Efficient Continuous-Flow Benzotriazole Activation and Coupling of Amino Acids

Sofie Seghers[1], Frederik E.A. Van Waes[1], Ana Cukalovic[1], Jean-Christophe M. Monbaliu[2], Jeroen De Visscher[1], Joris W. Thybaut[3], Thomas S.A. Heugebaert[1] and Christian V. Stevens[1]*

[1]SynBioC Research Group, Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium
[2]Center for Integrated Technology and Organic Synthesis, Department of Chemistry, University of Liège, 4000 Liège, Belgium
[3]Laboratory for Chemical Technology, Department of Chemical Engineering and Technical Chemistry, Faculty of Engineering and Architecture, Ghent University, Technologiepark 914, 9052 Gent, Belgium

Received: 23 June 2015; accepted: 21 July 2015

Despite extensive research into peptide synthesis, coupling of amino acids with weakly nucleophilic heterocyclic amines remains a challenge. The need for microwave technology to promote this coupling interferes with the scalability of the process. By applying the microwave-to-flow paradigm, a library of (α-aminoacyl)amino-substituted heterocycles was continuously produced at near quantitative conversions and the reaction was scaled up successfully. Various *N*-Cbz-protected amino acids were activated using BtH/$SOCl_2$ under continuous-flow conditions with excellent yields. Their coupling with heterocyclic amines was accomplished in MeCN–NMP on a preparative scale. However, performing both steps in-line resulted in an inconvenient work-up. Therefore, a two-step approach was taken, isolating the intermediate Bt-activated amino acid via simple filtration. This allows for a solvent switch to DMSO for the coupling reaction which led to excellent conversions for a broad range of substrates.

Keywords: continuous flow, microreactor, amino acids, benzotriazole, amide bond formation

1. Introduction

Peptidomimetics and peptide conjugates are of considerable interest in the design and the optimization of new leads in the pharmaceutical and agrochemical industry [1]. In particular, oligopeptidoylamino-substituted heterocycles have served as model for the development of a wide variety of biologically active molecules, including efflux pump inhibitors [2], peptide deformylase inhibitors [3], cytotoxic compounds [4], inhibitors of apoptosis [5], inhibitors of tumor necrosis factor-α converting enzymes [6], anti-inflammatory [7] and antisickling [8] compounds, pesticides [9], and for the design of biosensors [10].

The synthesis of these oligopeptidoylamino-substituted heterocycles usually requires high temperatures and prolonged reaction times and involves an acylation step using a variety of coupling reagents, e.g., 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) [3], *N*,*N*′-dicyclohexylcarbodiimide (DCC) [6b] or *1H*-Benzotriazole (BtH) [11]. BtH has a pronounced range of benefits: having a rather low toxicity, excellent leaving group characteristics, a high tolerance to other functional groups, as well as being easy to handle, soluble in organic solvents, and efficiently coupling with carboxylic substrates [11, 12]. Moreover, BtH is less expensive than other Bt containing coupling reagents, such as HATU or HBTU.

Katritzky and coworkers proved the strength of the benzotriazole methodology for the coupling of *N*-protected α-aminoacylbenzotriazoles with several heterocyclic amines [13]. This coupling is a much less explored area than peptide synthesis, as the *N*-acylation of such weakly nucleophilic heterocyclic amines is not a straightforward reaction using conventional coupling methods in batch [14]. A library of these (*N*-protected α-aminoacyl)amino heterocyclic compounds was generated by Katritzky et al. after short reaction times under microwave heating (30 min, 70 °C), however, mostly with modest yields at millimole scale. Microwave irradiation has proven to be very efficient in chemical synthesis, when compared to conventional heating [15]. Rapid and efficient heating of the entire reaction mixture is achieved by direct coupling of the microwave energy with the molecule dipoles (microwave dielectric heating). Using sealed vessels, microwave-heated reaction mixtures can reach temperatures above the boiling point of the solvent applied. This can result in a considerable decrease of the required reaction time as compared to the classically heated reactions, which are limited to the solvent reflux temperature. Furthermore, microwave-enhanced processes often showed to be superior to the classical process conditions regarding side-product formation. Microwave heating is, however, mostly unsuitable for scale-up: the heating efficiency rapidly drops when increasing the reaction scale above gram quantities, and many conditions of volume, temperature, and solvent are virtually out of scope. A recent trend to circumvent these hurdles utilizes the translation of microwave to microreactor technology ("microwave-to-flow" paradigm) [16]. Considering the common features of these two technologies – exquisite control of reaction parameters, efficient heating, and possibility of high-temperature and high-pressure conditions – it is possible to perform this transition and open up a route for the scale-up of microwave-heated chemical processes [17].

In view of the aim of our research group to facilitate reactions in flow that are difficult to scale [18], we report the continuous-flow adaptation of the Katritzky Bt-activation of amino acids, and the subsequent amide coupling reaction, leading to increased yields and efficient scale-up (Scheme 1).

Solution-phase peptide bond formation in flow, using β-amino acids as starting materials for the synthesis of β-peptide-based antibiotics, was reported previously by Wiles and Watts [19] and Seeberger et al. [20] Watts et al. made use of electroosmotic flow to mobilize the reagents. *N*-Boc- or *N*-Fmoc-protected amino acids were used as such in a DCC-activated coupling reaction with a second amino acid, protected as the Dmab ester, or were pre-activated as the pentafluorophenyl ester via an EDC coupling reaction in batch, prior to the dipeptide synthesis under continuous-flow conditions [19] Acid fluorides, prepared in batch, were selected as activated forms of *N*-Boc- or *N*-Fmoc-protected amino acids by Seeberger et al. [20].

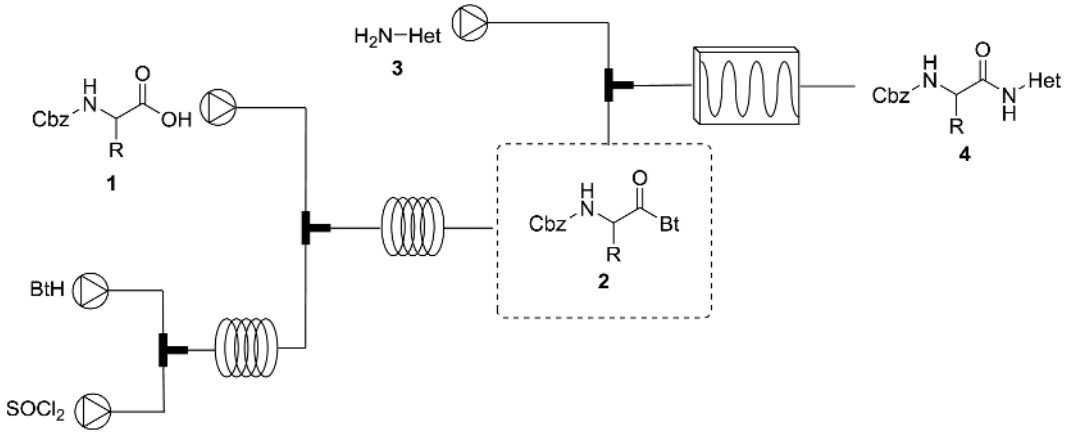
Ley et al. published a synthesis strategy using polymer-supported reagents, also making use of a continuous-flow Bt-activation of *N*-protected amino acids and subsequent coupling with a

