Continuous flow supercritical CO_2 platform for *in-situ* synthesis and purification of small molecules for drug discovery

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ABSTRACT: The use of supercritical CO_2 ($scCO_2$) as an enabling technology paves the way to an efficient in-line integration of the synthesis and purification of organic molecules. The $scCO_2$ platform presented here provides a streamline process to produce a molecular diverse family of triazoles, common drug precursors, by 1,3-dipolar Copper-catalyzed azide-alkyne cycloaddition (CuAAC), so-called Huisgen reaction also decreasing the environmental impact by significantly reducing the use of traditional solvents. To exemplify potential of this $scCO_2$ platform, a side from the preparation of a family of triazoles, the synthesis and purification of Rufinamide, a drug used to treat seizures associated with Lennox-Gastaut syndrome, is also reported.

KEYWORDS: Supercritical CO_2, flow chemistry, triazole, 1,3-dipolar cycloaddition, click chemistry, in-line/on-line.

INTRODUCTION

The need for a sustainable and cost-effective synthesis of druggable molecules is an essential goal of the innovative pharmaceutical industries being the solvent reduction/substitution one of the green chemistry challenges. In a typical synthesis of a drug, the solvent use represents the largest contributor to analyses of green chemistry metrics such as Process Mass Intensity (PMI).[1] Indeed, ACS GCI Pharmaceutical Roundtable has identified the Solvent Minimization as key challenge. A large quantity of organic solvents is used not only in the synthetic steps to solubilize reaction components but also more importantly in the separation and purification of the resulting reaction mixtures. Indeed, the separation steps contribute a range of approximately 40-90% of the process mass intensity of a synthesis. In terms of energy utilization, distillation and drying steps alone often consume greater than 50% of the energy requirements of a process.[2] As a result, both waste generation and the energy demand involve during the separation steps often overpass those needed for the reaction synthesis.

In the last few years, continuous flow processes are becoming a standard in the development and manufacture of Active Pharmaceutical Ingredients (APIs).[3,4] Indeed, pharmaceutical industry is undergoing a transition to continuous processes to benefit from the reduced lead time, cost, and footprint, as well as, from the improved quality associated with this methodology.[5,6] However, one key factor, often overlooked, is that reactions tend to be run under relatively dilute conditions to ensure homogeneity, leading to increased usage of solvents.

In the search of a solution to mitigate the environmental impact of the discovery and manufacturing of druggable molecules, the use of supercritical carbon dioxide ($scCO_2$) has been envisioned. $scCO_2$ offers multiple and diverse advantages as replacement for traditional solvents. CO_2 is abundant, non-toxic, non-flammable, inert and cost-effective compound. Although being a

greenhouse gas, the CO_2 used in Chemistry Research activities is not generated for this purpose but recycled from a previous use (usually by other industries) and, therefore it does not contribute to increase the greenhouse effect being a substance of great potential as a green solvent.[7] Indeed, $scCO_2$ Although being a greenhouse gas, the CO_2 used in Chemistry Research activities is not generated for this purpose but recycled from a previous use (usually by other industries) and, therefore it does not contribute to increase the greenhouse effect being a substance of great potential as a green solvent (extraction,[8] dyeing,[9] drying,[10] materials processing,[11] etc) and, therefore, it can be considered as a mature green solvent.[12] Furthermore, when chemical transformations are performed in $scCO_2$, the reaction environment can be tuned by manipulating pressure and/or temperature leading to unique chemical and process advantages,[13] including faster reaction rates better productivity and selectivity, or process intensification and safety, among many others.[14]

Both CO_2 and continuous flow processes can coin to be inherently safer due to a myriad of reasons including lower reaction volumes, dilution of high explosive reagents (i.e. hydrogen, oxygen, azide) better temperature control (i.e. avoiding exotherms), and ability to accommodate higher pressures without risk [15] Indeed, the potential of $scCO_2$ platform can be used to rapidly access high-value compounds under flow conditions in telescopic and a multigram scale processes.[16,17] In addition, SFC-CO_2 has been used as chromatographic mobile phases for several decades currently being a well-established technique used together with high-performance liquid chromatography (HPLC) for the purification of compounds of wide structural diversity and at any scale (from mg to Kg), mostly in the chiral environment.[18] Therefore, the use of $scCO_2$ enables the coupling of both, the synthesis and the purification steps, in a continuous streamline process either

by manipulating the phase behaviour of the systems or by the coupling of supercritical fluid chromatography (SFC).[19]

Here we report an example of this reaction and purification integration that offers an efficient way to produce a molecular diverse family of 1,2,3-triazoles with improved overall efficiency of the process and significant reduced consumption of organic solvents.

RESULTS AND DISCUSSION

The device and the initial catalyst screening for Huisgen 1,3-dipolar cycloaddition under $scCO_2$. The design of the $scCO_2$ flow platform used in this work is schematically represented in the Figure 1. The in-house built system is composed by two modules. The first module is used for the synthesis and consists of a $scCO_2$ pump, an automatic 6-port valve injector with a reagents loop and, a reactor. The system can accommodate a tubular reactor or, alternatively, a fix-bed reactor according to the specific needs of the reaction. The remaining components can be shared in all configurations. A solution of the reagents is injected into the $scCO_2$ flow stream using an injector reagent port. The residence time is controlled by the flow of $scCO_2$. This simple reaction flow module featured a high reaction speed that allowed reagents, conditions, and catalyst to be screened in less than 5 minutes (from reagent injection to product collection). The purification module is directly connected to the reaction module by a set of valves and consists of two additional pumps (for the $scCO_2$ and the modifier) and a preparative chromatographic column. The pressure and temperature of the whole system are controlled by a back-pressure regulator and an oven, respectively, with all the parameters being monitored by corresponding sensors.

