Part II
Bulk and Fine Chemistry

3
Liquid- and Liquid–Liquid-phase Reactions – Aliphatic Substitution Reactions

Paul Watts and Charlotte Wiles

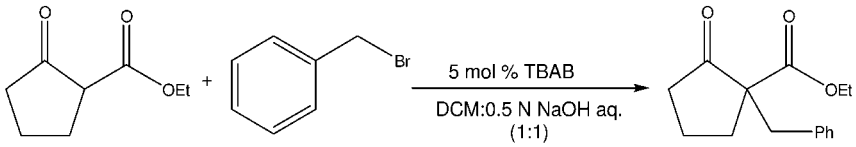
Substitutions are an important class of organic reactions, which proceed through numerous mechanistic pathways to afford an array of synthetically useful compounds. The mechanistic route is largely dependent on the substrate, reagent, leaving group and reaction conditions. With this scope for diversity in mind, this chapter describes a selection of the reactions performed in microfabricated reactors, focusing on the inherent advantages compared with batch techniques.

3.1
Nucleophilic Substitution at Saturated Carbon

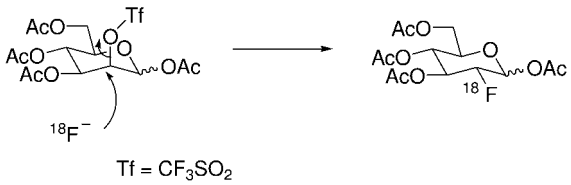
Several groups have investigated the advantages associated with conducting nucleophilic substitution at saturated carbons under similar flow conditions. Ueno *et al.* [1] demonstrated an example of aliphatic nucleophilic substitution conducted under biphasic conditions (channel dimensions 200 μm wide × 100 μm deep × 45.0 cm long), whereby the effect of reagent residence time on the proportion of ethyl 2-oxocyclopentanecarboxylate alkylated with benzyl bromide was investigated. To perform a reaction, a solution of substrate and alkyl halide (0.30 and 0.45 M, respectively) in dichloromethane (DCM) was added from one inlet and an aqueous solution of sodium hydroxide (0.50 N) and tetrabutylammonium bromide (TBAB) (1.5×10^{-2} M) from a second inlet (Scheme 3.1). Maintaining the reactor at room temperature, the authors studied the effect of residence time on the conversion to product, collecting the reaction products in aqueous NH_4Cl, prior to analysis by HPLC. Operating the reactor at a flow rate of 5.9 μL min^{-1} (residence time of 2 min), the authors reported a 75.0% yield of the desired product, representing an increase of 26.0% compared with a stirred batch reactor, over the same reaction period. By reducing the flow rate to 1.2 μL min^{-1}, and hence increasing the residence time to 10 min, the authors reported an increase in conversion to 96.0%. They subsequently investigated the reactions of numerous aliphatic alkyl bromides, along with an α-cyanoketone, reporting in all cases enhanced yields compared with analogous batch reactions; an observation that was attributed to the large interfacial area

Micro Process Engineering, Vol. 2: Devices, Reactions and Applications.
Edited by V. Hessel, A. Renken, J.C. Schouten, and J.-I. Yoshida

ISBN: 978-3-527-31550-5



Scheme 3.1 Biphasic alkylation of ethyl 2-oxocyclopentanecarboxylate under continuous flow.

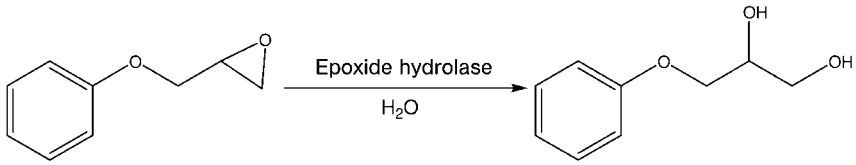


Scheme 3.2 Nucleophilic substitution reaction for the synthesis of 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG).

obtained within the microchannel. Furthermore, the use of a biphasic reaction system is advantageous as the product generated in the reactor can be readily separated from inorganic residues and therefore does not require additional purification prior to use.