* Author for correspondence: chris.stevens@ugent.bechris.stevens@ugent.be

DOI: 10.1556/1846.2015.00029

First published online: 28 November 2015

Scheme 1. Envisioned continuous-flow synthesis of *N*-Cbz-(α-aminoacyl)amino-substituted heterocycles.



second amino acid. Several dipeptides were synthesized as such in good yields (61–83%) [21]. The authors developed an innovative procedure to tackle the problem of the continuous synthesis of peptides utilizing advanced approaches such as immobilized reagents, scavengers, and catch and release protocols. However, this entails the need for expensive polymer supported reagents and extra synthetic steps. Moreover, as the activation and coupling step are separated in time, it is not a fully continuous-flow process. More recently, an efficient solid-phase continuous-flow synthesis of peptides was reported by Fülöp et al., using HATU as coupling reagent [22].

2. Results and Discussion

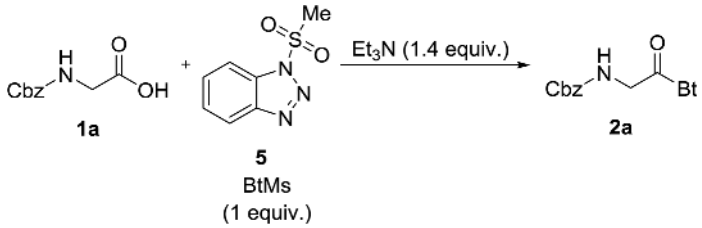
2.1. Continuous Bt-Activation of *N*-Cbz-α-Amino Acids. In the first step, the continuous-flow activation of *N*-Cbz-protected amino acids was studied. Performing both the activation and coupling of amino acids in a continuous-flow mode would facilitate a high throughput and the fully automated synthesis of peptide bonds. Protection of amino acids by a Cbz-group was preferred over other protecting groups, e.g., Boc or Fmoc protection, in view of the higher yields of the Bt-activation in literature [23]. Two Bt-activation methods were evaluated: on the one hand, 1-(methanesulfonyl)benzotriazole (BtMs) was used, and on the other hand, BtS(O)Bt was generated in situ by reaction of thionyl chloride and 1*H*-benzotriazole.

In the first attempt to produce the benzotriazole-activated *N*-Cbz-protected α-amino acids **2** under flow conditions, BtMs **5** was synthesized by reaction of benzotriazole with methanesulfonyl chloride (MsCl) in about 90% isolated yield [24]. In order to transfer the Bt-activation step using BtMs to the continuous-flow concept, different solvents and reaction conditions were evaluated in batch to ensure optimal solubility of all reaction partners. Comparable yields were obtained in dry THF and dry MeCN, while the use of dry NMP led to a complex reaction mixture (CRM), as observed by ^{1}H-NMR analysis (Table 1) [25]. All three solvents resulted in clear reaction mixtures.

The Bt-activation was then evaluated in a continuous-flow mode in dry MeCN, using a small-scale borosilicate glass chip reactor (internal volume [IV]: 10 μL; channel width: 300 μm; channel depth: 60 μm; see Supporting Information [SI] for a full system description). The conversions (as determined by external standardization) for this series of optimization experiments are summarized in Table 2.

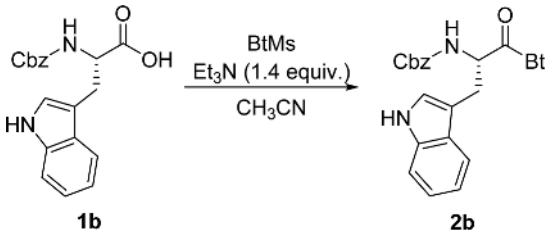
In the first series of flow experiments, the influence of the reaction temperature was evaluated (Table 2, entries 1–4). An increase of the reaction temperature generally resulted in an increase of the conversion although the effect was moderate. At higher temperatures, more free BtH was observed in the liquid chromatography–mass spectrometry (LC–MS) spectrum, indicating a higher degree of degradation. Using an excess of BtMs resulted in a considerable increase of the conversion (Table 2, entries 2 and 5, 3 and 9). The highest conversion was obtained with a residence time of 15 min at 90 °C (Table 2, entry 6). A further increase of the reaction time resulted in a decrease of the conversion (Table 2, entry 7). A lower conversion was obtained at higher reaction temperatures when an excess of BtMs was used (Table 2, entries 6 and 10, 7 and 11), and a maximum of 81% was reached for the activation of Cbz-L-Trp **1b** using 1.25 equivalents of BtMs combined with a residence time of 15 min at 90 °C (Table 2, entry 6). It should be noted that, in all these flow experiments, a limited amount

Table 1. Preliminary batch experiments for the activation with BtMs **5**



Entry	Solvent	*T* (°C)	Yield (%)
1	THF	Δ	18
2	THF (dry)	Δ	57
3	MeCN (dry)	70	53
4	MeCN (dry)	Δ	55
5	NMP (dry)	70	CRM

Table 2. Optimization of the Bt-activation of Cbz-L-Trp **1b** in flow



1b **2b**

Entry	*T* (°C)	BtMs (equiv.)	RT (min)	Conversion (%)[a]
1	80	1	10	46
2	90	1	10	56
3	100	1	10	52
4	120	1	10	63
5	90	1.25	10	74
6	90	1.25	15	81
7	90	1.25	20	67
8	100	1.25	5	56
9	100	1.25	10	74
10	100	1.25	15	63
11	100	1.25	20	58

[a] Towards **2b**, as determined quantitatively by LC–MS with external standard (see SI).

(about 6%) of unidentified side products were detected on LC–MS. However, no correlation between side product formation and the reaction temperature or residence time was observed.

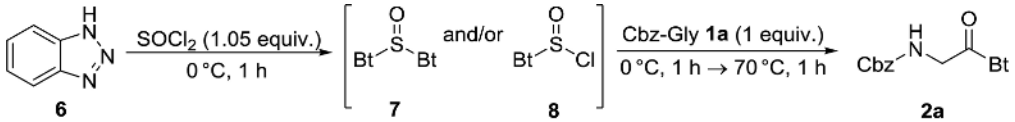
Next, the alternate activation using *in situ* generated BtS(O)Bt was studied. Again, a series of batch experiments with different solvents was performed in order to determine suitable reaction conditions for the continuous-flow experiments (Scheme 2 and Table 3).