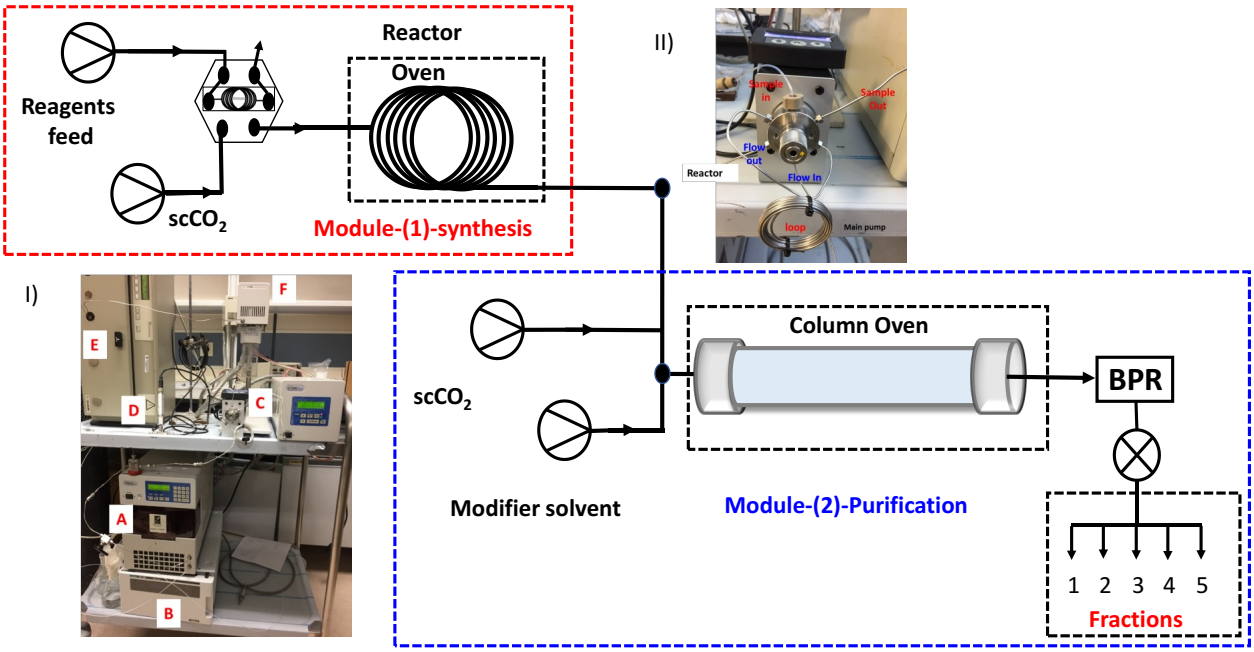


Figure 1. Flow platform for the in-line $scCO_2$ synthesis and purification. **Module-(1)-synthesis**: A: CO_2 pump (Jasco PU-2080-CO_2 Plus), flow rate range: 0.001~10 mL/min, maximum pressure: 300 bar; B: HPLC liquid pump (Jasco PU-4180) flow rate range: 0.001~10mL/min, maximum usable pressure: 300 bar; C: the reaction injector. 6 port 2-pos valve, u-electric, TTL 1/16*.75mm, 225 ºC/400 psi gas, N60/E Universal electric actuator Interface: TTL (contact closure). Injector loop: 1.55 mL; D: pressure transducer; E: HPLC oven and tubular reactor (stain-steel coil) of external diameter 1/16 inches (0.15875 cm), wall size 0.0508 cm, internal diameter 0.05715 cm. Length 6 m Total volume: 1.54 cm^3; F: Back Pressure Regulator (Jasco BP-4340). **Module-(2)-purification**: CO_2 pump (Jasco PU-2080-CO_2 Plus) flow rate range: 1~10 mL/min, maximum pressure: 300 bar; HPLC liquid pump (Jasco PU-4180 HPLC Pump), flow rate range: 1~5 mL/min, maximum pressure: 300 bar; HPLC chromatographic oven (Gilson MODEL 831 Temperature Regulator), Separation column: benzene sulfonamide 100A 5u, 250 x 21.2 mm (Princenton chromatography), Back Pressure Regulator (Jasco BP-4340). Safety maximum pressure limit set at 180 bar.

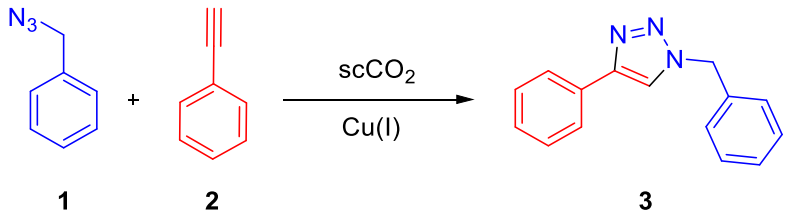
The 1,3-dipolar Copper-catalyzed azide-alkyne cycloaddition (CuAAC), so-called Huisgen reaction,[20] was selected as model reaction to evaluate the potential of this platform. This reaction is the most convenient protocol for the synthesis of functional 1,2,3-triazoles, which are well-known scaffolds with widespread occurrence in medicinal compounds.[21,22] 1,2,3-Triazole rings formed from azides and terminal acetylenes via the Cu(I)-catalyzed cycloaddition reaction are often found to have a variety of biological properties, including anti-HIV,[23] antiallergic,[24] antifungal,[25] anticancer,[26] and antibiotic activity.[27]

Different types of copper catalysts have been reported for the preparation of 1,2,3-triazoles under flow conditions. [28] Since the work reported by Bogdan and Sach copper tubular reactors have been used for the continuous flow synthesis of 1,2,3-disubstituted triazoles.[29] Although copper tubes have been used as catalytic reactors to carry out this reaction, Füllöp et al. have demonstrated that copper powder is an efficient, cheap, and available source of Cu (I) as catalyst for this reaction under high-pressure continuous flow conditions.[30]

Regarding the CuAAC reaction in supercritical carbon dioxide, there are only few examples reported in the literature. The reaction has been used for the modification of polymer using Cu(I), [31] and for the synthesis of family of triazoles under relative mild condition using both metallic copper wires[32] and Cu (II) salts in absence of ligand.[33]

Based on these antecedents, we decided to evaluate the cycloaddition reaction between the benzyl azide (**1**) and phenylacetylene (**2**) as benchmark reaction using the in-house built continuous flow $scCO_2$ reaction module 1 platform (Scheme 1). In our setup, the $scCO_2$ stream modulates residence times, while the modifier (organic solvent) helps solubilising the reaction reagents and products. The copper metallic powder was initially evaluated as possible catalytic system for the synthesis of the triazole **3**. The initial experiments were performed by injecting 1.55 mL of a solution of 400

mM of the reagents **1** and **2** in acetonitrile (ACN) through a fixed-bed reactor (length 11 cm of ¼ inch) loaded with Cu(0) (3,854 g of Cu (0)) using $scCO_2$ as carrier solvent (1 mL/min) at 90 °C and 85 bar.



1 **2** **3**

Scheme 1. Selected benchmark Copper-catalyzed azide−alkyne cycloaddition.

Under these conditions up to 80% of yield of the desired product was observed when the reaction crude was concentrated and analysis by ^{1}H-NMR. Encourage by this promising result, the reaction was carried out injecting different concentration of reagents ranging from 100 to 400 mM. The Figure SI.1 depicts the yield calculated by ^{1}H-NMR form the reaction crude after evaporation of the acetonitrile.

These results showed that the yield increased with the reagents concentration. This is in good agreement with the expected mechanism reported for reaction in presence of Cu turnings. The generally accepted mechanistic hypothesis concerning the CuAAC reaction assumes that the genuine catalytically active species in the cycloadditions using Cu(0) metal is in fact Cu(I) species leached from the surface.[34] Keeping temperature, pressure, and flow rate constant, the only changes made were related to the composition of the reaction mixture processed through copper bed. Kappe et al. has stabilised that leaching induced by the azide cycloaddition partner **1** was 20 times higher than with phenylacetylene **2**, indicating a strong complexation of the organic azide with the Cu(I) or Cu(II) species present in the Cu surface.[34] Stable complexes between organic azides and Cu(I/II) ions have been reported in the literature providing support for the observed leaching of Cu by azide **1** under experiments. Our results can be related with leaching of Cu metal

when the reaction mixture was passed through the Cu, higher is the concentration used higher the leaching and therefore the yield observed by ^{1}H-NMR.

Although these results were very promising, we found that they were misleading. Indeed, yields lower than 25% were found, even for the best conditions, when the same samples were directly analysed by HPLC instead of ^{1}H-NMR. The higher yield calculated by ^{1}H-NMR can be attributed to a non-efficient quenching of catalytic active species in the solution. The reduced pressure and temperature used in the rota-evaporator favoured the evolution of the reaction as the catalyst concentration increase, under solvent evaporation, and copper can be complexed with the triazole formed. These factors push forward the conversion of the reactants into the wanted product leading to higher yields than those calculated by the HPLC of the samples directly obtained from the reactor. The direct analysis of the reaction solution by HPLC avoids this issue. The reaction solution was diluted and directly analysed by HPLC. The samples prepared in this form were stable not observing any appreciable evolution of the reaction even after 24 hours of their preparation. Thus, the samples coming from the continuous flow reactor should be diluted and analysed within 24 hours after their preparation.

