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Multistep Continuous-Flow Synthesis in Medicinal Chemistry: Discovery and Preliminary Structure–Activity Relationships of CCR8 Ligands

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Abstract: A three-step continuous-flow synthesis system and its application to the assembly of a new series of chemokine receptor ligands directly from commercial building blocks is reported. No scavenger columns or solvent switches are necessary to recover the desired test compounds, which were obtained in overall yields of 49–94%. The system is modular and flexible, and the individual steps of the sequence can be interchanged with similar outcome, extending the scope of the chemistry. Biological evaluation confirmed activity on the chemokine CCR8 receptor and provided initial structure–activity-relationship (SAR) information for this new ligand series, with the most potent member displaying full agonist activity with single-digit nanomolar potency. To the best of our knowledge, this represents the first published example of efficient use of multistep flow synthesis combined with biological testing and SAR studies in medicinal chemistry.

Keywords: chemokine receptor ligands · flow chemistry · medicinal chemistry · small molecule agonist · structure–activity relationships

Introduction

Organic synthesis by using continuous-flow methods has received a great deal of attention in recent years due to the potential advantages over traditional batch synthesis that this technology has to offer.[1] Some of the more prominent features include precise and rapid heating and cooling, safer handling of exothermic reactions and explosive or toxic reagents, safe and convenient handling of reactions under high pressure, easy implementation of immobilized catalysts, and facile scale-up. The improved safety and more accurate temperature control have made continuous-flow synthesis an attractive option for the manufacture of active pharmaceutical ingredients.[2] Telescoping a multistep synthesis into a single continuous-flow process by coupling several flow reactions in series is one of the most intriguing possibilities, enabling in principle the convenient synthesis of complex products from simple starting materials in a single continuous flow in a fraction of the time required for batch synthesis. Although the initial development of an efficient multistep flow synthesis protocol may require some effort, once developed, such a system allows for multistep library synthesis of screening compounds and follow-up by repeated or scaled-up synthesis of interesting single library compounds without the need for any further development. A number of multistep flow synthesis systems have been reported,[1a,c,2e,3] but the potential of such systems in medicinal chemistry is still unrealized.

Although continuous-flow synthesis offers a number of potential advantages, important challenges remain. Precipitation causing clogging and blockage should be taken into account in the design of flow synthesis systems. Multistep systems present additional important issues that must be addressed. First, different reactions frequently require different solvents, which demands disruption of the continuous stream since there are as yet no satisfactory means of achieving continuous solvent switching. Second, byproducts may interfere with downstream steps. A third significant challenge is the "third-stream problem", that is, timing the introduction of additional reagents downstream, which is complicated by dispersion in the first step(s).[4]

Herein, we report an efficient three-step flow synthesis in which all of these challenges have been overcome by careful selection of the reactions. The system has been designed to synthesize new ligands directed towards the chemokine receptor CCR8, a potential drug target expressed on monocytes, splenocytes, and thymocytes, and implicated in allergic disease.[5] Relatively few exogenous ligands have been described for this receptor.[6] Inspired by known CCR8 ligands (Figure 1), we have designed a three-step continuous-flow

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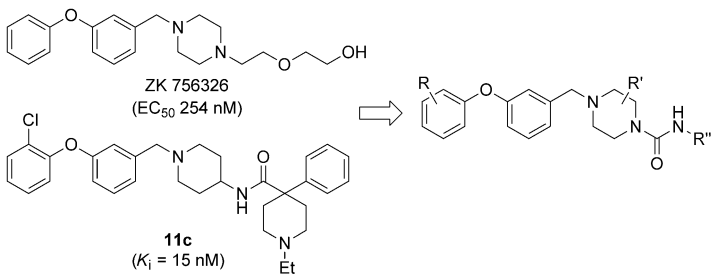


Figure 1. Inspirational CCR8 ligands.

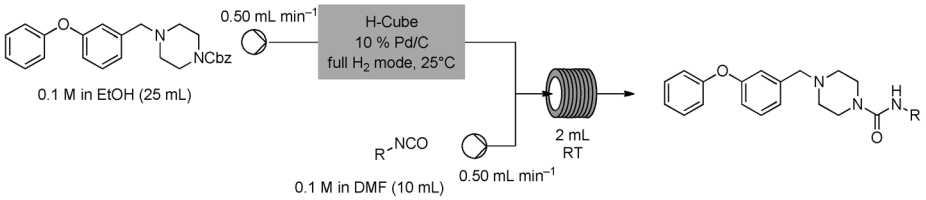
system for the synthesis of related structures incorporating a urea group.[7] In the flow system presented herein, reactions that can be performed in DMF have been chosen to avoid problems related to precipitation and solvent switching. Furthermore, the reactions give either no or unproblematic stoichiometric byproducts, thus solving the second of the aforementioned problems. The "third-stream problem" has been addressed by ensuring a moderate excess of the third building block over an appropriate time period. In the optimized version of the system, all scavenger resins have been eliminated. This results in a functional system capable of assembling three variable building blocks into diverse test compounds in a fraction of the time required for batch synthesis. We also report that the compounds synthesized by this system indeed act as potent CCR8 modulators, and we describe preliminary structure–activity-relationship studies of the 4-benzylpiperazine-1-carboxamide compound series.

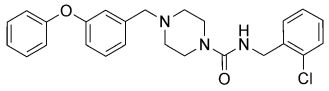
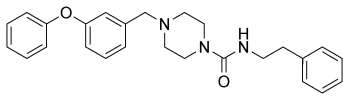
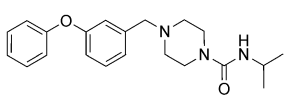
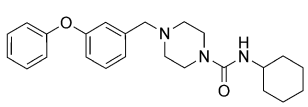
Results and Discussion

