

23 Fully open the valve on the back-pressure regulator with a 1/10-inch screwdriver in a counterclockwise motion. This will vent all the gas from the back-pressure regulator, setting the pressure to atmospheric pressure (**Fig. 5c**).

▲ **Critical Step** Failure to vent the previous pressure within the back-pressure regulator may lead to a higher-than-intended pressure due to prior pressure carryover.

24 Connect the end of the gas-delivery tube to the back-pressure regulator and tighten it by hand (**Fig. 5c**).

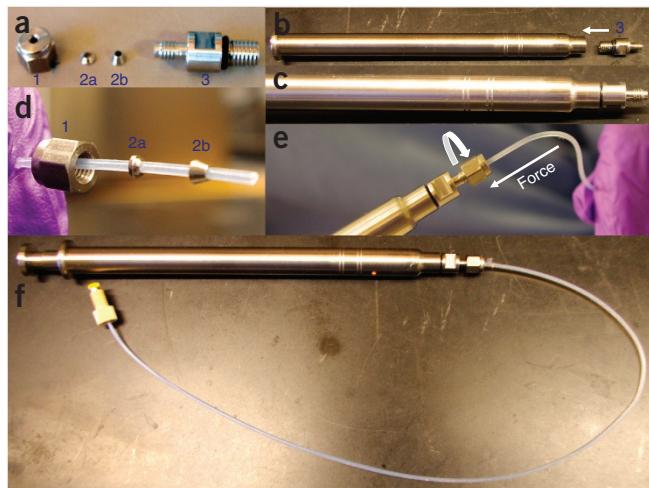


Figure 4 | Stainless-steel syringe assembly. (a) Lay the components of the stainless-steel syringe kit out to ensure that all components are present (the syringe and its plunger are not shown). (b) Screw the syringe connector (component 3) into the syringe and tighten it. (c) A photograph of the stainless-steel syringe and the syringe connector joined. (d) The correct order and orientation of the components arranged onto the PFA tubing as described in Step 16. (e) Ensure that the PFA tubing is forced into the syringe connector (component 3) when tightening the metal nut, as described in Steps 17 and 18. (f) The finished stainless-steel syringe. Note that the ferrule set, super flangeless nut, and the PFA tubing can be replaced as needed (see Troubleshooting section for Steps 18 and 19).

PROTOCOL

25 Fully open the needle valve on the compressed gas tank and allow pressure equilibration into the back-pressure regulator for 10 s.

▲ **Critical Step** Failure to allow full equilibration will lead to an inaccurate back pressure.

26 Using a 1/10-inch screwdriver, close the valve on the back-pressure regulator by screwing it in a clockwise motion (**Fig. 5c**).

27 Close the needle valve on the compressed gas tank.

▲ **Critical Step** Closing the needle valve on the compressed gas tank before closing the valve on the back-pressure regulator can result in an inaccurate pressure setting.

? TROUBLESHOOTING

28 Unscrew the back-pressure regulator from the gas-delivery tube. The back-pressure regulator is now charged at the required pressure and is ready for use.

? TROUBLESHOOTING

29 To change the pressure in the back-pressure regulator, follow Steps 22–28. Typically, a higher back pressure should be used if vaporization of the solvent or reagent is observed. A lower back pressure should be used if the pumps are not capable of infusing reagents, i.e., if the pumps stall.

30 | Assembly of a generic continuous flow system

(Steps 30–50, 20 min; **Supplementary Video 4**)

Cut a 15-cm long piece of DuPont 0.02-inch-inner-diameter PFA tubing using polymer-tube cutters to ensure that both ends have been cut flat.

▲ **Critical Step** Use polymer-tube cutters to cut the PFA tubing to reduce the chance of the reactor leaking. Polymer-tube cutters provide a flush cut, ensuring optimal sealing of the fitting.

31 To one end of this tubing, add a super flangeless nut and a ferrule set, as previously described in Steps 2–7.

? TROUBLESHOOTING

32 Insert the super flangeless nut from the PFA tubing into the outlet port of the pressurized back-pressure regulator. First, tighten the nut using the extender tool for standard head nuts and then using pliers.

▲ **Critical Step** Make sure that the tubing is attached to the correct port of the back-pressure regulator, as indicated by the arrow (**Fig. 6a**). Furthermore, overtightening the super flangeless nut can lead to damage to the PFA tubing and cracking of the super flangeless nut.

33 Gather the previously made reactor coil. Screw a PEEK T-junction mixer with an inner diameter of 0.02-inch to one end of the reactor coil. Tighten the nut using the extender tool for standard head nuts (**Fig. 6b**).

▲ **Critical Step** Overtightening the super flangeless nut can lead to damage to the PFA tubing and cracking of the super flangeless nut.

34 Attach the other end of the reactor coil to the back-pressure regulator (**Fig. 6d**). To do this, insert the super flangeless nut from the reactor coil into the remaining inlet port of the back-pressure regulator. First, tighten the nut using the extender tool for standard head nuts, and then use pliers.

▲ **Critical Step** Overtightening the nut can lead to damage to the PFA tubing and cracking of the nut.



Figure 5 | Pressurization of the back-pressure regulator. (a) Attach the gas-delivery tube to the needle valve on a compressed gas tank, as shown. (b) Set the pressure in the compressed-gas regulator to the pressure required in the back-pressure regulator; a value of 100 psig is shown here. (c) Open the valve on the back-pressure regulator and attach it to the gas-delivery tube. Open the needle valve and allow equilibration for 10 s. Close the back-pressure regulator valve and then close the needle valve. Once the back-pressure regulator is unscrewed from the gas-delivery tube, it is ready for use.

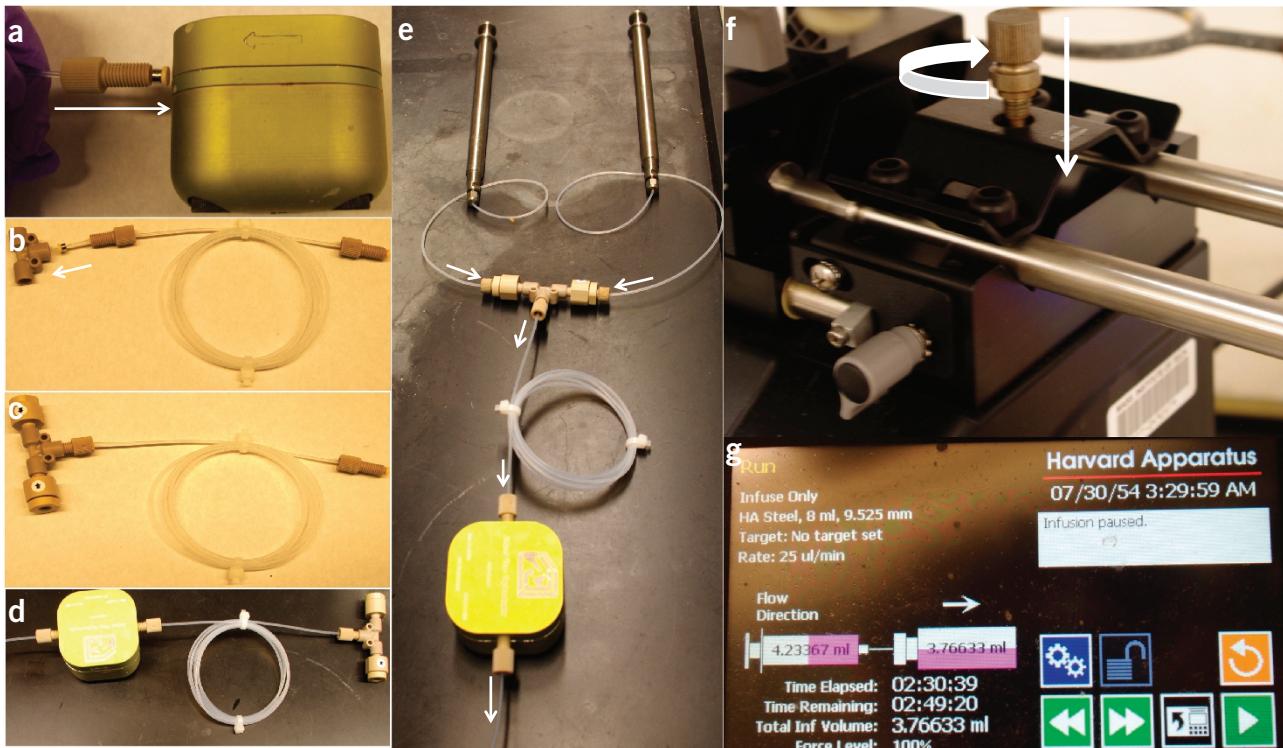


Figure 6 | Continuous flow system assembly. (a) Insert the super flangeless nut from the 15-cm PFA tubing into the back-pressure outlet valve as directed by the arrow. Tighten the super flangeless nut with pliers to ensure a tight seal. (b) Connect the required reactor to be used to a PEEK T-junction mixer and tighten the nut with the extender tool for standard head nuts. (c) Attach standard 1/4–28 in-line check valves to the PEEK T-junction mixer and ensure that the arrows on the check valves are pointing in the direction of the fluid flow. (d) Attach the super flangeless nut from the remaining end of the reactor coil to the back-pressure regulator inlet port, and tighten the nut with pliers. (e) Attach the stainless-steel syringes to standard 1/4–28 in-line check valves and tighten with pliers. (f) Insert the stainless-steel syringes into the Harvard syringe pumps as shown and compress the pad to secure the syringes. (g) Program the syringe pump to infuse at a particular flow rate (titled ‘Rate’ here).

35 | Attach standard 1/4–28 in-line check valves to both remaining ports of the PEEK T-junction mixer. First, tighten the nut using the extender tool for standard head nuts, and then use pliers (Fig. 6c). In-line check valves ensure that the fluid travels in the correct direction during system operation.

▲ **CRITICAL STEP** If no check valves are used, then pressure changes can force solutions back into the stainless-steel syringes during system equilibration; check valves ensure the correct fluid direction while system equilibration occurs. After equilibration, the fluid will proceed in the direction of least pressure resistance (the exit). If you are using a particularly corrosive solution, use nonmetallic 1/4–28 in-line check valves.