The use of THF, CH_2Cl_2, or MeCN resulted in the formation of a turbid reaction mixture, although good isolated yields were obtained (Table 3, entries 1–3, 75–80% yield). Also the use of DMF resulted in the formation of precipitate. In case dry DMSO was used as a solvent in batch, no conversion was detected on LC–MS. A homogeneous reaction mixture without precipitation of the formed salts was observed with NMP. However, isolation of the end product **2a** was difficult due to the high boiling point of NMP (Tb=202 °C). Evaporation of the solvent under mild vacuum was not possible, and distillation of the solvent under high-vacuum conditions (2 mbar) resulted in a complex residue. As such, solvent mixtures THF–NMP and MeCN–NMP were evaluated for the activation (Table 3, entries 7–14). Using these solvent mixtures, clean conversions were observed, and in view of the ease of isolation, the solvent mixture with the lowest amount of NMP was selected. Lower amounts of NMP could be used in MeCN before turbidity was observed (Table 3, entry 10), and a near quantitative conversion (99%) was reached after 10 min at 0 °C.

Having determined the optimal solvent mixture, the Bt-activation of amino acids under continuous-flow conditions was optimized (Scheme 3 and Table 4). These experiments were performed in a PFA tube reactor (ID: 508 μm) at 0 °C.

Changing the residence times in both tube reactors had little influence on the conversion (Table 4, entries 1–3). Comparable conversions were obtained with residence times varying from 2 to 10 min (resp. 1–5 min) for the first (resp. second) tube reactor. A slight increase of the excess of $SOCl_2$ eventually led to an excellent conversion (Table 4, entry 4). Subsequently,

Scheme 2. Activation of Cbz-Gly **1a** with BtH–$SOCl_2$

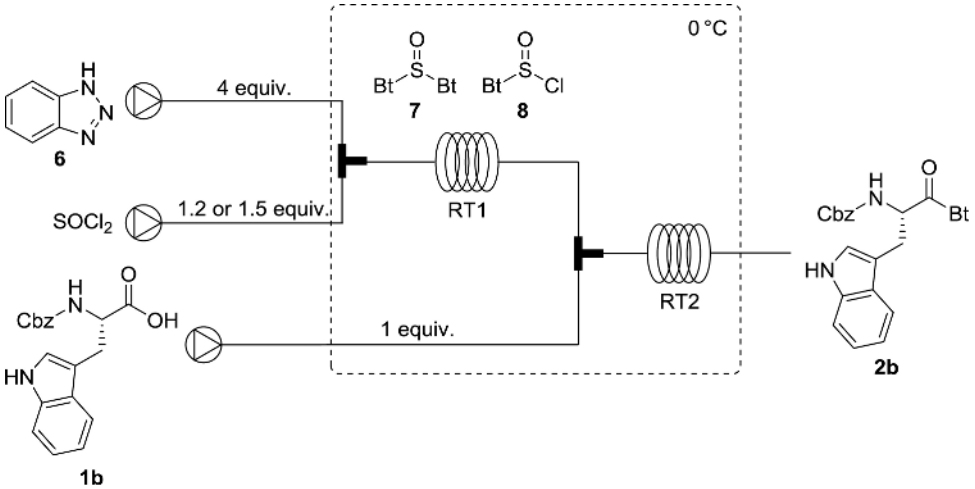


6 (4 equiv.) **7** **8** **2a**

Table 3. Evaluation of different solvents for the activation with BtH–$SOCl_2$

Entry	Substrate	Solvent	Observation	Entry	Substrate	Solvent	Observation
1	**1a**	Dry THF	Turbid	8	**1b**	MeCN–NMP (2:1)	Clear
2	**1a**	Dry CH_2Cl_2	Turbid	9	**1b**	MeCN–NMP (4:1)	Clear
3	**1a**	Dry MeCN	Turbid	10	**1b**	MeCN–NMP (6:1)	Clear
4	**1a**	Dry DMF	Turbid	11	**1b**	MeCN–NMP (9:1)	Turbid
5	**1a**	Dry DMSO	Clear	12	**1b**	THF–NMP (1:1)	Clear
6	**1a**	Dry NMP	Clear	13	**1b**	THF–NMP (2:1)	Clear
7	**1b**	MeCN–NMP (1:1)	Clear	14	**1b**	THF–NMP (4:1)	Turbid

Scheme 3. Activation of Cbz-L-Trp **1b** with BtH–$SOCl_2$ under continuous-flow conditions



6 **7** **8** **1b** **2b**

Table 4. Activation of Cbz-L-Trp **1b** with BtH–$SOCl_2$ under continuous-flow conditions

Entry	RT_1 (min)	RT_2 (min)	$SOCl_2$ (equiv.)	Conversion (%)[a]
1	2	1	1.2	83
2	4	2	1.2	84
3	10	5	1.2	89
4	2	1	1.5	94

[a] Towards **2b**, as determined quantitatively by LC–MS with external standard (see SI).

different amino acids were activated using the continuous-flow procedure. The end products were easily isolated as they precipitated when the reactor output was collected in H_2O. The crude precipitate contained only trace amounts of NMP. In order to remove these solvent traces, it was washed with H_2O, which entailed a small amount of product loss.

For each derivative, a sample was collected over a period of 30 min. Table 5 gives an overview of the crude yield (before the removal of traces of NMP) and purified yield (after complete removal of NMP) for the synthesized derivatives. The obtained isolated yields in batch are also displayed. No racemization was observed for enantiomeric pure starting materials based on comparison of the optical rotation of the synthesized derivatives with literature data [23b, 23c].

In order to monitor the yield during the course of the operation, the continuous-flow Bt-activation of three different derivatives was run for several hours. Samples were collected over a period of one hour, and the isolated yield of each sample was determined (Figure 1). Comparable isolated yields were obtained over a period of 6 h, indicating stable operation.

2.2. Telescoping the Coupling Step with the Continuous Bt-Activation. In the second phase of this study, the alignment of both activation and coupling step of the *N*-Cbz-protected amino acids with heterocyclic amines was considered. Therefore, the coupling of Cbz-D,L-Phe-Bt **2d** and 2-aminopyridine **3a** was evaluated in MeCN–NMP (6:1) (Scheme 4). These experiments were performed on a glass modular mesoreactor system (IV: 6.5 mL; see Supporting Information for a full description). The reaction temperature was raised in comparison to the reported batch procedure in order to speed up the coupling reaction and to limit the residence time.

The first series of experiments with 1.05 equivalents of 2-aminopyridine **3a** resulted in moderate conversions of 47–64% depending on the residence time (Table 6, entries 1–3).

