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A Multistep Continuous-Flow System for Rapid On-Demand Synthesis of Receptor Ligands

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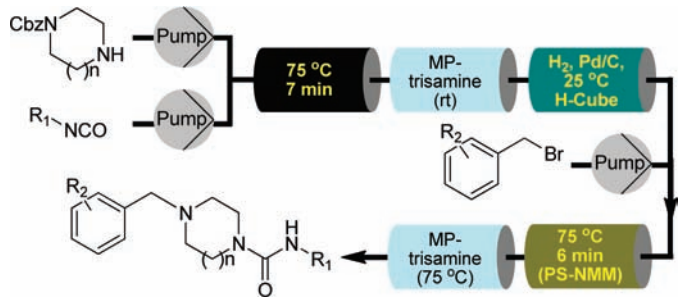
ABSTRACT



A multistep continuous-flow system for synthesis of receptor ligands by assembly of three variable building blocks in a single unbroken flow is described. The sequence consists of three reactions and two scavenger steps, where a Cbz-protected diamine is reacted with an isocyanate, deprotected, and reacted further with an alkylating agent.

Continuous-flow reactions, well established in the process industry, have recently also received increased attention as a tool for small-scale organic synthesis.[1] Micro- and meso-flow reactors in synthesis offer numerous potential advantages over traditional batch synthesis. For example, (1) mixing of reagents in a microflow system can be dramatically more efficient than in a reaction flask, (2) a microflow system offers precise heating and cooling of the reaction flow over a very wide temperature and pressure span, (3) exothermic and run-away reactions usually pose a negligible risk in a flow system due to the small reactor volume, (4) reactions and processes can be coupled in series and in parallel, (5) flow systems are ideal for immobilized catalysts, (6) flow chemistry is ideal for automation, and (7) once a flow system is optimized for a reaction, the reaction is easily rerun with the same or analogous reagents and can be scaled up within reasonable limits by simply running the flow reaction for a longer period of time. A host of diverse reactions have been performed in single-step continuous-flow systems. In the more advanced systems, of which there are relatively few examples, several reactors or processes are coupled in series to enable multistep synthesis in a single continuous flow.

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[‡] H. Lundbeck A/S.

(1) Reviews on flow chemistry: (a) Mason, B. P.; Price, K. E.; Steinbacher, J. L.; Bogdan, A. R.; McQuade, D. T. *Chem. Rev.* **2007**, *107*, 2300–2318. (b) Yoshida, J.; Nagaki, A.; Yamada, T. *Chem.—Eur. J.* **2008**, *14*, 7450–7459. (c) Kirschning, A. *Beilstein J. Org. Chem.* **2009**, *5*, 15. and accompanying reviews. (d) Wiles, C.; Watts, P. *Eur. J. Org. Chem.* **2008**, 1655–1671. (e) Ahmed-Omer, B.; Brandt, J. C.; Wirth, T. *Org. Biomol. Chem.* **2007**, *5*, 733–740. (f) Kirschning, A.; Solodenko, W.; Mennecke, K. *Chem.—Eur. J.* **2006**, *12*, 5972–5990. (g) Jas, G.; Kirschning, A. *Chem.—Eur. J.* **2003**, *9*, 5708–5723. (h) Jahnisch, K.; Hessel, V.; Lowe, H.; Baerns, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 406–446. (i) Belder, D. *Angew. Chem., Int. Ed.* **2009**, *48*, 3736–3737. (j) Geyer, K.; Codee, J. D.; Seeberger, P. H. *Chem.—Eur. J.* **2006**, *12*, 8434–8442. (k) Baxendale, I. R.; Hayward, J. J.; Ley, S. V. *Comb. Chem. High Throughput Screening* **2007**, *10*, 802–836. (l) Fukuyama, T.; Rahman, M. T.; Sato, M.; Ryu, I. *Synlett* **2008**, *2008*, 151–163. (m) Benito-López, F.; Egberink, R. J. M.; Reinhoudt, D. N.; Verboom, W. *Tetrahedron* **2008**, *64*, 10023–10040. (n) Glasnov, T. N.; Kappe, C. O. *Macromol. Rapid Commun.* **2007**, *28*, 395–410.