36 | Fill the stainless-steel syringes with the appropriate solution. This is achieved by pulling back the syringe plunger with the open end of the PFA tubing resting in the fluid to be loaded. Withdraw the syringe plunger until the desired volume of fluid is reached. Examining the graduated marker on the syringe plunger will allow you to see what volume has been loaded. Remove the PFA tubing from the solution and expel any residual air by gently applying pressure to the syringe plunger and gently tapping the syringe.

? TROUBLESHOOTING

37 | Attach the super flangeless nut from the stainless-steel syringes’ PFA tubing to standard 1/4–28 in-line check valves. Tighten these super flangeless nuts with the extender tool for standard head nuts, and then use pliers (Fig. 6e).

38 | Load the stainless-steel syringes into the syringe pumps ready for infusion (Fig. 6f). It is recommended that all nuts used in the continuous flow system be tightened with pliers before system initiation.

39 | If the reactor is to be heated or cooled, place the reactor coil into a bath, and allow it to reach the required temperature. In our experience, it is possible to heat PEEK fittings to ~150 °C for extended periods of time without their failure. However, if possible, leave the PEEK fitting outside of the oil bath to extend its lifetime; the plastic part of the

PROTOCOL

ferrule swells with extended exposure to heat. To ensure that the reactor coil does not move in the oil bath, use binder clips to attach the reactor to the side of the oil bath. If you are using cryogenic temperatures, use the appropriate cryogenic bath solution to achieve the desired temperature.

▲ **Critical Step** Ensure that the binder clip is not compressing the tubing, as this could lead to reactor clogging.

? TROUBLESHOOTING

40 Set the correct stainless-steel syringe size on the screen of the pump; in this case, it is 8 ml (**Fig. 6g**). Next, set the required flow rate and then start syringe-pump infusion.

41 Collect the fluid exiting from the back-pressure regulator into a collection vial.

▲ **Critical Step** It is important to immediately quench or analyze the solution exiting from the continuous flow system in order to accurately determine the effect of the reaction conditions.

42 Before collecting the results for analysis, ensure that three residence times have elapsed, as this ensures that the samples are representative of the continuous flow system (i.e., the system has reached steady state). For example, if the residence time of the fluid is 5 min, do not start collection of samples for analysis before 15 min of equilibration time has elapsed. Be mindful that all components of the continuous flow system contribute to the total residence time (back-pressure regulator, T junction, and unions). To calculate the residence time of additional components, measure the internal volume of the component by injecting known volumes of water through the component. To achieve this, fill a 5-ml plastic syringe with water and inject it through the component until water emerges from the outlet. The volume of water that has left the syringe is the internal volume of the component.

43 Once the reagents in the syringes have been used up, or enough volume has been collected for analysis, stop syringe-pump infusions, and let the reactor cool to ambient temperature.

44 *System cleanup* (Steps 44–50; ~20 min). Unscrew one of the stainless-steel syringes from the standard 1/4–28 in-line check valves and exit the contents into the correct waste receptacle.

45 Wash the syringe, re grease the O-rings with Krytox grease, and add the reaction solvent (~7 ml) to the stainless-steel syringe as described in Step 7. Load this stainless-steel syringe back into the syringe pump.

46 Attach the super flangeless nut from the stainless-steel syringe pumps to the inlet of the standard 1/4–28 in-line check valve, tighten it, and then start infusion. Flowing this fluid through the system will clean the reactor coil by pushing the reactants out of the system.

▲ **Critical Step** Cooling the system to ambient temperature is fine if the reagents and products are soluble at room temperature. If they are not, the reactor can clog. In this case, clean the system at a temperature at which the reagents/products are soluble.

47 Once all of the solvent has been injected into the system, ensure that both syringes have stopped infusing and the syringe pump is turned off. Unscrew the stainless-steel syringes from the standard 1/4–28 in-line check valves and eject their contents into the correct waste receptacle.

! Caution Disconnecting a high-pressure system can result in uncontrollable fluid ejection. To avoid this, first open the valve of the back-pressure regulator to reduce the system pressure to ambient pressure. The solution will exit the exit port of the back-pressure regulator. This solution must be collected as waste.

48 Wash the stainless-steel syringes with water and soap, and then dry them with acetone. Before using them again, ensure that Krytox grease has been added to the O-rings of the stainless-steel syringe plunger.

49 Disconnect the reactor coil from the back-pressure regulator and remove it from the bath. Lay the reactor on paper towels to absorb the oil and unscrew the PEEK T-junction mixer.

50 Blow compressed air through the reactor coil to remove any residual solvent.

Section 2—Advanced procedures ● TIMING ~10 min–24 h

51 At this stage, there are a number of ways in which the continuous flow system can be made more complex. After users have tried the basic continuous flow system, more intense mixing may be required. Although solutions mix efficiently in the

PEEK T-junction mixer, some situations generate large slugs of two immiscible fluids (slug flow). Additional active mixing through a static mixer or a packed-bed reactor can increase reaction efficiencies. In the first instance, incorporate a static mixer after the PEEK T-junction mixer and before the reactor coil. However, if the system is still ineffective, pass the fluid through a packed-bed reactor housing sand, stainless steel, or glass beads. Packed-bed reactors provide efficient and sustained mixing, and they have been used in several continuous flow syntheses³⁷. Here, we describe the assembly of a static mixer (option A, **Supplementary Video 5**) and packed-bed reactor housing sand (option B, **Supplementary Videos 6–9**).

If users wish to perform multistep continuous flow transformations, the purification of intermediate compounds is vital to ensuring high reaction yields. Liquid–liquid extractions can be achieved in-line by injecting water into the continuous flow stream through a mixer and then directing the stream into a liquid–liquid separator. Here, the organic phase passes through a semipermeable membrane and enters new PFA tubing. The aqueous phase does not pass through the membrane and instead enters separate PFA tubing. If water-soluble salts or impurities are present, they will remain in the aqueous phase, purifying the organic stream to some extent. The organic phase (or aqueous phase) can be channeled into another reactor coil for a sequential reaction or can be collected. Option C thus describes the assembly and cleaning of a Zaiput Flow Technology liquid–liquid separator.

A subsequent reaction in a multistep continuous flow sequence can require a solvent with decreased water content. Using a liquid–liquid separator in a prior step can result in an organic solvent having an H₂O content of 1 to >100,000 p.p.m. (e.g., 2-methyl THF). Passing the organic phase through a drying unit can decrease the H₂O content to ~300 p.p.m. The H₂O content of the organic solvent entering the drying unit will determine the lifetime of the unit. As a rough guide, 2-methyl THF with an H₂O content of ~5,000 p.p.m. can be dried to 350 p.p.m. for a lifetime of ~6 h, whereas an H₂O content of 50,000 p.p.m. can be dried to 350 p.p.m. for ~90 min. The performance of the drying unit is dependent upon the length of the column, and the shape and size of the molecular sieves housed within it. Ensuring that the organic phase is as dry as possible before entering the drying unit ensures its maximal lifetime. Option D thus describes the assembly and use of a drying unit.

(A) Assembly of a static mixer ● TIMING ~10 min

- (i) Cut a 10-cm piece of DuPont 0.062-inch-inner-diameter, 1/8-inch-outer-diameter PFA tubing using polymer-tube cutters.
- ▲ **Critical Step** Use polymer-tube cutters to cut the PFA tubing to reduce the chance of the reactor leaking. Polymer-tube cutters provide a flush cut, ensuring optimal sealing of the fitting.
- (ii) Remove the Teflon static mixer from its packaging (**Fig. 7a**) and insert it into the PFA tubing using tweezers (**Fig. 7c**). **! Caution** The static mixer is sensitive, and too much pressure will bend and snap it—be careful.
- (iii) Using the end of the tweezers, gently push the Teflon static mixer down the PFA tubing until a headroom of ~1 cm is visible (**Fig. 7d**). If only a small static mixer is required, then proceed to the next step. If more extensive mixing is required, add more static mixers on top of each other to create a larger mixing region. If more than three static mixers are required, we suggested trying a packed-bed reactor (see below).
- ? Troubleshooting**
- (iv) Add a ferrule set for 1/8-inch-outer-diameter tubing (components 2 and 3, **Fig. 7b**) and a super flangeless nut for ≤1/8-inch-outer-diameter tubing (component 1, **Fig. 7b**) to the PFA tubing using tweezers. Ensure that the orientation of the components is the same as shown in **Figure 7e**. Tighten and secure the ferrule set as described in Steps 2–7 to finish the static mixer (**Fig. 7f**).

(B) Assembly of a packed-bed reactor ● TIMING ~30 min

- (i) Gather two Swagelok stainless zero-volume-reducing unions having an outside diameter of 1/4-inch and lay the parts out as displayed in **Figure 8a**; see also **Supplementary Videos 6–9**.
- (ii) Mount the seamless stainless-steel tubing into a bench vise and mark out a 10-cm portion (**Fig. 8b**). Place the metal tube cutter around the point to be cut, and tighten it. Rotate the metal tube cutter around the stainless-steel tubing, and at every ten turns, tighten the tube cutter further to ensure that the blade of the tube cutter remains in contact with the stainless-steel tubing (**Supplementary Video 6**).

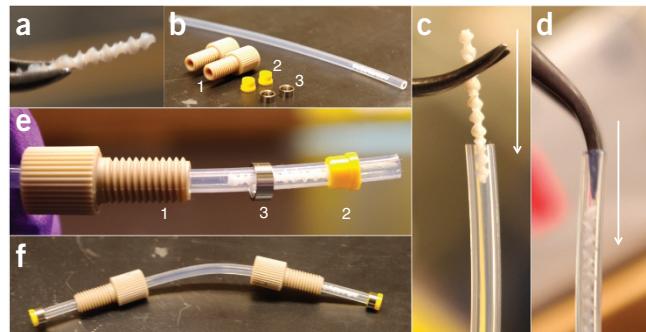


Figure 7 | Assembly of a static mixer. (a) The Teflon static mixer after being removed from packaging. (b) PFA tubing housing the static mixer, two 1/8-inch super flangeless nuts (component 1), and a 1/8-inch ferrule set (components 2 and 3). (c) Insert the Teflon static mixer into the PFA tubing using tweezers (Step 51A(iii)). (d) Further insert the Teflon static mixer using the end of the tweezers until it is ~1 cm into the PFA tubing (Step 51A(iii)). (e) The correct order and orientation of the components as described in Step (iv). (f) The complete static mixer.