2-Deoxy-2-[^{18}F]fluoro-D-glucose (FDG) has been used as a probe in positron emission tomography (PET). FDG has been synthesized by the nucleophilic substitution of mannosyl triflate with [^{18}F]fluoride ion, which is produced by irradiation of [^{18}O]water with 10–18 MeV protons in a cyclotron (Scheme 3.2). Because the half-life of ^{18}F is 110 min, the synthesis of FDG should be carried out very quickly. Therefore, the use of a microflow system was examined for the FDG synthesis and a microfluidic chip reactor was found to be effective for the purpose [2].

In addition to the many chemically catalyzed reactions performed in microfluidic systems, Belder *et al.* [3] investigated the biocatalytic hydrolysis of glycidyl phenyl ether to the respective (±)-diol (Scheme 3.3). The complexity of the transformation was further increased by integrating an on-line separation step, permitting the synthesis, separation and detection of a pair of enantiomers in a single integrated glass device. To achieve this, the authors introduced an aqueous solution of epoxide hydrolase from one inlet and a solution of glycidyl phenyl ether from a second inlet; the reagents subsequently mixed in a meandering channel, to afford the (±)-diol. Chiral electrophoretic separation of the enantiomers followed and the enantiomeric excess was determined via on-chip fluorescence detection. Using this approach, the authors were able to investigate the enantioselectivity of three epoxide hydrolase



Scheme 3.3 Biocatalytic hydrolysis of glycidyl phenyl ether.

mutants, obtaining conversions in the range 22.0–43.0% and enantiomeric excesses (*ees*) of 49.0–95.0%. In addition to the ability to perform biocatalytic transformations in a microfluidic environment, the most important facet of this investigation is the ability to integrate synthesis, separation and detection within a single planar device, an advantage that will no doubt aid in technology transfer to mainstream synthetic laboratories.

3.2
Nucleophilic Substitution at Carbonyl Carbon

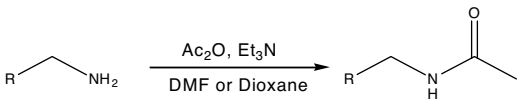
3.2.1
Amide Synthesis

One of the most widely investigated reactions performed in microreactors is the acylation of amines, and this section serves to illustrate a range of the methodologies and reactor types investigated to date.

In 2002, Schwalbe *et al.* [4] demonstrated the synthetic viability of performing aliphatic nucleophilic substitution reactions in a metal microreactor, reporting the high-throughput synthesis of aliphatic amides. Employing a microreaction unit with a total internal volume of 2.0 mL, they investigated the effect of reagent residence time on the acetylation of numerous amines using DMF or dioxane as the reaction solvent. As Table 3.1 illustrates, under optimized reaction conditions excellent yields are obtained in all cases, with reactor throughputs ranging from 4.4 to 68.3 $g\,h^{-1}$.

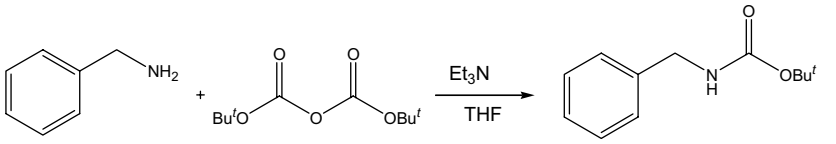
As an extension to this, the same group subsequently investigated the synthesis of *N*-Boc-benzylamine in a CYTOS laboratory system coupled with in-line IR spectroscopic analysis [5]. To perform a reaction, a mixed solution of benzylamine and triethylamine (each 0.8 M in THF) was introduced into the reactor from one inlet and

Table 3.1 A selection of the results obtained for the acylation of aliphatic amines in a metal microreactor (under pressure-driven flow).



Amine	Residence time (min)	Yield (%)	Throughput ($g\,h^{-1}$)
1-Propylamine	13.0	98.0	6.4
1-Hexylamine	4.0	100.0	27.8
1-Heptylamine	1.0	94.0	6.7
Cyclopentylamine	2.0	100.0	49.5
Cyclohexylamine	13.0	89.0	8.2

Table 3.2 Rapid reaction optimization demonstrated for the synthesis of *N*-Boc-benzylamine.



