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Practical Considerations and Examples in Adapting Amidations to Continuous Flow Processing in the Early Development

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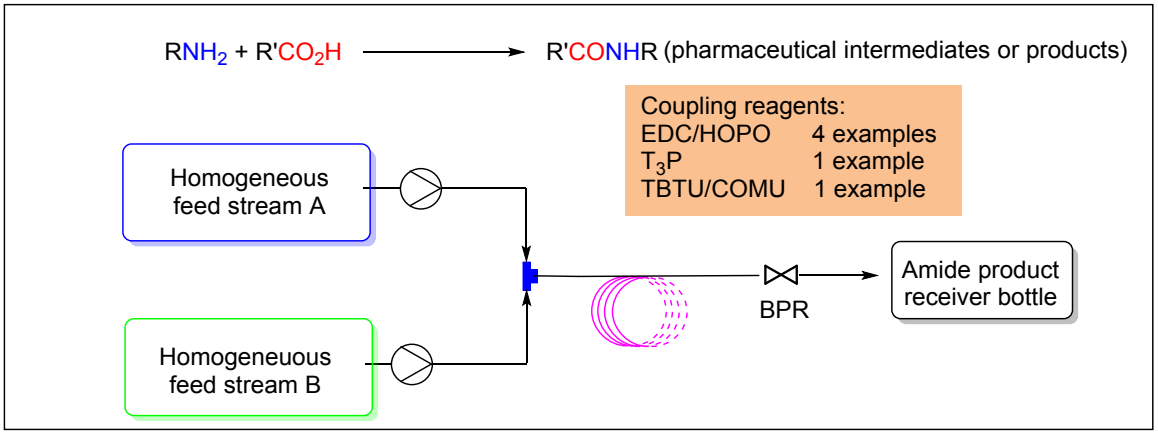


Abstract: Amidation is among the most frequently executed reactions in pharmaceutical research and development. We have explored the feasibility of adapting amidations to plug flow reactor (PFR) process conditions for preparation of early development compounds. Among coupling reagents possessing good thermal stability, carbodiimides and T3P have been selected, as they are readily soluble, require no pre-activation, offer excellent reaction kinetics and enable convenient product isolation. A carbodiimide/2-hydroxylpyridine oxide (HOPO) protocol was demonstrated in four case studies with homogeneous feed and reaction streams readily adaptable to a PFR design. In a head to head comparison, T3P was also found readily adaptable to a PFR flow process and gave comparable yields. The EDC/HOPO method works well for amidations that do not involve substrates highly sensitive to racemization; its water compatibility makes it the reagent of choice when the amine reactant is in a salt form, since water can be added as a co-solvent to aid solubility. For substrates extremely sensitive to racemization, we have shown one successful example of peptide coupling using TBTU or COMU under PFR continuous flow conditions.

Keywords: Flow amidation, continuous process, peptide coupling, early development.

1. Introduction

In the recent decade, continuous processing has attracted considerable interest within the pharmaceutical industry. Continuous flow technology offers many advantages over batch methods including precise control of stoichiometry, reaction time and temperature, high reproducibility, and often better yields thanks to improved reaction control.[1] Continuous processing has been applied in the handling of hazardous reactions, unstable reaction intermediates, high temperature/pressure and the expansion of chemical design spaces in the pharma industry.[2] In addition, there has always been a focus on process intensification to gain production efficiency, reduce manufacturing facility footprints, shorten development timelines, and improve quality control.[3] Amidation, as a common, well-studied chemical transformation, is traditionally carried out under batch conditions, and in general does not present scale up concerns. Numerous coupling reagents have been developed to address special needs of reaction rates, epimerization, and efficiency. Recently, a Pfizer process safety lab published a detailed thermal stability assessment of the most commonly used peptide coupling reagents.[4] As amidation is among the most frequently executed

reactions[5] in the pharma industry, we were interested in understanding the generality of adapting these reactions to flow processes, and looked for opportunities to apply the technology in the early phase of development. While solid-phase supported peptide synthesis via continuous processing has been well documented in the literature,[6] solution phase flow amidations have been reported more sporadically. Polster and colleagues demonstrated a Schotten-Baumann reaction by coupling an acid chloride with an amine using a plug flow reactor (PFR).[7] A continuous stirred-tank reactor (CSTR) process for the scale-up of a highly potent drug candidate was described by White and coworkers from the same Eli Lilly process chemistry group.[8] In the latter example, application of continuous processes approaches showed clear benefits in reducing potential worker exposure by reducing unit operations and allowing commercial-scale API production in laboratory fume hoods.