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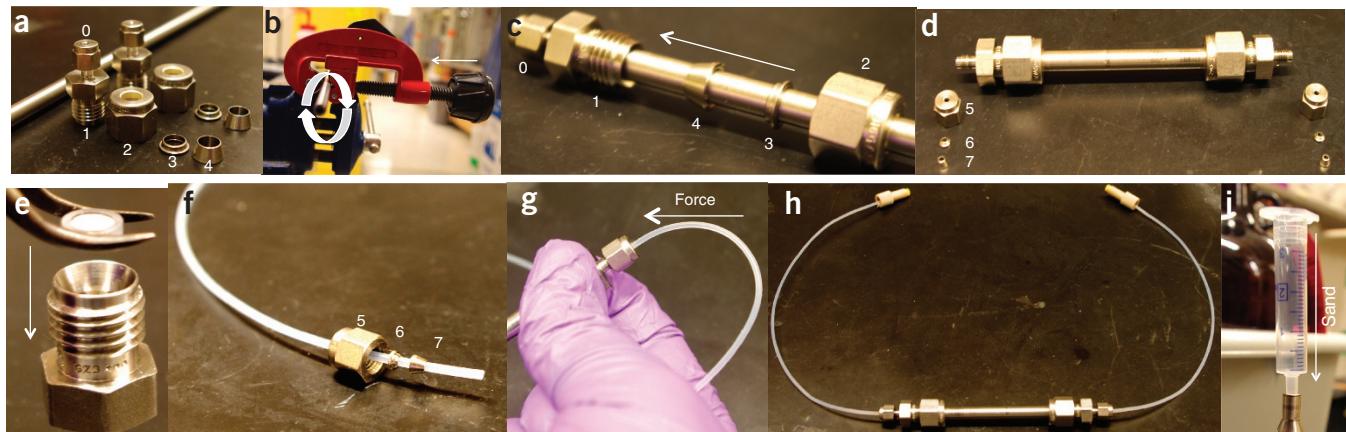


Figure 8 | Packed-bed reactor assembly. (a) The components of the Swagelok stainless-steel zero-volume reducing union laid out in a workable order, with the seamless stainless-steel tubing in the background. (b) After the seamless stainless-steel tubing has been clamped into place, use a metal tube to cut the stainless-steel tubing to the required length. (c) The correct order and orientation in which to place the components of the Swagelok stainless-steel zero-volume reducing union onto the stainless-steel tubing. (d) After unscrewing the small metal nut (component 0 in c) from the Swagelok union (component 1 in c), place the ferrule set in this workable order. (e) Place the 10- μm stainless-steel frit into the Swagelok union (component 1) using tweezers. (f) The correct order and orientation in which to slide the components onto the PFA tubing. (g) Apply force to the PFA tubing when screwing the small metal nut (component 0) into the Swagelok union (component 1); this ensures good attachment. (h) The complete packed-bed reactor. Connecting the super flangeless nuts shown on both ends of the packed-bed reactor allows it to be inserted into any part of the continuous flow system. (i) Use a 3-ml plastic syringe to act as a funnel for the addition of a material to the packed-bed reactor.

Keep rotating the metal tube cutter around the stainless-steel tubing until the metal tube is cut. If the metal tubing is moving, then the tube cutters are too tight. In this case, loosen the tube cutters and tighten them less at every ten turns.

- (iii) File both ends of the cut stainless-steel tubing flat using a metal file (**Supplementary Video 7**). Then, deburr the opening using a deburring tool to ensure that the inside surface is flush with the rest of the stainless-steel tube.
- (iv) Unscrew the metal nut (component 0) from the union's 1/4-inch ports (component 1) and insert a 10- μm stainless-steel frit. Push the frit to the bottom using tweezers, and then reattach the metal nut (**Fig. 8e**).
▲ **Critical Step** Inserting the frit into the 1/4-inch ports (component 1) at this stage avoids incorrect spacing in Step 5.
- (v) To both ends of the stainless-steel tubing, add the Swagelok union parts in the order and orientation shown in **Figure 8c**. First, slide the metal nut (component 2) onto the tube with the threads pointing toward the end of the tube. Next, slide the ring component of the ferrule set (component 3) onto the tube, with the larger-diameter face pointing toward the threads of the metal nut. Then slide the cone component of the ferrule set (component 4) onto the tube, with the larger-diameter face pointing toward the ring component (component 3) and the metal nut (component 2). Finally, slide the Swagelok union (component 1) onto the tube, with the threads facing the ferrule set and the metal nut.
- (vi) Compress the components and tighten them by hand in a clockwise motion to secure them. Mount the stainless-steel tubing into the bench vise and use 1/2-inch and 9/16-inch wrenches to tighten the nut into the Swagelok union further.
▲ **Critical Step** The connection must be tight; the packed-bed mixer is under pressure.
- (vii) Unscrew the smaller metal nut (component 0) and lay the contained ferrule set out as shown in **Figure 8d**. Be careful not to lose the components inside, as they can sometimes slide out.
- (viii) Cut 2 \times 10-cm-long pieces of DuPont 0.02-inch-inner-diameter PFA tubing with a polymer-tube cutter to ensure that both ends of the tubing are flat. These will serve as the tubing connecting the packed-bed mixer to the other parts of the continuous flow system.
▲ **Critical Step** Use polymer-tube cutters to cut the PFA tubing to reduce the chance of the reactor leaking. Polymer-tube cutters provide a flush cut ensuring optimal sealing of the fitting.
- (ix) To one end of the PFA tubing, add components 5, 6, and 7 as shown in **Figure 8f** (**Supplementary Video 8**). First, slide the metal nut (component 5) onto the PFA tubing, with the thread facing the end of the PFA tubing. Next, slide on the metal ring (component 6), with the larger-diameter side facing the threads of the metal nut and the smaller diameter facing the end of the PFA tubing. Finally, add the metal cone (component 7), with the larger-diameter side facing the threads of the metal nut and the metal ring and the smaller face pointing toward the end of the PFA tubing.

(x) Insert this end of the PFA tubing into the Swagelok union (component 1) and apply pressure while simultaneously finger-tightening. Mount the packed-bed reactor back into the bench vise and tighten the fittings using 9/16-inch and 5/16-inch wrenches (**Fig. 8g**).

? TROUBLESHOOTING

(xi) Repeat Step 51B(ix and x) on the other side of the stainless-steel tube to ensure that both sides of the packed-bed reactor now have PFA-tubing attachments.

(xii) To both ends of the PFA tubing, add a super flangeless nut and a ferrule set using the method previously described in Steps 2–7 (**Fig. 8i**).

(xiii) Unscrew the metal nut (component 2) from the Swagelok union (component 1) to reveal an opening into the stainless-steel tube.

(xiv) To create a funnel for loading, take a 3-ml disposable plastic syringe and completely remove the plunger. Insert the small end of the syringe into the stainless-steel tube so that it is held firm.

(xv) Add sand to the stainless-steel tube while simultaneously tapping the tube and the syringe with a spatula to ensure good packing and sand release (**Supplementary Video 9**). Other materials, such as stainless-steel chippings, can be used in the packed-bed reactor; however, the use of very small packing materials can cause a large pressure increase, leading to syringe-pump failure. As a general rule, use a material that will require ten units or more to span the diameter of the stainless-steel tubing. For example, if the internal diameter of the packed-bed reactor is 0.180 inch, then, sand particles with a diameter of ≤ 0.0180 inch should be used.

(xvi) Use the flat back end of a drill piece to pat down the sand to ensure complete and firm packing.

(xvii) Place your finger on top of the filled stainless-steel tubing and subject the surrounding nuts to compressed air to remove any loose sand.

▲ CRITICAL STEP Sand caught in the metal nut can lead to an inefficient seal.

(xviii) Screw the metal nut (component 2) into the Swagelok union (component 1) using 1/2-inch and 9/16-inch wrenches to ensure a tight fitting. The packed-bed reactor is now complete and ready to use (**Fig. 8h**). To change the material in the packed-bed reactor, repeat Step 51B(xii–xviii), ensuring that the sand is disposed of as solid waste and that the column is washed with the solvent of choice. Unpacking the column is best when the material contained within the column is dry.

? TROUBLESHOOTING

(C) Use of an in-line liquid–liquid separator

● TIMING ~30 min

▲ CRITICAL If you are using a new in-line liquid–liquid separator, proceed to Step 51C(xi). If you need to check and clean one that has been used before, start at Step 51C(i).

(i) Remove the six screws (three per side) from both sides of the separator using a 3/32-inch Allen key as shown in **Figure 9a**.

(ii) Open the separator, being careful to avoid any residual fluid that may be present.

(iii) If the membrane is fouled, use tweezers to remove it. Discard the fouled membrane as solid waste (**Fig. 9b**). In this sense, ‘fouled’ describes a chemical degradation or perturbation of the membrane. For example, solids can collect on the membrane, rendering it inefficient, or chemicals can coat the membrane, leading to failure. Regular inspection of the membrane is advised.

(iv) Unscrew the metal disk surrounding the diaphragm with a 5/64-inch Allen key (**Fig. 9b**).

(v) Carefully remove the diaphragm with tweezers.

▲ CRITICAL STEP Damaging this part will cause the separator to fail.