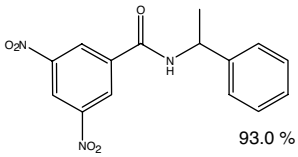
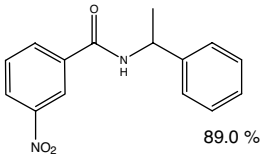
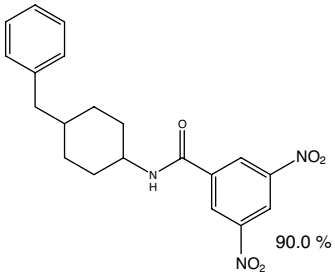
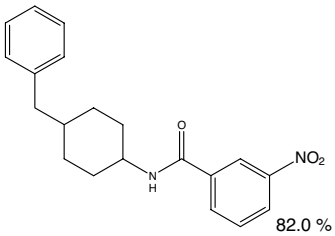
Reactor volume (mL)	Residence time (min)	Yield (%)
2.0	0.5	70.0
17.0	4.3	93.0
32.0	8.0	97.0
47.0	11.8	98.0

a solution of di-*tert*-butyl dicarbonate (0.8 M in THF) from a second inlet. Maintaining an overall flow rate of 2.0 mL min^{-1}, the authors optimized the reaction by altering the reaction volume of the microreaction unit from 2.0 to 47.0 mL, allowing residence times ranging from 0.5 to 11.8 min to be investigated. As summarized in Table 3.2, using this approach, an increase from 70.0 to 98.0% was observed, illustrating the ease and speed with which continuous flow reactions can be optimized.

In the same year, Kitamori and coworkers [6] reported the use of a glass microreactor (channel dimensions 240 μm wide × 60 μm deep), coupled to a series of displacement pumps, in which a 2 × 2 parallel amide synthesis was performed. They found that by introducing the acid chlorides into the reactor as solutions in ethyl acetate (1.0×10^{-2} M) and the amines (1.0×10^{-2} M) in 0.1 M aqueous sodium hydroxide, they were able to perform phase transfer reactions efficiently. With this in mind, the reactions of DL-1-phenylethylamine and 4-amino-1-benzylpiperidine with 3,5-dinitrobenzoyl chloride and 3-nitrobenzoyl chloride, to afford the reaction products illustrated in Table 3.3, were investigated. Operating all reagent streams at 50.0 μL min^{-1}, yields of >80.0% were reported for all four amides and, although problems were encountered with obtaining equal flow distributions at the junctions of fluidic interconnections, no cross-contamination was observed between the parallel reactions. The main advantage of this technique, however, is the biphasic nature of the reaction that ensures the reaction products are retained in the organic phase while any inorganic salts, or by-products, are removed in the aqueous phase. Consequently, through careful optimization of the reactions, all starting materials can be consumed, permitting the isolation of pure amides from a continuously flowing process.

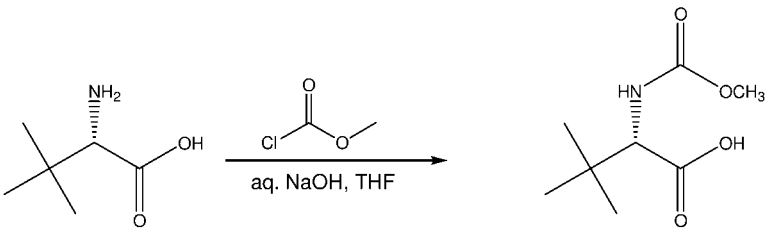
Employing a CYTOS microreaction system, Zhang *et al.* [7] investigated the synthesis of carbamates under continuous flow as a means of rapidly preparing drug-like compounds for early clinical trials. Focusing the investigation on the exothermic reaction between methyl chloroformate and L-*tert*-leucine, in the presence of NaOH, they reported the controlled synthesis of *N*-methoxycarbonyl-L-*tert*-leucine

Table 3.3 Illustration of the 2 × 2 array performed in a parallel, phase transfer microreactor.