Continuous-flow synthesis: Our first-generation system comprised three consecutive steps in a continuous flow: isocyanate addition to a carbobenzyloxy (Cbz)-monoprotected diamine, removal of the protecting group by catalytic hydrogenation, and alkylation of the liberated amine with benzyl bromide or a similar reagent (Scheme 1).[7] To ensure complete conversion, an excess of isocyanate was used in the urea formation, and a polystyrene (PS)-trisamine scavenger column was inserted directly after the reactor to remove the excess. The final N-alkylation step was carried out in a PS-NMM (NMM = *N*-methylmorpholine) column and then the mixture proceeded to a PS-trisamine column to scavenge the excess alkylating reagent. This system demonstrated a significant advantage of flow chemistry: the synthesis of target compounds directly from commercial starting materials in three continuous steps with no handling of intermediates and a capacity of two compounds per day. However, the productivity was hampered by the three columns containing PS-supported scavenger resins. Since the scavenger resins are consumed, they must be replaced or regenerated after the synthesis of every few compounds, which is expensive, requires manual intervention, and lowers the productivity of the system. For a multistep system, the dispersion of reactants and intermediates caused by the scavenger columns is especially problematic. The frequency of resin replacement can be reduced by using larger columns, but this typically results in unacceptable dispersion. Furthermore, the presence of the scavenger columns necessitates long washing cycles between the synthesis of each compound, adding a significant unproductive-time penalty to each synthesis. Notably, the scavenger columns did not eliminate the need for final chromatographic purification of the test compounds.

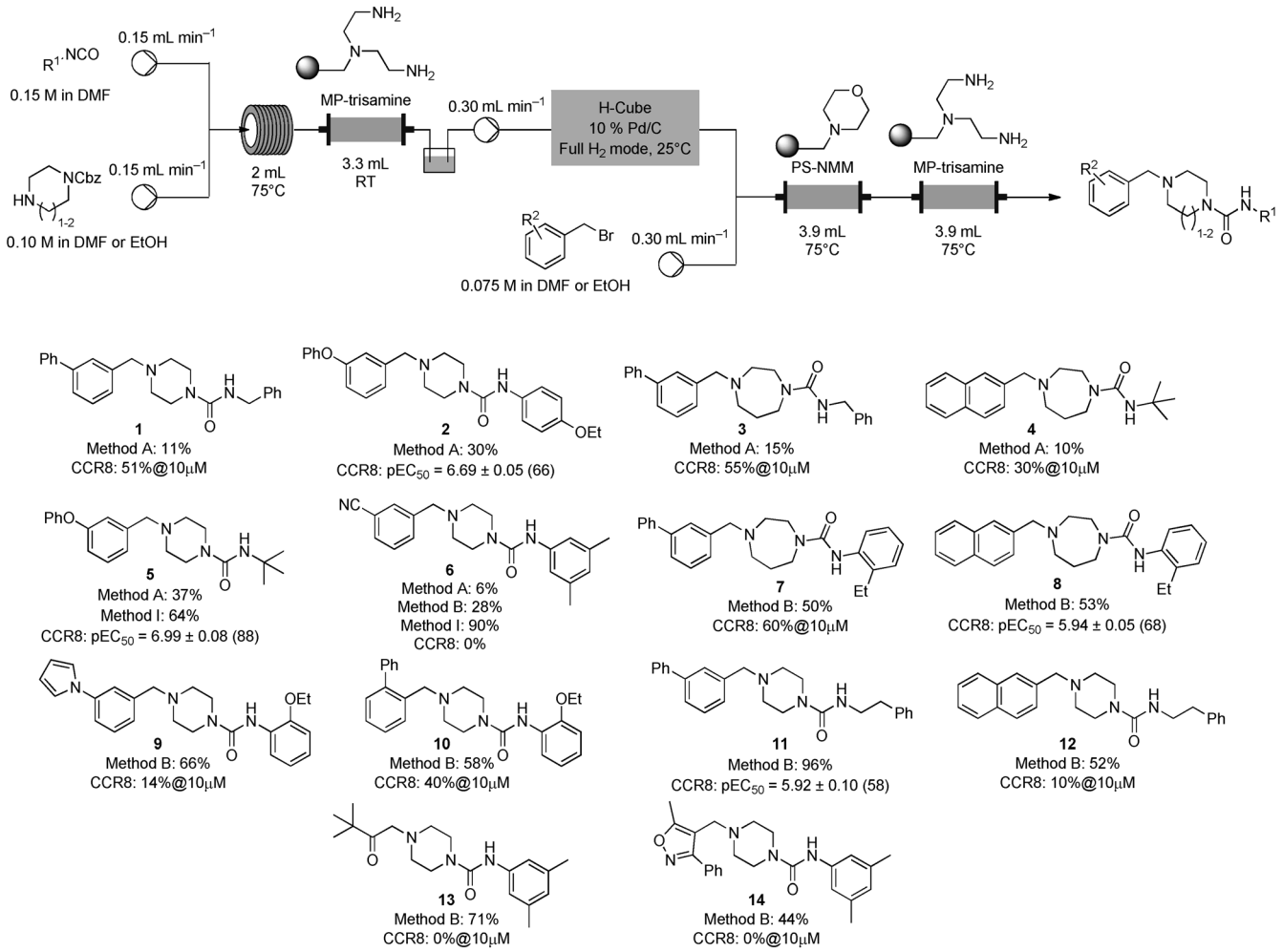
In further optimization of the system, a critical question was whether the benefit of the scavenger columns outweighed their disadvantages. We reasoned that if stoichiometric amounts of reagents were to be used and complete conversion could be achieved, there would be no need for scavengers. A simplified two-step model system was investigated by using a preformed alkylated and Cbz-protected piperazine as the substrate (Table 1). The choice of starting material and reaction sequence was based on the assumption that complete conversion by using stoichiometric amounts of reagents would most likely be easier to achieve for the isocyanate addition and hydrogenolysis steps than for the N-alkyl-

Table 1. Two-step flow system.



Product		Purity [%][a]	pEC_{50} (EC_{50} [nM]; E_{max} [%])[b]
15		99	6.61 ± 0.34 (245; 68)
16		99	7.39 ± 0.10 (41; 62)
17		99	7.46 ± 0.18 (34; 74)
18		94	7.62 ± 0.20 (24; 85)

[a] Purity by ELSD (evaporative light scattering detector), ^{1}H and ^{13}C NMR spectroscopy. [b] Activation of COS-7 cells transfected with CCR8 and promiscuous Gqi4myr protein in an inositol triphosphate-turnover assay; E_{max} is given as a percentage of maximal induced activation by CCL1.