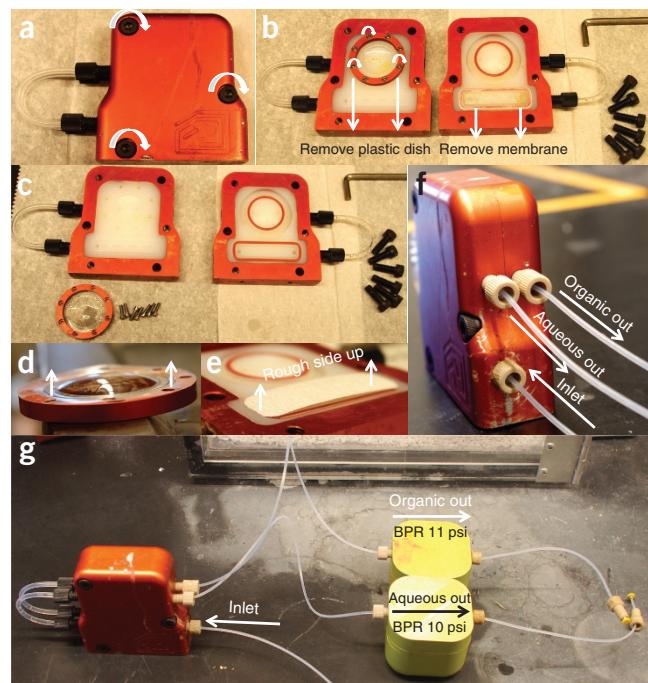


Figure 9 | Assembly of a liquid–liquid separator and its incorporation into a continuous flow system. (a) Unscrew the three screws on both sides of the separator using a 3/32-inch Allen key. (b) Remove the fouled membrane and discard as solid waste. Unscrew the circular metal housing using a 5/64-inch Allen key. (c) This fully deconstructed separator can be cleaned with hot soapy water, then ethanol, and then acetone. (d) When constructing the separator, ensure that the raised face of the diaphragm is facing toward the separator. (e) When replacing the membrane, ensure that the rough side of the membrane is facing upward. (f) The stream to be separated enters the separator from the bottom. The organic phase passes through the membrane into another channel and is collected on the opposite side of the separator. The aqueous phase remains in the same channel and thus remains on the same side of the separator. (g) The separator incorporated into a continuous flow system. Normally, a slight excess back-pressure on the organic stream facilitates separation. For further information, see <http://www.zaiput.com>.

PROTOCOL

- (vi) Wash all of the components and the device with soapy water, ethanol, and finally acetone. Leave the device to dry for a few hours, using a paper towel to remove the majority of the water (**Fig. 9c**).
- (vii) Place a new precut porosity membrane onto the device as shown in **Figure 9e**. Alternatively, sheets of porosity membrane can be purchased and then cut to the correct size using a scalpel.
- ▲ **Critical Step** The rough side of the membrane must face upward. If this membrane is placed on the component with the seal facing the wrong way, separation can fail.
- (viii) Insert the diaphragm (a new one, if the old one is damaged) back into the metal disk, ensuring that when it is placed back into the device, the raised face is pointing toward the concave part of the separator (**Fig. 9d**).
- (ix) Screw the metal disk into place, using a cross-tightening pattern to avoid overtightening the screws.
- (x) Put the lid of the separator into place and insert all six screws with a 3/32-inch Allen key. Use a cross-tightening pattern and tighten each screw gently to ensure an even fit.
- (xi) Cut 3 × 10-cm pieces of DuPont 0.02-inch-inner-diameter PFA tubing and cut both ends using polymer-tube cutters.
- ▲ **Critical Step** Use polymer-tube cutters to cut the PFA tubing to reduce the chance of the reactor leaking. Polymer-tube cutters provide a flush cut, ensuring optimal sealing.
- (xii) Attach the super flangeless nuts and ferrule sets to both ends of the PFA tubes using the method previously described in Steps 2–7.
- (xiii) Attach the PFA tubes to the three separator inlets. Insert the super flangeless nut into the entry of the separator and tighten it with pliers to ensure a tight fitting (**Fig. 9f**).
- ▲ **Critical Step** These super flangeless nuts must be tight; otherwise, the separator will leak.
- (xiv) Attach a 10-psig back-pressure regulator to the aqueous stream and a 11-psig charged back-pressure regulator to the organic stream as shown in **Figure 9g**.

▲ **Critical Step** These values are not absolute, and, depending on the continuous flow setup, the values will vary.

We suggest operating at the lowest back pressure possible, but if a higher back pressure is required, do not exceed 290 psi. In the first instance, if you are using a separator at the end of a system, determine whether separation is achieved with no back pressure on either of the exiting streams. Importantly, the pressure on the two sides must be nearly equal. Thus, if the organic side leads into a packed-bed reactor and a drying unit, this will add pressure to the organic side, which must be counteracted by placing a back pressure on the aqueous side.

? TROUBLESHOOTING

- (xv) Flow toluene (50 ml) and then water (50 ml) through the system to equilibrate the device at a flow rate of 200 $\mu\text{l min}^{-1}$.

▲ **Critical Step** Precharging the membrane in this manner will improve separation efficiency.

(D) Assembly of a drying unit ● TIMING ~24 h

- (i) Add 10 g of 4-Å molecular sieves to a 100-ml glass beaker and heat the beaker at 140 °C for at least 16 h. Step 51D(ii–vi) can be performed while heating the 4-Å molecular sieves.
- (ii) Unpack the contents of an Omnitfit chromatography column kit (150-mm length). Remove the retaining cap from the column, and fully extend the adjustable end piece (**Fig. 10a**).
- (iii) Cut 2 × 15-cm pieces of DuPont 0.02-inch-inner-diameter PFA tubing and cut flat the ends of the tubing with a polymer-tube cutter.
- ▲ **Critical Step** Use polymer-tube cutters to cut the PFA tubing to reduce the chance of the reactor leaking. Polymer-tube cutters provide a flush cut, ensuring optimal sealing of the fitting.

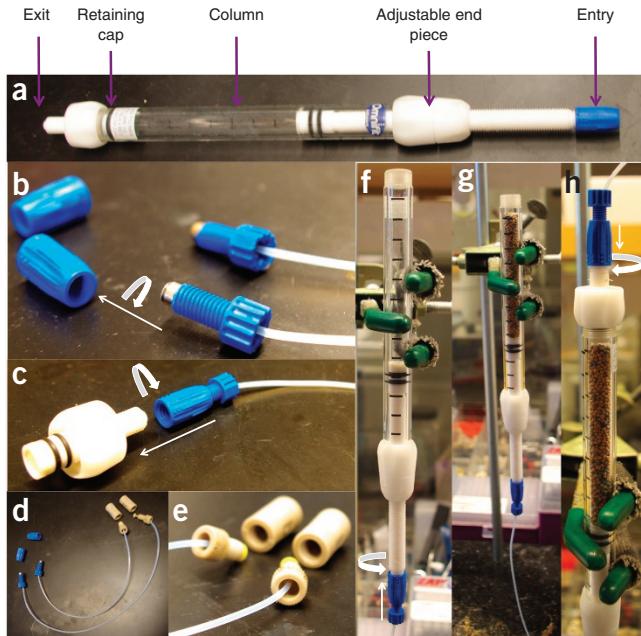


Figure 10 | Assembly of a drying unit. (a) A 150-mm Omnitfit column as received. The key components described in this protocol are highlighted. (b) Screw the blue nut from one of the PFA tubes into the blue connection cap and tighten it. (c) Screw one of the connection-cap-modified PFA tubes to the retaining cap and tighten it. (d,e) The components used to create the modified PFA tubing (d) are described in Step 51D(iv). The two modified PFA tubes, 2 × connection caps, and two low-pressure unions are shown (e). (f) The Omnitfit column with PFA tubing is attached to the adjustable end piece. (g) Fill the Omnitfit column with activated 4-Å molecular sieves to 95% of the volume, as described in Step 51D(ix). Note that the choice of molecular sieves depends on the solvent to be dried. (h) Insert the retaining cap and tighten it to complete the drying column.

- (iv) To both pieces of 15-cm PFA tubing, attach a blue plastic nut with a ferrule set (contained in the column kit, but a super flangeless nut can also be used) to one end of the tubing, and a super flangeless nut with a ferrule set to the other end of the PFA tubing, using the method previously described in Steps 2–7 (**Fig. 10d,e**).
- (v) Attach one of the 15-cm PFA tubes to the connection cap (found in the column kit) by inserting the blue plastic nut from the PFA tubing into the blue connection cap and tightening it using pliers. Attach the connection cap to the fully extendable end piece and tighten it (**Fig. 10f**).
- (vi) Thread the nut from the extendable end piece onto the glass column. Turn the nut in a clockwise motion until the connection is firm but not tight (**Fig. 10f**).
- (vii) Twist the upper portion of the nut counterclockwise to lower the variable-length section. Once the desired depth has been achieved, fully tighten the lower nut onto the glass column in a clockwise manner. To remove the extendable end piece, reverse Step 51D (vi and vii).
- (viii) Attach the other 15-cm PFA tube to a connection cap by inserting the blue plastic nut from the PFA tubing into the blue connection cap and tightening it using pliers. Attach the connection cap to the retaining cap and tighten it (**Fig. 10b,c**).
- (ix) Fill the chromatography column to 95% volume (130-mm deep) with 4-Å molecular sieves directly from the oven and immediately cap the column with the retaining head as shown in **Figure 10h**.
- ! CAUTION** Use laboratory-grade oven gloves, as the glass beaker containing the molecular sieves is extremely hot. We suggest clamping the column in an upright position to make this step easier (**Fig. 10g**).
- (x) Screw the retaining head into the column until it is firm.
- ! CAUTION** Overtightening will cause the glass column to crack.
- (xi) Connect the PFA tubing attached to the adjustable end piece to another part of the continuous flow system by inserting the super flangeless nut into a low-pressure PEEK union for 1/16-inch-outer-diameter tubing. The other side of this low-pressure PEEK union can be connected to any part of the continuous flow system.

Section 3—Preparation of sulfonamide as an example reaction: assembly of the continuous flow system ● TIMING ~30 min

▲ CRITICAL The reaction detailed below is used in our laboratory to train researchers in continuous flow synthesis.

In this experiment, dibenzylamine (**1**) and 4-chlorobenzenesulfonyl chloride (**2**) react to yield sulfonamide (**3**) in a single reactor with an in-line quench (**Fig. 11**). Several skills, including reactor construction, charging of a back-pressure regulator, the assembly of stainless-steel syringes, the assembly of a continuous flow system with multiple junctions, and yield determination, are described.

52| Assemble a reactor coil constructed from 0.02-inch-inner-diameter DuPont tubing with a length of 1,524 cm (50 feet, 3.089 ml) by following Steps 1–11.

53| To one end of the constructed reactor coil, attach a PEEK Y-assembly mixer for 1/16-inch-outer-diameter tubing (0.60-inch inner diameter) and finger-tighten the joint. Further tighten this joint with the extender tool for standard head nuts.