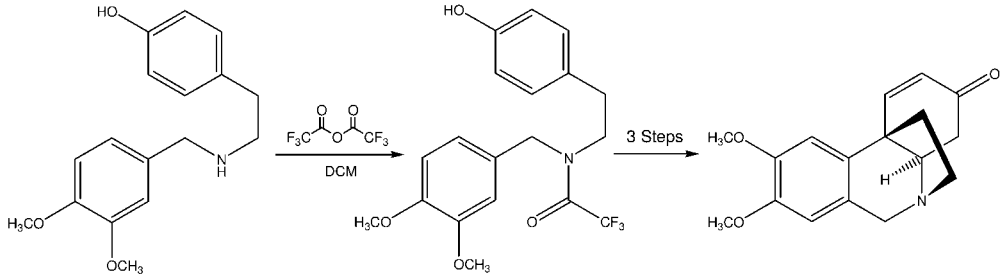
	3,5-Dinitrobenzoyl chloride	3-Nitrobenzoyl chloride
DL-1-Phenylethylamine	 93.0 %	 89.0 %
4-Amino-1-benzylpiperidine	 90.0 %	 82.0 %

(Scheme 3.4). Monitoring the reaction conducted in a traditional reaction set-up by calorimetry, they observed a significant increase in reaction temperature on addition of methyl chloroformate. When conducting such reactions on a large scale, if any heat removal or stirring mechanisms fail, this could lead to thermal runaway. As a means of increasing overall process safety, the authors investigated the ability to perform such reactions in a continuous manner, allowing a controlled reaction temperature to be achieved, removing the risk associated with sudden temperature fluctuations.

Employing a metal microreaction unit, with an overall volume of 35 mL, a premixed solution of aqueous NaOH (4.5 mmol mL^{-1}) and L-*tert*-leucine (1.25 mmol mL^{-1}) was introduced into the reactor at 4.0 mL min^{-1} and a solution of methyl chloroformate in THF (6.0 mmol mL^{-1}) at 1.0 mL min^{-1}; affording a residence time of 7 min. Maintaining the reactor at 35 °C, reactions were performed for 32 min and the reaction products worked-up off-line, affording *N*-methoxycarbonyl-L-*tert*-leucine in 91.0% yield, with a throughput of 112.5 g h^{-1}. The authors attributed the excellent



Scheme 3.4 Controlled synthesis of *N*-methoxycarbonyl-L-*tert*-leucine.

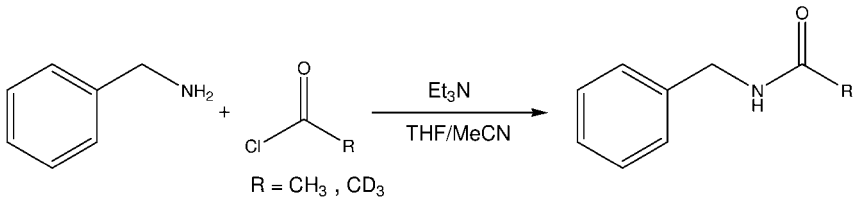


Scheme 3.5 Synthesis of amide, a key reaction intermediate in the synthesis of the naturally occurring alkoxide oxomaritidine.

reaction control to the efficient heat transfer properties obtained within microfluidic systems, along with the ability to deliver methyl chloroformate constantly to the reactor, thus controlling the exothermicity of the reaction.

As part of a recent study into the continuous flow synthesis of an alkaloid natural product, oxomaritidine, Ley and coworkers [8] reported the use of a glass microreactor to perform the trifluoroacetylation of a secondary amine, affording the key reaction intermediate (Scheme 3.5). To perform a reaction, the authors pumped a solution of amine in DCM and trifluoroacetic anhydride (5 equiv.) through the heated (80 °C) microreactor at a flow rate of 35.0 μL min^{-1}, affording a reagent residence time of 3.5 min. Under the aforementioned reaction conditions, near quantitative acylation to afford *N*-(3,4-dimethoxybenzyl)-2,2,2-trifluoro-*N*-[2-(4-hydroxyphenyl) ethyl]acetamide was achieved. Treatment of the crude reaction product with a polymer-supported primary amine followed, sequestering any residual anhydride and trifluoroacetic acid formed, allowing in-line product purification. The resulting amide underwent oxidative phenolic coupling and base-promoted cleavage of the amide bond, allowing spontaneous 1,4-conjugate addition to occur, to afford the desired alkaloid in an overall yield of 40.0% (NMR purity >90.0%).