On the basis of these results, a new experiment using a fix-bed reactor loaded with fresh Cu(0) was carried out again but using lower flow rate of the $scCO_2$ as carrier (0.5 mL/min) and higher temperature and pressures to favour the reaction. The results obtained are summarised in the Table S.1 (entry corresponds with a consecutive injection using the same fix-bed reactor). In the first injection up to 48% of the desired triazole **3** was obtained. When the reaction mixture was concentrated and analysed by ^{1}H-NMR, an overestimated 91% yield was observed. Unfortunately, when the additional reagents solution was injected into the systems under the same conditions a

lower yield was obtained. Indicating that the copper suffered of some deactivation/passivation process. Indeed, complete loss activity was observed in the third sequential injection.

The Figure SI.2 showed the final aspect of the Cu(0) used in continuous flow reactions with $scCO_2$ and ACN compared to fresh Cu(0). It seems clear that CO_2 induce some copper surface modification leading to catalyst deactivation. Indeed, when 75 mg of recycled cupper was used for the batch reaction (0.5 mL of 400 mM reagents **1** and **2** / 70 ºC) 95% yield was observed if using ACN as solvent while not activity was observed if using $scCO_2$ which confirmed the passivation of the copper by CO_2.

Table 1. Results of the model reaction with consecutive injections using of $Cu(OAc)_2 \cdot H_2O$.[a]

Entry	**[1] (mol/L)**	**Catalyst**	**Yield (%)**[b]
1	0.4	$Cu(OAc)_2 \cdot H_2O$	10[c]
2	0.2	$Cu(OAc)_2 \cdot H_2O$	77
3	0.2	$Cu(OAc)_2 \cdot H_2O$	82
4	0.2	$Cu(OAc)_2 \cdot H_2O$	86
5	0.2	$Cu(OAc)_2 \cdot H_2O$	96
6	0.2	$Cu(OAc)_2 \cdot H_2O$	72
7[d]	0.2	$Cu(OAc)_2 \cdot H_2O$	67
8	0.2	$CuCl_2$	23

[a] Consecutive injection of 1.55 mL of 400-200 mM solution of **1** and **2** in ACN using 0.5 ml/min of $scCO_2$ as carrier at 100 ºC and 85 bars, 1% mol cat. [b] Yield calculated by HPLC. [c] Reactor blockage. Solid product. 1% mol. [d] 0.5% mol cat.

In view of the results obtained with Cu(0), we decided to evaluate the use $Cu(OAc)_2 \cdot H_2O$ as homogeneous possible alternative catalyst.[33] For this, the fix-bed reactor was substituted by 1/16" coil reactor. The Table 1 summarized the results obtained injecting a 400 mM solution of the reagents in ACN and 1 % mol $Cu(OAc)_2 \cdot H_2O$ in the $scCO_2$ stream (0.5 mL/min) at 100 ^oC and 85

bar. Under this condition a blockage of the reactor was observed (Entry 1, Table 2). This suggested a high conversion of the reagents to the final product **3**, known to be solid.[35] Indeed, pure **3** was only detected in the MeOH solution resulting from reactor clean-up. To avoid this issue, the concentration of the solution of the reagents **1** and **2** was reduced by a half, to 200 mM. In the new conditions, a 77% yield of the product was obtained (Entry 2, Table 2). Three additional injections were, then, sequentially carried out. For these injections, the yield for **3** was progressively improved from 82 to 96% (Entry 3-5, Table 2) which was explained by the undesired carry-over of product between sequential injections. When two additional injections were carried out reducing the amount of Cu(II) from 1% to 0.5% mol, slight reduction of the yield was observed (Entry 6-7, Table 2).

The catalytic efficiency of the system can be attributed to *in situ* Cu(I) generation from the air-stable Cu(II) salt, which is supported by the change observed in the colour solution from blue/greenish ,characteristic of the Cu(II) transition, to yellow (Figure SI.3).

The effectiveness of $Cu(OAc)_2$ in enabling rapid CuAAC reactions in $scCO_2$ offers promises in developing CuAAC-based bioconjugation protocols without the sensitivity to molecular oxygen. It should be noted that, at ambient temperature, the same 200 mM solution only yielded 2% of **3** in 1 hour when the reaction was run under batch conditions without $scCO_2$. In contrast, the yield increased only yielded to 2% during one hour in comparison with the 3.08 minutes of residence time under flow reaction. The yield can be improved until 77% by heating at 70 ^{o}C for four hours. It should be also mentioned that the using the same conditions than in the Table 1 (entry 2, 100 ^{o}C and 85 bar) but using ACN as solvent carrier instead of $scCO_2$ only 50% yield was obtained.

Those results could be explained by the reduction of the $Cu(OAc)_2$ catalyst in the presence of the phenylacetylene by Eglinton oxidative homocoupling (Figure SI.3).[36] The terminal alkyne could

be deprotonated by the action of the $CH_3CO_2^-$ species then, oxidized by Cu(II) to form the alkynyl radical that could afterwards dimerize to deliver the corresponding 1,3-diyne and Cu(I). Indeed, when the reaction was performed under identical conditions of those detailed in entry 8-Table 2 but using $CuCl_2$ instead of $Cu(OAc)_2 \cdot H_2O$, only 23% yield was obtained. This result highlights the importance of the copper counterion and the possible mechanism involving the phenylacetylene dimerization.

In view of these results, the effect of P and T over reaction efficiency was evaluated by multiple injection of the reagents **1** and **2** using 0.5 mL/min of $scCO_2$ as carrier (Figure 2) The results suggested that both variables can be used to adjust the reaction conditions and optimise the reaction efficiency higher temperature led to higher yield (up to 92%). Same yield could be obtained at lower temperature if increasing the pressure and confirmed that the catalytic efficiency could be easily manipulated by adjusting those two parameters.

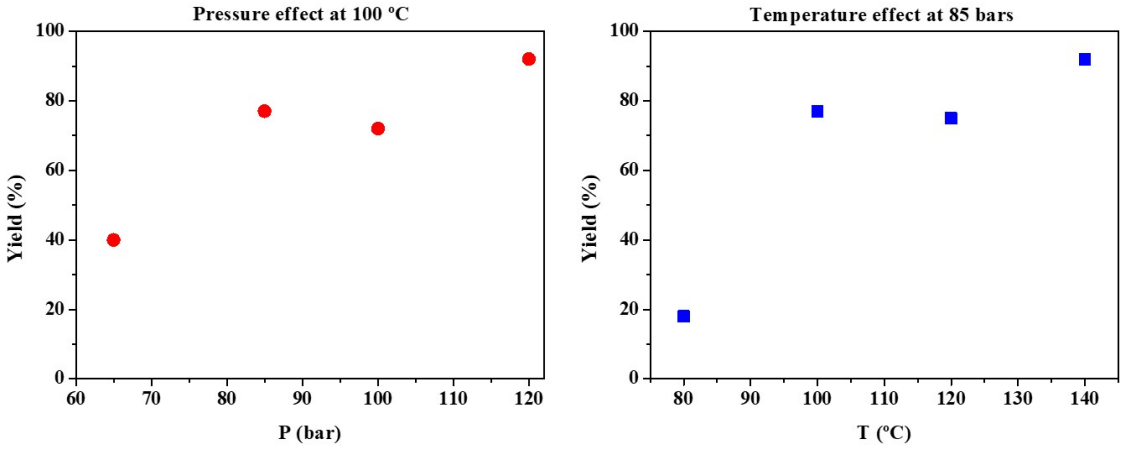
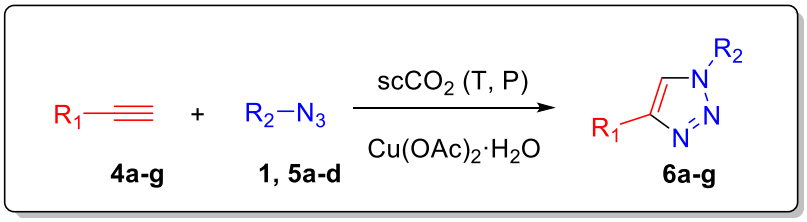


