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Continuous-Flow Microliter Microwave Irradiation in the Synthesis of Isoxazole Derivatives: An Optimization Procedure

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Abstract: An efficient method was developed for the synthesis of 3,4,5-trisubstituted and 3,5-disubstituted isoxazoles by using continuous-flow microwave-heated microreactors. A study on the separate effects of the temperature, continuous-flow regime, and microwave irradiation showed that the continuous-flow regime had important effects for less reactive diketones, where microwave heating enhanced the reaction, permitting the formation of 5-methyl-3-phenylisoxazole, which was not formed in the absence of microwaves.

Key words: heterocycles, cyclizations, condensation, microwaves, continuous-flow reactors, microreactors

Microreaction technology, which has recently been used in both academia and industry as a tool to develop a wide variety of synthetic organic transformations, has several advantages over commonly used batch methods.[1] Microreactors are miniaturized reaction systems, fabricated by techniques from microtechnology and precision engineering, that are used to study chemical reactions on a microscale.[2] Microreactors consists of three-dimensional structures (microchannels) with diameters typically less than 1000 μm. The physical properties of microreactors, such as their short diffusion distances, small internal volumes, and high surface-to-volume ratios, permit improvements in the yields and selectivities of many reactions, mainly as a result of improved mass and heat transfer, more efficient mixing, and regular flow profiles. Over the past 15 years, microreactors have entered the field of flow chemistry, providing additional advantages such as improved safety, better reproducibility, ease of automation, and precise control of reaction conditions.[3]

The dielectric heating induced by microwave irradiation can result reduced reaction times, high product yields, and improved selectivity for a wide variety of chemical transformations.[4] Microwave irradiation has been recently applied to microreactor technology as an alternative mode of heating, thereby combining the unique heating mechanism of microwave irradiation with the typical advantages of continuous-flow microreactors.[5] Furthermore, the technique of continuous-flow microwave-assisted organic synthesis permits scaling up of microwave processes in a manner that, because of the low depth of penetration (a few centimeters) of microwaves at the established frequency of 2.45 GHz, would be impossible in large-scale batch reactors.[5]

The optimization of chemical processes to reduce costs and to eliminate unnecessary labor in purification of products and removal of byproducts is a key aspect of research that is especially important when dealing with microwave chemistry, where the analysis and optimization of the reaction process can take longer to perform than the reaction itself. With a focus on the process of optimization of a microwave-assisted chemical reaction, we have recently reported on the design and fabrication of a microliter-microwave reactor coupled with a custom-made nanoliter-scale NMR spectrometer.[6] This permits optimization to be performed on a very small reaction volume and in a short time, thereby reducing the quantity of waste produced, improving the safety of the process, and minimizing the consumption of reagents, solvents, and energy. Previous optimization methods have involved, for example, on-chip methodology[7] or on-line HPLC analyses, where various algorithms can be used to automate the optimization process.[8]

Here in is described an efficient method for the synthesis of 3,5-substituted and 3,4,5-substituted 1,2-isoxazole derivatives, together with an exhaustive optimization of the reaction conditions. The method involved the use of continuous-flow microwave irradiation of a microreactor. Complete conversions were possible within a few minutes without using excessively high temperatures. In addition, a modification of the setup afforded a good rate of production of compound of interests by scaling up the process. Because the isoxazole motif is present in numerous natural products of medicinal value,[9] there is interest in the development of highly regioselective syntheses of isoxazole derivatives from simple starting materials. Efficient syntheses of 3-substituted and 3,5-substituted isoxazoles have recently been reported that use α,β-unsaturated aldehydes or ketones.[10] Haswell and co-workers described the use of electroosmotic flow to induce flow of reagent solutions within microchannels for the preparation of pyrazole and isoxazole derivatives.[11]

Preliminary data for this reaction show that three factors affect the conversion rate of the product: the temperature, the use of microwave heating, and the flow regime. Complete conversion in the preparation of 3,5-dimethylisoxazole (**4**) is achieved by using a combination of microwave

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heating and a continuous flow regime at 80 °C. However, we were interested in determining the influence of each parameter separately to check whether the effect of microwave irradiation is important in attaining full conversion, because continuous-flow reactors appear to be perfectly suited to mimicking the rapid heating achievable in microwave chemistry.[12] We therefore examined the individual effects of microwave irradiation in comparison with conventional heating, of operating at room temperature as opposed to a higher temperature (80 °C), and of adopting a continuous-flow regime as opposed to batch mode. We carried out a step-by-step analysis to test all possible combinations of these conditions by varying one factor at a time to determine its influence while keeping other variables constant. This allowed us to evaluate the independent effects of the temperature, the flow regime, and the heating process on the conversion rate and permitted the identification of the most effective combination of continuous-flow and microwave irradiation conditions.[5]