More recently, Hooper and Watts [9] investigated the incorporation of deuterium labels into an array of small organic compounds via the base-mediated acylation of primary amines. Unlike the previous examples described here, the use of acetic-d_6 anhydride proved undesirable as it led to the generation of acetic-d_3 acid as the by-product, a problem that was circumvented through the use of acetyl-d_3 chloride (Scheme 3.6). To perform a reaction, the authors employed two borosilicate glass microreactors connected in series (reactor 1 = 201 μm wide × 75 μm deep × 2.0 cm



Scheme 3.6 Incorporation of deuterium labels within a microreactor system.

long and reactor 2 = 158 μm wide × 60 μm deep × 1.5 cm long) and manipulated reagents and products using a syringe pump. To reduce development costs, reactions were initially optimized using the unlabelled reagent; substitution with acetyl-d_3 chloride therefore permitted the rapid synthesis of the deuterated analogue.

To allow long-term operation of the fluidic system, the authors found it necessary to employ a mixed solvent system, obtaining a balance between product solubility and minimal degradation of the acylating agents. With this in mind, solutions of benzylamine (0.1 M in MeCN) and triethylamine (0.1 M in MeCN) were introduced into the reactor from separate inlets and mixed prior to the addition of the acyl halide (0.05 M in THF). Reaction products were subsequently collected in a quench solution of aqueous MeCN prior to analysis by HPLC. Using this approach, the optimal reaction conditions for the synthesis of *N*-benzylacetamide (95.3% conversion) were found to be an overall flow rate of 40 μL min^{-1} and a residence time of 2.6 s. To demonstrate the ease of method transfer, the authors substituted acetyl-d_3 chloride, as the acylating agent, reporting the synthesis of the deuterated amide in 98.0% yield (98.2% conversion), with a throughput of 8.95 mg h^{-1}.

Building on the many successful acylations performed within single microfabricated devices, Kitamori and coworkers [10] reported the synthesis of *N*-(3,5-dinitrobenzoyl)-α-phenylethylamine (Table 3.3) in a Pyrex glass pile-up reactor, demonstrating the ability to increase reaction throughput by operating multiple microreactors in parallel. The device consisted of ten glass layers, each containing an etched channel network (reaction channel dimensions 360 μm wide × 120 μm deep × 47.0 cm long), with fluidic control to all 10 microchannels achieved through a single inlet. Employing previously optimized conditions [3], the pile-up reactor was capable of synthesizing the desired amide at a rate of 1.98 g h^{-1}, affording an annual throughput of 1/60 t yr^{-1}.

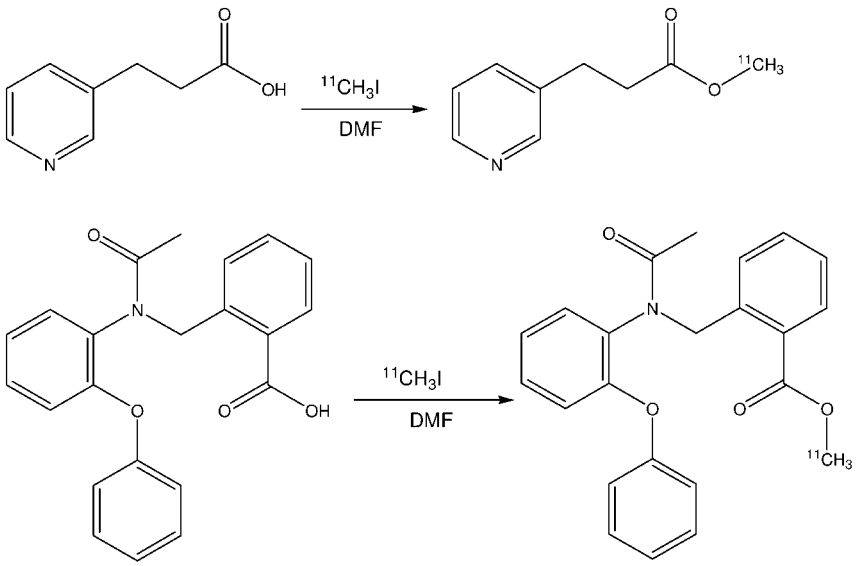
More recently, Schenk *et al.* [11] investigated a means of increasing fluidic stability, when employing a single liquid feed to multiple reaction units; they reported, for the first time, a liquid flow splitter unit capable of delivering multiple reagents, from central reservoirs, to six microdevices [12]. In order to benchmark the technique, the authors performed all reactions under conditions analogous to those employed in typical stirred batch reactors and selected the well-documented synthesis of *N*-butylacetamide as a model.