2. Practical considerations in flow amidations

In this study, our primary objective was to explore the feasibility of applying continuous processing technologies to coupling carboxylic acids with amines to form the

corresponding amides in early development programs. With emphasis on simplicity, convenience and speed, we considered the following:

(1) Pre-activation of the carboxylic acid. Though a pre-activation step can be carried out in a continuous process and telescoped to the amidation, in general this introduces operational complexity as the stoichiometry of the coupling reagent(s) is often critical. Complete conversion of the carboxylic acid and consumption of the activation reagent are necessary to avoid impurity formation from reaction of the amine with the coupling reagent. When an amidation reaction requires a pre-activation of the carboxylic acid, either the acid or the amine is used in slight excess to factor in reagent quality and the presence of adventitious water. Commonly used pre-activated acid derivatives include:

a) Acid chloride. As exemplified in both afore-mentioned Lilly publications, acid chlorides are often isolated before proceeding to the amidation step. Commonly used chlorinating reagents are oxalyl chloride, thionyl chloride, cyanuric chloride, phosphorus trichloride or phosphorous pentachloride. As the chlorinating agent is generally used in excess, and it reacts with the

amine reactant, it's difficult to directly telescope to the amidation reaction under continuous flow.

b) Mixed anhydride. They are routinely used in solution phase amidations under batch conditions. One of the most commonly used activating agents is pivaloyl chloride.[9] Cole and coworker preformed the pivaloyl mixed anhydride under batch conditions, and subsequently used isolated intermediate in a CSTR process for the manufacture of an active pharmaceutical ingredient (API).[10] Nevertheless pivaloyl chloride and its byproduct pivalic acid carry a pungent odor detectable even at very low levels, which makes its use undesirable for the late steps in API manufacture. Furthermore, pivaloyl chloride is a highly acutely toxic (HAT) reagent.[11] Isobutyl chloroformate and the related family of reagents are also deemed generally unsafe for use on large scale.[4] Phosphoric[12] or sulfuric mixed[13] anhydrides are also known, but less commonly utilized. In recent years, the use of propylphosphonic anhydride (T3P) has gained a great deal of popularity;[14] the corresponding phosphonic acid mixed anhydride is formed *in situ*, and the reaction is mostly frequently

carried out in the same vessel without the need to pre-form the mixed anhydride.

c) Acyl imidazole. This is a popular method of activation and can be readily prepared by reacting the carboxylic acid with carbonyl diimidazole (CDI).[15] The pre-activation requires precise control of stoichiometry and process analytical technology (PAT) control to monitor the conversion. While an under-charge of CDI leads to incomplete reaction, an over-charge of the reagent often leads to urea byproduct formation. Another major hurdle in application of CDI in continuous processes is its poor solubility in commonly used reaction solvents (*vide infra*).

(2) Solubility/solvent selection. A homogeneous reaction is highly desirable in adaptation to a flow process. Green chemistry principles are applied in solvent selection[16] with ease of work up and product isolation also taken into account. In a continuous flow design, coupling reagents should be readily soluble at ambient temperature in typical processing solvent(s). In this aspect, CDI, with low to moderate solubility[17] in common aprotic solvents, is limited to reactions carried out in an

aprotic polar solvent, e.g. DMF, DMSO, and sulfolane when adapting to a PFR flow process. The use of a large amount of solvent generally impedes product isolation, and is therefore avoided if more efficient alternatives are available.

(3) Thermal safety. Per the recent reagent selection guideline for amide coupling endorsed by the Pfizer process safety lab,[4] our initial focus was on the "preferred" reagent category, largely determined by a thermal safety assessment. If it were necessary to employ a non-preferred reagent, a comprehensive process safety evaluation is completed prior to scale up.

(4) Flow equipment. With simplicity and convenience in mind, it is desirable to have fewer feed streams/pumps and PAT requirements for reasons of lower risk of equipment failure. Therefore, a flow system with two feed streams is targeted whenever feasible.[18] A PFR design is preferred since it is more readily adaptable from various laboratory scales to production scale, particularly in the early developmental phases. Reactions that generate gases (CO_2 from CDI activation, CO and HCl from oxalyl chloride) are more demanding of reactor design, and preference is given to alternatives that are friendly to flow adaptation. Furthermore, reagents such as acid chlorides that are corrosive to pump heads/seals are disfavored.