Table 5. Bt-activation of amino acids under continuous-flow conditions

Entry	End product **2**	Crude yield (%)	Purified yield (%)[a]	Calculated throughput (g/day)
1	Cbz-Gly-Bt **2a**	Quantitative	99 (98)	50
2	Cbz-L-Phe-Bt **2c**	99	94 (82)	61
3	Cbz-D,L-Phe-Bt **2d**	99	97 (64)	63
4	Cbz-L-Ala-Bt **2e**	85	80 (67)	42
5	Cbz-D,L-Ala-Bt **2f**	82	76 (61)	40
6	Cbz-L-Met-Bt **2 g**	97	71 (66)	44
7	Cbz-L-Trp-Bt **2b**	91	85 (58)	60
8	Cbz-D,L-Trp-Bt **2 h**	94	87 (66)	62

[a] Yields obtained in batch indicated in parentheses.

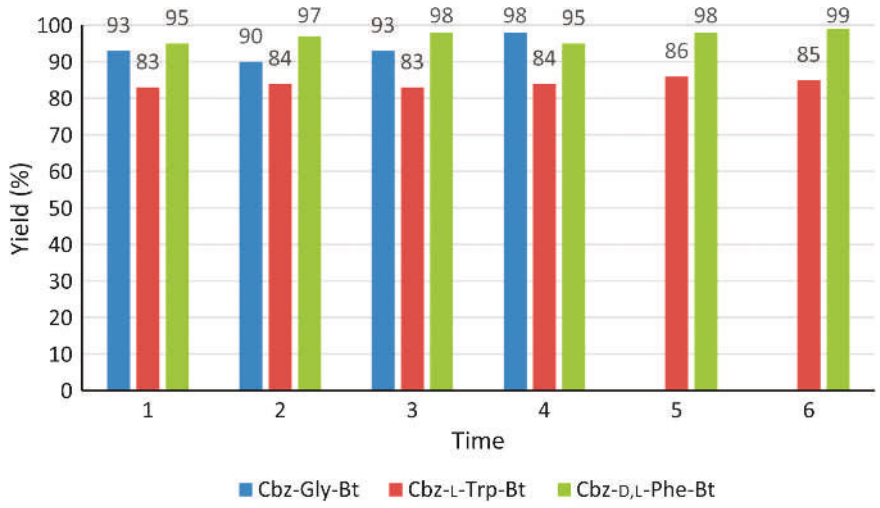


Figure 1. Isolated yield of the process over time

Scheme 4. Continuous-flow coupling of Cbz-D,L-Phe-Bt **2d** and 2-aminopyridine **3a**

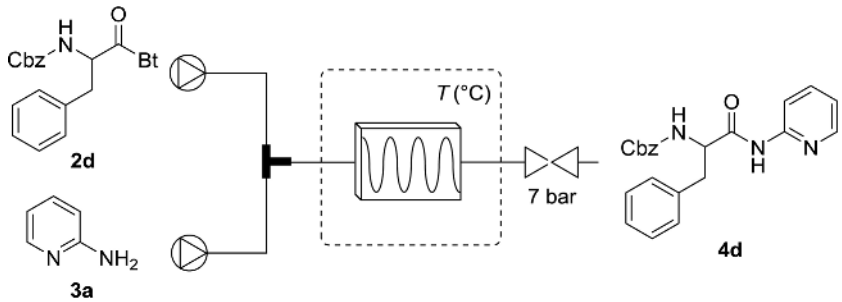


Table 6. Coupling of Cbz-D,L-Phe-Bt **2d** and 2-aminopyridine **3a** in flow in MeCN–NMP (6:1)

Entry	**2d** (M)	**3a** (equiv.)	RT (min)	*T* (°C)	Conversion (%)[a]
1	0.05	1.05	10.16	130	47
2	0.05	1.05	15.5	130	57
3	0.05	1.05	20.33	130	64
4	0.05	1.2	10.16	130	51
5	0.05	1.2	20.33	130	68
6	0.05	1.2	29.5	130	80
7	0.075	1.2	10.16	130	60
8	0.075	1.2	29.5	130	86
9	0.025	1.2	10.16	130	36
10	0.025	1.2	20.33	130	53
11	0.025	1.2	29.5	130	65

[a] As determined by LC–MS (see SI).

Increasing the equivalents of 2-aminopyridine **3a** resulted in slightly higher conversions (Table 6, entries 4 and 5). An optimal conversion of 86% was reached with a residence time of 30 min, and an increased concentration of the starting material (Table 6, entry 8). The end product was easily isolated via precipitate formation in 75% yield. Note that the optimal concentration of starting compound **2d** equals the concentration at the outlet of the continuous-flow Bt-activation step.

Hence, attempts were made to perform the Bt-activation of Cbz-D,L-Phe-OH **1d** in-line with the coupling with 2-aminopyridine **3a** (Scheme 5). The Bt-activation was carried out in two 0.3 mL PFA-tube reactors (ID: 508 μm), with a residence time of respectively 2 and 1 min (vide supra). The reaction mixture was then pumped over an alkaline resin in an attempt to trap the released HCl and the excess of $SOCl_2$ which could restrain the conversion in the subsequent coupling reaction. Because of the rapid saturation of this resin, however, another approach was needed. The dosage of 2-aminopyridine **3a** was raised to 2 equivalents to compensate for the excess of $SOCl_2$, and 3 equivalents of triethylamine were added to capture HCl as the corresponding soluble salt. The coupling reaction was performed on a large scale, using a glass modular mesoreactor system (IV: 17.1 mL; see Supporting Information for a full description). As observed on LC–MS, an excellent conversion to the end product **4d** was obtained. The work-up, however, was severely hampered by the large excess of benzotriazole and NMP in the reaction mixture. An aqueous work-up, which entailed product losses, and a problematic chromatographic purification step were required. As the isolation of the intermediate Bt-activated amino acid can be performed easily via filtration, a two-step approach appeared to be far more convenient and further integration was not pursued.

Moreover, the proposed two-step approach allows for a free selection of solvent for the coupling reaction and, thus, facilitates maximizing the yields (see below). Further demonstrating the need for a solvent switch, when evaluating the coupling reaction of *N*-Cbz-Gly-Bt **2a** and a more sterically hindered amine 5-amino-3-methyl-1-phenylpyrazole **3b** in MeCN–NMP (6:1), barely any conversion was obtained.

2.3. Continuous Coupling of *N*-Cbz-α-Aminoacylbenzotriazoles and Heterocyclic Amines. In the last phase of this study, the continuous-flow coupling of *N*-Cbz-α-aminoacylbenzotriazoles **2** and heterocyclic amines **3** (which are difficult to couple) was further optimized. This was initially performed on a small-scale borosilicate glass chip reactor (IV: 10 μL; see Supporting Information for a full description).