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Most described systems of this type are designed to carry out a single reaction that requires several operations or perform several simple transformations on a single starting material.[2] Only a small handful of multistep systems have been described which can assemble several building blocks to more complex molecules.[3,4] One outstanding example is the synthesis of oxomaritidine from two simple starting materials in six flow steps, where only one intermediate product handling operation by the user was required in connection with change of solvent.[3a,5] Another recent example describes the conversion of an acid chloride to an isocyanate by a Curtius reaction, and subsequent reaction with alcohols to produce carbamates in a single flow.[3d] This system is relevant to our system, since implementation of a Curtius step would allow acid chlorides rather than isocyanates as starting materials, and thereby expand the number of easily accessible structures. A third notable flow system performs two amide couplings, an asymmetric chlorination and a nucleophilic substitution in a single continuous flow carried out in a system of gravity columns.[4a] The scarcety of reports of multistep continuous-flow syntheses is probably related to the practical challenges that appear when such systems are assembled, which include the frequent need for different solvents in different reactions, accumulation of byproduct and impurities which affect downstream reactions, timing of reactant flows, build-up of high back-pressure over several packed columns in series, precipitation and clogging of tubes and columns, and chromatographic separation of dissolved reagents in packed columns. Another reason may be the high-tech flavor that the field has, with discussions often focusing on lab chips and microstructured devices, giving the impression that considerable investment is necessary to enter the field.

Multistep continuous-flow systems appear to be particularly suitable for medicinal chemistry, where a large number of analogues in the optimization process can be synthesized by the same reaction sequence consisting of a few relatively simple steps, like amide couplings, reductive aminations, catalytic hydrogenations, and metal-catalyzed coupling reactions.[6] Previous flow systems which assemble three building blocks have been described, but these have only been exemplified by the synthesis of a single compound, and the building blocks are in all cases assembled by amide bond formations.[4] Here, we describe the construction of a continuous-flow sequence designed for rapid synthesis of new ligands in medicinal chemistry by combination of three building blocks, and we demonstrate its potential by synthesis of a library of diverse drug-like test compounds. Targeting the chemokine receptor CCR8, which is of interest for treatment of various inflammatory and allergic conditions,[7] our goal was to construct a system for on-demand production of compounds with pharmacophores corresponding to known CCR8 ligands (Figure 1). Three reactions were selected to

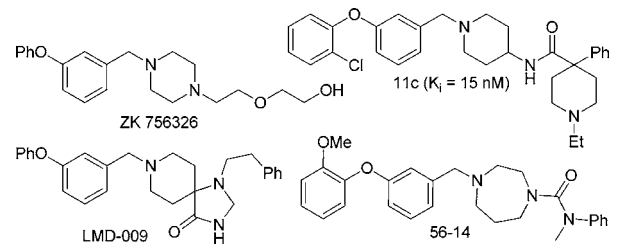

Figure 1. Inspirational chemokine receptor CCR8 ligands.[8]

accomplish this: the reaction of an amine with an isocyanate, a Cbz-deprotection, and alkylation of a secondary amine.

A stock solution stream of a Cbz-protected diamine is mixed with an isocyanate solution stream in a T-piece, and

(2) For example: (a) Lee, C. C.; Sui, G.; Elizarov, A.; Shu, C. J.; Shin, Y. S.; Dooley, A. N.; Huang, J.; Daridon, A.; Wyatt, P.; Stout, D.; Kolb, H. C.; Witte, O. N.; Satyamurthy, N.; Heath, J. R.; Phelps, M. E.; Quake, S. R.; Tseng, H. R. *Science* **2005**, *310*, 1793–1796. (b) Baumann, M.; Baxendale, I. R.; Ley, S. V. *Synlett* **2008**, 2111–2114. (c) Gustafsson, T.; Gilmour, R.; Seeberger, P. H. *Chem. Commun.* **2008**, 3022–3024. (d) van der Linden, J. J. M.; Hilberink, P. W.; Kronenburg, C. M. P.; Kemperman, G. J. *Org. Process Res. Dev.* **2008**, *12*, 911–920. (e) Fukuyama, T.; Kobayashi, M.; Rahman, M. T.; Kamata, N.; Ryu, I. *Org. Lett.* **2008**, *10*, 533–536. (f) Nagaki, A.; Kim, H.; Yoshida, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 7833–7836. (g) Usutani, H.; Tomida, Y.; Nagaki, A.; Okamoto, H.; Nokami, T.; Yoshida, J. *J. Am. Chem. Soc.* **2007**, *129*, 3046–3047.

