BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902023e22764

Article

Continuous flow Aza-Michael reaction for preparing the fast-acting synthetic opioid drug Remifentanil

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Remifentanil is a modern fentanyl analogue with ultrashort-action granted by an esterase-labile methyl propanoate chain. Here, we present the development of a continuous flow methodology for the key N-alkylation step of remifentanil preparation in a biphasic, "slug-flow" regime. We screened parameters under microwave-assisted reactions, translated conditions to flow settings, and obtained remifentanil under 15-min residence time in a 1-mL microreactor, with a space-time yield of 89 mg/mL·h and 94% yield.

Keywords: Remifentanil. Opioids. Organic synthesis. Continuous flow synthesis.

INTRODUCTION

Remifentanil (Figure 1, structure 2) is a fast-acting synthetic μ-opioid that can provide rapid onset and offset of anesthetic effects (Bürkle, Dunbar, Van Aken, 1996). This is important for the clinical management of pain, especially during preoperative analgesia and mechanical ventilation of intubated patients. This drug and fentanyl are estimated to have equivalent potency (Figure 1, structure 1), but, like other μ-opioids, remifentanil causes relevant side effects such as bradycardia and respiratory depression. Nevertheless, thanks to its fast *in-vivo* metabolization, remifentanil offers patients much faster recovery times, a feature that may arguably diminish its potential for abuse. Remifentanil has similar structure to carfentanil (Figure 1, structure **3**), one of the most potent fentanyl analogues designed by Janssen in the 1960s (Janssen, Gardocki, 1964; Raffa *et al*., 2018). However, unlike carfentanil, remifentanil, as devised by Feldman *et al.* at Glaxo Inc. in the 1990s, bears a methyl propanoate chain instead of an alkylaryl moiety at the nitrogen of the piperidine ring (Feldman, 2020), so it is rapidly metabolized by non-specific esterases in the bloodstream and tissues and converted to the inactive remifentanil acid.

Continuous flow technologies have gained growing attention from organic and medicinal chemists and from the fine chemistry industry and producers of active pharmaceutical ingredients (APIs) (de Souza *et al*., 2018; Porta, Benaglia, Puglisi, 2016; Aguillon *et al*., 2020; Murie *et al*., 2021). Continuous flow techniques have allowed chemists to develop up-scalable setups aimed at producing important APIs and natural products on demand (Adamo *et al*., 2016; Pastre, Browne, Ley, 2013), promoting faster processes with lower footprint, especially during the multi-step preparation of bioactive compounds (Bloemendal *et al*., 2020). Regarding our studies on the synthesis, stability, and metabolism of important analgesics belonging to the class of fentanyl opioids, we have been searching for an innovative methodology to convert norcarfentanil hydrochloride (Figure 2, structure 4) to remifentanil through an aza-Michael addition reaction carried out in flow in a multiphase solvent system. In this work, we have developed a continuous flow methodology that focuses on a key step of remifentanil preparation. The methodology allowed highly pure API to be prepared from norcarfentanil in a biphasic, "slug-flow" regime.

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MATERIAL AND METHODS

General

The NMR spectra were recorded at ambient temperature on Bruker®: DRX-400 (^{1}H, ^{13}C[^{1}H]) and DRX-500 (^{1}H,^{13}C[^{1}H]) spectrometers. The ^{1}H and ^{13}C chemical shifts are reported in ppm by using the residual solvent signal of the deuterated solvents (Deuterated chloroform: δH=7.26 ppm, δC=77.16 ppm and deuterated methanol: δH=3.49 ppm, δC= 49.86 ppm). All the chemical shifts are reported in parts per million relative to tetramethylsilane (δH = 0.00 ppm). All the coupling constants are reported in Hz. Chromatographic analyses coupled to mass spectrometry (GC–MS analyses) were performed on a Shimadzu® GCMS-QP2010 operating with electron ionization (EI: 70 eV) and equipped with an automatic injector. The employed column was ZB-5ms (5% Phenyl-Arylene, 95% Dimethylpolysiloxane). The detected ions are presented as *m/z* ratio and percentage intensities (%).