(5) Product isolation. The ease of workup/product isolation needs to be considered in any process development program. Therefore, N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) and the associated family of coupling reagents are not deemed favorable as byproducts of the coupling reagent can be difficult to remove.

Among the Pfizer process safety lab preferred coupling reagent list, methanesulfonyl chloride toluenesulfonyl choride, , phosphorus oxychloride (POCl3) oxalyl chloride, carbonyl diimidazole (CDI), pivaloyl chloride (PivCl), cyanuric chloride (TCT), 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), diphenyl phosphoryl chloride (DPC), diphenylphosphinyl chloride (DPPCl), diethyl phosphorocyanidate (DEPC), diphenyl phosphite (DPP), and pentafluorophenyl diphenylphosphinate (FDPP) require pre-activation. The dihydroquinone family (EEDQ, **2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline** or IIDQ, and 1-tert-butoxy-2-butoxycarbonyl-1,2-dihydroisoquinoline or BBDI) can lead to impurity formation,[19] and generates the quinoline byproduct that adds complexity in the product isolation. The imidazolium reagents (bromo-tris-pyrrolidino-phosphonium hexafluorophosphate or PyBrOP, chlorotripyrrolidinophosphonium hexafluorophosphate or PyClOP, 1-(chloro-1-pyrrolidinylmethylene)pyrrolidinium hexafluorophosphate or PyCIU, 2-chloro-1,3-dimethylimidazolinium Hexafluorophosphate or CIP) are not widely popular among process chemists as pyrrolidide derivatives were often observed as byproducts, which is attributed to the presence of pyrrolidine as a contaminant in commercial sources, and the reagents typically require crystallized before use.[20] It has been reported that PyBroP activation of Boc-amino acids can give moderate yields due to formation of the N-carboxyanhydride (NCA).[21] The use of tetramethylfluoroformamidinium hexafluorophosphate (TFFH) in a scale up facility will necessitate the use of Hastelloy reactors.[22] Tetrachloro-N-hydroxyphthalimide tetramethyluronium hexafluorophosphate (CITU) was not considered during the study since it was introduced after this work was completed; it does not appear widely available on bulk scale. With these realities in mind, the number of reagents to consider dramatically decreases to the carbodiimides and T3P. In the carbodiimide family, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) is particularly attractive due to fast reaction kinetics

and the ease of product isolation; the urea byproduct is water soluble.[23] The combination of carbodimides with 2-hydroxylpyridine oxide (HOPO) is known to be a powerful amidation protocol.[24] HOPO is regularly used as an additive to increase reaction kinetics and suppress epimerization.[25] General considerations of EDC/HOPO[26] and T3P in their adaptability to flow processes are summarized in Table 1.

Table 1. EDC/HOPO and T3P in Flow Adaptability Considerations

Attributes \ Reagent	EDC/HOPO	T3P
Cost per mol (based on SciFinder™)	EDC $48 HOPO $16	$255
Water compatibility	Yes; a big advantage as amines are often in organic salt forms and water is generally needed as a co-solvent for solubility.	No
Critical stoichiometry	No; in general excess of the reagent does not present risk to reaction performance. If the reaction is not fully converted, additional reagent can be added.	
Homogeneous feed streams	Yes; water can be used as co-solvent if needed.	Yes; in many solvents.
Risk of racemization (for substrate of concern)	Low/Medium	Low/Medium
Thermal hazard	Low[26]	Low
Ease of workup/ product isolation	Good; often direct drop isolation	Good
Flow adaptation feasibility	**High**	**High**

3. Results and discussion

Several Pfizer development portfolio examples were chosen to measure flow adaptability potential based on the above analysis. In this study, the workflow involved:

(1) Identification of stable and homogenous feed and effluent streams, as they are critical for the development of a continuous flow processes in a PFR.[7] Solubility screening of the reactants, product and reagents. Different combinations of

reactants/reagent can lead to different solubility behavior. Chemical and thermal stability of the feed streams is also essential in devising the feed streams.

(2) Optimization of the reaction conditions and studying batch reaction kinetics under various temperatures with an aim to keep reaction/residence time within 60 min.[27] ChemGlass pressure relief vials[28] were used if the reaction were kept under pressure.

(3) Isolation of the product without chromatography, ideally using direct drop isolation.

(4) Demonstration of the reactions by flow on multi-gram scales.