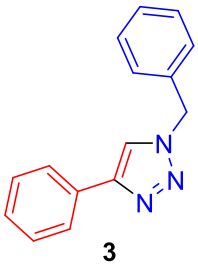
Figure 2. Effect of the pressure and temperature for the model reaction under flow conditions (200 mM solution of reagents **1** and **2** in ACN, 1% of $Cu(OAc)_2 \cdot H_2O$, 0.5 mL/min $scCO_2$).

Chemistry exemplification. Once a suitable catalytic system was found, the applicability of the methodology for the preparation of a family of compounds of wider structural diversity was

assayed. In a first study, the reaction of the benzyl azide with different alkynes was evaluated (Figure 3). The different alkyl azides (**5a-d**) were prepared with a new efficient polymer-supported azide as efficient polymeric reagent,[37] that yielded the corresponding acetonitrile solution of azide (**5a-d**) under mild nonaqueous conditions and, upon fast and easy filtration of the polymeric reagent. The 200 mM acetonitrile solution of the corresponding alkyne (**4a-g**) and the azide (**5a-d**) in a 1:1.2 equivalent ratio together with 1% mol of $Cu(OAc)_2 \cdot H_2O$ was injected to the reaction platform using $scCO_2$ as carrier (0.5 mL/min).

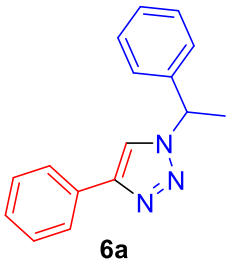
The Figure 3 summarised results obtained for the preparation of eight 1,2,3-triazoles with structural diversity. The temperature and pressure were fine-tuned to produce the targeted compound in good to excellent yields in only 3.08 minutes of residence time.





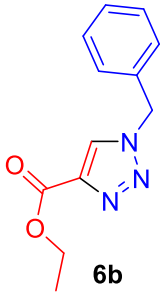
3

92% (100 °C, 120 bar)
92% (140 °C, 85 bar)



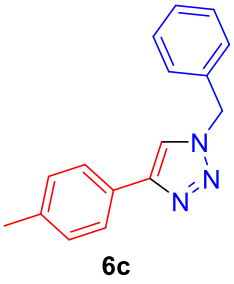
6a

47% (100 °C, 120 bar)
70% (140 °C, 100 bar)



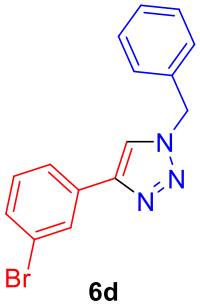
6b

99% (100 °C, 85 bar)
99% (140 °C, 100 bar)



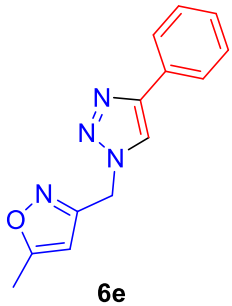
6c

37% (100 °C, 85 bar)
99% (140 °C, 100 bar)



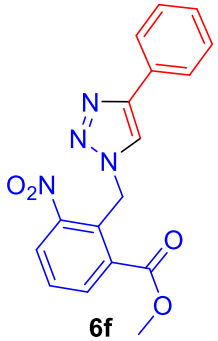
6d

25% (100 °C,85 bar)
53% (140 °C, 100 bar)



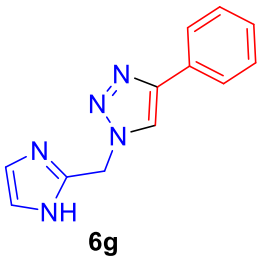
6e

39% (100 °C, 85 bar)
65% (140 °C, 120 bar)



6f

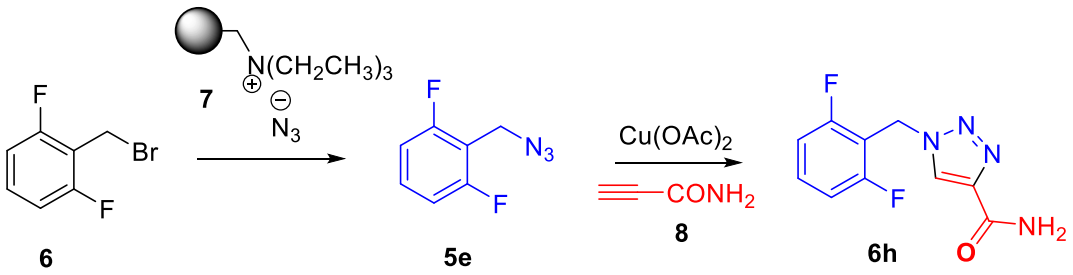
50% (100 °C, 85 bar)
95% (140 °C, 120 bar)



6g

90% (100 °C, 85 bar)

Figure 3. Family of 1,2,3-triazoles obtained from the reaction of a solution 200 mM of azide and alkyne in ACN catalysed by 1% of $Cu(OAc)_2 \cdot H_2O$ using $scCO_2$ as carrier at 0.5 mL/min. The temperature (Celsius degrees) and pressure (bar) values detailed are those yielding the best results.



Scheme 2. Schematic synthesis of rufinamide used in this work.

The platform was next evaluated for the synthesis of Rufinamide, a drug used in the treatment of seizures associated with Lennox-Gastaut syndrome.[38]

Table 2. Yields of Rufinamide obtained in consecutive injections of 1.55 mL solution of **5e** and **8** in ACN using 0.5 ml/min of $scCO_2$ as carrier and $Cu(OAc)_2 \cdot H_2O$ as catalyst.[a]

Entry	T (ºC)	P (bar)	Yield 6h (%)[b]
1	100	85	26
2	140	85	43
3	140	120	52
4	140	140	40
5[c]	70	-	13
6[d]	70	-	17

[a] 200 mM of **5e** and **8** in ACN, $Cu(OAc)_2 \cdot H_2O$ 1% mol. [b] Yield calculated by HPLC. [c] *Batch* conditions: 400 mM of **5e** and **8** in ACN, $Cu(OAc)_2 \cdot H_2O$ 1% mol, atmospheric pressure and 1h. [d] *Batch* conditions: 400 mM of **5e** and **8** in ACN, $Cu(OAc)_2 \cdot H_2O$ 1% mol, atmospheric pressure and 4h.