Microwave-assisted reactions

Reactions under microwave irradiation for methodological screening were carried out in septum-sealed glass vials in an Anton Paar GmbH - Monowave 300 device. 4-Piperidinone was used as model substrate for evaluating the aza-Michael reaction conditions. To a 30-mL microwave vial containing water (10 mL) and tetrahydrofuran (THF) or methanol (10 mL), 4-piperidone hydrochloride monohydrate (500 mg, 3.26 mmol) and potassium carbonate (K_2CO_3, 700 mg, 5 mmol, 1.5 equiv.) were added. The vial was sealed, and the mixture was stirred until it was completely homogenized. The reaction mixture was irradiated until 50 °C for 15 min. After that, the mixture was partitioned with brine (10 mL) and extracted with ethyl acetate (2 x 15 mL). The organic phase was separated, washed with brine, and dried with magnesium sulfate, to afford methyl 3-(4-oxopiperidin-1-yl)propanoate without need for further purification. The product was a clear yellow oil with 76% isolated yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.66 (s, 1H), 2.78 (t, *J* = 7.2 Hz, 1H), 2.73 (t, *J* = 5.9 Hz, 2H), 2.50 (t, *J* = 7.1 Hz, 1H), 2.40 (t, *J* = 5.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 208.8, 172.8, 52.9, 52.6, 51.8, 41.2, 32.7. GC-MS m/z (%): 112.1 (100), 42.1 (41), 56.1 (16), 84.1 (12), 55 (10), 113.1 (8), 98.1 (7), 185.1 (6), 57.1 (4), 59 (4), 41.1 (3).

Continuous flow reactions

To pump the reagent stock solutions, one or two Syrris® Asia Syringe pumps were used, equipped with 250/500 µL or 0.5/1 mL syringe pairs. An Asia Heater, equipped with either an Asia Tube Reactor (stainless steel, 4 mL) or an Asia Microreactor (glass, 1 mL), was employed, with tubing and connections (1.57 mm i.d.) composed of PTFE and PEEK polymers, respectively. At the end of the continuous flow setups, a back pressure regulator (BPR) with 516-kPa rated cartridge was used. When remifentanil was prepared in continuous flow with the use a microchip reactor, the system comprised two channels ("A" and "B") with stock solutions as follows: Channel A, Norcarfentanil hydrochloride (**4**) (0.125 mol/L) and K_2CO_3 (0.250 mol/L) in water; and Channel B, methyl acrylate (0.15 mol/L) in THF. This system gave an isolated yield of 94%, with a calculated space-time yield (STY) of 67 mg/mL·h. Remifentanil was prepared in a stainless-steel tube; the setup consisted of three channels ("A", "B", and "C"), a 4-mL stainless-steel reactor, and a 516-kPa BPR at the end of the system. Channel A: Norcarfentanil hydrochloride (0.5 mol/L) in water. Channel B: K_2CO_3 (1 mol/L) in water. Channel C: Methyl acrylate (0.6 mol/L) in THF. The reactor was heated to 75 °C, and the flow rates were set to a residence time (Res. T.) of 20 min. After a steady state was reached (at least 25 min), reaction samples were collected and extracted as described earlier and analyzed, and the yield was calculated. Under these conditions, remifentanil (**2**) was obtained in 80% isolated yield, and the calculated STY was 112 mg/mL·h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65–6.67 (m, 5H), 3.67 (s, 3H), 3.52 (s, 3H), 2.52 (t, *J* = 7.5 Hz, 2H), 2.49 (d, *J* = 11.9 Hz, 2H), 2.36–2.26 (m, 4H), 2.15 (d, *J* = 13.0 Hz, 2H), 1.75 (q, *J* = 7.4 Hz, 2H), 1.48 (td, *J* = 13.0, 4.1 Hz, 2H), 0.83 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.13, 173.99, 172.93, 139.51, 130.71, 129.33, 128.70, 62.84, 53.33, 52.07, 51.58, 49.66, 33.55, 32.19, 29.09, 9.19. GC-MS m/z (%): 168.1

(100), 227.1 (69), 212.1 (49), 42.1 (35), 303.2 (30), 57.1 (27), 140.1 (25), 142.1 (22), 59.1 (17), 113.1 (14), 228.1 (13).