▲ CRITICAL STEP Overtightening the nut can lead to damage to the PFA tubing and cracking of the nut. A Y mixer was purposefully chosen in this example to stop solid formation upon mixing of compounds **1** and **2**.

54| Set a back-pressure regulator to 200 psig by following Steps 20–28, and attach it to the other end of the reactor coil. First, finger-tighten the regulator, and then further tighten it with the extender tool for standard head nuts and then with pliers (**Fig. 12**).

▲ CRITICAL STEP Overtightening the nut can lead to damage to the PFA tubing and cracking of the nut.

55| Place the reactor coil into an oil bath and secure the exit and entry of the reactor coil with binder clips (**Fig. 12f**). Check that the silicone oil covers the reactor to ensure even heating.

▲ CRITICAL STEP Make sure that the binder clips are not pressing upon the reactor tubing, as this can lead to a clog.

56| Secure the first PEEK Y-assembly mixer by holding it in place with a clamp (**Fig. 12f**).

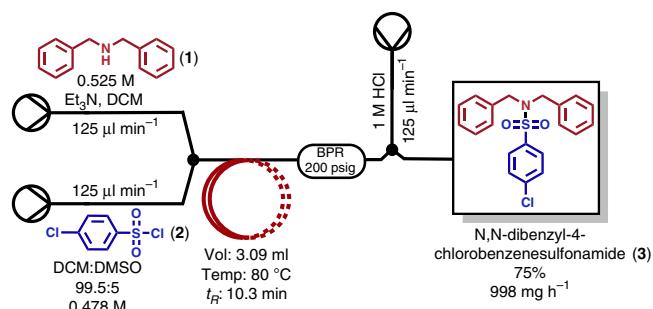


Figure 11 | Synthesis of *N,N*-dibenzyl-4-chlorobenzenesulfonamide (**3**) from dibenzylamine (**1**) and 4-chlorobenzenesulfonyl chloride (**2**). This continuous flow synthesis produces 998 mg h⁻¹ *N,N*-dibenzyl-4-chlorobenzenesulfonamide (**3**) through the use of a single reactor coil and an in-line quenching.

PROTOCOL

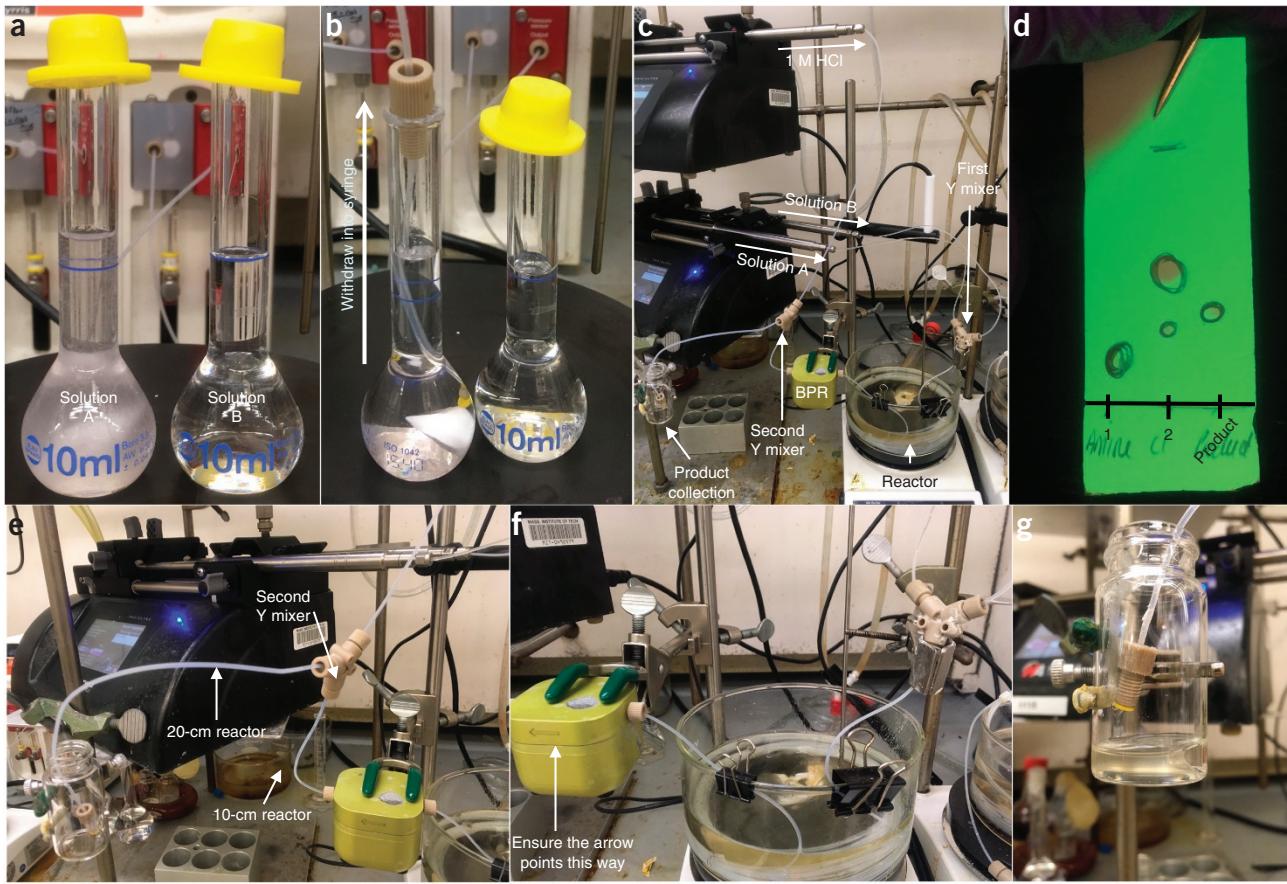


Figure 12 | Continuous flow reactor setup for the synthesis of sulfonamide (3). (a) Solutions A and B before mixing. (b) Solutions A and B after mixing, showing complete sample homogeneity. (c) The reactor setup highlighting the three stainless-steel syringes, two Y mixers, the reactor, back-pressure regulator, and the product-collection flask. (d) TLC of dibenzylamine (1) and 4-chlorobenzenesulfonyl chloride (2) against the product in a 1:10 ethyl acetate/hexanes, solution. (e) A photograph of the quenching stage of the reaction. (f) The reactor is placed into an oil bath and is held in place using binder clips. Note the orientation of the exit of the reactor into the back-pressure regulator. (g) The product-collection flask showing that the crude-reaction mixture is a light-brown color.

57| Secure the back-pressure regulator with a clamp (**Fig. 12c,f**).

▲ **CRITICAL STEP** Ensure that the exit of the reactor is attached to the inlet of the back-pressure regulator in the orientation shown in **Figure 12f**.

58| Assemble a 10-cm-long reactor from 0.02-inch-inner-diameter DuPont tubing with super flangeless nuts and ferrule sets for 1/16-inch-outer-diameter tubing on both ends by following Steps 1–11.

59| Insert one end of the 10-cm reactor into the exit of the back-pressure regulator and finger-tighten the nut. Further tighten this joint with the extender tool for standard head nuts and then with pliers (**Fig. 12e**).

▲ **CRITICAL STEP** Overtightening the nut can lead to damage to the PFA tubing and cracking of the nut.

60| Insert the other end of the 10-cm reactor into a second PEEK Y-assembly mixer for 1/16-inch-outer-diameter tubing (0.60-inch through hole), and finger-tighten the nut (**Fig. 12e**). Further tighten this joint with the extender tool for standard head nuts.

▲ **CRITICAL STEP** Overtightening the nut can lead to damage to the PFA tubing and cracking of the nut.

61| Assemble a 20-cm-long reactor from 0.02-inch-inner-diameter DuPont tubing with super flangeless nuts, and ferrule sets for 1/16-inch-outer-diameter tubing by following Steps 1–11.

62| Insert one end of the 20-cm reactor into the second PEEK Y-assembly mixer and finger-tighten the nut (**Fig. 12e**). Further tighten this joint with the extender tool for standard head nuts.

▲ **CRITICAL STEP** Overtightening the nut can lead to damage to the PFA tubing and cracking of the nut.

63 Insert the other end of the 20-cm reactor into a 25-ml glass beaker; this will serve as the collection vial during system equilibration.

Preparation of chemical solutions and loading into stainless-steel syringes • TIMING ~20 min

64 Charge a weighing boat with 4-chlorobenzenesulfonyl chloride (**2**; 1.00 g, 4.78 mmol, 1.00 equiv.). Transfer the 4-chlorobenzenesulfonyl chloride to a 10-ml volumetric flask, add DMSO (50 µl), and then stopper the flask. This is flask A.

65 Charge a 10-ml volumetric flask with dibenzylamine (**1**; 1.083 g, 5.26 mmol, 1.10 equiv.) and triethylamine (1344 µl, 9.57 mmol, 2.00 equiv.) and stopper the flask. This is flask B.

66 Add anhydrous DCM to both flasks using a disposable 10-ml plastic syringe and a hypodermic needle under typical air-free conditions to create ~9-ml final-volume solutions (Fig. 12a).

67 Add 1/2-inch PTFE stir bars to both solutions A and B, and stir the solutions at 300 r.p.m. for 10 min at RT. This will completely dissolve compound **2** and ensure mixture homogeneity (Fig. 12b). Adjust the solutions to 10 ml by adding anhydrous DCM using a disposable 10-ml plastic syringe and a hypodermic needle under typical air-free conditions.

▲ **CRITICAL STEP** Dissolving solids changes the volume of the solutions. Adding only 9 ml of DCM before dissolution ensures that any volume change will not exceed 10 ml.

68 Withdraw 8 ml of solution A into a stainless-steel syringe by following Step 36. Load and secure the stainless-steel syringe into a Harvard syringe pump. Attach the tubing from the exit of the stainless-steel syringe to the first PEEK Y-assembly mixer at the entry of the reactor and finger-tighten the nut (Fig. 12c). Further tighten this joint with the extender tool for standard head nuts.

▲ **CRITICAL STEP** Overtightening the nut can lead to damage to the PFA tubing and cracking of the nut.

69 Repeat Step 67 with the solution from flask B, and insert the stainless-steel syringe into the same Harvard syringe pump.