It is gratifying to find out that for all reactions investigated, a fully homogeneous reaction system was attainable. In each example, reaction screening was performed under batch conditions for optimal stoichiometry, solvent selections, choice of reagents and residence time. Much effort was directed to assuring a homogeneous reaction mixture and high reaction conversion in each case. In Entry 1 (Table 2), exactly 1.0 equiv each of the reactants (**1** and **2**) were used with 1.2 equiv of EDC. When triethylamine was used as a base, a carbamimidate impurity[29] was formed as a major

impurity at as much as ~11% (by UPLC area). However, when the base was switched to sodium bicarbonate, a clean reaction was observed. (Table 2). After evaporation of most THF and acetone, the product (**3**) was isolated in 90.5% yield in a 10 g flow demonstration run. In both entries 1 and 2 (Table 1), the amine reactant was a hydrochloride salt, and had limited solubility in most aprotic organic solvents except the very polar DMSO or sulfolane. The compatibility of EDC with water[30] was a huge advantage by allowing water as a co-solvent in the reaction, which afforded a homogeneous reaction system, a critical feature in adapting the reaction to PFR flow processes. In Entry 2 (Table 2) reaction, chemoselectivity was important for both the acid and amine reactants, as competing functionalities were present (the hydroxyl group of glycolic acid and the heteroaryl-NH_2 of amine **5**). To suppress side reactions from the self-condensation from glycolic acid, we chose an aqueous biphasic solvent system. As EDC is water compatible (Table 1), the EDC/HOPO aqueous coupling conditions were considered optimal for these coupling partners; most other conditions screened gave impurities from competing side-reactions.[31] The reaction was complete in 5 min at 20 °C to give the product in 99.0% purity by UPLC in the reaction mixture

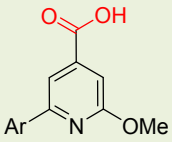
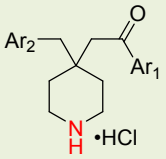
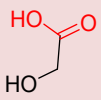
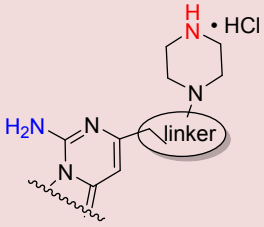
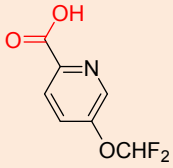
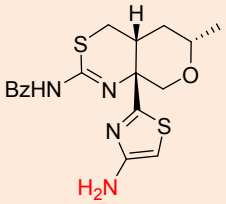
(as glycolic acid is a very cheap commodity chemical and readily removed in the aqueous phase, 3.0 equiv was used to speed up the reaction). The product isolation was easily accomplished by removal of THF and filtration to give a 92% yield of compound **6** in a 5 g flow run (**Figure 1**). The reaction in Entry 3a (Table 2) worked well using either T3P or EDC as the coupling reagent. The flow adaptation for T3P facilitated amidation was a simple modification from the batch conditions. The reaction solvent was changed from EtOAc to DCM to obtain a homogeneous reaction mixture, this was necessary due to the limited solubility of nicotinic acid **7** in EtOAc. T3P in acetonitrile and DMF worked reasonably well for the reaction, but was not optimal for product isolation. The workup and isolation involved the removal of DCM by evaporation and granulation in aqueous iPrOH; 93% isolated yield was obtained in a 10 g flow run as illustrated in **Figure 1**. The reaction could also be readily adapted using the EDC/HOPO method, although the reaction kinetics were slower than using T3P; a THF/water/acetone solvent mixture provided a homogeneity. In a 10 gram flow run, the reaction was carried out at 90 °C for a 45 min residence time to give 91.6% isolated yield (Entry 3b, Table 2). The amidation in entry 4 (Table 2) had been carried

out in multi-kilogram scale under batch conditions. T3P, used as the coupling reagent, was the major cost contributor in this reaction as both reactants are cheap commodity chemicals. This reaction could be simply adapted to the EDC/HOPO method, but the EDC byproduct, ethyl-(N',N'-dimethylamino)propyl urea, had similar solubility versus the product at the desirable pH range, complicating product isolation. Use of N, N′-Diisopropylcarbodiimide (DIC) as the coupling reagent circumvented the issue successfully. The reaction remained fully homogeneous in a solvent mixture of THF/water (10 mL/g each). At 75 °C in 30 min, the reaction was converted cleanly. The physical and chemical properties of the product necessitated a more tedious product isolation. Thus, the DIC byproduct (diisopropyl urea) was removed by filtration after evaporation of THF, the resulting aqueous filtrate was adjusted to pH 11, and was then back-extracted with nBuOH to enable the final isolation. After concentration of the extract and granulation in acetonitrile, 70.3% yield was isolated in a 10 g flow demo run. Higher yield could potentially be achieved with some optimization as *in situ* yield was excellent.