(3) Multistep flow systems combining two building blocks: (a) Baxendale, I. R.; Deeley, J.; Griffiths-Jones, C. M.; Ley, S. V.; Saaby, S.; Tranmer, G. K. *Chem. Commun.* **2006**, 2566–2568. (b) Baxendale, I. R.; Griffiths-Jones, C. M.; Ley, S. V.; Tranmer, G. K. *Synlett* **2006**, 427–430. (c) Baxendale, I. R.; Ley, S. V.; Mansfield, A. C.; Smith, C. D. *Angew. Chem., Int. Ed.* **2009**, *48*, 4017–4021. (d) Sahoo, H. R.; Kralj, J. G.; Jensen, K. F. *Angew. Chem., Int. Ed.* **2007**, *46*, 5704–5708. (e) Baumann, M.; Baxendale, I. R.; Ley, S. V.; Nikbin, N.; Smith, C. D.; Tierney, J. P. *Org. Biomol. Chem.* **2008**, *6*, 1577–1586. (f) Baumann, M.; Baxendale, I. R.; Ley, S. V.; Nikbin, N.; Smith, C. D. *Org. Biomol. Chem.* **2008**, *6*, 1587–1593. (g) Grant, D.; Dahl, R.; Cosford, N. D. *J. Org. Chem.* **2008**, *73*, 7219–7223. (h) Baxendale, I. R.; Ley, S. V.; Smith, C. D.; Tamborini, L.; Voica, A. F. *J. Comb. Chem.* **2008**, *10*, 851–857. (i) Gustafsson, T.; Ponten, F.; Seeberger, P. H. *Chem. Commun.* **2008**, 1100–1102. (j) Griffiths-Jones, C. M.; Hopkin, M. D.; Jonsson, D.; Ley, S. V.; Tapolczay, D. J.; Vickerstaffe, E.; Ladlow, M. *J. Comb. Chem.* **2007**, *9*, 422–430. (k) Hafez, A. M.; Taggi, A. E.; Dudding, T.; Lectka, T. *J. Am. Chem. Soc.* **2001**, *123*, 10853–10859.

(4) Multistep flow systems combining three building blocks: (a) France, S.; Bernstein, D.; Weatherwax, A.; Lectka, T. *Org. Lett.* **2005**, *7*, 3009–3012. (b) Watts, P.; Wiles, C.; Haswell, S. J.; Pombo-Villar, E. *Tetrahedron* **2002**, *58*, 5427–5439. (c) Baxendale, I. R.; Ley, S. V.; Smith, C. D.; Tranmer, G. K. *Chem. Commun.* **2006**, 4835–4837.

(5) Kundig, P. *Science* **2006**, *314*, 430–431.

(6) For examples of flow systems with medicinal chemistry applications, see: (a) Hamper, B. C.; Tesfu, E. *Synlett* **2007**, 2257–2261. (b) Bogdan, A. R.; Sach, N. W. *Adv. Synth. Catal.* **2009**, *351*, 849–854.

(7) (a) Pease, J. E.; Horuk, R. *Expert Opin. Ther. Pat.* **2009**, *19*, 199–221. (b) Mitsuyama, E.; Kunori, Y.; Kamimura, T.; Kaminuma, O. *Mini-Rev. Med. Chem.* **2006**, *6*, 463–466.