70 Withdraw 8 ml of a 1 M HCl solution into a stainless-steel syringe by following Step 36. Insert this stainless-steel syringe into a different Harvard syringe pump. Secure the stainless-steel syringe and attach the tubing from the exit of the stainless-steel syringe to the second PEEK Y-assembly mixer at the end of the continuous flow system (Fig. 12e). Further tighten this joint with the extender tool for standard head nuts.

▲ **CRITICAL STEP** Overtightening the nut can lead to damage to the PFA tubing and cracking of the nut.

71 Before starting the experiment, ensure that all connections are secure and that the stainless-steel syringes are properly secured into the Harvard syringe pumps.

Performing the experiment • TIMING ~40 min

72 Set the temperature of the oil bath to 80 °C with a stir rate of 400 r.p.m. Wait until the temperature of the oil bath is stable for at least 10 mins.

73 Set both Harvard syringe pumps to infuse at 125 µl min⁻¹, and start the infusion (Fig. 6g).

74 Allow the system to equilibrate for at least 35 min.

75 When the system is ready for collection, first, set a timer for 10.3 min (allows for 1.00 ml (0.4784 mmol) of the rate-limiting reagent to pass through the system). Second, exchange the equilibration beaker with a new 25-ml glass beaker and immediately start the timer. Reserve the equilibration beaker for later use.

76 After 10.3 min, immediately remove the collection beaker and replace it with the equilibration beaker used previously. The fluid collected contains a theoretical maximum yield of 0.4784 mmol.

77 Transfer the collection fluid to a 125-ml separatory funnel. Rinse the collection vial with DCM (5 × 5 ml) and transfer the washings to the separatory funnel.

PROTOCOL

78 | Add 25 ml of 1 M HCl to the separatory funnel; stopper the separatory funnel and then shake it. This is referred to as a typical organic liquid–liquid separation. After shaking, allow the layers to separate and collect the lower organic layer; discard the aqueous layer as waste, as this contains residual dibenzylamine.

79 | Transfer the organic layer back to the separatory funnel and add 25 ml of 1 M NaCl. Perform a liquid–liquid extraction. Collect the lower layer (organic) and discard the aqueous layer as waste.

80 | Transfer the organic layer back to the separatory funnel and add 25 ml of deionized H₂O. Perform a liquid–liquid extraction. Collect the lower layer (organic) and discard the aqueous layer as waste.

81 | Add ~700 mg of MgSO₄ to the organic layer and swirl the mixture for 2 min to dry the solution. The solution will achieve a snow-globe effect once it is dry. If 700 mg of MgSO₄ does not achieve this effect, add small portions of MgSO₄ until it is achieved.

82 | Filter the solution into a 250-ml round-bottom flask using a glass powder funnel and qualitative filter paper.

83 | Carefully remove the organic solvent under reduced pressure using a rotatory evaporator; a white powder will result.

84 | Take a small quantity of this solid (~1 mg) and add it to 5 ml of ethyl acetate. Perform thin-layer chromatography (TLC) analysis of the product ($R_f = 0.368$) using ethyl acetate/hexanes at a 1:10 ratio against both 4-chlorobenzenesulfonyl chloride (**2**; $R_f = 0.500$) and dibenzylamine (**1**; $R_f = 0.205$). The TLC should indicate a single product under UV radiation (Fig. 12d).

85 | Perform column chromatography (25-g silica column, height = 7.0 cm, diameter = 3.0 cm) with an 8% (vol/vol) solution of ethyl acetate in hexanes. The product will elute at 6–9 column volumes.

86 | Combine the fractions containing the desired product and remove the solvent under reduced pressure using a rotary evaporator. Dissolve the resulting solid into a small amount of DCM (~5 ml) and transfer the solution to a preweighed 25-g vial.

87 | Remove the solvent under reduced pressure using a rotatory evaporator until the product is dry. Further dry the product under vacuum at 40 °C for 2 h. Weigh the 25-g vial to obtain the mass of the product. The theoretical yield for this transformation is 177.5 mg.

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 1**.

TABLE 1 | Troubleshooting table.

Step	Problem	Possible reason	Solution
7	The PFA tubing disconnects from the super flangeless nut after tightening	Components were not correctly oriented on the PFA before tightening	Attach a new ferrule set in the order and orientation shown in Figure 3a,b . Do not reuse the original ferrule set
		The ferrule was not positioned around the PFA tubing when the super flangeless nut was tightened, or the super flangeless nut was not tightened enough	When tightening the super flangeless nut, apply pressure to the PFA tubing as shown in Figure 3c to ensure that the ferrule is set around the PFA tubing. Do not reuse the ferrule
18	The PFA tubing disconnects from the syringe connector	The ferrule set was not around the PFA tubing when tightened	When tightening the metal nut into the syringe connector, apply pressure to the PFA tubing to ensure that the ferrule is set around the PFA tubing

(continued)

TABLE 1 | Troubleshooting table (continued).

Step	Problem	Possible reason	Solution
19	The PFA tubing disconnects from the super flangeless nut after tightening	Components were not correctly oriented on the PFA before tightening The ferrule was not around the PFA tubing when the super flangeless nut was tightened into the union, or the super flangeless nut was not tightened enough A failure of the Swagelok-to-PFA connection is common. Mechanical stress leads to the stainless steel biting into the PFA tubing	Attach a new ferrule set in the order and orientation shown in Figure 3a,b . Do not reuse the original ferrule set When tightening the super flangeless nut, apply pressure to the PFA tubing as shown in Figure 3c to ensure that the ferrule is set around the PFA tubing. Do not reuse the ferrule This region of the tubing should be inspected before each use; replace the tubing if wear is observed
27	The syringe plunger has become sticky and is hard to operate when being filled with fluid	The grease has been removed from the O-rings	Ensure that regulator maintenance is conducted. Grease the O-rings of the syringe plunger with Krytox grease during maintenance
28	The gas-delivery tube detaches from the compressed air tank valve at high pressures	The gas-delivery tube is not secure	Tighten the metal nut at the end of the gas-delivery tube to ensure a firm fit between the back-pressure regulator and the gas-delivery tube
31	The back-pressure regulator does not allow fluid through when not connected to a flow system (i.e., stand-alone)	The device is broken	Contact Zaiput Flow Technologies for advice
	The PFA tubing disconnects from the super flangeless nut after tightening	The fluid from the continuous flow system is entering the wrong side of the separator Components were not correctly oriented on the PFA before tightening The ferrule was not around the PFA tubing when the super flangeless nut was tightened into the union, or the super flangeless nut was not tightened enough	Switch the entry and exit tubes of the back-pressure regulator. The engraved arrow on the back-pressure regulator shows the correct direction of fluid flow Attach a new ferrule set in the order and orientation shown in Figure 3a,b . Do not reuse the original ferrule set When tightening the super flangeless nut, apply pressure to the PFA tubing as shown in Figure 3c to ensure that the ferrule is set around the PFA tubing. Do not reuse the ferrule
36	Difficulties while loading air- or moisture-sensitive reagents into stainless-steel syringes under appropriate conditions?	Stainless-steel syringes require the reagents to be drawn up by the user, so, this must be carried out under air-free conditions	(i) Prepare solutions as you would for typical syntheses, i.e., in a round-bottom flask or in a screw-top vial. Next, screw a quick-stop Luer in-line check valve (IDEX Health & Science, cat. no. P-655) to the super flangeless nut of the stainless-steel syringe and join it to a suitable needle. Using this needle, load the solutions into the syringes using the same method as you would use to remove anhydrous solvent from a solvent bottle (standard air-sensitive syringe techniques) (ii) Syrris Asia pumps avoid the need to transfer solutions from Schlenk flasks to syringes. Attaching a quick-stop Luer in-line check valve and a needle to the pump allows direct infusion into the continuous flow system. However, such pumps cost ~\$20,000 USD (iii) When using reagents that are highly air-sensitive, it should be noted that PFA tubing allows slow permeation of air and moisture, which will lead to poor yields. Use stainless-steel tubing for the reactor coil

(continued)

PROTOCOL

TABLE 1 | Troubleshooting table (continued).

Step	Problem	Possible reason	Solution
39	The reactor coil does not submerge in the oil bath	Orientation or buoyancy of the reactor is wrong	Clip the entrance and exit of the reactor coil to the oil bath. Ensure that the orientation of the reactor coil leaves it fully submerged in oil/fluid. Make sure that the clips do not press upon the reactor tubing, as this can cause a clog. Furthermore, a lead weight can be used to weigh down the coil in the bath
	A reactor temperature of >150 °C is required, or the PEEK mixers must be submerged in the oil bath	PEEK nuts and PFA tubing can fail at higher temperatures	Use stainless-steel mixers and tubing to handle extreme temperatures (IDEX Health & Science, cat. no. U-428). The manufacturer recommends a PFA tubing temperature limit of 80 °C. We have used PFA tubing at 200 °C at 200 psig without fail, although safety should be the prime concern here. Note: If PEEK fittings are heated and cooled subsequently, the connections must be retightened or leaking will occur. If you are using high reactor temperatures, we suggest using tubing with a smaller inner-diameter (0.01-inch or 0.02-inch), given the thicker tubing wall
	Syringe pumps stop injecting the fluid into the continuous flow system	The continuous flow system is clogged and/or the syringes can deliver fluids only up to certain pressures. This limit depends on the type of pump used	<ul style="list-style-type: none"> (i) Working backward from the last piece of apparatus in the continuous flow system, unscrew the apparatus and see whether the syringe pumps are able to inject. This will help identify where the clog is (ii) If solid formation is located in the reactor coil, replace the reactor coil or declog the reactor. Then try diluting the reaction, placing the reactor coil into a sonicating bath, or using larger-diameter tubing to handle the solids. If you are increasing the internal diameter of the tubing, we suggest using mixers with through holes of the same internal diameter (iii) If the packed-bed reactor is clogged, change the stainless-steel frits to ones with larger porosities (IDEX Health & Science). Furthermore, we have experienced that using stainless-steel and other small-diameter packing materials can induce an unworkable back pressure. Switching to sand maintains great mixing but lowers the pressure the packed-bed reactor places on the system (iv) In-line check valves can break under high pressure, stopping fluid flow. Change and/or test the valves periodically (v) Operating the continuous flow system under a high back pressure can lead to pump failure, if the back-pressure regulator is causing system failure. Check the regulator for solid formation, or lower the pressure it places on the system, if possible (vi) If solid formation occurs in the back-pressure regulator, sonicate the solution or dilute the reaction before it enters the back-pressure regulator. If the back-pressure regulator is clogged, disassemble it to clear the clog. Solids in the back-pressure regulator can perforate the diaphragm. Contact Zaiput Flow Technologies for replacement of the diaphragm and parts

(continued)

TABLE 1 | Troubleshooting table (continued).

