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Efficient Continuous Flow Synthesis of Hydroxamic Acids and Suberoylanilide Hydroxamic Acid Preparation

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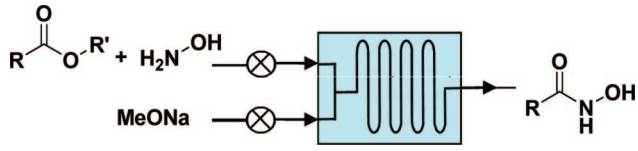

A continuous flow tubing reactor can be used to readily transform methyl or ethyl carboxylic esters into the corresponding hydroxamic acids. Flow rate, reactor volume, and temperature were optimized for the preparation of a small collection of hydroxamic acids. Synthetic advantages were identified as an increased reaction rate and higher product purity. This method was also successfully applied to the multistep preparation of suberoylanilide hydroxamic acid, a potent HDAC inhibitor used in anticancer therapy.

In recent years pharmaceutical and biotech companies are under high pressure to produce a steady stream of innovative, well-differentiated drugs with a reduced cost both for discovery and development. With the aim at increasing the productivity of original and highly pure molecules as potential modulators of therapeutic targets, different and novel technologies (related to synthesis, workup, and isolation) were developed.[1] Among these new technologies, continuous flow organic synthesis, after an extensive investigation at the academic level, is now being applied in fine chemistry with the transfer of many classes of reaction successfully reported.[2] More recently pharmaceutical companies are embracing flow methodology in drug discovery programs, attracted by its potential advantages over the existing batch techniques.[3] Theoretical and practical benefits associated with performing reactions under micro/meso continuous flow have been demonstrated for a number of common organic transformations, ranging from liquid−liquid to solid−liquid−gas systems.[4] Established advantages of continuous flow chemistry processes include precise control of variables such as temperature, pressure, concentration, residence time, and heat transfer. All of these aspects significantly affect the reaction outcome, improving yield and selectivity.[5] Moreover, the possibility of carrying out reactions in superheated solvents allows novel thermal regimes previously inaccessible within conventional apparatus.[6] By rapid and efficient heat dispersion, large exotherms can be minimized, producing safer and more selective processes.[7]

Here we report a general and efficient procedure for the conversion of esters into the corresponding hydroxamic acids with good yields and purities using a commercially available continuous flow reactor.[8]

Hydroxamic acids occur in several molecules with a wide spectrum of biological activities[9] such as antibacterial, antifungal, antiinflammatory, antiasthmatic, and anticancer properties. In particular this moiety is present in potent matrix metalloproteinase[10] and hystone deacetylase[11] inhibitors because hydroxamic acids are strong bidentate metal-ion-chelating agents that interact with zinc(II)-containing proteins. Given the importance of this functionality, the development of new methodologies for a general and efficient synthesis are still of great interest, although several methods have been developed and published so far.[12] As a part of a medicinal chemistry project, a simple conversion of ester into hydroxamic acid was envisaged as a suitable and convenient synthetic method for the preparation of a collection of compounds featuring this particular moiety. We first investigated the use of methylbenzoate (**1a**) to optimize the parameters (flow rate, residence time, and temperature). A mixture of **1a** (0.5 M in MeOH) and hydroxylamine (1:10 ratio) was simultaneously pumped into the flow reactor with a solution of MeONa (0.5 M in MeOH) of MeONa, allowing efficient mixing in the PTFE tubing at the selected temperature. The optimization of the experimental parameters was systematically

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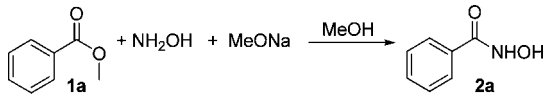
TABLE 1. Optimization of Experimental Parameters



entry	flow rate (mL/min)	residence time (min)	T (°C)	conversion (%)[a]
1	1	5	50	52
2	1	5	70	65
3	1	5	80	58[b]
4	0.5	20	60	76
5	0.5	20	70	74
6	0.5	30	70	80

[a] Conversion was determined by LC/MS at 215 nM. [b] Corresponding carboxylic acid was present as byproduct.