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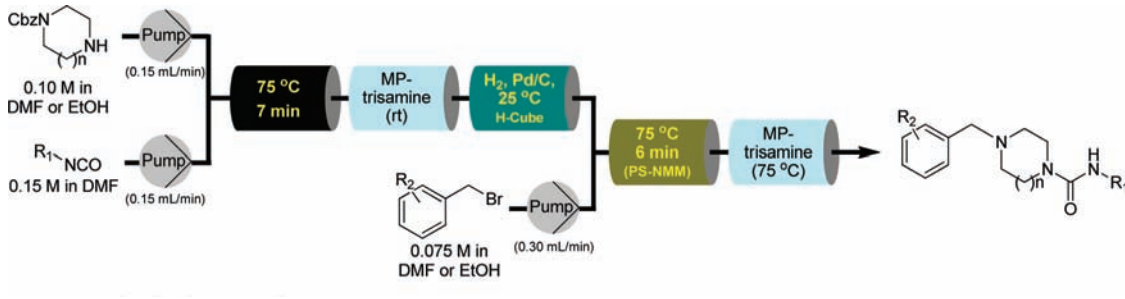
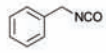
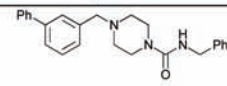
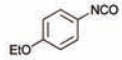
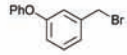
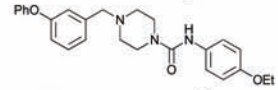
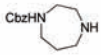
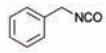
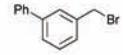
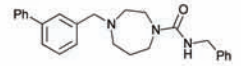
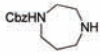
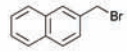
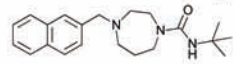
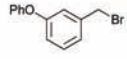
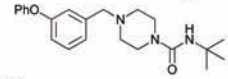
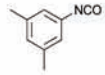
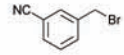
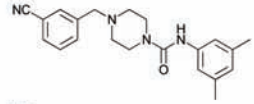
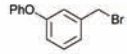
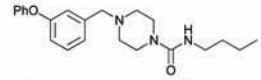
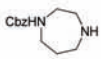
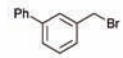
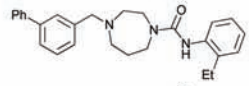
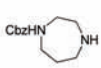
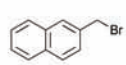
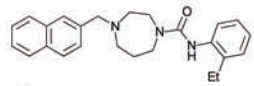
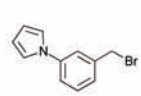
(9) (a) Jones, R. V.; Godorhazy, L.; Varga, N.; Szalay, D.; Urge, L.; Darvas, F. *J. Comb. Chem.* **2006**, *8*, 110–116. (b) Franckevicius, V.; Knudsen, K. R.; Ladlow, M.; Longbottom, D. A.; Ley, S. V. *Synlett* **2006**, 889–892.

(10) (a) Brinkman, J. A.; Cheung, A. W.-H.; Firooznia, F.; Guertin, K. R.; Marcopulos, N.; Qi, L.; Racha, J. K.; Sarabu, R.; Tan, J.; Tilley, J. W. Patent WO2007134958, 2007. (b) Chen, M.; Claret, E.; Cleve, A.; Davey, D.; Guilford, W.; Khim, S.-K.; Kirkland, T.; Kochanny, M. J.; Liang, A.; Light, D.; Parkinson, J.; Vogel, D.; Wei, G. P.; Ye, B.; Ye, H. US patent US2007155727, 2007. (c) Jikyo, T.; Shudo, K. Patent WO2006028161 , 2006. (d) Campbell, M.; Mason, A. M.; Pinto, I. L.; Pollard, D. R.; Smith, I. E. D. Patent WO2006085108, 2006. (e) Tassoni, E.; Giannessi, F.; Dell' Uomo, N.; Gallo, G.; Conti, R.; Tinti, M. O. Patent WO2007096251, 2007. (f) Ahn, K.; Johnson, D. S.; Fitzgerald, L. R.; Liimatta, M.; Arendse, A.; Stevenson, T.; Lund, E. T.; Nugent, R. A.; Nomanbhoy, T. K.; Alexander, J. P.; Cravatt, B. F. *Biochemistry* **2007**, *46*, 13019–13030.