Scheme 1. First-generation three-step flow system. Method A: stock solutions of Cbz-diamine and alkylating agent in ethanol and the required isocyanate in DMF. Method B: all stock solutions in DMF.[7] Method I: see Table 2. Yields are after purification by semi-automatic flash chromatography (Combiflash Companion) and are reproduced from ref. [7]. Activation of COS-7 cells transfected with CCR8 and promiscuous Gqi4myr protein in an inositol triphosphate-turnover assay; E_{max} is given as a percentage of maximal induced activation by CCL1 at 10 μM concentration of test compound.

ation step. The system was constructed by using an H-Cube, with its integral pump feeding Cbz-phenoxybenzylpiperazine through the hydrogenation module and an additional pump feeding the isocyanate solution into a T-piece, where it was mixed with the deprotected piperazine. A steady-state 1:1 stoichiometry between the piperazine and the isocyanate was maintained by using 0.1 M reagent solutions and both pumps running at 0.50 mL min^{-1}, giving a reaction time of 2 min at RT. We were pleased to observe that this simple system facilitated quantitative conversion to the products, and that the compounds produced were of a suitable quality for direct testing without the need for a final purification (Table 1).

We next turned our attention to the N-alkylation step. Based on the results obtained with the two-step system, it seemed likely that the sometimes low to moderate yields and formation of side products seen with the first-generation three-step system were related to this step and possibly to the scavenger columns. Poor conversion might result from a mismatching of concentration–time profiles of the intermediate stream and the alkylating agent stream. Such mismatching could occur as a result of excessive dispersion in the first two steps. The problem of matching the concentration and timing of a third stream to that of a reaction mixture exiting a flow reactor, the concentration–time profile of which may have been changed by dispersion, is a recognized challenge in multistep flow synthesis (the "third-stream problem").[4] One solution to this problem has been the use of rather sophisticated equipment to measure reactant concentrations in-line, for example, by IR or UV detection, to automatically control the flow rate of the third stream.[4a–d] We opted for the more low-tech solution of simply adjusting the concentration–time profile of the third reagent stream to overlap with that of the intermediate stream so that at least a stoichiometric amount of the third reagent would be present at all times. To reduce costs and increase capacity, the PS-NMM base in the column reactor was replaced by a mixture of sodium carbonate and sand. We found that feeding a threefold excess of the alkylating agent relative to the amine/isocyanate solution of the first step with a 40 min

delay ensured that the alkylating agent was in excess at all times and resulted in complete conversion (at the same concentration, 0.050 M, and flow rate, 0.20 mL min^{-1}, at 75 °C with a residence time of 12.5 min). Since a final purification was necessary under any circumstances and excess alkylating agent could thus be easily removed, the second PS-trisamine scavenger column was also removed.

With these improvements implemented, a modified three-step system was configured by using stoichiometric amounts of the two first reagents and with the scavenger columns removed; see Table 2, configuration I. A 0.05 M solution of the starting amine and isocyanate in DMF was pumped at a flow rate of 0.20 mL min^{-1} through a 5 mL tube reactor at 100 °C, from which it proceeded to the H-Cube (full H_2 mode, 80 °C, catalyst cartridge: 10% Pd/C). The output stream from the H-Cube was fed into a gas–liquid separator to vent out excess hydrogen gas and carbon dioxide. The stream containing the deprotected piperazine was combined with a solution of the alkylating agent (both pumped at 0.20 mL min^{-1}) by using a T-piece and the mixture was fed through the sodium carbonate/sand column at 75 °C with a residence time of 12.5 min. We were delighted to observe that when the synthesis of **6** was performed with the improved system (Table 2, configuration I), a 90% yield was obtained, as compared with 6 or 28% yields with the initial system (Scheme 1). The use of unsubstituted 3-phenoxybenzyl bromide, Cbz-piperazine, and benzyl isocyanate gave a 94% overall yield (**19**, Table 2). The yield dropped to 49% with homopiperazine (**20**, Table 2), but increased to 85% and above when reverting to Cbz-piperazine and introducing small substituents on the isocyanate or the alkylating agent (**21**, **22**, Table 2). Substituents on the piperazine ring introduce steric hindrance and can potentially interfere with both the urea formation and the alkylation step. However, methyl groups in both positions were well tolerated, resulting in 62–89% yields of the respective products (**23**, **24**, **27**, **29**, **30**, Table 2). Gratifyingly, piperazines with the larger phenyl substituent also performed well, giving 57–66% yields (**25**, **26**, Table 2). The use of 4-(Cbz-amino)piperidine instead of Cbz-piperazines as the central building block resulted in 50–82% yields (**31**, **32**, Table 2). Compounds **29**–**33** were produced with configuration II, see below.

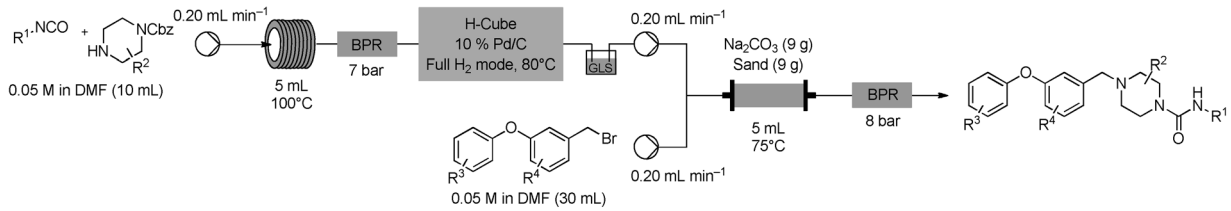
It is noteworthy that the above results were obtained with a 1:1 ratio of the Cbz-diamine and isocyanate building blocks, with only the alkylating agent in the third step being used in excess (3 equiv). Removal of the scavenger resins drastically reduced the cost and down time associated with their periodic replacement, and did not complicate the final purification (except in the case of compound **33**; see below). Another improvement was achieved by taking dispersion into account and compensating for it in the third step as described above. No effort to control dispersion was made in the first-generation system, which resulted in significant portions of starting materials being wasted in the leading and trailing edges of the main cut. The yields reported for our first-generation system (Scheme 1) are based on the product collected over a typical period of 10 min at steady-state operation, whereas the yields from the second-generation system (Table 2) are based on the total amounts injected. The yields reported in Tables 1 and 2 are therefore not directly comparable.