TABLE 2. Comparison of Reaction Carried out in Batch, under Microwave Irradiation, and Using the Flow Reactor

entry	experimental condition	conversion (%)[a]
1	batch reaction	58
2	sealed tube with MW irradiation	72
3	flow system	80

[a] Conversion was determined by LC/MS at 215 nM.

investigated by varying flow rate, temperature, and reactor volume. This process was greatly facilitated by continuous flow conditions and a significant number of runs were rapidly conducted in a sequential manner (Table 1).

The temperature of 70 °C resulted in the best compromise to have good conversion without the formation of the corresponding carboxylic acid. This byproduct was significantly present when the reaction was performed at 80 °C. The conversion into the hydroxamic acid was improved by prolonging the residence time by lowering the flow rate and increasing the volume of the reactor. When the reaction was performed at 70 °C for 30 min, 80% conversion was achieved by LC/MS, resulting in 82% yield of isolated **2a** (entry 6). For comparative purposes the reaction was run both as a standard batch process and in a sealed tube under microwave irradiation, applying the same conditions of temperature, concentration of reagents, and reaction time as optimized in the flow protocol (Table 2).

We found that such conditions gave moderate conversion using conventional equipment (58%, entry 1) and comparable result under microwave irradiation (72%, entry 2). The precise control of temperature distribution and the efficient heating and mixing associated with flow technique minimized the formation of the corresponding carboxylic acid as a byproduct and ensured that this simple chemical transformation proceeded at faster rates compared to the batch system.

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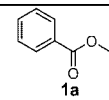
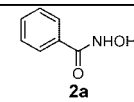
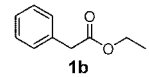
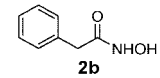
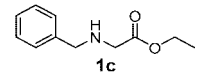
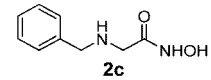
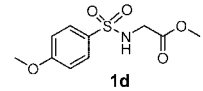
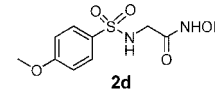
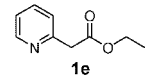
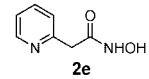
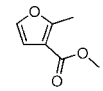
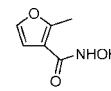
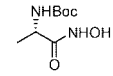
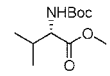
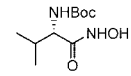
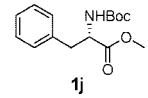
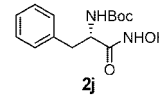
TABLE 3. Conversion of Esters into Hydroxamic Acids under Flow Conditions

entry	ester	hydroxamic acid	yield (%)[a]
1	 1a	 2a	82
2	 1b	 2b	96
3	 1c	 2c	96
4	 1d	 2d	95
5	 1e	 2e	97
6	 1f	 2f	52
7	 1g	 2g	100
8	 1h	 2h	81
9	 1j	 2j	83

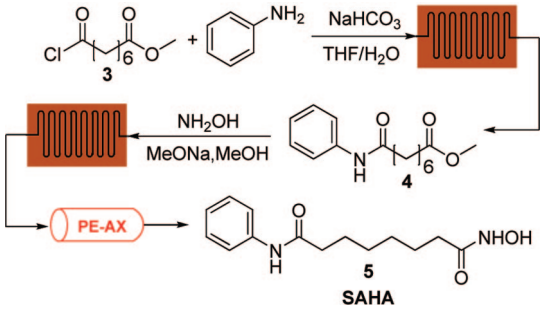
[a] Isolated yields; purity >95% (LC/MS).

To verify the effectiveness and reproducibility of the optimized reaction conditions, the synthesis of a small collection of hydroxamic acids was undertaken. The employed flow protocol was based upon the optimized conditions described for the synthesis of **2a** without further optimization (i.e., 70 °C, flow rate 0.5 mL/min, residence time 30 min) in a 250 mg scale. All of the desired hydroxamic products were obtained in good to excellent yields (Table 3) with no detection of the corresponding carboxylic acids byproduct.

The data reported in Table 3 clearly show that the formation of hydroxamic acids from esters is quite general and occurs smoothly in the continuous flow system. In fact simple aryl or alkylaryl esters (entries 1 and 2), simple amino esters (entry 3) and Boc-protected (entries 7−9) amino esters, sulfonamido-ester (entry 4), and heteroaryl esters (entries 5 and 6) were suitable substrates for the reaction. Moreover the optimized reaction conditions were successfully applied to enantiomerically pure esters without loss of stereochemical integrity. The specific optical rotation for the hydroxamic acid of *N*-Boc-alanine methyl ester **2g** was found to be identical to that reported in literature for the enantiopure compound {$[\alpha]^{20}_D$ −28; lit. $[\alpha]^{20}_D$ −29 (*c* 1, MeOH)}.[12a]

With compounds **1b**, **1d**, and **1j** the obtained results show an improvement if compared to the microwave-assisted process described in the literature.[12a] Noticeably for compound **1d** the isolated yield was increased from 33% to 95%; in the other cases an average improvement of about 20% was observed.