Table 1. Three-Step Continuous-Flow Synthesizer



entry	Cbz-diamine	isocyanate	alkylating agent	product	method[a]	yield[b]
1					A	6 mg (11%)
2					A	20 mg (30%)
3					A	15 mg (15%)
4					A	9 mg (10%)
5					A	20 mg (37%)
6					A/B	5 mg (6%) / 22 mg (28%)
7					B	16 mg (30%)
8					B	31 mg (50%)
9					B	31 mg (53%)
10					B	40 mg (66%)
11					B	36 mg (58%)
12					B	57 mg (96%)
13					B	29 mg (52%)
14					B	35 mg (71%)
15					B	27 mg (44%)

[a] Method A: Stock solutions of Cbz-diamine and alkylating agent in ethanol. Method B: All stock solutions in DMF. [b] Yields are after purification by semiautomatic flash chromatography (Combiflash Companion).

the mixture stream is heated to 75 °C (Table 1). Excess isocyanate is scavenged in a column with trisamine on macroporous polystyrene (MP-trisamine). The flow continues into an H-Cube hydrogenator, where the Cbz-group is removed by hydrogenation over palladium.[9] The byproducts from this reaction, toluene and carbon dioxide, cause no problems in the downstream processes. In the final step the secondary amine is alkylated by a benzyl bromide or another appropriate alkylating agent in the presence of polystyrene-bound *N*-methylmorpholine. Excess alkylating agent is removed by leading the flow through a final column with MP-trisamine at 75 °C. The outlet flow is concentrated, and the residue is purified semiautomatically in a Combiflash system. To the best of our knowledge, this represents the first examples of tertiary amine synthesis in flow by *N*-alkylation with alkyl halides, as well as the first synthesis of ureas from isocyanates in flow, although examples related to the latter reaction exist.[3d,e]

The H-Cube hydrogenation is known to work well with ethanol, and the system was initially fed with stock solutions of Cbz-diamines and benzyl bromides in this solvent. The flow system was able to produce compounds of good quality in useful amounts, but yields were generally low (Table 1, entries 1−6), partly ascribed to the competing reaction between isocyanates and ethanol. The solvent was therefore changed to DMF in all stock solutions (Table 1, entries 6−15). Fortunately, this worked well with the H-Cube, and the yields generally improved to on average 50−60% over the complete sequence and up to 96% in one example (entry 12). The solvent change also brought other advantages, since DMF is a powerful solvent and eluent for most organic compounds, minimizing problems related to precipitation and clogging of the system and retention on packed columns. The residence time in the optimized system is approximately 45 min. With synthesis proceeding for 10−15 min followed by flushing for 45 min, the system can deliver approximately one crude product per hour.

Once the three-step flow sequence was assembled and optimized, it proved to be a powerful and robust tool for rapid production of diverse compounds. Sequential production with a minimum of effort compared to batch or parallel synthesis contributes to minimizing the turnaround time of the critical design−synthesis−screening cycle. Although the examples in Table 1 are mostly limited to compounds that fit the targeted pharmacophore, the diverse selection of isocyanates and alkylating agents demonstrate that the system is potentially useful for synthesis of highly diverse compounds. The system also has potential applications beyond targeting the CCR8 receptor. For example, ligands for a large number of diverse receptors, enzymes, and ion channels have the *N*-benzyl-(homo)piperazine-urea scaffold, including adenosine A_{2B} antagonists,[10a] leukotriene A_4 hydrolase inhibitors,[10b] serotonin 5-HT_3 antagonists,[10c] nicotinic acid HM74A agonists,[10d] carnitine palmitoyl transferase inhibitors,[10e] and fatty acid amide hydrolase inhibitors,[10f] suggesting that our multistep system can be useful for synthesis and optimization of ligands for vastly diverse targets.

Apart from the H-Cube, our flow system consists entirely of inexpensive and readily available components. It is conveniently assembled and modified, and the modules can be easily rearranged. The implemented reactions were carefully selected, with special attention to any stoichiometric byproducts. Addition reactions are generally preferable, and reactions which produce inert byproducts are also usually good choices. Scavenging of stoichiometric amounts of byproduct is possible, but less economic and practical.

In summary, we have demonstrated the value of multistep flow synthesis in medicinal chemistry with a three-step flow system which conveniently assemble three varied building blocks in a single continuous flow. This represents the first demonstration of robustness and usefulness of such a system by the synthesis of a diverse library of test compounds. An important aim of this report is to demonstrate that the general medicinal chemistry laboratory with only minor investments can construct simple systems for flow synthesis, which can be very effective and flexible tools for rapid synthesis of desired lead analogues in the drug discovery and optimization process.

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Supporting Information Available: Experimental procedures, description of flow equipment, and ^{1}H and ^{13}C NMR of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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