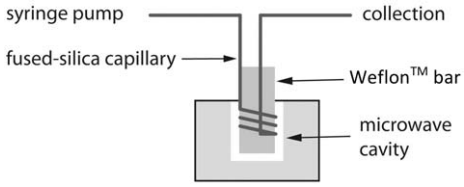
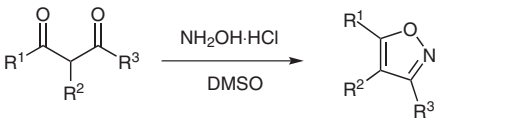


Figure 1 Illustration of the experimental set-up used for continuous-flow microwave-assisted organic synthesis. The reagents were loaded into the capillary system which was located inside the microwave cavity with the aid of a graphite-impregnated Teflon (Weflon™) bar.

Initially, the reactions conditions were optimized to provide the shortest possible reaction time (for lower costs) by using the most appropriate temperature range and design of the microreactor/microwave system to facilitate the translation of these reactions into high-throughput chemistry for extension to the synthesis of a small library of pyrazole and isoxazole derivatives.[13] In addition, short residence times were chosen to simplify studies on the influence of the other parameters. We therefore fabricated a custom-built flow-cell that could be used to adapt a conventional microwave oven (Discover, CEM Corp.) for continuous-flow processing. A 70-cm-long fused-silica capillary with external and internal diameters of 349 and 100 μm, respectively, was wound in and out of the microwave cavity and wrapped around a bar of graphite-impregnated Teflon (Weflon™) containing the reaction mixture, thereby defining a reaction volume of 3 μL that could be exposed to microwave irradiation (Figure 1). For conventionally heated continuous-flow reactions, a capillary of the same length and diameter as that used for the microwave reactions was introduced into a sand bath once the desired temperature had become constant. Flow in the system was controlled by a high-force pump and a gas-chromatography syringe (SGE Analytical Science). An appropriate flow-rate provided an optimum reaction time of only 3–6 minutes for complete conversion. Batch-mode reactions were carried out, either by using a 10-mL reaction vessel and a microwave reactor (Discover, CEM Corp.) or a heated sand bath for microwave or conventional heating, respectively.

In all cases, the percentage conversion was determined by direct analysis of samples of the effluent from the capillary (for continuous-flow conditions) or of samples from the reaction vessel (for batch-mode reactions) by ^{1}H NMR spectroscopy in an offline mode.

Various commercially available 1,3-diketones were treated with hydroxylamine hydrochloride in dimethyl sulfoxide to give the corresponding 1,2-isoxazoles (Scheme 1). The optimization process was performed on three model reactions in an attempt to identify any common behavior.



4: R^1 = Me; R^2 = H; R^3 = Me
5: R^1 = Me; R^2 = Me; R^3 = Me
6a: R^1 = Ph; R^2 = H; R^3 = Me
6b: R^1 = Me; R^2 = H; R^3 = Ph

Scheme 1 General reaction scheme for the preparation of three 1,2-isoxazoles

Table 1 summarizes the conversion rates obtained for isoxazole derivatives **4**, **5**, **6a**, and **6b**, for each combination of experimental conditions, as described above. Three variables were tested: the temperature, the reaction regime (batch or flow), and heating mode (microwave or conventional). We therefore performed eight (2^3) experiments to cover each of the possible combinations (Table 1, entries 1–8).

To determine which factors were the most relevant (temperature, microwave heating, or flow regime), we had to perform a detailed comparison between pairs of entries that differed only in terms of one factor (for example, entry 1 versus entry 2), thereby eliminating the influence of other factors that could have led to confusion in the analysis of data. For instance, the effect of temperature was determined by comparing entries 1 and 2, in which both reactions were performed in a batchwise manner in the absence of microwave irradiation. Thus, comparison of entries 1 and 3 showed the effect of the flow regime, and comparison of entries 1 and 5 showed the effect of microwave heating. Table 2 lists the effects that were identified by comparing the entries as discussed above. This analysis showed that the reaction to form compound **4** shows similar tendencies to the reaction to form compound **5**, whereas the reaction to form compound **6** showed a different behavior. The temperature and flow regime had the greatest effects on the formation of **4** and **5**, whereas microwave heating and the flow regime were the most significant factors in the formation of compound **6**. In addition, for all the isoxazole derivatives, continuous-flow had three times the effect of microwave heating (Table 2, entries 2 and 3), supporting recent studies in the literature.[12] On the other hand, microwave heating produced

high conversions to isoxazoles **6a** and **6b**, the only case in which the effect of microwave heating was significant (entry 3). In fact, regioisomer **6b** was obtained only in the presence of microwave irradiation (Table 1, entries 1–5, 6, and 8) at particular temperatures; the nature of the flow regime did not affect the formation of isomer **6b** (Table 1, entries 1 and 3 and entries 6 and 8).