SCHEME 1. Two-Step Synthesis of Suberoylanilide Hydroxamic Acid (SAHA, 5)



Simple scale-up makes the continuous flow technique very advantageous when compared to MW systems, as up to now MW heating has proved to be unsuitable for large scale processes. Moreover the risks associated with failing to scale-up a process are limited using continuous flow. The reaction condition setup on the microreactor can be directly transferred to production on larger scale without the need for reoptimization by simply using the flow reactor for an extended time or by employing multichannel parallel reactors (numbering-up process).[13]

A demonstration of this preparative capability was readily obtained. Applying the optimized conditions and doubling the concentration of the starting materials, 4.3 g of *N*-hydroxy-2-phenylacetamide **2b** was straightforwardly produced after 1.5 h (output 2.9 g/h). Yield and purity were similar to that of the smaller scale, proving that the reaction conditions identified for the production of a few milligrams can be transferred to a larger scale without any further optimization.

In addition to the easy scalability of the protocol, the careful control of temperature exotherms and the small volumes employed by flow reactor allow the safer use of the highly toxic and potentially explosive hydroxylamine.[14]

On the basis of the good results obtained in the development of the continuous flow synthesis of hydroxamic acids, this new methodology was applied to the synthesis of suberoylanilide hydroxamic acid (SAHA, Scheme 1). This compound, one of the early histone deacetylase (HDAC) inhibitors discovered by Breslow and colleagues,[15] was approved by the U.S. FDA in October, 2006 for the treatment of patients with cutaneous T-cell lymphoma (CTCL).[16] Despite the several synthetic procedures for the preparation of SAHA that have been reported,[17] as far as we know, none of them described the synthesis under flow conditions. Our two-step sequence entails the conversion of the commercially available methyl suberoyl chloride **3** into methyl suberanilate **4** under Schotten−Baumann conditions, followed by the transformation of ester by aqueous hydroxylamine in presence of sodium methoxide (Scheme 1).

TABLE 4. First Step (Scheme 1) Optimization Results

entry	[A] (M)	[B] (M)	flow rate (mL/min)	residence time (min)	*T* (°C)	**4** (%)[a]
1	0.05	0.05	2	2.5	rt	>95
2	1	1	3.5	1.4	rt	>95

[a] Conversion was determined by LC/MS at 215 nM.

TABLE 5. Second Step (Scheme 1) Optimization Results

entry	MeONa (equiv)	flow rate (mL/min)	residence time (min)	*T* (°C)	**4** (%)[a]	**5** (%)[a]	**6** (%)[a]
1	1	0.33	30	70	73	19	8
2	1	0.33	30	90	39	49	12
3	2	0.33	30	90	17	70	13
4	2	0.4	50	90	3	83	15

[a] Conversion was determined by LC/MS at 215 nM.

In the first step a solution of suberoyl chloride in THF and a mixture of aniline and sodium carbonate in THF/H_2O were simultaneously pumped at room temperature into the reactor. In the optimized protocol the conversion of the acyl chloride into the corresponding amide resulted in clean and quantitative yield in less than 2 min (Table 4).

No product isolation at this intermediate stage was necessary: the output of the reaction was evaporated, and the obtained amido ester was dissolved in methanol and used directly in the second step without further purification.

Unfortunately the optimized reaction conditions to obtain hydroxamic acid described above gave only low conversion to **5** (SAHA). A new optimization process was necessary (Table 5), and the best conditions were achieved by flowing a solution of the crude methyl suberanilate and aqueous hydroxylamine in methanol together with a stream of 2 equiv of sodium methoxide in MeOH at 90 °C for 50 min.