RESULTS AND DISCUSSION

During our search for a novel and efficient methodology for preparing fentanyl analogues, we investigated reaction conditions under microwave irradiation that could be the translated to continuous flow settings, in what has been called a "microwave to flow" investigative approach (Glasnov, Kappe, 2011). An important aspect of continuous flow methodologies is that, while in flow, reactions are carried out in microchannels, which can greatly enhance mass and heat transfer processes (Kockmann *et al.*, 2008; Mandrelli *et al.*, 2017). However, this limits flow reactions conducted in homogeneous media: the presence, or formation, of solids in the reaction mixture likely results in setup clogging and failure (Pieber, Gilmore, Seeberger, 2017). Therefore, a sound strategy when developing preparation methods in continuous flow is to screen reaction conditions in conventional batch settings and searching for optimal combinations of solvent systems, reagent concentrations, and heating that could then be employed in a flow setup. To accomplish this, we began our investigation by reacting a simple model substrate, 4-piperidinone, with different equivalents of methyl acrylate in the presence of inorganic bases, in various combinations of solvent systems. We carried out this reaction, an example of an aza-Michael addition, in batch under reflux for several hours, to achieve full conversion. This procedure can be greatly accelerated if conducted under microwave irradiation. Thus, after screening for the reaction conditions, we found that 4-piperidone hydrochloride could be reacted with methyl acrylate in a water/THF biphasic system, in the presence of two equiv. of a base, in a biphasic solvent system under microwave irradiation ranging from 50 to 75 °C, to obtain 76% isolated yield. It is important to mention that, in batch processes of greater volumes, the presence of biphasic systems can be highly detrimental to reaction yields because contact between reactants in different phases is limited, which often requires the use of phase transfer catalysts (PTCs), i.e. surfactants (Malet-Sanz, Susanne, 2012). Under flow, however, biphasic solvent systems result in a "slug-flow" regime, which can greatly increase the contact surface between phases and enhance such reactions (Figure 1, C). Therefore, we proposed that this type of reaction could be successfully performed in flow in the absence of a PTC.