The synthesis of Rufinamide (**6h**) was performed by the reaction between the azide (**5e**) and the alkyne (**8**) as shown in Scheme 2. A supported tetra-alkyl ammonium azide was used to produce the azide **4h**. A solution of 200 mM of **5e** and **8** in ACN containing 1% mol of $Cu(OAc)_2 \cdot H_2O$ was, then, injected in a flow stream of $scCO_2$ at 0.5 mL/min. The pressure and the temperature were used to enhance the yield of the targeted compound (Table 2). The highest value (52%) was

obtained for a residence time of 3.08 minutes (Entry 3, Table 2) at 140 ^{o}C and 120 bar a significant improvement *versus* the 17% yield obtained when running the reaction under batch conditions (Entry 6, Table 2).

Furthermore, when the reaction under flow and batch conditions were compared, the introduction of the $scCO_2$ as carrier also led to significant improvements in productivity. The Table 3 summarized the comparison of the productivity (g /L x h) calculated for four selected 1,2,3-triazole either under conventional or $scCO_2$ conditions. In the case of the benchmark reaction to produce **3** (Scheme 1), *batch* conditions at 70 ^{o}C and atmospheric pressure showed 59% and 73% yield safter 1 and 2 hours, respectively (Entries 1 and 2, Table 3). However, under continuous flow conditions using $scCO_2$ as carrier, 99% yield was observed for a residence time of 3.08 minutes, which leads to an enhancement of productivity by an order of magnitude in comparison with those obtained under batch conditions (Entries 1 and 2 *vs* entries 3 and 4, Table 3). Larger effects were found for the triazoles **6b** and **6c**.

Although the yields and productivities for Rufinamide were lower than for other triazoles, still a significant improvement was observed in comparison with the values obtained when the reaction was carried out under conventional conditions using ACN as a solvent. It should be pointed out that batch experiments were performed at 400 mM, while for flow experiments the concentration was reduced to 200 mM to avoid any precipitation and blockage of the reactors due to the solid nature of the triazole produced and their limited solubility.

The enhancement on efficiency observed when using CO_2 as carrier can be related with the higher temperature and pressure used under continuous flow conditions. However, when our benchmark reaction between **1** and **2** was performed in flow under the same experimental conditions but using acetonitrile as carrier solvent instead of $scCO_2$, only 50% yield corresponding with a productivity

of 521 g x L^{-1} x h^{-1} was obtained in comparison with the 92% and 952 g x L^{-1} x h^{-1} values achieved respectively in $scCO_2$ (Entries 4 *vs* 5, Table 3). This result suggests the ACN injected solution of the reagents leads to a CO_2-expanded liquid,[39] which can enhance reaction rates as reported for other catalysed reactions. [40]

Table 3. Comparison $scCO_2$ vs conventional solvent.

Entry	Product	Conditions[a]	T (ºC)	P (bar)	Time (h)	Yield (%)[b]	Productivity (g x L^{-1} x h^{-1})
1	**3**	*batch*	70	-	1	59	51
2			70	-	2	73	16
3		flow $scCO_2$	100	120	-	92	959
4			140	85	-	92	959
5		flow ACN	100	85	-	50	521
6	**6b**	*batch*	70	-	1	80	74
7			70	-	4	95	22
8		flow $scCO_2$	100	85	-	99	1099
9			140	100	-	99	1099
10	**6c**	*batch*	70	-	1	20	9
11			70	-	2	50	12
12		flow $scCO_2$	100	85	-	37	442
13			140	100	-	99	1184
14	**6h**	*batch*	70	-	1	13	6
15			70	-	4	17	2
16		flow $scCO_2$	140	85	-	43	491
17			140	120	-	52	594

[a] Flow conditions: 200 mM of azide and alkyne in ACN catalysed by 1% of $Cu(OAc)_2 \cdot H_2O$ using $scCO_2$ as carrier at 0.5 mL/min; *Batch* conditions: 400 mM of azide and alkyne in ACN catalysed by 1% of $Cu(OAc)_2 \cdot H_2O$. [b] Yield calculated by HPLC.

These results demonstrate that the continuous flow platform using $scCO_2$ as carrier can lead to the synthesis of a family of 1,2,3-triazole molecules or in a significantly improved green, cost-effective and, time- efficient manner compared to the traditional batch procedures using conventional solvents These results also confirm the suitability of the solvent-$scCO_2$ platform to carry out the synthesis of relative complex molecules due to the advantages of the CO_2-expanded liquid in dissolving all the reaction components (reagents and homogeneous catalyst).

On-line reaction and separation in supercritical conditions. Once demonstrated the suitability of the platform for the synthesis of a family of triazoles, the integration of both reaction and purification steps was tested for the preparation of the rufinamide. A simple separation setup was built in-house by connecting the Module-1-synthesis and Module-2-purification specified in Figure 1. The purification module consisted mainly of a $scCO_2$ pump that provides the $scCO_2$ mobile phase and HPLC pump for the SCF phase modifier, in this case methanol and a chromatographic column. Both pumps were limited to a maximum pressure of 300 bar and a flow rate of 5 mL/min. It should be noted that these maximum set-up conditions may constrain the separation efficiency in comparation with a semipreparative or preparative $scCO_2$ systems able to work at much higher flow rates and pressures.[18] However, even with these experimental constrains of our set-up, we decided to evaluate the possible synthesis and purification of the triazole under flow and online conditions. Indeed, our approach simply aimed to be a Proof of Concept of the feasibility of a reaction-integration in-line process, without paying attention at the yield or the quality of the isolated fractions as the conditions used were not optimized but were simple those allowed by the available configuration.