The suggested two-step process tolerates the introduction of a new solvent in the second step in view of increased conversions. Following the target reaction as presented by Katritzky et al., initial reactions were performed using DMF as solvent [13]. The optimization process is outlined in Table 7. When performing the reaction of glycine **2a** and 5-amino-3-methyl-1-phenylpyrazole **3b** in DMF and in DMSO under the same conditions, a similar conversion was measured and no influence of the solvent was noticed (Table 7, entries 4 and 5). Other lower boiling point solvents from this class (acetone, acetonitrile, and dichloromethane), alone or in combination with DMSO or DMF, resulted in much lower or no conversion. Therefore, DMF was replaced by DMSO as solvent in all consecutive trials. Note that, in a control experiment, the use of DMSO under the reported microwave heating conditions resulted in a drop of the conversion compared to the reported results for DMF (*i.e.,* 20% instead of 40% for **2a** and **3b**).

Excellent conversions were obtained for the coupling of a library of *N*-protected α-aminoacylbenzotriazoles and several heterocyclic amines in DMSO, when compared to the use of MeCN–NMP (6:1) as solvent mixture. For the coupling of Cbz-D,L-Phe-Bt **2d** and 2-aminopyridine **3a**, the conversion was further increased by 13% in DMSO compared to MeCN–NMP, despite the lower residence time and the equimolar amount of 2-aminopyridine **3a** (Table 6, entry 8, and Table 7,

Scheme 5. Reactor set-up for the in-line Bt-activation of **1d** and subsequent coupling with **3a**

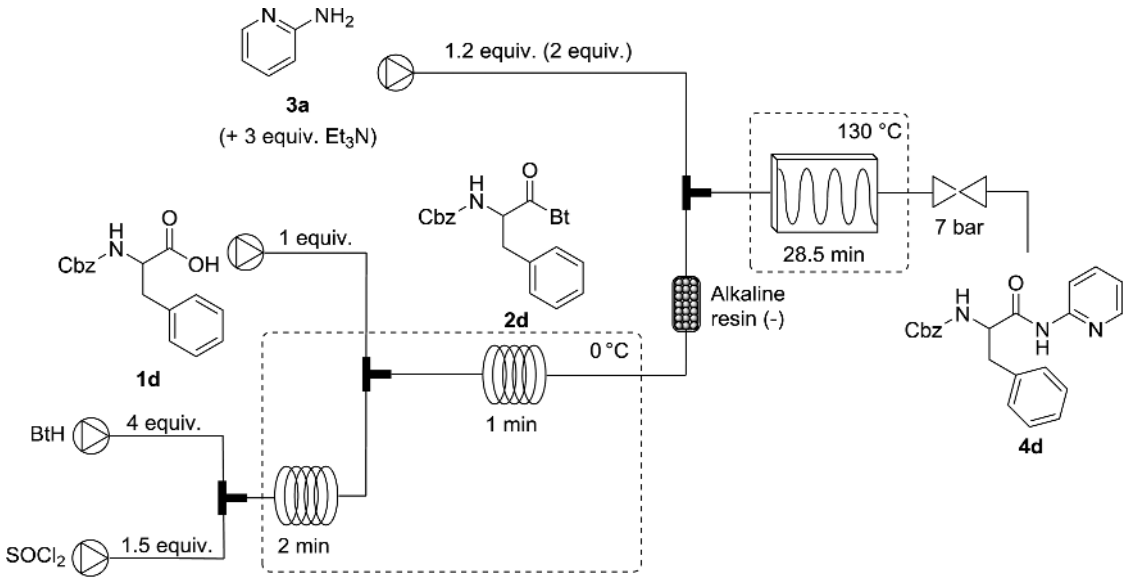
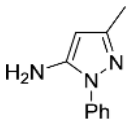
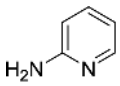
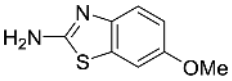
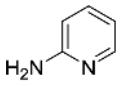
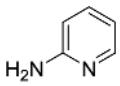
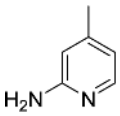
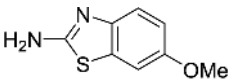
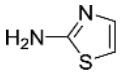


Table 7. Continuous production of *N*-Cbz-(α-aminoacyl)amino-substituted heterocycles **4** (10 μL scale)

Entry	Substrate[a]	Het-NH_2[b]	Solvent	RT (min)	*T* (°C)	Conv. (%)[c]	Reported yield [13] (%)[d]
1	Cbz-Gly-Bt **2a**	 **3b**	MeCN–NMP	50.0	130	<1	40
2	Cbz-Gly-Bt **2a**		DMF–acetone	10.0	70	<1	
3	Cbz-Gly-Bt **2a**		DMF	100	70	10	
4	Cbz-Gly-Bt **2a**		DMF	100	130	40	
5	Cbz-Gly-Bt **2a**		DMSO	100	130	40	
6	Cbz-L-Phe-Bt **2c**	 **3a**	DMF	15.2	130	99	55
7	Cbz-L-Phe-Bt **2c**		DMF	12.5	130	95	
8	Cbz-L-Phe-Bt **2c**		DMSO	15.2	130	99	
9	Cbz-L-Ala-Bt **2e**	 **3c**	DMSO	10	100	77	98
10	Cbz-L-Ala-Bt **2e**		DMSO	20	100	88	
11	Cbz-L-Ala-Bt **2e**		DMSO	10	130	94	
12	Cbz-L-Ala-Bt **2e**		DMSO	20	130	99	
13	Cbz-L-Met-Bt **2 g**	 **3a**	DMSO	25	130	99	68
14	Cbz-L-Met-Bt **2 g**		DMSO	25	100	93	
15	Cbz-L-Met-Bt **2 g**		DMSO	15.2	130	99	
16	Cbz-D,L-Phe-Bt **2d**	 **3a**	DMSO	10	130	95	70
17	Cbz-D,L-Phe-Bt **2d**		DMSO	15.2	130	95	
18	Cbz-D,L-Phe-Bt **2d**		DMSO	20	130	98	
19	Cbz-D,L-Phe-Bt **2d**		DMSO	5	130	82	
20	Cbz-L-Pro-Bt **2i**	 **3d**	DMSO	50	100	99	98
21	CBz-D,L-Ala-Bt **2f**	 **3c**	DMSO	20	130	99	78
22	Cbz-D,L-Ala-Bt **2f**		DMSO	10	130	99	
23	Cbz-L-Trp-Bt **2b**	 **3e**	DMSO	15.2	130	99	81
24	Cbz-L-Trp-Bt **2b**		DMSO	10	130	99	
25	Cbz-D,L-Trp-Bt **2 h**		DMSO	25	130	94	66
26	Cbz-D,L-Trp-Bt **2 h**		DMSO	33	130	96	

[a] See SI for starting concentrations.
[b] 1.05 equiv. **3**.
[c] As determined by LC–MS (see SI).
[d] Isolated yield of the microwave-heated batch procedure.

entry 18). Furthermore, in case of the coupling of *N*-Cbz-Gly-Bt **2a** and 5-amino-3-methyl-1-phenylpyrazole **3b**, less than 1% conversion was observed in MeCN–NMP, while in DMSO, 40% conversion was achieved (Table 7, entry 5).