While the use of EDC/HOPO or T3P proved to be appropriate for routine amidations, this could lead to racemization for some sensitive substrates (e.g. entry 5, Table 2). This calls for the use of coupling reagents that are not preferred as judged solely by thermal stability. In the scenario where both coupling partners are expensive (e.g. cyclic peptide (**14**) and tripeptide (**13**)), and limited quantities are available for process

development, a flow process offers the benefit of mitigating scale up risk; *i.e.* the entire amount of materials are not committed up front. Reaction performance may be determined in the very beginning of the run with either online or offline analysis. The reaction can be fine-tuned if necessary, to maximize yield and minimize formation of impurities. It was demonstrated that both 1-cyano-2-ethoxy-2-oxoethylidenaminooxy)-dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) and 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) worked remarkably well for this peptide coupling (entry 5, Table 2). The diastereomeric impurity was detected at less than 0.2% under both flow and batch conditions. With less than 10 grams of Polymixin cyclic peptide **14**, we were able to develop a process that was ready to be scaled up.[32] This was particularly important, as both availability of starting materials and time for process development are important constraints in early drug development phases.

Table 2. Amidation Reaction Conditions Adaptable to Flow[33]

Entry	Acid (Compound No. and equiv. used)		Amine (Compound No. and equiv. used)		Feed streams and Reaction Conversions	Product (Isolated Yield)
1		**1** 1.0 equiv		**2** 1.0 equiv	Feed stream A: **1, 2** and $NaHCO_3$ (2.0 eq) in THF/water (8/2 vol[34]). Feed stream B: EDC (1.2 eq), HOPO (1.0 eq) in THF/water/acetone (2/3/3 vol). Conditions: 40 °C, 2 min 22%; 55 °C 15 min, 83%; 85 °C, 45 min, 100%.	**3** (90.5%)
2		4 3.0 equiv		**5** 1.0 equiv	Feed stream A: **5** in THF/water (5/0.5 vol). Feed stream B: **4**, EDC (2.2 eq), HOPO (1.0 eq) in THF/water (5/4.5 vol). Conditions: 20 °C, 1 min, 77%; 20 °C, 5 min. 100%.	**6** (92.0%)
3a		**7** 1.0 equiv		**8** 1.05 equiv	Feed stream A**: 7,** Et_3N (4.2 eq) in THF/water (8/1 vol). Feed stream B: **8**, 3.0 eq EDC, HOPO (1.0 eq) in THF/water/aceteone (2/4/6 vol). Conditions: 75 °C 35 min, 97%; 90 °C 30 min, 99%	**9**[35] (91.6%)
3b					Feed stream A: **7, 8** in Et_3N (4.2 eq) in DCM (10 vol). Feed stream B: 50 wt% T3P (2.5 eq) in EtOAc.	**9** (93.0%)