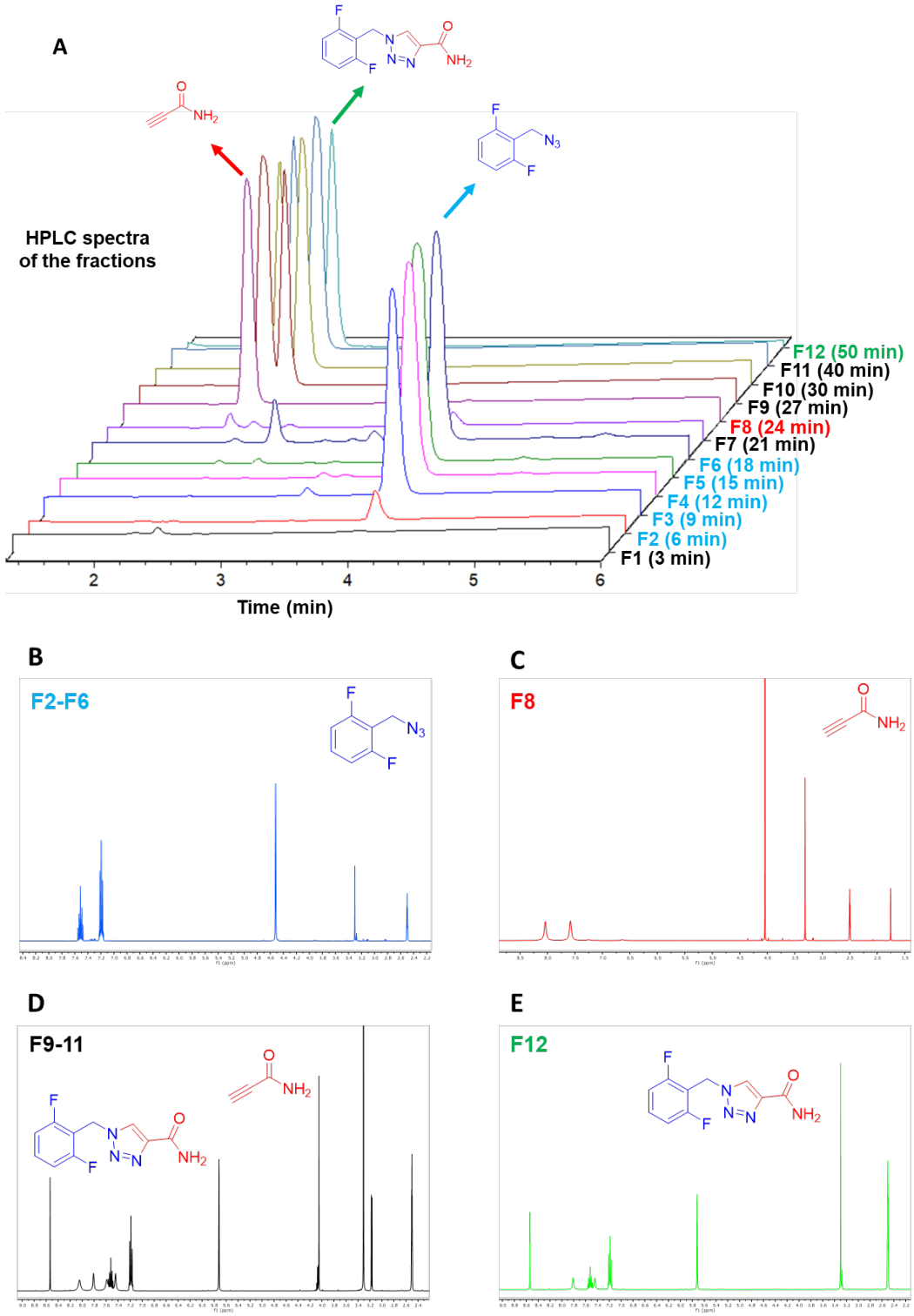


Figure 4. HPLC and ^{1}H-NMR spectra for the fractions collected in the inline reaction-purification flow experiment. Conditions: 1.55 mL injection of a solution of 100 mM of **5e** and **8** in ACN containing 1% of of $Cu(AcO)_2 \cdot H_2O$.

In the synthetic module, the temperature and the pressure were set at 140 ºC and 100 bar; respectively while the flow rate of the CO_2 carrier was fixed at 0.5 mL/min. At t = 0 of the reaction time the pump for CO_2 and the cosolvent in the purification setup were turn off. In the purification setup, the column oven was setup at 40 °C while the back pressure regulator was fixed at 100 bar. When the entire platform was stable in terms of P and T, 1.55 mL of a solution of 100 mM of **5e** and **8** in ACN containing 1% of $Cu(AcO)_2 \cdot H_2O$ was injected. Initial attempts at higher concentration (200 mM) led to the blockage of the system due to the low solubility of rufinamide. Considering the low flow rate and polarity of $scCO_2$ phase used in the synthetic module as well as the dimensions of the semi-preparative column selected for the in-line separation, the reaction crude was expected to be retained on top of the column. After 10 minutes reaction time, a new injection was repeated to load enough reaction crude material on the top of the purification column. Thus, once loaded the column with enough reaction crude, the pumps of the purification module were turn on to 5 mL/min and 1 mL/min flow rate for CO_2 and MeOH respectively, for the isocratic elution of the retained reaction components. Effluent was collected as 3 minutes fractions and for half an hour (F1-F10), while the last fractions (F11-F13) larger 10 min fractions were collected using a 1 mL/min of MeOH and 4 mL/min of CO_2.

The different fractions were analysed by HPLC and concentrated to yield a residue that was weighed then, analysed by ^{1}H-NMR (Figure 4). The analysis of the samples showed that the initial F2-F6 fractions consisted of the unreacted azide **6h** while fraction (F8) contained the alkyne. Fractions F9 to F11showed a mixture of the final product and the alkyne **8**. In the final sample (F12) the pure rufinamide was contained in F12. The total yield of the reaction considering all the fractions containing Rufinamide was *ca*. 40%. Thus, the results produced in the system setup reported herein met the basic goal of proving the proof about the feasibility of using $scCO_2$ for

integrated in-line synthesis and purification. Clearly, improved results could be easily achieved if proper SFC instrumentation was available.

CONCLUSIONS

The work herein reported highlights the potential of using $scCO_2$ to integrate both the synthesis and the inline purification of druggable molecules in a single process by taking advantage of the unique properties of $scCO_2$ as carrier/solvent. Such integration is a win–win situation proving significant advantages. First, environmental benefits, as the use of the continuous flow set-up provided a reduction of 80% of solvent when the reaction used $scCO_2$ compared to traditional ACN. Then, chemical benefits because under the same reactions' conditions, a much higher yield was obtained in $scCO_2$ (77% *vs* 50%). This result led to a significant productivity enhancement (orders of magnitude) when reaction was carried out under flow vs batch conditions. Finally, the integration of the reaction and the separation steps when using $scCO_2$ in both steps can facilitate and streamline drug synthesis and process development in medicinal chemistry, as highlighted for the synthesis and isolation of Rufinamide, and opens new avenues in the production of the target compounds in a much faster, cheaper, more efficient, and greener way.

EXPERIMENTAL SECTION

General Information. All reagents were obtained from Sigma-Aldrich or Scharlab and were used without further purification. ^{1}H-NMR experiments were carried out using a Bruker Avance III HD 400 MHz spectrometer. The chemical shifts are given in delta (δ) values (ppm). FTIR spectra were acquired with a MIRacle single reflection ATR diamond/ZnSe accessory in a JASCO FT/IR-6200 instrument. High-performance liquid chromatography (HPLC) analyses were carried out in an

Agilent 1100 Series chromatograph UV detector at 210 nm using a ProntoSil 120-5-C18 AQ 5µm column (25 cm × 4.6 mm I.D.) and anthracene as internal standard (5.836 min) (ACN/H_2O 0.1%TFA (step 1: 80:20 to 50:50 during 8 min, step 2: 50:50 during 15 in), flow: 1.2 mL/min, T: 25 °C; injection volume: 1 µL). Supported tetra-alkyl ammonium azide (**7**) was prepared as previously reported at reference 41.

Synthesis of azides

(Azidomethyl)benzene (**1**): 190 µL of benzyl bromide (1.6 mmol) were added to a suspension of 0.436 g supported polymeric azide **7** (2.4 mmol) in ACN (8 mL). The resulting mixture was stirred during 48 hours at room temperature and 150 rpm. Then, the polymer was filtered off and the resulting solution was evaporated to yield an oil of compound **1** (> 99% of yield by NMR) used without any further purification. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.93 (s, 2H, CH_2), 7.28 (m, 5H, Ph) ppm; HPLC: 3.992 min for compound **1**.

(1-azidoethyl)benzene (**5a**): 0.273 g of (1-bromoethyl)benzene (1.6mmol) was added to a suspension of 0.436 g supported polymeric azide **7** (2.4 mmol) in ACN (8 mL). The resulting mixture was stirred during 48 hours at room temperature and 150 rpm. Then, the polymer was filtered off and solution of the azide **5a** used without purification (200 mM of compound **5a**, > 99% of yield). HPLC: 4.733 min for compound **5a**.

3-(azidomethyl)-5-methylisoxazole (**5b**): obtained as **5a** but using 0.285 g of 3-(bromomethyl)-5-methylisoxazole (1.6 mmol) to yield to 200 mM of compound **5b** in ACN, > 99% of yield. HPLC: 3.016 min for compound **5b**.