Table 1 Summary of the Conversions Obtained for the Three Isoxazole Derivatives for Each Possible Combination of Experimental Conditions

Entry	Temp (°C)	Reaction regime	Heating mode[a]	Conv. to **4** (%)	Conv. to **5** (%)	Conv. to **6a/6b** (%)
1	25	batch	CH	6	7	1/0
2	80	batch	CH	39	35	6/0
3	25	flow	CH	23	19	33/0
4	80	flow	CH	43	43	23/0
5	25	batch	MW	6	6	11/0
6	80	batch	MW	86	75	57/13
7	25	flow	MW	67	52	37/0
8	80	flow	MW	96	67	76/10

[a] CH: conventional heating; MW: microwave heating.

The effect of the flow regime was more marked in the formation of compounds **6**, especially **6a** (Table 2, entry 2 and Table 1, entries 1 and 3). The same trend was observed with the use of microwave heating. Presumably, the less-reactive conjugated ketone 1-phenylbutane-1,3-dione (**3**) requires an additional input of energy to generate the transitions state for the reaction. This energy is best supplied by the volumetric heating induced by microwaves or by the efficient heat transfer induced in continuous-flow processing.[14] In fact, complete conversion rates for compounds **5** and **6** were only obtained by increasing the temperature to 120 °C and the residence time to six minutes under continuous-flow microwave-heating conditions, thereby demonstrating the lower reactivity of ketones **2** and **3**, which, theoretically, have a lower density of positive charge on the carbonyl carbon because of the presence of the extra methyl or phenyl groups in substrates **2** and **3**, respectively. We aim to perform theoretical calculations to rationalize the observed differences and to produce a general rule for predicting the enhancement effects of microwave irradiation on microreactors.[12]

Having established the optimal conditions for flow chemistry, we scaled the reaction up to a microliter scale by using a custom-built glass flow reactor with an internal volume of 350 μL; this was more than one hundred times the volume of the previous continuous-flow reactor used in the optimization study. We selected continuous-flow microwave-assisted experimental conditions because they gave the highest conversion rates in the optimization experiments. A commercial microwave reactor (Model 52; Resonance Instruments, Inc.) was used. This reactor has a small microwave cavity separated from the magnetron by a two-meter long coaxial cable, facilitating its use for continuous-flow studies.

Table 2 Conversion Data Ratios Extracted from Table 1, Illustrating the Corresponding Effects

Entry	Effects	Isoxazole **4**	Isoxazole **5**	Isoxazoles **6a/6b**
1	temperature	6.5	5	6
2	flow regime	4	3	33
3	microwave heating	1	1	11

Scale-up reactions were carried out for the synthesis of 3,4,5-trimethylisoxazole (**5**). The reaction was performed under exactly the same conditions as the previous optimized conditions and reached complete conversion, as expected. Thus, with a residence time of six minutes, a temperature of 120 °C and a flow rate of 58 μL/min, we obtained a total of 1.15 g/h of isoxazole **5**.

Pentane-2,4-dione (**1**), 3-methylpentane-2,4-dione (**2**), 1-phenylbutane-1,3-dione (**3**), and $NH_2OH{\cdot}HCl$ were commercially available and were used without further purification. ^1H and ^{13}C NMR spectra were recorded at 298 K in DMSO-d_6 on a Varian Inova 500-MHz spectrometer at 499.7 or 125.67 MHz, respectively.

Continuous-Flow Microwave Reactions; General Procedure

Microwave irradiations were performed in a microwave reactor (Discover, CEM Corp.) in which the usual reaction vessel was replaced with a customized flow cell. The temperature was monitored during the reaction by using the standard IR pyrometer in the microwave reactor. The appropriate solutions of the 1,3-diketone and $NH_2OH{\cdot}HCl$ in DMSO were loaded into the fused-silica capillary that defined the reaction volume by using a gas-chromatography syringe (SGE Analytical Science). The 70 cm long fused-silica capillary with an outside diameter of 349 μm and an internal diameter of 100 μm was wrapped around a Weflon™ bar to facilitate heating of the small sample volume. The capillary was wound in and out of the microwave cavity to define a reaction volume of 3 μL. A flow rate of the high-force pump (PHD 2000–71-2000, Harvard Apparatus Inc.) that controlled the low flow rate in the system was adjusted to 1 μL/min, corresponding to a residence time of 3 min. To reach full conversions for compounds **5** and **6**, the flow rate was reduced to give a longer residence time (6 min). When the reaction temperature was 25 °C under microwave irradiation, the reactor operated at a power of 1–7 W. The percentage conversion rate was determined by analyzing samples of the effluent from the capillary directly by ^1H NMR spectroscopy in the offline mode.