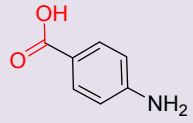
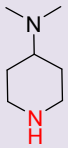
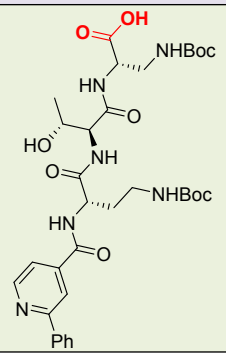
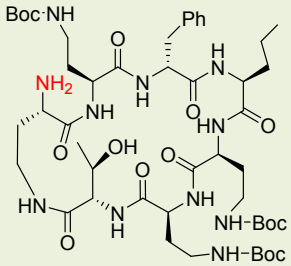
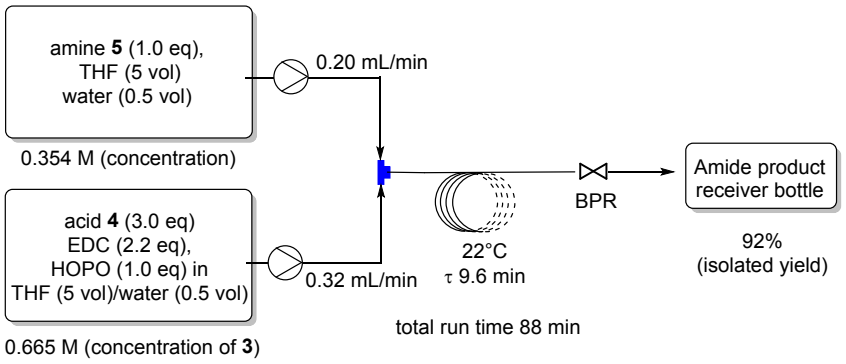
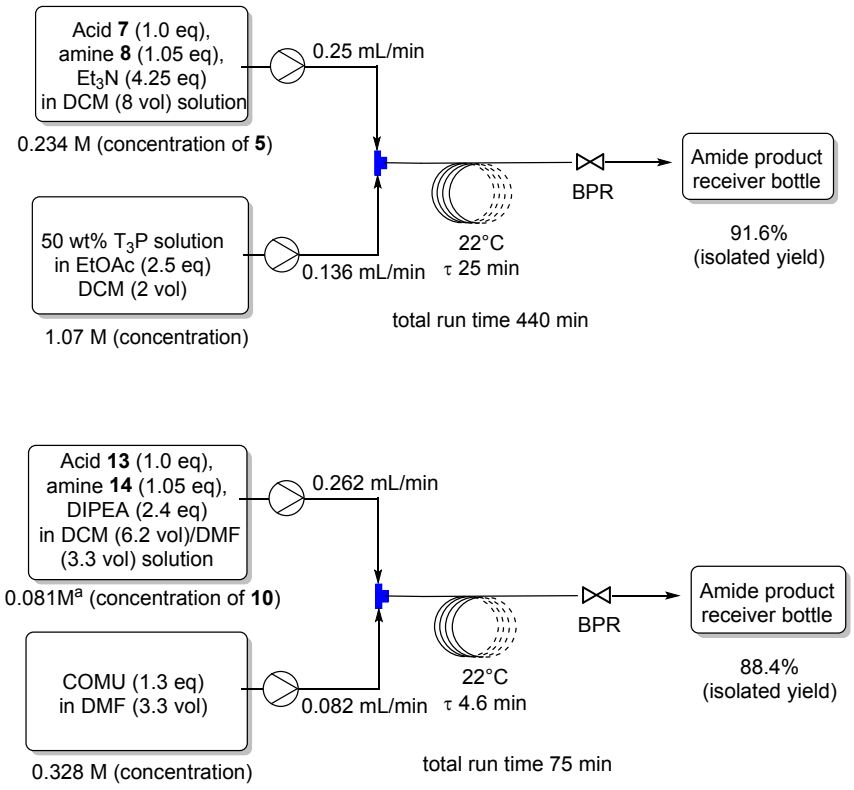
					Conditions: 22 °C, 2 min, 94%; 5 min, 98%; 20 min, 99%	
4		**10** 1.0 equiv		**11** 1.0 equiv	Feed stream A: **10, 11** and HOPO (1.0 eq) in THF/water (10/10 vol). Feed stream B: DIC (2.0 eq). Conditions: 20 °C, 2 h, 50%; 75 °C, 30 min, 100%.	**12**[36] (70.3%)
5		**13** 1.0 equiv		**14** 1.05 equiv	Feed stream A: **13, 14** and DIPEA (2.4 eq) in DCM (6.2 vol)/ DMF (3.3 vol). Feed stream B: COMU (1.3 eq) in DMF (3.3 vol). Conditions: 22 °C, 1 min, 90%; 4 min, 100%.	**15**[37] (88.4%)

Figure 1. Examples of Laboratory PFR Flow Diagrams





[a] The low molar concentration was attributed to the high molecular weight of **10**.

4. Conclusion

We have made a brief assessment to develop a general method to adapt amidations to PFR flow processes. Among coupling reagents exhibiting acceptable thermal stability, we have shortlisted carbodiimides and T3P as reagents of choice considering minimal pre-activation requirements, reasonable solubility, adaptability to a range of flow equipment and straightforward product isolation. EDC/HOPO coupling reagents were demonstrated to be effective in PFR flow processes, and in the four case studies,

homogeneous feed and reactions streams could be achieved. In a head to head comparison, T3P was also found readily adaptable flow, providing comparable yields, although halogenated (less green) DCM reaction solvent was required to assure solubility. With its low cost and coupling efficiency, EDC/HOPO is deemed an attractive choice for amidations that do not involve substrates that are highly sensitive to racemization; its water compatibility offers the additional advantage of allowing water to be used as a co-solvent to dissolve amine reactants in salt forms. The workup and isolation typically involve evaporation of organic solvent, and the product can often crystallize[38]directly from the resultant aqueous mixture. For substrates prone to epimerization, we have shown one successful example of peptide coupling using TBTU or COMU[39] via PFR continuous flow.