One remaining limitation that we sought to address is the need for the urea substituent to withstand catalytic hydrogenation with a Pd/C catalyst. Under these conditions, aromatic halides and other readily reduced functional groups will not survive. Reversing the sequence of the steps, that is, placing the isocyanate addition after the H-Cube, would provide a solution to this problem. This should permit the presence of reduction-sensitive functionalities in either R^1 or R^2 (but not in both at the same time) depending on the order of the steps. Performing the alkylation step first implies formation of HBr early in the sequence, with a risk of poor conversion and precipitation downstream in the case of incomplete scavenging of this acid. To investigate the feasibility of a reversed system, we swapped the tube reactor and sodium carbonate/sand column along with the corresponding reagents (Table 2, configuration II). As before, the first step was performed by using equimolar amounts of reagents whereas for the third step a threefold excess of isocyanate was used. Employing the same flow rates as in the original system, the residence time in the alkylation reactor was doubled while the residence time in the isocyanate addition reactor was halved due to the third stream. We were pleased to find that the reversed version of the system worked just as well as the original sequence (compare, for example, **21** with **33** in Table 2). The unsubstituted 3-phenylbenzyl and piperazine building blocks performed well with 4-chlorobenzyl isocyanate, but the lack of a final PS-trisamine scavenger column prevented direct chromatographic separation of excess isocyanate from the final product in one case, that of compound **33**. However, this issue was solved by collecting the final product in a flask containing diethylenetriamine (DTA), thus converting excess isocyanate into a polar urea that was readily removed in the final chromatography.

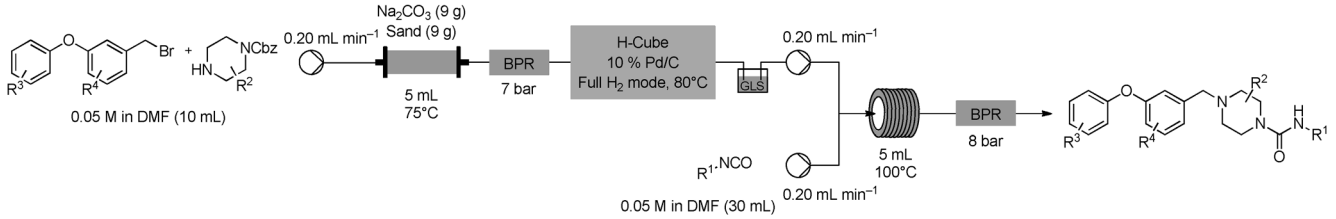
Biological testing: The compounds were tested in an inositol phosphate turnover assay (IP_3 assay) in COS-7 cells transiently transfected with human CCR8. Screening of the 4-benzylpiperazine-1-carboxamide compounds from the first-generation system resulted in the identification of several active compounds serving as partial or full agonists (Scheme 1). In agreement with the inspirational structures (Figure 1),[6a,b] a western 3-phenoxyphenyl moiety (**2** and **5**) afforded the most potent compounds with EC_{50} around 100 nM, whereas 3-biphenyl (**1**, **3**, **7**, and **11**) and 2-naphthyl (compounds **4**, **8**, and **12**) compounds were also active with EC_{50} values of the most potent compounds (**8** and **11**) around 1 μM. The 3-biphenyl/2-naphthyl pairs **7**/**8** and **11**/**12** demonstrate that preference for either group depends on the rest of the molecule. Simple substituted benzyl (**6**), 2-biphenylmethyl (**10**), non-aromatic (**13**), or heteroaromatic (**9**, **14**) groups that deviated much from this pattern were mostly inactive, although it cannot be excluded that the eastern 3,5-dimethylphenyl or

Table 2. Improved three-step flow system.[a]

Configuration I



Configuration II



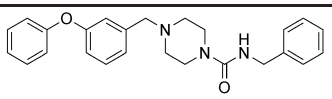
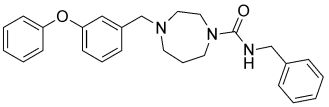
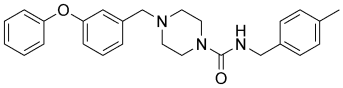
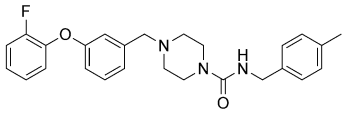
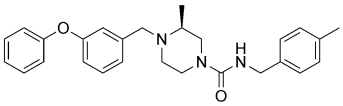
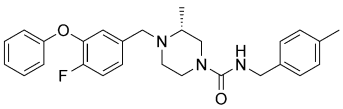
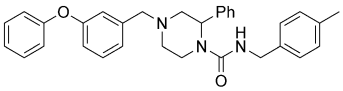
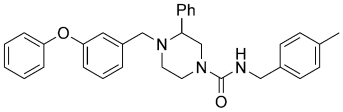
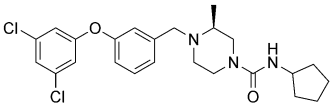
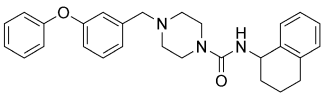
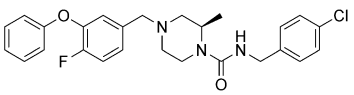
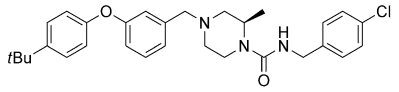
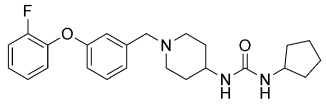
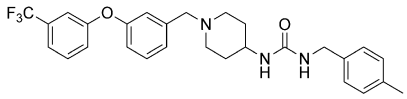
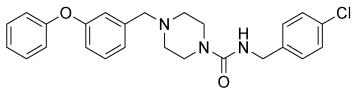
Product		Method	Yield [%]	pEC_{50} (EC_{50} [nM]; E_{max} [%])[b]
19		I	94	7.10 ± 0.21 (80; 92)
20		I	49	6.37 ± 0.06 (422; 88)
21		I	85	6.13 ± 0.02 (743; 81)
22		I	92	7.54 ± 0.09 (29; 38)
23		I	62	6.94 ± 0.03 (114; 46)
24		I	87	5.86 ± 0.16 (1370; 48)
25		I	66	5.41 ± 0.32 (3870; 10)
26		I	57	4 %[b]
27		I	62	6.85 ± 0.63 (142; 52)
28		I	84	8.46 ± 0.25 (3; 60)
29		II	79	7.44 ± 0.42 (37; 32)