With the aim of avoiding a time-consuming workup procedure and extensive manual purification of the final compound, an integrated sequential flow synthetic pathway was set up, employing an immobilized scavenger.[18] The reaction stream was then directly passed through a short packed column[19] containing silica-supported quaternary amine (ISOLUTE PE-AX)[20] for the selective removal of the carboxylic acid byproduct **6** (8-oxo-8-(phenylamino)octanoic acid). The solution containing the product and traces of the starting material was collected, and after solvent evaporation, crystallization from MeOH afforded SAHA in 84% yield and 99% purity (80% yield over two steps).

In conclusion, we have developed a simple and high-yielding method for the synthesis of hydroxamic acids directly from the corresponding carboxylic esters using a flow reactor. The synthetic protocol is amenable both for preparation of compound collections and for the scale-up of single derivatives. Various reaction conditions can be screened very easily and rapidly to set up a more efficient protocol by optimizing temperature,

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(19) Commercially available Omnifit glass chromatography columns with adjustable height endpieces.

(20) ISOLUTE PE-AX Sorbent is commercially available.

stoichiometry, and residence time. Continuous operations provide an attractive approach for handling hazardous processes and reagents in a safer manner. Remarkably, the method was successfully employed with enolizable esters, such as chiral amino acids, with no trace of racemization at the α carbon. Furthermore once the optimum reaction conditions are established the reaction can be scaled up to provide virtually any amount of product by running the apparatus for longer periods of time. In particular we have demonstrated the possibility of preparing 2.9 g/h of **2b**. Moreover the two-step synthesis of SAHA was developed and the purification of final product was achieved using immobilized scavengers.

Experimental Section

The flow reactor was configured using a combination of an R-2 Pump Module and R-4 Reactor Module.[8]

General Reaction Procedure for the Synthesis of Hydroxamic Acids. Reagent stock bottle A: ester (1 equiv); 50% aq hydroxylamine (10 equiv), 0.5 M solution in MeOH. Reagent stock bottle B: MeONa (1 eq), 0.5 M solution in MeOH. Using the automated injection system, both solutions were transferred at a constant flow rate (0.166 mL/min) into a preheated PTFE tubing reactor (reactor volume 10 mL) maintained at 70 °C. The reaction took place inside the reactor, and the product was collected in an appropriate product stock bottle. The solvent was evaporated, and the product was purified by a suitable method. The reactions were performed on a 250 mg scale.

Synthesis of Suberoylalanide Hydroxamic Acid. First Step. Preparation of Suberanilic Acid Methyl Ester. Reagent stock bottle A: methyl suberoyl chloride (200 mg, 0.97 mmol, 1 equiv) 1 M solution in THF. Reagent stock bottle B: aniline (62.4 mg, 0.97 mmol, 1 equiv); $NaHCO_3$ (81.2 mg, 0.97 mmol, 1 eq), 1 M solution in 1:1 THF/H_2O. Using the automated injection system, both solutions were transferred at a constant flow rate (3.5 mL/min) inside a PTFE tubing reactor (reactor volume 10 mL) at room temperature. The reaction took place into the reactor, and the product was collected in a suitable product stock bottle. The solvent was evaporated, and the crude of methyl suberanilate was used for the next step without further purification.

Second Step. Preparation of Suberoylalanide Hydroxamic Acid (SAHA). Two tubing reactors (10 mL each) were installed and connected together in series. The second reactor was connected to a packed column with solid-supported scavenger. Reagent stock bottle C: methyl suberanilate (255 mg, 0.97 mmol, 1 equiv); 50% aq hydroxylamine (0.58 mL, 9.7 mmol, 10 equiv), 0.5 M solution in MeOH. Reagent stock bottle D: MeONa (105 mg, 1.95 mmol, 2 equiv), 1 M solution in MeOH. Using the automated injection system, both solutions were transferred at a constant flow rate (0.2 mL/min) into preheated PTFE tubing reactors (total reactor volume 20 mL) maintained at 90 °C. The tubing reactor was connected to an Omnifit glass column (6.6 mm i.d.) packed with silica-supported quaternary amine (1.5 g)[20] to trap the carboxylic acid byproduct. After this purification step, the product was collected in an appropriate product stock bottle. The solvent was evaporated, water (10 mL) was added, and pH was adjusted to 7 with acetic acid. The precipitated solid was filtered and dissolved in hot MeOH, and then the solution was cooled to 0 °C. The formed solid was filtered to give 219 mg of suberoylalanide hydroxamic acid. Overall yield 80%.

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Supporting Information Available: Experimental procedures and characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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