Continuous-Flow Conventionally Heated Reactions; General Procedure

The appropriate solutions of the 1,3-diketone and $NH_2OH{\cdot}HCl$ in DMSO were loaded into a fused-silica capillary that defined the reaction volume by using a gas-chromatography syringe (SGE Analytical Science). The 38 cm long fused silica capillary with an outside diameter of 349 μm and an internal diameter of 100 μm was introduced into a sand bath to heat up the small sample volume. The heated capillary length defined a reaction volume of 3 μL. The flow rate of the high-force pump (PHD 2000 – 71-2000, Harvard Apparatus Inc.) was adjusted to 1 μL/min, corresponding to a residence time of 3 min. The percentage conversion rate was determined by

analyzing samples of the effluent from the capillary directly by ^{1}H NMR spectroscopy in the offline mode.

Microwave-Heated Batch Reactions; General Procedure
Microwave-assisted reactions were performed in a microwave reactor (Discover, CEM Corp.). The temperature during the reaction was monitored by using the standard IR pyrometer in the microwave reactor. The appropriate solutions of the 1,3-diketone and $NH_2OH{\cdot}HCl$ in DMSO were mixed and stirred in a conventional microwave reaction vessel. The mixture was irradiated for 3 min at the appropriate temperature (25 or 80 °C). When the reaction temperature was 25 °C under microwave irradiation, the reactor operated at a power of 1–7 W. The percentage conversion rate was determined by analyzing samples of the effluent from the capillary directly by ^{1}H NMR spectroscopy in the offline mode.

Conventionally Heated Batch Reactions; General Procedure
The appropriate solutions of the 1,3-diketone and $NH_2OH{\cdot}HCl$ in DMSO were mixed and stirred in a small reaction vessel (10 mL) and heated for 3 min to the appropriate temperature (25 or 80 °C) in a sand bath. The percentage conversion rate was determined by analyzing samples of the effluent from the capillary directly by ^{1}H NMR spectroscopy in the offline mode.

3,5-Dimethylisoxazole (4)[15,16]
A solution of pentane-2,4-dione (**1**; 3 mmol, 3 M) and $NH_2OH{\cdot}HCl$ (3.33 mmol, 3.3 M) in DMSO was exposed to the appropriate experimental conditions. The percentage conversions to the product are listed in Table 1. The spectroscopic properties of the product agreed with those reported in the literature.

3,4,5-Trimethylisoxazole (5)[16]
A mixture of 3-methylpentane-2,4-dione (**2**; 3 mmol, 3 M) and $NH_2OH{\cdot}HCl$ (3.33 mmol, 3.3 M) in DMSO was exposed to the corresponding experimental conditions. The percentage conversions to the product are listed in Table 1. The spectroscopic properties of the product agreed with those reported in the literature.

3-Methyl-5-phenylisoxazole (6a) and 5-Methyl-3-phenylisoxazole (6b)[17]
A mixture of 1-phenylbutane-1,3-dione (**3**; 3 mmol, 3M) and $NH_2OH{\cdot}HCl$ (3.33 mmol, 3.3 M) in DMSO was exposed to the corresponding experimental conditions. The percentage conversions to the relevant products are listed in Table 1. The spectroscopic properties of the product agreed with those reported in the literature.

Scaled-Up Continuous-Flow Microwave-Heated Reactions; General Procedure
The scaled-up reactions were performed in a commercial microwave reactor (Model 521, Resonance Instruments Inc.) by using a customized glass flow-cell with an internal volume of 350 μL. The temperature during the reaction was monitored by using the standard IR pyrometer in the microwave reactor. The percentage conversion rate was determined by analyzing samples of the effluent from the capillary directly by ^{1}H NMR spectroscopy in the offline mode.

3,4,5-Trimethylisoxazole (5)[16]
A mixture of 3-methylpentane-2,4-dione (**2**; 2.1 mmol, 3 M) and $NH_2OH{\cdot}HCl$ (3.3 M) in DMSO was heated to 120 °C for a residence time of 6 min at a flow rate of 58 μL/min. The rate of production of the product was 1.15 g/h.

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