To perform a parallel reaction, a mixed solution of triethylamine and butylamine (each 0.22 M) in THF was fed into the reactors from one inlet, a constant stream of THF was introduced from a second inlet and a solution of acetyl chloride (0.2 M) in THF from a third inlet. Using this reactor set-up, the solvent stream was maintained at three times the flow rate of the reagent streams and the reaction products were quenched in water at the outlet. The reaction products were subsequently filtered, to remove the ammonium salt, and concentrated *in vacuo* prior to analysis by GC–MS. Under the aforementioned conditions, the authors reported the ability to synthesize *N*-butylacetamide in high purity (93.5%), demonstrating good reproducibility across the six reaction units (RSD = 4.9%). Further work is currently under way to improve the reaction methodology in order to permit continuous operation of the reactor without fouling.

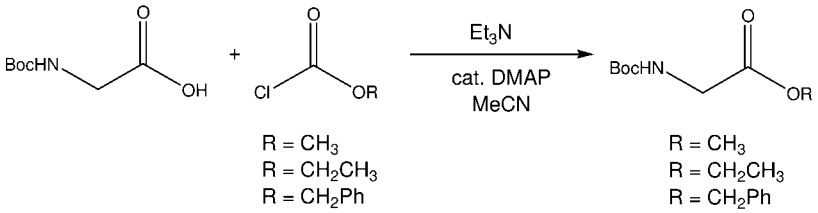
3.2.2
Ester Synthesis

Owing to the reduced reaction times and rapid reaction optimization obtained in microfluidic systems, one of the most application-driven areas of microreaction technology is the miniaturization of synthetic radiochemistry, where time constraints govern the synthetic techniques used. With this in mind, Lu *et al.* [13] demonstrated the use of a borosilicate glass microreactor (channel dimensions 220 μm wide × 60 μm deep × 1.4 cm long) for the rapid synthesis of a series of radiolabelled compounds (Scheme 3.7a). Using pressure-driven flow, a premixed solution of 3-pyridin-3-ylpropionic acid and tetra-*n*-butylammonium hydroxide (TBAOH) (each 0.01 M in DMF) was introduced from inlet A and a solution of $^{11}CH_3I$ (0.01 M in DMF) from inlet B. Reaction products were quenched upon collection in MeCN prior to off-line analysis, and purification, by HPLC. Operating the reactor at a flow rate of 1.0 μL min^{-1} (residence time = 12 s), the respective labelled ester was obtained with a radiochemical yield (RCY) of 88.0%. This provided an overall processing time of 10 min, which is comparable to reaction times currently employed in PET tracer synthesis. Among other examples, a peripheral benzodiazepine receptor (PBR) ligand was also synthesized via the ^{11}C of carboxylic acid. Again, an optimized flow rate of 1.0 μL min^{-1} was employed, affording the desired PBR ligand with an RCY of 65.0% (Scheme 3.7b).

Although many techniques for the synthesis of esters have been reported, due to the extremes of pH and elevated reaction temperatures employed few are mild enough to be performed in electrokinetic systems or on acid-sensitive compounds.



Scheme 3.7 Facile technique for the ^{11}C-methylation of an array of carboxylic acids in a pressure-driven microfluidic device.



Scheme 3.8 Esterification of Boc-glycine in an EOF-driven microreactor.

With this in mind, Haswell and coworkers [14] reported the catalytic conversion of a series of *in situ*-generated mixed anhydrides to esters in an EOF-driven glass microreactor (channel dimensions 350 µm wide × 52 µm deep × 2.5 cm long). To perform a microreaction, solutions of triethylamine (1.00 M), premixed Boc-glycine and methyl chloroformate (each 1.00 M) and 4-dimethylaminopyridine (DMAP) (0.50 M) in anhydrous MeCN were mobilized through the microreactor (385, 417 and 364 $V\,cm^{-1}$) for 20 min and the reaction products were collected at the common ground electrode (0 Volts) in MeCN. Analysis of the resulting reaction mixture by GC–MS confirmed quantitative conversion of the Boc-glycine to the respective methyl ester, with no sign of residual anhydride. The authors subsequently demonstrated the generality of the technique, synthesizing the ethyl and benzyl esters of Boc-glycine (Scheme 3.8), along with the esterification of an array of aromatic carboxylic acids. In all cases, excellent conversions ranging from 91.0 to 100.0% were obtained using this mild synthetic technique.