Step	Problem	Possible reason	Solution
			<ul style="list-style-type: none"> (vii) If solid formation is occurring in the union connecting two parts of the continuous flow system, use a larger-internal-diameter union or dilute the reaction (viii) If solid formation is occurring in the in-line liquid–liquid separator, try another solvent and/or dilute the reaction (ix) Solutions can react in the syringe, causing precipitation. Check for solid formation in the syringe. If present, separate the reactive components into separate syringes (x) The length of the continuous flow system is too long for the capabilities of the syringe pumps. Shorten the length of the continuous flow system, or remove the apparatus placing large pressures (i.e., packed-bed reactors housing stainless-steel chippings) on the system (xi) Check the pressure ratings of the syringe pumps. Harvard syringes have a factory force level setting of 50%; change this to 100%. Syrris syringe pumps have a factory setting of 15 bar; change this to 25 bar (xii) The mixers have become blocked because of solid formation upon mixing. Change the mixers to have larger inner-diameter channels. For example, change to a 0.06-inch through-hole T mixer. If the problem persists, try a range of mixers and then proceed to a tube-in-tube mixer (xiii) If the continuous flow stream is viscous, dilute it with a solvent to decrease back pressure
	Syringe pump breaks	Although far less likely than a reactor clog, sometimes, syringe pumps break and need to be repaired	Send the pump to be repaired
	The PFA tubing in the continuous flow system bursts	<p>The pressure of the system is too high for the specific PFA tubing. The system could have also been heated or cooled, beyond the limit of the specific tubing. Finally, the tubing could be damaged because of mechanical stress such as kinking</p> <p>Solid formation can lead to a clog. This will in turn increase the pressure of the system, leading to a rupture of the PFA tubing</p> <p>The reaction releases a large volume of gas that markedly increases the pressure of the system</p>	<ul style="list-style-type: none"> (i) If you are using tubing with an inner diameter >0.02 inch, change to smaller-diameter tubing; the smaller-diameter tubing has a thicker wall, making it more resistant to higher pressures and temperatures. Note that 0.01-inch-inner-diameter tubing is available for a more extreme option (ii) Switch to stainless-steel tubing for the reactor coil. This is constructed in much the same way as a packed-bed reactor is constructed (iii) Ensure that the reactor is not kinked or twisted <p>Reaction-engineer the transformation so that solid formation is avoided or limited. This could be done by dilution, variation in the reagents, or changes to the reaction temperature</p> <ul style="list-style-type: none"> (i) Dilute the reaction (ii) Lower the back-pressure regulator to decrease the total system pressure (iii) If gas release is due to reagent degradation, contemplate different reaction conditions, such as a lower reaction temperature

(continued)

PROTOCOL

TABLE 1 | Troubleshooting table (continued).

Step	Problem	Possible reason	Solution
	Lower yields are observed when the continuous flow system is made longer or more complex	In general, a larger system has higher pressures; thus, if you are operating at low flow rates ($\sim 10 \mu\text{l min}^{-1}$), the syringe driver can slip, reducing the amount of reagent added to the continuous flow system. This will reduce the yield	Use higher flow rates to avoid the syringe slipping at lower flow rates. An increase in the length of the reactor coils will have to occur for the residence time to remain the same. Furthermore, ensure that the syringe size is correctly input into the syringe pump
51A(iii)	Using three static mixers does not provide enough mixing	Long pieces of PFA tubing between back-pressure regulators, packed-bed reactors, T mixers, and drying units can create complex pressure gradients. Instead of flowing in one direction, it can recycle around. This can lead to lower yields or over-reactivity	(i) Reduce the dead volume in the system. Have the shortest amount of PFA tubing possible between the parts of apparatus (ii) If you are adding several solvents at different T mixers, combine all these solvents together into one stream for mixing with the primary continuous flow stream. This means that instead of several T mixers, there is only one mixer, reducing the possibility of complex pressure gradients
51B(x)	The part labeled 0 in Figure 8a snaps when the nut is tightened	Static mixers provide only a certain level of mixing	Switch to a packed-bed reactor housing sand, stainless-steel chippings, or glass beads. This will increase the amount of micromixing in the system. As mentioned previously, be careful of the back pressure this places on the system
51B(xviii)	When tightening the nut between part 2 and part 1 in Figure 8a , a crunching noise is heard	This is the weakest point of the packed-bed reactor. Overtightening will cause it to bend and snap	Be careful when tightening this joint. Ensure that the wrench is at 90° to the packed-bed reactor when tightening. Proceed carefully
51C(xiv)	The separator is not separating the organic and aqueous layers	There is sand present in the joint	While covering the entrance to the packed-bed reactor, tap the reactor to loosen sand or other materials. Subject the nut to compressed air to blow away loose debris
		There is too much pressure on the stream where no fluid is exiting	Change the back-pressure regulators to either lower the pressure on the side where no fluid is exiting or increase the pressure on the side where the fluid is exiting. Through this process, you will find the ideal pressures for optimal separation
		The membrane is fouled or is compromised with solids	Replace the membrane
		The pore size of the membrane is too small or too large for the flow rate being used	Change to a different-diameter pore-size membrane. The specific type is specified in Step 51C(i-x)
		The flow rate going through the separator is too high	Lower the flow rate or use a higher-capacity separator
		The organic layer is miscible with water	Add a nonmiscible organic solvent for the extraction process

TIMING

Steps 1–11, assembly of a reactor coil: ~10 min

Steps 12–19, assembly of a stainless-steel syringe: ~10 min

Steps 20–50, assembly of a basic continuous flow system: ~30 min

Step 51A, assembly of a static mixer: ~10 min

Step 51B, assembly of a packed-bed reactor: ~30 min
 Step 51C, use of an in-line liquid–liquid separator: ~30 min
 Step 51D, assembly of a drying unit: ~24 h
 Steps 52–87, example reaction: ~90 min

ANTICIPATED RESULTS

Sulfonamide (**Fig. 11**) is isolated as a white solid ($R_f = 0.368$ in 1:10 EtOAc/hexanes), 133.1 mg, yield: 75%. ^1H NMR (500 MHz, CDCl_3 , 20 °C): δ_{H} = 7.75 (dt, $^3\text{J}(\text{H},\text{H})$ = 8.66 Hz, $^4\text{J}(\text{H},\text{H})$ = 1.95 Hz, 2H; Aryl-**H**), 7.47 (dt, $^3\text{J}(\text{H},\text{H})$ = 8.70 Hz, $^4\text{J}(\text{H},\text{H})$ = 1.98 Hz, 2H; Aryl-**H**), 7.26–7.23 (m, 6H; Aryl-**H**), 7.10–7.05 (m, 4H; Aryl-**H**), 4.34 (s, 4H; CH_2); ^{13}C NMR (150 MHz, CDCl_3 , 20 °C): $\delta_{\text{C}} = 139.4$, 139.0, 135.4, 129.4, 128.7, 128.6, 128.0, 50.7; HRMS: m/z calcd. for $\text{C}_{20}\text{H}_{18}\text{ClNO}_2\text{S}$ [H⁺adduct]; 372.0820; found 372.0834.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS J.B. and T.F.J. wrote the manuscript.