Experimental Section

General Methods. Flow experiments were performed using a Vapourtec R4 reactor equipped with a T-mixer and the desired reactor volume and a 100 psi back pressure regulator. The Vapourtec R4 reactor was initially equilibrated with exactly the same makeup of the solvent or solvent mixture for each feed line under the reaction temperature. The feed streams were then switched to the reactant/reagent lines with the intended flow rates as shown in the diagrams. The reaction was monitored offline by UPLC during the continuous flow. At the end of the reactant/reagent feed completion, the feed was continued with exactly the same makeup of the solvent or solvent mixture for at least 10 mL. The effluent collected was then worked up and isolated as described. An Agilent SB-CN column (2.1 x 50 mm) with mobile phases A (0.05% TFA in water) and B (acetonitrile). Flow rate 0.65 mL/min, 0 - 2.9 min, ramp from 5% B to 100% B; 2.9 – 3.4 min, stay at 100% B; 3.4 - 3.5 min, ramp to 5% B; 3.5 – 4.0 min, 5% B.

N-(2-((4aR,6S,8aR)-2-benzamido-6-methyl-4,4a,5,6-tetrahydropyrano[3,4-d][1,3]thiazin-8a(8H)-yl)thiazol-4-yl)-5-(difluoromethoxy)picolinamide (**9**): Two feed streams were

prepared as follows: Stream A – amine **8**[35] (10.0 g, 25.7 mmol) was dissolved in a solvent mixture of THF (80 mL) and water (10 mL). Triethylamine (11.1 g, 109 mmol) was added. A homogenous solution was obtained after stirring for 5 min. Stream B – acid **7**[35] (5.22 g, 27.0 mmol) was dissolved in THF (20 mL). Water (40 mL) and 2-Hydroxypyridine N-Oxide (2.86 g, 25.7 mmol) were added, followed by acetone (60 mL). EDC (15.1 g, 77.2 mmol) was subsequently added. The resulting mixture was stirred for 10 min to give a homogeneous solution. Vapourtec reactor R4 with a 10 mL stainless steel coil equipped with a 100 psi back pressure regulator was equilibrated with the exactly the same makeup of the solvent mixture for the respective feed lines. Upon reaching steady temperature and pressure, the reaction system feeds were switched over from solvent lines to reagents lines, and the flow rates were set at 0.148 mL/min (A) and 0.183 mL/min (B) with a target residence time of 30 min. At the end of the run, the feeds were switched over to solvents again until at least 10 mL were pushed out. The effluent was concentrated by rotovap under reduced pressure (75 mmHg, 30 °C), the product crystallized out directly. The slurry was filtered and rinsed with water, then dried in a vacuum oven to give **9** as a white solid (13.2 g, 91.6%). The spectroscopic data is identical to those reported.[35]

(4-aminophenyl)(4-(dimethylamino)piperidin-1-yl)methanone (12): 4-Aminobenzoic Acid (10.0 g, 72.9 mmol), 2-Hydroxypyridine N-oxide (8.20 g, 72.9 mmol) and 4-(Dimethylamino)piperidine (9.84 g, 72.9 mmol) were dissolved in a mixture of THF (100 mL) and water (100 mL). 1,3-Diisopropylcarbodiimide (18.8g, 146 mmol, 18.8 g) was added (Note: In batch reaction kinetics study, there was no meaningful reaction at ambient temperature, therefore it was decided to feed the reaction a single stream in the flow demonstration run). The reaction mixture was a homogenous solution at RT. After the reactor coils were equilibrated the solvent makeup of the reaction under the reaction temperature. The reaction solution was fed at 1 mL/min to a 20 mL coil (with static mixer inserts) followed by a 10 mL standard TFE coil both at 75°C with targeted residence time of 30 min (no BPR was attached). After the run is complete, the run was continued with the solvents for at least 45 mL. The collected effluent was rotorvapped to remove THF under reduced pressure (60°C, 20 mmHg). The resulting slurry was cooled to RT, and filtered to remove the diisopropyl urea as white solid. The aqueous filtrate was pH adjusted to ~11 with 1N NaOH solution, then extracted with BuOH (1 x 10 vol and 1 x 5 vol). The combined BuOH solution was washed with brine solution (1 x