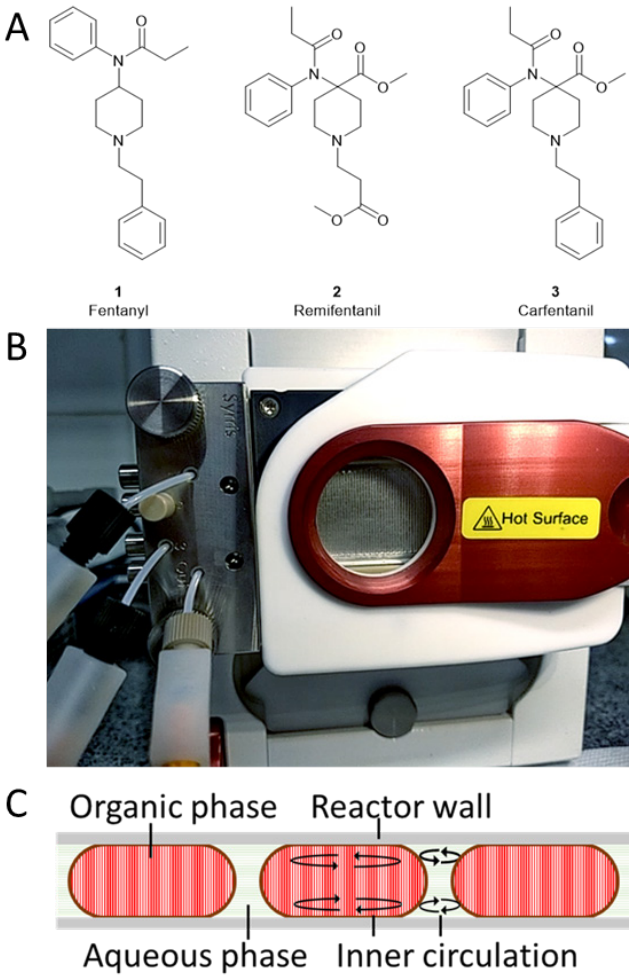
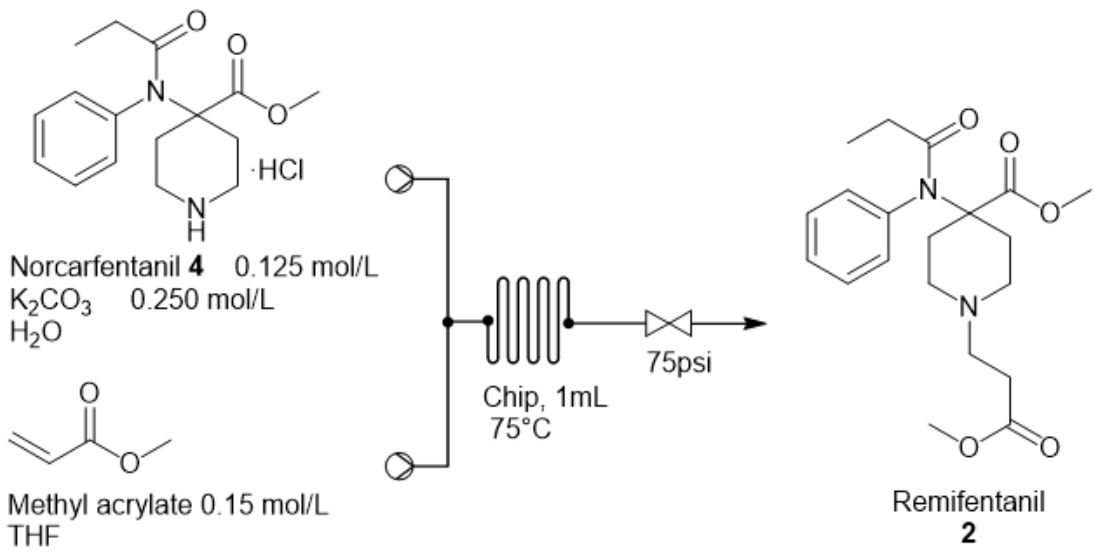


FIGURE 1 - A: structures of fentanyl, remifentanil, and carfentanil. B: a glass chip microreactor with a slug-flow reaction being carried out. C: diagram of a slug-flow regime.

After we established the reaction conditions under microwave heating and successfully accomplished the alkylation of our model substrate, we devised a dual-channel setup to investigate the aza-Michael reaction under flow conditions for application in remifentanil preparation. With this setup, we pumped a stock aqueous solution of norcarfentanil hydrochloride (0.125 M) and K_2CO_3 (0.250 M) and a stock THF solution of methyl acrylate (0.15 M) through dedicated channels. After

meeting in a regular T-Union, the reaction mixture was pumped through a glass microchip reactor (1-mL internal volume), heated to 75 °C. We attached a 75-psi rated spring BPR at the end of the system to prevent the reaction mixture from boiling (Figure 2, top diagram). We set the flow rates of the system with a view to evaluating total residence times ranging from 2.5 to 20 min. At each flow rate, we allowed sufficient system runtime (>1.3x residence times) so that steady-system states would be achieved. Then, we collected reaction volumes and neutralized and extracted them to evaluate outcomes after workup.



Entry	Res. T. (min)	Isolated Yield (%)	STY (mg/mL·h)
1	2.5	57	319
2	5	64	180
3	10	88	124
4	15	94	89
5	20	94	67

FIGURE 2 - Flow setup diagram for the aza-Michael reaction for preparing remifentanil and space-time yields under different reaction residence times.