Table 2. (Continued)

Product		Method	Yield [%]	pEC_{50} (EC_{50} [nM]; E_{max} [%])[b]
30		II	89	1 %[b]
31		II	50	6.69 ± 0.12 (203; 78)
32		II	82	7.52 ± 0.07 (30; 81)
33		II/DTA[c]	74	6.34 ± 0.13 (462; 83)

[a] Method I refers to synthesis by the general procedure for configuration I. Method II refers to synthesis by the general procedure for configuration II. BPR = back-pressure regulator; GLS = gas–liquid separator. [b] Activation of 10 μM concentration. [c] DTA = diethylenetriamine.

2-ethoxyphenyl substituents also contributed to the low activity. The eastern part of the molecule could in general accommodate a broader variety of structures, with the *N*′-urea substituents of the most active compounds comprising substituted phenyl (**2**, **8**), phenylethyl (**11**), and aliphatic (**5**) groups.

The favored 3-phenoxybenzylpiperazine was chosen as the substrate for the exploratory two-step system (Table 1), as this scaffold was expected to be active by comparison with the inspirational compounds. Appending the 2-chlorobenzylurea (**15**) produced a CCR8 agonist with an EC_{50} of 245 nM (Table 1). Ureas substituted with phenylethyl (**16**), isopropyl (**17**), or cyclohexyl (**18**) group exhibited potencies that were further increased by an order of magnitude.

The compounds synthesized by the optimized three-step system were also mostly based on the 3-phenoxybenzylpiperazine scaffold. Compared to the unsubstituted *N*-benzylpiperazine **19**, the corresponding homopiperazine **20** proved to be less potent. The same was true for the products with a *para*-methyl- (**21**) or *para*-chloro-substituted (**33**) eastern *N*-benzyl moiety. However, this information was not available prior to the synthesis of the remaining analogues, most of which bear an eastern *N*-benzyl group with a *para*-methyl or *para*-chloro substituent. An *ortho*-fluoro substituent on the western terminal phenoxy analogue (**22**) resulted in increased potency and a reduction in efficacy to 38 % relative to the endogenous agonist CCL1. An (*S*)-methyl substituent on the piperazine ring of **21** flanking the phenoxybenzyl moiety (**23**) also increased the potency to the level seen for the unsubstituted analogue **19**. A corresponding methyl substituent in combination with western 3,5-dichlorophenoxy and eastern *N*-cyclopentyl moieties (**27**) resulted in similar activity. Adding a *para*-fluoro substituent to the western benzyl group of **21** in combination with an (*R*)-methyl group flanking the phenoxybenzyl moiety of the piperazine (**24**) resulted in substantial reductions in both potency and efficacy, whereas an (*R*)-methyl group flanking the urea moiety in combination with an eastern *para*-chloro substituent (**29**) increased the potency (EC_{50}) to 37 nM. A *tert*-butyl substituent at the *para*-position of the western phenoxy moiety (**30**) resulted in a complete loss of activity. The larger phenyl substituent on the piperazine ring reduced the potency tenfold when flanking the urea moiety (**25**), and resulted in almost complete loss of activity even at a concentration of 10 μM when flanking the phenoxybenzyl moiety (**26**). Unsubstituted 3-phenoxybenzylpiperazines with western *N*-benzyl (**19**) and *N*-cyclohexyl (**18**) substituents exhibited potent activity. Gratifyingly, combining the two in **28** resulted in a highly potent CCR8 agonist with an EC_{50} of 3 nM, the highest potency observed for any of the tested compounds.

The central 4-aminopiperidine moiety occurs in related CCR8 ligands (e.g., **11c** in Figure 1), and the mono-Cbz-protected derivative was confirmed to be a successful substrate in the flow system. Compound **31**, and more particularly **32**, were among the most potent, but did not rival **28**.

Conclusion

Multistep flow-synthesis systems show great promise in medicinal chemistry, but their full potential has not yet been realized. This is likely due to the challenges related to precipitation of intermediates or products, the need for different solvents for different reactions, interference of byproducts or excess reagents with downstream steps, and timing of reagent addition in downstream steps. In this report, we have shown how these limitations can be overcome in an efficient and inexpensive manner. By combining reactions that do not produce byproducts likely to be problematic in downstream steps and that can all be performed in DMF, we have devised an efficient three-step continuous-flow system for the synthesis of diverse test compounds from commercial building blocks. In the initial version of the system, three polystyrene-based scavenger columns were used to remove excess reagents and to scavenge acid produced in the alkylation step. This, however, led to significant problems with

dispersion and feeding of the third building block, in addition to the need to replace the resin between every few reactions. The optimized system does not contain any scavenger resins; instead, an Na_2CO_3/sand column is used, with adjusted stoichiometry of the reagents. This resulted in a system for the practical and efficient synthesis of ligand analogues in generally excellent yields over the three steps. The substrate scope of the system has been demonstrated to be broad for all three building blocks. For example, the application of 4-(Cbz-amino)piperidine in place of Cbz-piperazine allowed access to structures closely related to those of previously reported CCR8 ligands. Testing on CCR8 established that the 3-phenoxybenzylpiperazine scaffold generally displayed the highest potency. Various small substituents were generally well tolerated, but only in a few cases produced substantially increased potency. A larger degree of variation was accommodated in the eastern part of the compounds. Fusing the eastern parts of two potent compounds led to the identification of the most potent *N*-tetraline **28**, a single-digit nanomolar CCR8 agonist. The multistep flow system has thus been demonstrated as an efficient tool in the optimization of receptor ligands and implies drastic reductions in both time and labor compared to batch synthesis after implementation. To the best of our knowledge, this represents the first example of efficient use of a multistep flow system for the synthesis of test compounds in the optimization process of an active medicinal chemistry project.