3.3 Conclusion

Nucleophilic substitution reactions are highly important in organic synthesis. By conducting these within continuous flow reactors, it is observed that the products are generated in higher yield and selectively compared to the corresponding batch reactions. It is evident from the literature examples cited in this chapter that the reactions can be easily scaled within commercial micro reactor systems to enable kg quantities of product to be readily produced.

References

1 M. Ueno, H. Hisamoto, T. Kitamori, S. Kobayashi, Phase-transfer alkylation reactions using micro reactors, *Chem. Commun.* **2003**, 936–937.

2 C.-C. Lee, G. Sui, A. Elizarov, C. J. Shu, Y.-S. Shin, A. N. Dooley, J. Huang, A. Daridon, P. Wyatt, D. Stout, H. C. Kolb, O. N. Witte, N. Satyamurthy, J. R. Heath, M. E. Phelps, S. R. Quake, H.-R. Tseng, Multistep Synthesis of a Radiolabeled Imaging Probe Using Integrated Microfluidics, *Science* **2005**, *310*, 1793–1796.

3 D. Belder, M. Ludwig, L. Wang, M. T. Reetz, Enantioselective catalysis and

analysis on a chip, *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 2463–2466.

4 T. Schwalbe, V. Autze, G. Wille, Chemical synthesis in micro reactors, *Chimia* **2002**, *56*, 636–646.

5 T. Schwalbe, V. Autze, M. Hohmann, W. Stirner, Novel innovation systems for a cellular approach to continuous process chemistry from discovery to market, *Org. Proc. Res. Dev.* **2004**, *8*, 440–454.

6 Y. Kikutani, T. Horucji, K. Uchiyama, J. Hisamoto, M. Tokeshi, T. Kitamori, Glass microchip with three-dimensional microchannel network for 2x2 parallel synthesis, *Lab Chip* **2002**, *2*, 188–192.

7 X. Zhang, S. Stefanick, F. J. Villani, Application of microreactor technology in process development, *Org. Proc. Res. Dev.* **2004**, *8*, 455–460.

8 I. R. Baxendale, J. Deeley, C. M. Griffiths-Jones, S. V. Ley, S. Saaby, G. K. Tranmer, A flow process for the multi-step synthesis of the alkaloid natural product oxomaritidine: a new paradigm for molecular assembly *Chem. Commun.* **2006**, 2566–2568.

9 J. Hooper, P. Watts, Expedient synthesis of deuterium labelled amides within micro reactors, *J. Label. Compd. Radiopharm., in press*, **2007**, JLCR–06–0306.

10 Y. Kikutani, A. Hibara, K. Uchiyama, H. hisamoto, M. Tokeshi, T. Kitamori, Pile-up glass microreactor, *Lab Chip* **2002**, *2*, 193–196.

11 R. Schnek, V. Hessel, C. Hofmann, J. Kiss, H. Lowe, A. Ziogas, Numbering-up of micro devices: a first liquid-flow splitting unit, *Chem. Eng. J.* **2004**, *101*, 421–429.

12 R. Schenk, V. Hessel, C. Hofmann, H. Lowe, F. Schonfeld, Novel liquid flow splitting unit specifically made for numbering-up of liquid/liquid chemical microprocessing, *Chem. Eng. Technol.* **2003**, *26*, 1271–1280.

13 S. Lu, P. Watts, F. T. Chin, J. Hong, J. L. Musachio, E. Briard, V. Pike, Synthesis of 11C- and 18F-labeled carboxylic esters within a hydrodynamically-driven micro reactor, *Lab Chip* **2004**, *6*, 523–525.

14 C. Wiles, P. Watts, S. J. Haswell, E. Pombo-Villar, The preparation and reaction of enolates within micro reactors, *Tetrahedron* **2003**, *59*, 10757–10173.