Methyl 2-(azidomethyl)-3-nitrobenzoate (**5c**): obtained as **5a** but using 0.438 g of methyl 2-(bromomethyl)-3-nitrobenzoate (1.6 mmol) to yield to 200 mM of compound **5b** in ACN, > 99% of yield. HPLC: 3.453 min for compound **5c**.

2-(azidomethyl)-1H-imidazole (**5d**): obtained as **5a** but using 0.258 g of 2-(bromomethyl)-1H-imidazole (1.6 mmol) to yield to 200 mM of compound **5d** in ACN, > 99% of yield. HPLC: 1.880 min for compound **5d**.

2-(azidomethyl)-1,3-difluorobenzene (**5e**): obtained as **5a** but using 0.331 g of 2-(bromomethyl)-1,3-difluorobenzene **6** (1.6 mmol) to yield to 200 mM of compound **5e** in ACN, > 99% of yield. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.53 (s, 2H, CH_2), 7.20 (m, 2H, Ph), 7.52 (m, 2H, Ph) ppm; HPLC: 4.080 min for compound **5e**.

Synthesis of functional 1,2,3-triazoles

1-benzyl-4-phenyl-1H-1,2,3-triazole (**3**): *Batch conditions*: 0.5 mg of $Cu(AcO)_2 \cdot H_2O$ (0.0025 mmol) was added to 0.5 mL of 400 mM solution of (azidomethyl)benzene **1** and phenylacetilene **2** in ACN. The resulting solution was heated at 70 ºC during 2 hours under stirring. A sample of 0.5 mL of this solution was diluted with 2.5 mL of ACN and 0.5 mL of this solution was diluted with 2 mL of a solution of anthracene in ACN (5 mM, internal standard). This solution was injected in HPLC to calculate the yield. The rest of the reaction solution was concentrated, and the resulting crude analysed by ^{1}H-NMR.

Flow conditions: For the Cu(0) fixed-bed reactor flow reaction system: 1.55 mL of a 200 mM solution of (azidomethyl)benzene **1** and phenylacetylene **2** in ACN was injected in the flow steam of carrier solvent either $scCO_2$ (1 mL/min) or ACN (0.25 mL/min) using the system depicts in Figure 1 without the purification module and with a fixed-bed reactor (length 11 cm of ¼ inch)

loaded with Cu(0) (3,854 g of Cu (0)) at 90 ^{o}C and 85 bar. After 6 minutes the product was collected using a round flask in an ice bath. The obtained product was analysed by ^{1}H-NMR and HPLC. *CuCl or Cu(AcO)$_2$·H$_2$O homogeneous flow reactions*: 1.55 mL of a 200 mM solution of (azidomethyl)benzene **1**), phenylacetylene **2** (175 µL, 1.6 mmol) and CuCl (1% mol) 0.016 mmol) or Cu(AcO)$_2$·H$_2$O (3 mg, 0.016 mmol) in ACN was injected in the flow steam of carrier solvent scCO$_2$ (1 mL/min) using the system depicts in Figure 1 without the purification module and tubular reactor (stain-steel coil) of external diameter 1/16 inches (0.15875 cm), wall size 0.0508 cm, internal diameter 0.05715 cm. Length 6 m Total volume: 1.54 cm^3 at temperatures and pressures (Figure 3). After 3 minutes the product was collected using a round flask in an ice bath. The solution was analysed by HPLC and ^{1}H-NMR.

1-benzyl-4-phenyl-1H-1,2,3-triazole (**3**): ^{1}H-NMR (400 MHz, CDCl$_3$): δ 5.51 (s, 2H, CH$_2$), 7.30 (m, 10H, Ph), 7.52 (s, 1H, CH) ppm; HPLC: 3.821 min for compound **3**.

4-phenyl-1-(1-phenylethyl)-1H-1,2,3-triazole (**6a**): obtained as **3**. ^{1}H-NMR and HPLC (47-70% of yield). ^{1}H-NMR (400 MHz, CDCl$_3$): δ 2.11 (s, 3H, CH$_3$), 5.89 (s, 1H, CH), 7.37 (m, 10H, Ph), 8.12 (s, 1H, CH) ppm; HPLC: 3.872 min for compound **6a**.

Ethyl 1-benzyl-1H-1,2,3-triazole-4-carboxylate (**6b**): obtained as **3**. ^{1}H-NMR and HPLC (99% of yield). ^{1}H-NMR (400 MHz, CDCl$_3$): δ 1.36 (s, 3H, CH$_3$), 4.40 (s, 2H, CH$_2$), 5.5 (s, 2H, CH$_2$), 7.26 (m, 5e, Ph), 8.23 (s, 1H, CH) ppm; HPLC: 3.038 min for compound **6b**.

1-benzyl-4-(p-tolyl)-1H-1,2,3-triazole (**6c**): obtained as **3**. ^{1}H-NMR (400 MHz, CDCl$_3$): δ 5.49 (s, 2H, CH$_2$), 7.2 (m, 9H, Ph), 7.64 (s, 1H, CH) ppm; HPLC: 3.488 min for compound **6c**.

1-benzyl-4-(3-bromophenyl)-1H-1,2,3-triazole (**6d**): obtained as **3**. ^{1}H-NMR and HPLC (25-53% of yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 5.49 (s, 2H, CH_2), 7.21 (m, 9H, Ph), 7.62 (s, 1H, CH) ppm; HPLC: 5.857 min for compound **6d**.

5-methyl-3-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)isoxazole (**6e**): obtained as **3**. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.90 (s, 3H, CH_3), 4.48 (s, 2H, CH_2), 6.03 (s, 1H, CH), 7.39 (m, 3H, Ph), 7.74 (m, 2H, Ph), 8.00 (s, 1H, CH) ppm; HPLC: 3.907 min for compound **6e**.

Methyl 3-nitro-2-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)benzoate (**6f**): obtained as **3**. ^{1}H-NMR and HPLC (50-95% of yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.96 (s, 3H, CH_3), 6.20 (s, 2H, CH), 7.79 (m, 9H, Ph + CH) ppm; HPLC: 3.322 min for compound **6f**.

1-((1H-imidazol-2-yl)methyl)-4-phenyl-1H-1,2,3-triazole (**6g**): obtained as **3**. ^{1}H-NMR and HPLC (90% of yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 2H, CH_2), 6.55 (m, 6H, Ph+CH) ppm; HPLC: compound **6g** no observed and peak of the acetylene compound **4g** (3.825 min) used to calculate the yield.

1-(2,6-difluorobenzyl)-1H-1,2,3-triazole-4-carboxamide (**6h**): obtained as **3**. ^{1}H-NMR and HPLC (43-52% of yield). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 5.57 (s, 2H, CH_2), 7.17 (m, 2H, Ph), 7.81 (m, 1H, Ph), 8.2 (s, 2H, NH_2), 8.53 (s, 1H, CH) ppm; HPLC: 2.431 min for compound **6h**.