Experimental Section

General procedure for the two-step flow synthesis: Benzyl 4-(3-phenoxybenzyl)piperazine-1-carboxylate (2.5 mmol) was dissolved in EtOH (25 mL). The solution was pumped at a flow rate of 0.50 mL min^{-1} through an H-Cube (catalyst: 10% Pd/C, 30×4 mm cartridge, full H_2 mode, 25 °C). The required isocyanate (1.0 mmol) was dissolved in DMF (10 mL) and pumped by a second pump (0.50 mL min^{-1}) to mix with the intermediate from the H-Cube. The mixture continued through a stainless steel coiled reactor (2 mL, RT). The product was collected, diluted with water (20 mL), and extracted with EtOAc (3×15 mL). The combined extracts were dried over $MgSO_4$, filtered, and the solvent was evaporated under reduced pressure.

4-(3-Phenoxybenzyl)piperazine-1-carboxylic acid cyclohexylamide (18): The title compound was prepared according to the general procedure for the two-step flow synthesis by using cyclohexane isocyanate (126.1 mg, 1.01 mmol). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.29–7.23 (m, 2H), 7.19 (t, J = 7.8 Hz, 1H), 7.05–7.00 (m, 1H), 6.98 (d, J = 7.5 Hz, 1H), 6.96–6.89 (m, 3H), 6.83–6.78 (m, 1H), 4.54 (d, J = 7.3 Hz, 1H), 3.59–3.50 (m, 1H), 3.41 (s, 2H), 3.30–3.24 (m, 4H), 2.37–2.31 (m, 4H), 1.89–1.81 (m, 2H), 1.67–1.58 (m, 2H), 1.57–1.49 (m, 1H), 1.34–1.21 (m, 2H), 1.10–0.97 ppm (m, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 157.09, 157.06, 140.00, 129.62, 129.41, 123.78, 123.08, 119.27, 118.63, 117.40, 77.37, 77.16, 76.95, 62.43, 52.59, 49.34, 43.60, 33.79, 25.57, 25.05 ppm; HRMS: m/z calcd for $C_{24}H_{32}N_3O_2$ [M+H]: 394.2489; found: 394.2489.

General procedure for the three-step flow synthesis (configuration I): The required Cbz-protected diamine (0.50 mmol) and isocyanate (0.50 mmol) were dissolved in DMF (10.0 mL) in a pre-dried flask under argon. The solution was pumped at a flow rate of 0.20 mL min^{-1} through a coiled reactor (PFA, 5 mL, 100 °C) followed by an H-Cube (catalyst: 10% Pd/C, 30×4 mm cartridge, full H_2 mode, 80 °C). The product was collected in a flask (5 mL) to release excess H_2 and from there passed on by a second pump (0.20 mL min^{-1}). The alkylating agent (1.5 mmol) was dissolved in DMF (30 mL) and pumped by a third pump (0.20 mL min^{-1}, started 40 min after the first pump) to mix with the intermediate from the second pump. The mixture continued through a glass column filled with Na_2CO_3/sand (9+9 g, 5 mL, 75 °C). The solution was collected, the solvent was evaporated under reduced pressure, and the product was purified by column chromatography using a semi-automatic Combiflash system (24 g silica; eluent gradient: heptane/EtOAc).

4-(3-Phenoxybenzyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)piperazine-1-carboxamide (28): The title compound was prepared according to the general procedure (configuration I) from benzyl piperazine-1-carboxylate (109.6 mg, 0.497 mmol), 1-isocyanato-1,2,3,4-tetrahydronaphthalene (87.5 mg, 0.505 mmol), and 1-(bromomethyl)-3-phenoxybenzene (393.9 mg, 1.50 mmol). It was obtained in a yield of 185.6 mg (84%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.36 (t, J = 7.2 Hz, 3H), 7.30 (dd, J = 13.1, 5.1 Hz, 1H), 7.21–7.16 (m, 2H), 7.12 (dt, J = 11.2, 7.3 Hz, 3H), 7.08–7.01 (m, 3H), 6.93 (d, J = 8.1 Hz, 1H), 5.15–5.08 (m, 1H), 4.81 (d, J = 8.0 Hz, 1H), 3.53 (s, 2H), 3.43–3.32 (m, 4H), 2.89–2.72 (m, 2H), 2.46 (t, J = 4.7 Hz, 4H), 2.08 (dd, J = 12.8, 8.3 Hz, 1H), 1.85 ppm (d, J = 4.7 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 157.23, 157.20, 157.01, 140.05, 129.71, 129.52, 123.86, 123.18, 119.39, 118.76, 117.54, 62.50, 52.68, 50.68, 43.76, 29.46 ppm; HRMS: m/z calcd for $C_{28}H_{32}N_3O_2$ [M+H]: 442.2489; found: 442.2488.

General procedure for the three-step flow synthesis (configuration II): The Cbz-protected diamine (0.50 mmol) and alkylating agent (0.50 mmol) were dissolved in DMF (10.0 mL). The solution was pumped at a flow rate of 0.20 mL min^{-1} through a glass column filled with Na_2CO_3/sand (9+9 g, 5 mL, 75 °C) followed by an H-Cube (catalyst: 10% Pd/C, 30×4 mm cartridge, full H_2 mode, 80 °C). The product was collected in a flask (5 mL) to release excess H_2 and from there passed on by a second pump (0.20 mL min^{-1}). The isocyanate (1.5 mmol) was dissolved in DMF (30 mL) and pumped by a third pump (0.20 mL min^{-1}, started 40 min after the first pump) to mix with the intermediate from the second pump. The mixture continued through a coiled reactor (PFA, 5 mL, 100 °C). The solution was collected, the solvent was evaporated under reduced pressure, and the product was purified by column chromatography using a semi-automatic Combiflash system (24 g silica; eluent gradient: heptane/EtOAc).