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- Gutmann, B., Cantillo, D. & Kappe, C.O. Continuous flow technology—a tool for the safe manufacturing of active pharmaceutical ingredients. *Angew. Chem. Int. Ed. Engl.* **54**, 6688–6728 (2015).
- Movsisyan, M. *et al.* Taming hazardous chemistry by continuous flow technology. *Chem. Soc. Rev.* **45**, 4892–4928 (2016).
- Webb, D. & Jamison, T.F. Continuous flow multi-step organic synthesis. *Chem. Sci.* **1**, 675–680 (2010).
- Wiles, C. & Watts, P. Continuous flow reactors: a perspective. *Green Chem.* **14**, 38–54 (2012).
- Hartman, R.L., McMullen, J.P. & Jensen, K.F. Deciding whether to go with the flow: evaluating the merits of flow reactors for synthesis. *Angew. Chem. Int. Ed. Engl.* **50**, 7502–7519 (2011).
- Ley, S.V., Fitzpatrick, D.E., Ingham, R.J. & Myers, R.M. Organic synthesis: march of the machines. *Angew. Chem. Int. Ed. Engl.* **54**, 3449–3464 (2015).
- Newman, S.G. & Jensen, K.F. The role of flow in green chemistry and engineering. *Green Chem.* **15**, 1456–1472 (2013).
- Wiles, C. & Watts, P. Continuous process technology: a tool for sustainable production. *Green Chem.* **16**, 55–62 (2014).
- Britton, J. & Raston, C.L. Multi-step continuous flow synthesis. *Chem. Soc. Rev.* **46**, 1250–1271 (2017).
- Jähnisch, K., Hessel, V., Löwe, H. & Baerns, M. Chemistry in microstructured reactors. *Angew. Chem. Int. Ed. Engl.* **43**, 406–446 (2004).
- Sahoo, H.R., Kralj, J.G. & Jensen, K.F. Multistep continuous flow microchemical synthesis involving multiple reactions and separations. *Angew. Chem. Int. Ed. Engl.* **119**, 5806–5810 (2007).
- Wörz, O., Jäckel, K.P., Richter, T. & Wolf, A. Microreactors – a new efficient tool for reactor development. *Chem. Eng. Technol.* **24**, 138–142 (2001).
- Straathof, N.J.W., Su, Y., Hessel, V. & Noël, T. Accelerated gas-liquid visible light photoredox catalysis with continuous flow photochemical microreactors. *Nat. Protoc.* **11**, 10–21 (2016).
- Su, Y., Straathof, N.J.W., Hessel, V. & Noël, T. Photochemical transformations accelerated in continuous flow reactors: basic concepts and applications. *Chem. Eur. J.* **20**, 10562–10589 (2014).
- Garlets, Z.J., Nguyen, J.D. & Stephenson, C.R.J. The development of visible-light photoredox catalysis in flow. *Isr. J. Chem.* **54**, 351–360 (2014).
- Tucker, J.W., Zhang, Y., Jamison, T.F. & Stephenson, C.R.J. Visible-light photoredox catalysis in flow. *Angew. Chem. Int. Ed. Engl.* **51**, 4144–4147 (2012).
- Porta, R., Benaglia, M. & Puglisi, A. Flow chemistry: recent developments in the synthesis of pharmaceutical products. *Org. Process. Res. Dev.* **20**, 2–25 (2016).
- Malet-Sanz, L. & Susanne, F. Continuous flow synthesis. a pharma perspective. *J. Med. Chem.* **55**, 4062–4098 (2012).
- Schaber, S.D. *et al.* Economic analysis of integrated continuous and batch pharmaceutical manufacturing: a case study. *Ind. Eng. Chem. Res.* **50**, 10083–10092 (2011).
- Lee, S.L. *et al.* Modernizing pharmaceutical manufacturing: from batch to continuous production. *J. Pharm. Innov.* **10**, 191–199 (2015).
- Roberge, D.M. *et al.* Microreactor technology and continuous processes in the fine chemical and pharmaceutical industry: is the revolution underway? *Org. Process. Res. Dev.* **12**, 905–910 (2008).
- Wegner, J., Ceylan, S. & Kirschning, A. Flow chemistry – a key enabling technology for (multistep) organic synthesis. *Adv. Synth. Catal.* **354**, 17–57 (2012).
- Zhang, J., Gong, C., Zeng, X. & Xie, J. Continuous flow chemistry: new strategies for preparative inorganic chemistry. *Coord. Chem. Rev.* **324**, 39–53 (2016).
- Cambié, D., Bottecchia, C., Straathof, N.J.W., Hessel, V. & Noël, T. Applications of continuous flow photochemistry in organic synthesis, material science, and water treatment. *Chem. Rev.* **116**, 10276–10341 (2016).
- Peng, Y. *et al.* Room temperature batch and continuous flow synthesis of water-stable covalent organic frameworks (COFs). *Chem. Mater.* **28**, 5095–5101 (2016).
- Liu, Z. *et al.* Continuous flow synthesis of ZSM-5 zeolite on the order of seconds. *Proc. Natl. Acad. Sci. USA* **113**, 14267–14271 (2016).
- Hajba, L. & Guttman, A. Continuous flow biochemical reactors: biocatalysis, bioconversion, and bioanalytical applications utilizing immobilized microfluidic enzyme reactors. *J. Flow Chem.* **6**, 8–12 (2016).
- Planchestainer, M. *et al.* Continuous flow biocatalysis: production and in-line purification of amines by immobilised transaminase from *Halomonas elongata*. *Green Chem.* **19**, 372–375 (2017).
- Tang, X., Allemann, R.K. & Wirth, T. Optimising terpene synthesis with flow biocatalysis. *Eur. J. Org. Chem.* **2017**, 414–418 (2017).
- Britton, J., Raston, C.L. & Weiss, G.A. Rapid protein immobilization for thin film continuous flow biocatalysis. *Chem. Commun.* **52**, 10159–10162 (2016).
- Britton, J. & Raston, C.L. Rapid high conversion of high free fatty acid feedstock into biodiesel using continuous flow vortex fluidics. *RSC Adv.* **5**, 2276–2280 (2015).
- Britton, J. & Raston, C.L. Continuous flow vortex fluidic production of biodiesel. *RSC Adv.* **4**, 49850–49854 (2014).
- Choedkiatsakul, I., Ngaosuwan, K., Assabumrungrat, S., Mantegna, S. & Cravotto, G. Biodiesel production in a novel continuous flow microwave reactor. *Renew. Energ.* **83**, 25–29 (2015).
- Asadi, M., Hooper, J.F. & Lupton, D.W. Biodiesel synthesis using integrated acid and base catalysis in continuous flow. *Tetrahedron* **72**, 3729–3733 (2016).
- Roberge, D.M., Ducry, L., Bieler, N., Cretton, P. & Zimmermann, B. Microreactor technology: a revolution for the fine chemical and pharmaceutical industries? *Chem. Eng. Technol.* **28**, 318–323 (2005).

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36. Snead, D.R. & Jamison, T.F. A three-minute synthesis and purification of ibuprofen: pushing the limits of continuous flow processing. *Angew. Chem. Int. Ed. Engl.* **54**, 983–987 (2015).
37. Adamo, A. *et al.* On-demand continuous flow production of pharmaceuticals in a compact, reconfigurable system. *Science* **352**, 61–67 (2016).
38. Snead, D.R. & Jamison, T.F. End-to-end continuous flow synthesis and purification of diphenhydramine hydrochloride featuring atom economy, in-line separation, and flow of molten ammonium salts. *Chem. Sci.* **4**, 2822–2827 (2013).
39. Dai, C., Snead, D.R., Zhang, P. & Jamison, T.F. Continuous flow synthesis and purification of atropine with sequential in-line separations of structurally similar impurities. *J. Flow Chem.* **5**, 133–138 (2015).
40. Zhang, P., Russell, M.G. & Jamison, T.F. Continuous flow total synthesis of rufinamide. *Org. Process. Res. Dev.* **18**, 1567–1570 (2014).
41. Mascia, S. *et al.* End-to-end continuous manufacturing of pharmaceuticals: integrated synthesis, purification, and final dosage formation. *Angew. Chem. Int. Ed. Engl.* **52**, 12359–12363 (2013).
42. Heider, P.L. *et al.* Development of a multi-step synthesis and workup sequence for an integrated, continuous manufacturing process of a pharmaceutical. *Org. Process. Res. Dev.* **18**, 402–409 (2014).
43. Britton, J. & Jamison, T.F. A unified continuous flow assembly-line synthesis of highly substituted pyrazoles and pyrazolines. *Angew. Chem. Int. Ed. Engl.* **56**, 8823–8827 (2017).
44. McTeague, T.A. & Jamison, T.F. Photoredox activation of SF₆ for fluorination. *Angew. Chem. Int. Ed. Engl.* **55**, 15072–15075 (2016).
45. Andrade, L.H., Kroutil, W. & Jamison, T.F. Continuous flow synthesis of chiral amines in organic solvents: immobilization of *E. coli* cells containing both ω -transaminase and PLP. *Org. Lett.* **16**, 6092–6095 (2014).
46. Barnes, J.C. *et al.* Iterative exponential growth of stereo- and sequence-controlled polymers. *Nat. Chem.* **7**, 810–815 (2015).
47. Wu, J. *et al.* Continuous flow synthesis of ketones from carbon dioxide and organolithium or Grignard reagents. *Angew. Chem. Int. Ed. Engl.* **53**, 8416–8420 (2014).
48. Wu, J., Kozak, J.A., Simeon, F., Hatton, T.A. & Jamison, T.F. Mechanism-guided design of flow systems for multicomponent reactions: conversion of CO₂ and olefins to cyclic carbonates. *Chem. Sci.* **5**, 1227–1231 (2014).
49. Zhang, Y., Blackman, M.L., Leduc, A.B. & Jamison, T.F. Peptide fragment coupling using a continuous flow photochemical rearrangement of nitrones. *Angew. Chem. Int. Ed. Engl.* **52**, 4251–4255 (2013).
50. Kleinke, A.S. & Jamison, T.F. Hydrogen-free alkene reduction in continuous flow. *Org. Lett.* **15**, 710–713 (2013).
51. Shen, B. & Jamison, T.F. Rapid continuous synthesis of 5-deoxyribonucleosides in flow via Brønsted acid catalyzed glycosylation. *Org. Lett.* **14**, 3348–3351 (2012).
52. Shen, B., Bedore, M.W., Sniady, A. & Jamison, T.F. Continuous flow photocatalysis enhanced using an aluminum mirror: rapid and selective synthesis of 2-deoxy and 2,3-dideoxynucleosides. *Chem. Commun.* **48**, 7444–7446 (2012).
53. Webb, D. & Jamison, T.F. Diisobutylaluminum hydride reductions revitalized: a fast, robust, and selective continuous flow system for aldehyde synthesis. *Org. Lett.* **14**, 568–571 (2012).
54. Leduc, A.B. & Jamison, T.F. Continuous flow oxidation of alcohols and aldehydes utilizing bleach and catalytic tetrabutylammonium bromide. *Org. Process. Res. Dev.* **16**, 1082–1089 (2012).
55. Palde, P.B. & Jamison, T.F. Safe and efficient tetrazole synthesis in a continuous flow microreactor. *Angew. Chem. Int. Ed. Engl.* **50**, 3525–3528 (2011).
56. Sniady, A., Bedore, M.W. & Jamison, T.F. One-flow, multistep synthesis of nucleosides by Brønsted acid-catalyzed glycosylation. *Angew. Chem. Int. Ed. Engl.* **50**, 2155–2158 (2011).
57. Zhang, Y., Jamison, T.F., Patel, S. & Mainolfi, N. Continuous flow coupling and decarboxylation reactions promoted by copper tubing. *Org. Lett.* **13**, 280–283 (2011).
58. Yoshida, J.-i., Nagaki, A. & Yamada, T. Flash chemistry: fast chemical synthesis by using microreactors. *Chemistry* **14**, 7450–7459 (2008).
59. Yoshida, J.-i., Takahashi, Y. & Nagaki, A. Flash chemistry: flow chemistry that cannot be done in batch. *Chem. Commun.* **49**, 9896–9904 (2013).
60. Browne, D.L. *et al.* Continuous flow reaction monitoring using an on-line miniature mass spectrometer. *Rapid Commun.* **26**, 1999–2010 (2012).
61. Hall, A.M.R. *et al.* Practical aspects of real-time reaction monitoring using multi-nuclear high resolution FlowNMR spectroscopy. *Catal. Sci. Technol.* **6**, 8406–8417 (2016).
62. Brodmann, T., Koos, P., Metzger, A., Knochel, P. & Ley, S.V. Continuous preparation of arylmagnesium reagents in flow with inline IR monitoring. *Org. Process. Res. Dev.* **16**, 1102–1113 (2012).