The reaction temperature appeared to be essential for smooth conversion. When performing the reaction at 70 °C (Table 7, entries 2 and 3), as stated in the original procedure, a low conversion was observed. Increasing the temperature to 100 °C and 130 °C led to higher conversions. At 150 °C, conversions were similar to the results at 130 °C; however, LC–MS revealed the presence of a number of side products. Hence, the optimal reaction temperature proved to be 130 °C. Additionally, increasing residence times resulted in higher conversions.

Finally, the scale-up potential of this coupling reaction was demonstrated on the reaction of Cbz-L-Trp-Bt **2b** and 2-aminothiazole **3e**. An analogous larger scale glass modular

Table 8. Continuous coupling of Cbz-L-Trp-Bt **2b** and 2-aminothiazole **3e** (17.1 mL scale)

Substrate	Het-NH_2[a]	Conc. **2b** (mol/L)	Solvent	RT (min)	*T* (°C)	Conv. (%)	Yield (%)	Reported yield (%)[b]
Cbz-L-Trp-Bt **2b**	2e	0.5	DMSO	10	130	98	89	81

[a] 1.05 equiv. **3e**.
[b] Isolated yield of the microwave-heated batch procedure.

mesoreactor system (IV: 17.1 mL; see Supporting Information for a full system description) was used. This mesoreactor system makes use of static mixing elements in the channels, to counter the problem of the decreasing mixing efficiency as the channel diameter rises. To confirm the ease of scale-up, the previously optimized parameters were evaluated on a milliliter scale (Table 7, entry 24). A near quantitative conversion was achieved with a calculated throughput of 10.8 g/h, and the end product was isolated straightforwardly in 89% yield (Table 8).

3. Conclusion

A viable route for the production of the *N*-Cbz-(α-aminoacyl) amino-substituted heterocycles **4** using the benzotriazole methodology to activate amino acids in continuous flow was developed. Two methods were evaluated for the continuous Bt-activation of *N*-protected amino acids: BtMs and BtH–$SOCl_2$. When the appropriate solvent was used, both methods could be used under continuous-flow conditions without precipitate formation. After optimization of the Bt-activation of Cbz-L-Trp **1b** with BtMs **5**, a conversion of 81% was reached. However, activation with BtH–$SOCl_2$ in MeCN–NMP (6:1) resulted in an excellent conversion (94%). Moreover, work-up of the reaction mixture was very straightforward via filtration. Several derivatives were continuously synthesized in good isolated yields (71–99%). Telescoping the continuous Bt-activation and the subsequent coupling with a heterocyclic amine appeared to be not feasible as the use of MeCN–NMP (6:1) limits the conversion in the coupling step and the excess of BtH complicates the work-up. Therefore, a two-step approach was proposed, which allowed for a solvent switch to DMSO for the coupling reaction. This resulted in improved conversions (40–99%) in comparison to the microwave-heated batch process. In this way, the "microwave-to-flow" paradigm applies to the benzotriazole chemistry. Moreover, the ease of upscaling a microreactor process was confirmed for the coupling reaction of Cbz-L-Trp **1b** and 2-aminothiazole **3e** with an isolated yield of 89%.

4. Experimental Section

4.1. Activation of *N*-Cbz-Protected Amino Acids with BtMs in Flow. Experiments were performed with a Labtrix® Start microreactor (IV: 10 μL; see Supporting Information). Two solutions were prepared in advance: 0.125 M Cbz-L-Trp **1b** and 0.175 M Et_3N in MeCN (solution 1) and 0.156 M BtMs **5** in MeCN (solution 2). After rinsing the system with MeCN, the microreactor was primed with at least 5 system volumes at higher flow rate (10 μL/min). Two syringes were prewashed with 0.5 mL of the starting solutions each, filled with these solutions and connected to both inlets. The microreactor chip was heated to 90 °C, and both solutions were pumped through the microreactor with a flow rate of 0.33 μL/min corresponding with a residence time of 15 min and an excess of BtMs **5** (1.25 equiv.). After reaching steady state, a sample was collected and analyzed via LC–MS.

4.2. Activation of *N*-Cbz-Protected Amino Acids with BtH–$SOCl_2$ in Flow. The activation of amino acids with BtH–$SOCl_2$ was performed using a tube reactor in view of the corrosiveness of the reagents (Figure 2).

Both tube reactors were built using PFA-tubing (ID: 0.020″, OD: 1/16″) with an internal volume of 1.5 mL each. The reagent solutions were pumped through the tube reactor with a Syrris® Reagent Pump ($SOCl_2$ and BtH) and a Uniqsis® Binary Pump Module (Cbz-AA). The mixing of the reagents occurred in an ETFE T-connector. The reactor was cooled to the desired reaction temperature using an ice bath.

The reactor system was first rinsed with dry MeCN–NMP (6:1), and the following three solutions were prepared: (a) 0.45 M $SOCl_2$ in dry MeCN–NMP (6:1), (b) 1.2 M BtH **6** in dry MeCN–NMP (6:1), and (c) 0.15 M *N*-Cbz-AA **1** in dry MeCN–NMP (6:1). The tube reactor was cooled to 0 °C, and reagent A was also cooled to prevent degradation of $SOCl_2$. Reagents A and B were pumped through the tube reactor with a flow rate of 0.375 mL/min each, corresponding to a residence time of 2 min in the first tube reactor. The output of the first reactor was mixed with reagent C (0.75 mL/min). Hence, a residence time of 1 min and a stoichiometric ratio *N*-Cbz-AA–BtH–$SOCl_2$ 1:4:1.5 were obtained in the second tube reactor. After reaching steady

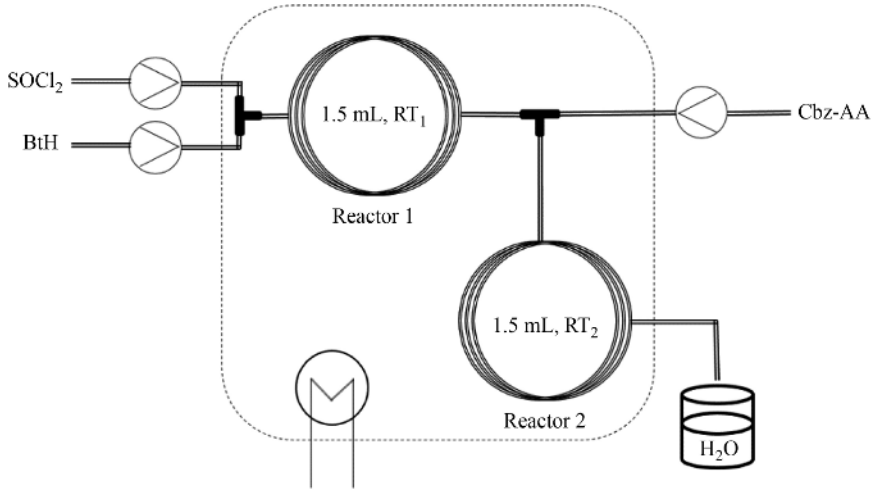


Figure 2. Activation of *N*-Cbz-protected amino acids with BtH–$SOCl_2$ using a tube reactor