Under a residence time of only 20 min, we achieved an isolated yield of 94% for remifentanil. Under this condition, we obtained a calculated STY of 67 mg/mL·h (Figure 2, entry 5). We were able to achieve an almost fivefold STY of 319 mg/mL·h under shorter residence times (Figure 2, entry 1). Nevertheless, reaction yields under these conditions were considerably lower, an aspect that may have to be accounted for, especially regarding the overall E-factor of the process if up-scaling is desired (Dallinger, Kappe, 2017; Sheldon, 2017). These results prompted us to explore how a flow system comprised of three channels, dedicated to stock solutions with higher concentrations, could improve reaction outcomes. Thus, we devised a new setting, equipped with a 4-mL stainless-steel coil reactor heated to 75 °C (Figure 3).

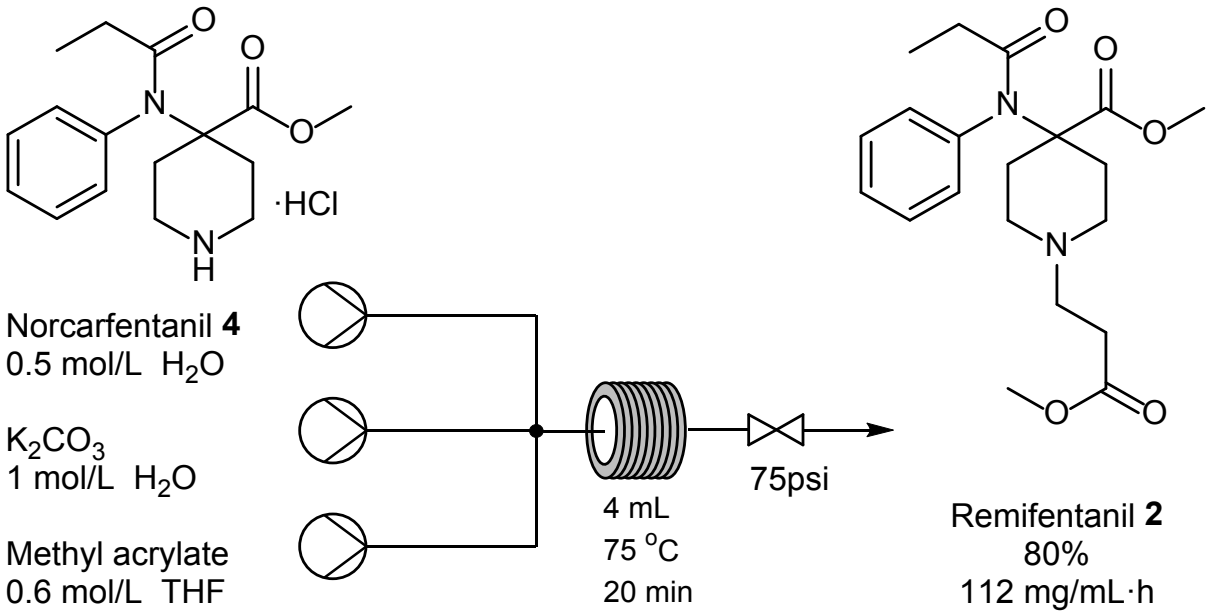


FIGURE 3 - Continuous flow setup scheme for preparing remifentanil.

With this setup, we configured the combined flow rate of the three pumps so that we would achieve a residence time of 20 min, and that stock solutions with fourfold reagent concentrations could be used without any pump stall. This allowed us to achieve STY of 112 mg/mL·h for remifentanil, with an isolated yield of 80% (Figure 3).

In summary, we have developed an efficient flow-based methodology that allows high-purity remifentanil to be continuously prepared from norcarfentanil hydrochloride. The use of continuous flow reactors in a biphasic "slug-flow" regime enables precise control of the aza-Michael reaction, providing faster and more efficient production of the API when compared to the corresponding batch process. The scope of the developed methodology and its application toward the synthesis of other important opioid drugs is currently being investigated in our laboratories.

ACKNOWLEDGMENTS

This work was made possible thanks to the support of Fundação de Amparo à Pesquisa de São Paulo, FAPESP– Grants 16/12718-1, 17/50188-7, 22/05327-7, Conselho Nacional de Desenvolvimento e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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Received for publication on 04th October 2022
Accepted for publication on 18th February 2023