(*R*)-4-{3-[4-(*tert*-Butyl)phenoxy]benzyl}-*N*-(4-chlorobenzyl)-2-methylpiperazine-1-carboxamide (30): The title compound was prepared according to the general procedure (configuration II) from (*R*)-benzyl 2-methylpiperazine-1-carboxylate (116.6 mg, 0.498 mmol), 1-bromomethyl-3-[4-(*tert*-butyl)phenoxy]benzene (162.3 mg, 0.508 mmol), and 4-chlorobenzyl isocyanate (259.8 mg, 1.55 mmol). It was obtained in a yield of 224.5 mg (89%). ^{1}H NMR (500 MHz, DMSO): δ = 7.39 (d, J = 8.6 Hz, 2H), 7.35 (d, J = 8.3 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 7.03 (d, J = 6.7 Hz, 2H), 6.95 (d, J = 8.3 Hz, 3H), 6.89 (d, J = 7.9 Hz, 1H), 4.22 (qd, J = 15.6, 5.7 Hz, 2H), 4.10 (s, 1H), 3.70 (d, J = 12.6 Hz, 1H), 3.53 (d, J = 13.9 Hz, 1H), 3.34 (d, J = 13.7 Hz, 1H), 2.92 (t, J = 11.3 Hz, 1H), 2.74 (d, J = 10.6 Hz, 1H), 2.55 (d, J = 11.0 Hz, 1H), 2.01 (dd, J = 10.8, 2.8 Hz, 1H), 1.92 (t, J = 10.2 Hz, 1H), 1.28 (s, 9H), 1.05 ppm (d, J = 6.5 Hz, 3H); ^{13}C NMR (126 MHz, DMSO): δ = 157.42, 157.04, 153.96, 145.89, 140.64, 140.24, 130.87, 129.66, 128.80, 127.97, 126.63, 122.86, 118.63, 117.28, 116.69, 61.22, 56.91, 52.91, 46.12, 42.80, 38.54, 34.00, 31.21, 15.23 ppm; HRMS: m/z calcd for $C_{30}H_{37}ClN_3O_2$ [M+H]: 506.2569; found: 506.2562.

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[1] a) J. Wegner, S. Ceylan, A. Kirschning, *Adv. Synth. Catal.* **2012**, *354*, 17–57; b) J. Wegner, S. Ceylan, A. Kirschning, *Chem. Commun.* **2011**, *47*, 4583–4592; c) D. Webb, T. F. Jamison, *Chem. Sci.* **2010**, *1*, 675–680; d) C. Wiles, P. Watts, *Chem. Commun.* **2011**, *47*, 6512–6535; e) R. L. Hartman, J. P. McMullen, K. F. Jensen, *Angew. Chem.*

2011, *123*, 7642–7661; *Angew. Chem. Int. Ed.* **2011**, *50*, 7502–7519; f) B. P. Mason, K. E. Price, J. L. Steinbacher, A. R. Bogdan, D. T. McQuade, *Chem. Rev.* **2007**, *107*, 2300–2318; g) L. Lengyel, V. Gyollai, T. Nagy, G. Dorman, P. Terleczky, V. Hada, K. Nogradi, F. Sebok, L. Urge, D. Ferenc, *Mol. Diversity* **2011**, *15*, 631–638; h) M. York, *Tetrahedron Lett.* **2011**, *52*, 6267–6270; i) A. R. Bogdan, N. W. Sach, *Adv. Synth. Catal.* **2009**, *351*, 849–854; j) P. Watts, C. Wiles, *Org. Biomol. Chem.* **2007**, *5*, 727–732.

[2] a) M. D. Hopkin, I. R. Baxendale, S. V. Ley, *Chem. Commun.* **2010**, *46*, 2450–2452; b) T. L. LaPorte, C. Wang, *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 738–745; c) M. Viviano, T. N. Glasnov, B. Reichart, G. Tekautz, C. O. Kappe, *Org. Process Res. Dev.* **2011**, *15*, 858–870; d) F. Lévesque, P. H. Seeberger, *Angew. Chem.* **2012**, *124*, 1738–1741; *Angew. Chem. Int. Ed.* **2012**, *51*, 1706–1709; e) A. R. Bogdan, S. L. Poe, D. C. Kubis, S. J. Broadwater, D. T. McQuade, *Angew. Chem.* **2009**, *121*, 8699–8702; *Angew. Chem. Int. Ed.* **2009**, *48*, 8547–8550.

[3] a) I. R. Baxendale, J. Deeley, C. M. Griffiths-Jones, S. V. Ley, S. Saaby, G. K. Tranmer, *Chem. Commun.* **2006**, 2566–2568; b) N. Pagano, A. Herath, N. Cosford, *J. Flow Chem.* **2012**, *1*, 28–31; c) W. Shu, L. Pellegatti, M. A. Oberli, S. L. Buchwald, *Angew. Chem.* **2011**, *123*, 10853–10857; *Angew. Chem. Int. Ed.* **2011**, *50*, 10665–10669; d) I. R. Baxendale, S. V. Ley, A. C. Mansfield, C. D. Smith, *Angew. Chem.* **2009**, *121*, 4077–4081; *Angew. Chem. Int. Ed.* **2009**, *48*, 4017–4021; e) H. R. Sahoo, J. G. Kralj, K. F. Jensen, *Angew. Chem.* **2007**, *119*, 5806–5810; *Angew. Chem. Int. Ed.* **2007**, *46*, 5704–5708; f) T. Noël, S. Kuhn, A. J. Musacchio, K. F. Jensen, S. L. Buchwald, *Angew. Chem.* **2011**, *123*, 6065–6068; *Angew. Chem. Int. Ed.* **2011**, *50*, 5943–5946; g) A. Herath, R. Dahl, N. D. P. Cosford, *Org. Lett.* **2010**, *12*, 412–415; h) P. F. Li, S. L. Buchwald, *Angew. Chem.* **2011**, *123*, 6520–6524; *Angew. Chem. Int. Ed.* **2011**, *50*, 6396–6400; i) L. Kupracz, J. Hartwig, J. Wegner, S. Ceylan, A. Kirschning, *Beilstein J. Org. Chem.* **2011**, *7*, 1441–1448; j) T. N. Glasnov, C. O. Kappe, *Adv. Synth. Catal.* **2010**, *352*, 3089–3097; k) M. Brasholz, J. M. Macdonald, S. Saubern, J. H. Ryan, A. B. Holmes, *Chem. Eur. J.* **2010**, *16*, 11471–11480; l) E. Riva, A. Rencurosi, S. Gagliardi, D. Passarella, M. Martinelli, *Chem. Eur. J.* **2011**, *17*, 6221–6226; m) A. Sniady, M. W. Bedore, T. F. Jamison, *Angew. Chem.* **2011**, *123*, 2203–2206; *Angew. Chem. Int. Ed.* **2011**, *50*, 2155–2158.