5 vol). The final BuOH phase was concentrated to remove most BuOH, 50 mL of MeCN was added. After stirring for 5 min, a slurry was obtained. It was granulated for 30 min at RT and filtered. The filtered solid was dried in a vacuum oven to give the desired product (12.7 g, 70%). The spectroscopic data were consistent with those of an authentic sample.[36]

Polymixin Analog (15): Two feed streams were prepared as follows: Stream A - tripeptide **13**[37] (1.01 g, 1.47 mmol) and PMBH-Boc3[37] (1.60 g, 1.54 mmol) were dissolved in DCM (10 mL) and DMF (5.3 mL) in the presence of iPr_2NEt (463.8 mg, 2.45 equiv). Stream B - COMU (816 mg, 1.30 equiv) was dissolved in DMF (5.3 mL). Vapourtec R4 was used for the flow run with 1.6 mL glass chip reactor (Uniqsis UQ5102) at RT equipped with 100 psi BPR. The flow rates were set at 0.262 mL/min (A) and 0.082 mL/min (B) with a targeted residence time of 4.6 min. The reaction workup and isolation were the same as reported earlier with 88% yield isolated.[37]

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17. CDI solubility screen in commonly used solvents.

Solvent	*Solubility in volumes (mL per mg CDI)*	*Comments*
Acetone, THF, MeCN	20	This data was collected using serial dilutions at 5, 10, 20, and 40 volumes.
MEK, EtOAc, PrOAc, 2-MeTHF, anisole	40	
MIBK, iPrOAc, BuOAc, iBuOAc, MTBE, t-AmOMe, toluene, heptane	>40	
DCM	10	

DMAc, DMF, DMSO, TFE	5	For example, a value listed as 10 volumes indicates the solubility is between 5 to 10 volumes.
ACN:THF(70:30)	10	
isosorbide dimethyl ether	10	

18. A single stream feed may also be preferred when the whole reaction mixture is thermally stable and shows no sign of reaction.

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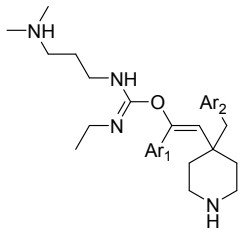
26. DSC onset temperature of HOPO was tested to be 274 °C with a very high thermal potential of -1780 $J \bullet g^{-1}$.

27. This is an arbitrarily picked time limit for a reasonable productivity. It is also possible to keep a longer reaction time if it becomes desirable.

28. The Chemglass pressure relief vials are rated for 150 psi. https://chemglass.com/reaction-vials-pressure-relief, accessed on Nov. 2, 2019.

29. The carbamimidate impurity was believed to be formed between the enolized aryl ketone (**2**, Table 1) with EDC with a proposed structure shown below. The use of a weaker base ($NaHCO_3$) effectively shut down its formation.



30. For EDC stability and $t_{1/2}$ data under aqueous conditions, see: Gilles, M. A.; Hudson, A. Q.; Borders, C. L., Stability of water-soluble carbodiimides in aqueous solution. *Anal. Biochem.* **1990,** *184* (2), 244-248.

31. A focused reaction screen was carried out including the following parameters: solvent (THF, or THF/water), additive (with or without HOPO), base (N-mehtyl morpholine, diisopropylethyamine, 1,4-diazabicyclo[2.2. 2]octane or no base). and coupling reagent (EDC, CDMT, T3P)

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33. Several factors were considered in the stoichiometry of reactants/reagents: complete conversion of the more precious reactant, competing side reactions and

purging factors in the product isolation. When a process is adapted for scale up use, the robustness of the reaction will be further examined including the stoichiometry (and margins of tolerance) of reactants/reagent.

34. Vol is defined as mL per gram of the reactant with the higher mass input. i.e. in entry 3, it is the amine reactant.

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36. Compound **12** is a commercially available material at multi-gram quantities. Also see: Gopalsamy, A.; Shi, M.; Bennett, E. M.; Zask, A.; Curran, K. J.; Venkatesan, A. M. 5,6,7,8-Tetrahydropyrido[4,3-D]pyrimidine compounds as mTOR , PI3 and hSMG-1 kinase inhibitors and their preparation and use in the treatment of diseases. *PCT Int. Appl.* **2010**, WO 2010120991 A1.

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38. For crystalline product only, this is often the case for pharmaceutical intermediates and products, particular in the GMP endgame.

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