Table 9. Concentrations of substrates **2**

Entry	Substrate	Conc. **2** (mol/L)
1	Cbz-Gly-Bt **2a**	0.5
2	Cbz-L-Phe-Bt **2c**	0.5
3	Cbz-L-Ala-Bt **2e**	0.15
4	Cbz-L-Met-Bt **2 g**	0.5
5	Cbz-D,L-Phe-Bt **2d**	0.25
6	Cbz-L-Pro-Bt **2i**	0.5
7	CBz-D,L-Ala-Bt **2f**	1
8	Cbz-L-Trp-Bt **2b**	0.5
9	Cbz-D,L-Trp-Bt **2 h**	0.25

state, a sample was collected in an excess of H_2O over a period of 30 min. A pale white precipitate (*N*-Cbz-AA-Bt **2**) was formed which was filtered and subsequently washed with H_2O to remove traces of NMP. Spectral data of the synthesized *N*-Cbz-AA-Bt **2** corresponded with literature [23b, 26].

4.3. Coupling of Cbz-D,L-Phe-Bt 2d and 2-Aminopyridine 3a in MeCN–NMP (6:1) in Flow. Experiments were performed with a KiloFlow® reactor (IV: 6.5 mL; see Supporting Information). The reactor was rinsed with dry MeCN–NMP (6:1), and the following solutions were prepared in advance: 0.075 M Cbz-D,L-Phe-Bt **2d** in dry MeCN–NMP (6:1) and 0.09 M 2-aminopyridine **3a** in dry MeCN–NMP (6:1).

The solution of **2d** was preheated to 50 °C. The reactor was heated to 130 °C, and both reagent solutions were pumped through the reactor at a flow rate of 0.11 mL/min. Hence, a residence time of 29.5 min and a stoichiometric ratio **2d**:**3a** 1:1.2 were obtained. After reaching steady state, a sample was collected and analyzed via LC–MS.

4.4. Coupling of an *N*-Cbz-α-Aminoacylbenzotriazole and a Heterocyclic Amine in DMSO in Flow. The optimization experiments were performed using a Labtrix® Start microreactor (IV: 10 μL). The system was prewashed with reaction solvent for at least 10 reactor volumes. Two syringes were filled with a first solution of *N*-Cbz-α-aminoacylbenzotriazole **2**, with a concentration as indicated in Table 9, and a second solution of 1.05 equivalents of the heterocyclic amine **3** in DMSO, and connected to both inlets. Next, the system was primed with at least 5 system volumes at higher flow rate (10 μL/min). The flow was then set to the desired value, and the system was equilibrated at the determined flow and temperature conditions. After at least 3 system volumes, a sample was collected and analyzed by LC–MS, after dilution in MeCN. The conversion was estimated by comparing the absorbance of free BtH and *N*-Cbz-α-aminoacylbenzotriazole at 254 nm (see Supporting Information). The complete characterization of all compounds has been published [13].

Next, the coupling reaction of Cbz-L-Trp-Bt **2b** and 2-aminothiazole **3e** was scaled up using the KiloFlow® mesoreactor under the optimal reaction conditions, as determined on a small scale. First, two 0.5 M reagent solutions were prepared. Twenty-five millimoles of Cbz-L-Trp-Bt **2b** (solution 1) and 1.05 equiv. of 2-aminothiazole **3e** (solution 2) were dissolved in DMSO to obtain 50 mL solutions. The reactor system was heated to 130 °C, and a BPR of 7 bar was applied. Both reagent solutions were pumped through the KiloFlow® reactor with a flow rate of 0.86 mL/min, which corresponds to a residence time of 10 min and a stoichiometric ratio Cbz-L-Trp-Bt **2b**:**3e** of 1:1.05. After reaching steady state, a sample was collected. Subsequently, DMSO was removed by distillation at high-vacuum conditions. The crude product was purified by column chromatography with hexane–ethyl acetate 3:2 to obtain the pure (α-aminoacyl)amino-substituted heterocycle (4.35 g, 10.34 mmol, 89%).

Acknowledgment. Financial support for this research from the Fund for Scientific Research Flanders (FWO Vlaanderen), VITO (Vlaamse Instelling voor Technologisch Onderzoek), and the Long Term Structural Methusalem Funding (BOF09/01 M00409) by the Flemish Government is gratefully acknowledged.

Supporting Information

Electronic Supplementary Material (ESM) is available in the online version at doi: 10.1556/1846.2015.00029.

References

1. (a) Grauer, A.; Konig, B. *Eur. J. Org. Chem.* **2009**, 5099–5111; (b) Lu, K.; Duan, Q. P.; Ma, L.; Zhao, D. X. *Bioconjugate Chem.* **2010**, *21*, 187–202; (c) Vagner, J.; Qu, H. C.; Hruby, V. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292–296.

2. Okandeji, B. O.; Greenwald, D. M.; Wroten, J.; Sello, J. K. *Bioorg. Med. Chem.* **2011**, *19*, 7679–7689.

3. Shi, W.; Ma, H. K.; Duan, Y. J.; Aubart, K.; Fang, Y. H.; Zonis, R.; Yang, L. P.; Hu, W. H., *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1060–1063.

4. Lee, J.; Bae, S.; Lee, S. H.; Choi, H.; Kim, Y. H.; Kim, S. J.; Park, G. T.; Moon, S. K.; Kim, D. H.; Lee, S.; Ahn, S. K.; Choi, N. S.; Lee, K. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6327–6330.

5. (a) Crisostomo, F. R. P.; Feng, Y. M.; Zhu, X. J.; Welsh, K.; An, J.; Reed, J. C.; Huang, Z. W. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6413–6418; (b) Zobel, K.; Wang, L.; Varfolomeev, E.; Franklin, M. C.; Elliott, L. O.; Wallweber, H. J. A.; Okawa, D. C.; Flygare, J. A.; Vucic, D.; Fairbrother, W. J.; Deshayes, K. *ACS Chem. Biol.* **2006**, *1*, 525–534.