[4] a) H. Lange, C. F. Carter, M. D. Hopkin, A. Burke, J. G. Goode, I. R. Baxendale, S. V. Ley, *Chem. Sci.* **2011**, *2*, 765–769; b) Z. Z. Qian, I. R. Baxendale, S. V. Ley, *Chem. Eur. J.* **2010**, *16*, 12342–12348; c) C. F. Carter, I. R. Baxendale, M. O'Brien, J. B. J. Pavey, S. V. Ley, *Org. Biomol. Chem.* **2009**, *7*, 4594–4597; d) C. F. Carter, H. Lange, S. V. Ley, I. R. Baxendale, B. Wittkamp, J. G. Goode, N. L. Gaunt, *Org. Process Res. Dev.* **2010**, *14*, 393–404; e) C. J. Smith, C. D. Smith, N. Nikbin, S. V. Ley, I. R. Baxendale, *Org. Biomol. Chem.* **2011**, *9*, 1927–1937.

[5] a) J. E. Pease, R. Horuk, *Expert Opin. Ther. Pat.* **2009**, *19*, 199–221; b) E. Mitsuyama, Y. Kunori, T. Kamimura, O. Kaminuma, *Mini-Rev. Med. Chem.* **2006**, *6*, 463–466; c) S. A. Islam, D. S. Chang, R. A. Colvin, M. H. Byrne, M. L. McCully, B. Moser, S. A. Lira, I. F. Charo, A. D. Luster, *Nat. Immunol.* **2011**, *12*, 167–186.

[6] a) C. A. Haskell, R. Horuk, M. Liang, M. Rosser, L. Dunning, I. Islam, L. Kremer, J. Gutierrez, G. Marquez, C. Martinez, M. J. Biscone, R. W. Doms, S. Ribeiro, *Mol. Pharmacol.* **2006**, *69*, 309–316; b) S. Ghosh, A. Elder, J. Guo, U. Mani, M. Patane, K. Carson, Q. Ye, R. Bennett, S. Chi, T. Jenkins, B. Guan, R. Kolbeck, S. Smith, C. Zhang, G. LaRosa, B. Jaffee, H. Yang, P. Eddy, C. Lu, V. Uttamsingh, R. Horlick, G. Harriman, D. Flynn, *J. Med. Chem.* **2006**, *49*, 2669–2672; c) P. C. Jensen, R. Nygaard, S. Thiele, A. Elder, G. M. Zhu, R. Kolbeck, S. Ghosh, T. W. Schwartz, M. M. Rosenkilde, *Mol. Pharmacol.* **2007**, *72*, 327–340; d) J. Jin, Y. Wang, F. Wang, J. K. Kerns, V. M. Vinader, A. P. Hancock, M. J. Lindon, G. I. Stevenson, D. M. Morrow, P. Rao, C. Nguyen, V. J. Barrett, C. Browning, G. Hartmann, D. P. Andrew, H. M. Sarau, J. J. Foley, A. J. Jurewicz, J. A. Fornwald, A. J. Harker, M. L. Moore, R. A. Rivero, K. E. Belmonte, H. E. Connor, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1722–1725; e) T. J. Jenkins, B. Guan, M. Dai, G. Li, T. E. Lightburn, S. Huang, B. S. Freeze, D. F. Burdi, S. Jacutin-Porte, R. Bennett, W. Chen, C. Minor, S. Ghosh, C. Blackburn, K. M. Gigstad, M. Jones, R. Kolbeck, W. Yin, S. Smith, D. Cardillo, T. D. Ocain, G. C. Harriman, *J. Med. Chem.* **2007**, *50*, 566–584; f) I. Shamovsky, C. de Graaf, L. Alderin, M. Bengtsson, H. k. Bladh, L. Börjesson, S. Connolly, H. J. Dyke, M. van den Heuvel, H. Johansson, B.-G. Josefsson, A. Kristoffersson, T. Linnanen, A. Lisius, R. Männikkö, B. Nordén, S. Price, L. Ripa, D. Rognan, A. Rosendahl, M. Skrinjar, K. Urbahns, *J. Med. Chem.* **2009**, *52*, 7706–7723; g) A. K. Karlsson, K. Walles, H. Bladh, S. Connolly, M. Skrinjar, A. Rosendahl, *Biochem. Biophys. Res. Commun.* **2011**, *407*, 764–771; h) P. Jensen, S. Thiele, A. Steen, A. Elder, R. Kolbeck, S. Ghosh, T. Frimurer, M. Rosenkilde, *Br. J. Pharmacol.* **2012**, *166*, 258–275.

[7] T. P. Petersen, A. Ritzen, T. Ulven, *Org. Lett.* **2009**, *11*, 5134–5137.

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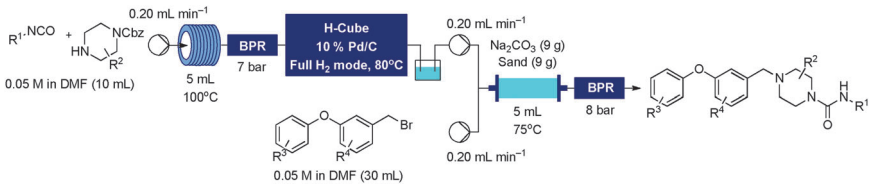

Multistep flow synthesis: Efficient assembly of chemokine receptor ligands in a three-step continuous-flow synthesis system is reported. The system combines three diverse building blocks in high yields and allows modifications in any part of the scaffold (see scheme; BPR = back-pressure regulator). The compounds represent a new series of CCR8 ligands, with the most potent member displaying full agonist activity and single-digit nanomolar potency.

Flow Synthesis

T. P. Petersen, S. Mirsharghi, P. C. Rummel, S. Thiele, M. M. Rosenkilde, A. Ritzén, T. Ulven** ..... ▮▮▮▮–▮▮▮▮

Multistep Continuous-Flow Synthesis in Medicinal Chemistry: Discovery and Preliminary Structure–Activity Relationships of CCR8 Ligands