6. (a) Hinkle, C. L.; Mohan, M. J.; Lin, P. Y.; Yeung, N.; Rasmussen, F.; Milla, M. E.; Moss, M. L. *Biochemistry* **2003**, *42*, 2127–2136; (b) Rabinowitz, M. H.; Andrews, R. C.; Becherer, J. D.; Bickett, D. M.; Bubacz, D. G.; Conway, J. G.; Cowan, D. J.; Gaul, M.; Glennon, K.; Lambert, M. H.; Leesnitzer, M. A.; McDougald, D. L.; Moss, M. L.; Musso, D. L.; Rizzolio, M. C. *J. Med. Chem.* **2001**, *44*, 4252–4267.

7. Duflos, M.; Courant, J.; Le Baut, G.; Grimaud, N.; Renard, P.; Manechez, D.; Caignard, D. H. *Eur. J. Med. Chem.* **1998**, *33*, 635–645.

8. Altman, J.; Gorecki, M.; Wilchek, M.; Votano, J. R.; Rich, A. *J. Med. Chem.* **1984**, *27*, 596–600.

9. Schnatterer, S.; Hawkins, D. W.; Jans, D.; Maier, M.; Kuhlmann, A.; Sanwald, E. F.; Thoenessen, M.-T.; Seeger, K. (Bayer Cropscience S.A., Fr) Preparation of pesticidal 5-(acylamino)pyrazole derivatives. WO 2004049803 A1, Jun 17, 2004.

10. Braxmeier, T.; Demarcus, M.; Fessmann, T.; McAteer, S.; Kilburn, J. D. *Chem. Eur. J.* **2001**, *7*, 1889–1898.

11. (a) Katritzky, A. R.; Lan, X. F.; Yang, J. Z.; Denisko, O. V. *Chem. Rev.* **1998**, *98*, 409–548; (b) Panda, S. S.; Hall, C. D.; Scriven, E.; Katritzky, A. R. *Aldrichimica Acta* **2013**, *46*, 43–55.

12. (a) Katritzky, A. R.; Abo-Dya, N. E.; Tala, S. R.; Gyanda, K.; Abdel-Samii, Z. K. *Org. Biomol. Chem.* **2009**, *7*, 4444–4447; (b) Katritzky, A. R.; Angrish, P.; Todadze, E. *Synlett* **2009**, 2392–2411; (c) Katritzky, A. R.; Narindoshvili, T.; Draghici, B.; Angrish, P. *J. Org. Chem.* **2008**, *73*, 511–516; (d) Katritzky, A. R.; Rachwal, S. *Chem. Rev.* **2010**, *110*, 1564–1610; (e) Katritzky, A. R.; Rachwal, S. *Chem. Rev.* **2011**, *111*, 7063–7120; (f) Katritzky, A. R.; Zhang, Y. M.; Singh, S. K. *Synthesis* **2003**, 2795–2798; (g) Katritzky, A. R.; Rogovoy, B. V. *Chem. Eur. J.* **2003**, *9*, 4586–4593.

13. Katritzky, A. R.; El-Gendy, B.; Todadze, E.; Abdel-Fattah, A. A. A. *J. Org. Chem.* **2008**, *73*, 5442–5445.

14. Quelever, G.; Burlet, S.; Garino, C.; Pietrancosta, N.; Laras, Y.; Kraus, J. L. *J. Comb. Chem.* **2004**, *6*, 695–698.

15. Leadbeater, N. E., *Microwave Heating as a Tool for Sustainable Chemistry.* CRC Press: Boca Raton, 2010; p 290.

16. Glasnov, T. N.; Kappe, C. O. *Chem. Eur. J.* **2011**, *17*, 11956–11968.

17. Wiles, C.; Watts, P., *Micro Reaction Technology in Organic Synthesis*, CRC Press: Boca Raton, 2011.

18. (a) Acke, D. R. J.; Stevens, C. V. *Green Chemistry* **2007**, *9*, 386–390; (b) Cukalovic, A.; Monbaliu, J.-C. R.; Stevens, C. Microreactor Technology as an Efficient Tool for Multicomponent Reactions. In *Synthesis of Heterocycles via Multicomponent Reactions I*; Orru, R. V. A.; Ruijter, E., Eds.; Springer: Berlin, Heidelberg, 2010; Vol. 23, pp 161–198; (c) Heugebaert, T. S.; Stevens, C. V.; Kappe, C. O. *ChemSusChem* **2015**, *8*, 1648–1651; (d) Van Waes, F. E. A.; Drabowicz, J.; Cukalovic, A.; Stevens, C. V. *Green Chem.* **2012**, *14*, 2776–2779; (e) Van Waes, F. E. A.; Seghers, S.; Dermaut, W.; Cappuyns, B.; Stevens, C. V. *J. Flow Chem.* **2014**, *4*, 118–124.

19. (a) Watts, P.; Wiles, C.; Haswell, S. J.; Pombo-Villar, E. *Tetrahedron* **2002**, *58*, 5427–5439; (b) Watts, P.; Wiles, C.; Haswell, S. J.; Pombo-Villar, E.; Styring, P. *Chem. Commun.* **2001**, 990–991; (c) Wiles, C.; Ngamsom, B. Continuous Flow Synthesis of β-Peptides within Labtrix® Micro Reactors 2010 (accessed Jan 07, 2015).

20. Flogel, O.; Codee, J. D. C.; Seebach, D.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 7000–7003.

21. Baxendale, I. R.; Ley, S. V.; Smith, C. D.; Tranmer, G. K. *Chem. Commun.* **2006**, (46), 4835–4837.

22. Mandity, I. M.; Olasz, B.; Otvos, S. B.; Fülöp, F. *ChemSusChem* **2014**, *7*, 3172–3176.

23. (a) Katritzky, A. R.; Angrish, P. *Steroids* **2006**, *71*, 660–669; (b) Katritzky, A. R.; Angrish, P.; Suzuki, K. *Synthesis* **2006**, 411–424; (c) Katritzky, A. R.; Suzuki, K.; Singh, S. K. *Synthesis* **2004**, 2645–2652; (d) Katritzky, A. R.; Todadze, E.; Cusido, J.; Angrish, P.; Shestopalov, A. A. *Chem. Biol. Drug. Des.* **2006**, *68*, 37–41; (e) Katritzky, A. R.; Vincek, A. S.; Suzuki, K. *Arkivoc* **2005**, 116–126.

24. Katritzky, A. R.; He, H. Y.; Suzuki, K. *J. Org. Chem.* **2000**, *65*, 8210–8213.

25. Katritzky, A. R.; Wang, M. Y.; Yang, H. F.; Zhang, S. M.; Akhmedov, N. G. *Arkivoc* **2002**, 134–142.

26. Katritzky, A. R.; Angrish, P.; Hur, D.; Suzuki, K. *Synthesis* **2005**, 397–402.