On-line reaction and purification of rufinamide (6h)

1.55 mL of a 200 mM solution of 2-(azidomethyl)-1,3-difluorobenzene **5e**, propiolamide **8** $Cu(AcO)_2 \cdot H_2O$ (1% mol) in ACN was injected in the flow steam of $scCO_2$ as carrier solvent (0.5 mL/min) using the system depicts in Figure 1 (Module 1 + Module 2) at 140 ºC and 100 bar. After 10 minutes reaction time, a new injection was carried out. Then and after additional 10 minutes,

the pumps of the purification module were turned on at a flow rate of 5 mL/min of CO_2 and 1 mL/min of MeOH. Samples were collected at the outlet of the BPR using a round flask in an ice bath for each fraction. The fractions were taken every 3 minutes for half an hour (F1-F10), then every 10 minutes for half an hour (F11-F13) and finally for 1 hour using a 1 mL/min of MeOH and 4 mL/min of CO_2. The obtained fractions were characterized by ^{1}H-NMR and HPLC (Figure 4).

ASSOCIATED CONTENT

The Supporting Information is available free of charge providing additional experiments (PDF).

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REFERENCES

(1) Jiménez-González, C.; Curzons, A. D.; Constable, D. J. C.; Cunningham, V. L. Cradle-to-gate life cycle inventory and assessment of pharmaceutical compounds, *Int. J. LCA* **2004**, 9, 114-121.

(2) Kiss, A. A.; Smith, R.; Rethinking energy use in distillation processes for a more sustainable chemical industry, *Energy* **2020**, 203, 117788.

(3) Fülöp, Z.; Szemesi, P.; Bana, P.; Éles, J.; Greiner, I. Evolution of flow-oriented design strategies in the continuous preparation of pharmaceuticals, *React. Chem. Eng*. **2020**, 5, 1527-1555.

(4) Hone, C. A.; Kappe, C. O. Towards the Standardization of Flow Chemistry Protocols for Organic Reactions, *Chem. Methods*. **2021**, 1, 454-467.

(5) Hu, C. Reactor design and selection for effective continuous manufacturing of pharmaceuticals. *J. Flow Chem*. **2021**, 11, 243-263.

(6) Hu, C.; Testa, C. J.; Born, S. C.; Wu, W.; Shvedova, K.; Sayin, R.; Halkude, B. S.; Casati, F.; Ramnath, A.; Hermant, P.; Takizawa, B.; O'Connor, T. F. Xiaochuan Yang, Sukumar Ramanujam, Salvatore Mascia, E-factor analysis of a pilot plant for end-to-end integrated continuous manufacturing (ICM) of pharmaceuticals, *Green Chem*. **2020**, 22, 4350-4356,

(7) Knez, Z.; Pantic, M.; Cor, D.; Novak, Z.; Knez, M. Are supercritical fluids solvents for the future?, *Chem. Eng. Processing - Process Intensification* **2019**, 141, 107532.

(8) Khaw, K.-Y.; Parat, M.-O.; Shaw, P. N.; Falconer, J. R. Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review, *Molecules* **2017**, 2, 1186.

(9) Goñi, M. L.; Gañán, N. A.; Martini, R. E. Supercritical CO_2-assisted dyeing and functionalization of polymeric materials: A review of recent advances (2015–2020), *J. of CO_2 Utilization* **2021**, 54, 101760.

(10) Zheng, S.; Hu, X.; Ibrahim, A.-R.; Tang, D.; Tan, Y.; Li, J. Supercritical fluid drying: classification and applications, *Recent Pat. Chem. Eng. Discontin*. **2010**, 230-244.

(11) Zhang, X.; Heinonena, S.; Levänen, E. Applications of supercritical carbon dioxide in materials processing and synthesis, *RSC Adv*. **2014**, 4, 61137-61152.

(12) Hayden, E. P. Supercritical Carbon Dioxide: Functions and Applications, *Nova Science Pub. Inc.* **2020**.

(13) Nunes da Ponte, M. Phase equilibrium-controlled chemical reaction kinetics in high pressure carbon dioxide, *J. Supercritical Fluids* **2009**, 47, 344-350.

(14) Beckman, E. J. Supercritical and near-critical CO_2 in green chemical synthesis and processing, *J. Supercritical Fluids* **2004**, 28, 121-191.

(15) Han, X.; Poliakoff, M. Continuous reactions in supercritical carbon dioxide: problems, solutions and possible ways forward, *Chem. Soc. Rev*. **2012**, 41, 1428-1436.

(16) Abreu, B. L.; Boufroura, H.; Moore, J. C.; Poliakoff, M.; George, M. W. Telescoped Continuous Flow Synthesis of 2-Substituted 1,4-Benzoquinones via Oxidative Dearomatisation of *para*-Substituted Phenols Using Singlet Oxygen in Supercritical CO_2, *Synthesis* **2022**, 54, A-G.

(17) Wu, L.; Abreu, B. L.; Blake, A. J.; Taylor, L. J.; Lewis, W.; Argent, S. P.; Poliakoff, M.; Boufroura, H.; George, M. W. Multigram Synthesis of Trioxanes Enabled by a Supercritical CO_2 Integrated Flow Process, *Org. Process Res. Dev.* **2021**, 25, 1873-1881.

(18) Taylor, L. T. Supercritical Fluid Chromatography, *Anal. Chem*. **2010**, 82, 4925-4935.

(19) Fitzpatrick, D. E.; Mutton, R. J.; Ley, S. V. In-line separation of multicomponent reaction mixtures using a new semi-continuous supercritical fluid chromatography system. *React. Chem. Eng*. **2018**, *3*, 799-806.

(20) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004-2021,

(21) Bonandi, E.; Christodoulou, M. S.; Fumagalli, G.; Perdicchia, D.; Rastelli, G.; Passarella, D. The 1,2,3-triazole ring as a bioisostere in medicinal chemistry. *Drug Discovery Today* **2017**, *22*, 1572-1581,

(22)Varala, R.; Bollikolla, H. B.; Kurmarayun, C. M. Synthesis of pharmacological relevant 1,2,3-triazole and its analogues-a review, *Current Organic Synthesis* **2021**, 18, 101-124.

(23) Feng, L.-S.; Zheng, M.-J.; Zhao, F.; Liu, D. 1,2,3-Triazole hybrids with anti-HIV-1 activity. *Arch Pharm.* **2021**, 354:e2000163, 1-10.

(24) Buckle, D. R.; Rockell, C. J. M.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1984**, *27*, 223-227.

(25) Aher, N. G.; Pore, V. S.; Mishra, N. N.; Kumar, A.; Shukla, P. K.; Sharma, A.; Bhat, M. K. *Bioorg. Med. Chem. Lett*. **2009**, 19, 759-763.

(26) Xu, Z.; Zhao, S.-J.; Liu, Y. 1,2,3-Triazole-containing hybrids as potential anticancer agents: Current developments, action mechanisms and structure-activity relationships, *Eur. J. Med. Chem.* **2019**, 183, 111700.

(27) Cao, Y.; Lu, H. Advances in the application of 1,2,4-triazole-containing hybrids as anti-tuberculosis agents, *Future Med. Chem.* **2021**, 13 (23), 2107-2124.

(28) Ötvös, S. B.; Fülöp, F. Flow chemistry as a versatile tool for the synthesis of triazoles, *Catal. Sci. Technol.* **2015**, 5, 4926-4941.

(29) Bogdan, A. R.; Sach, N. W. The Use of Copper Flow Reactor Technology for the Continuous Synthesis of 1,4- Disubstituted 1,2,3- Triazoles, *Adv. Synth. Catal.* **2009**, 351, 849-854.

(30) Ötvös, S. B.; Mándity, I. M.; Kiss, L.; Fülöp, F. Alkyne-Azide Cycloadditions with Copper Powder in a High- Pressure Continuous- Flow Reactor: High- Temperature Conditions versus the Role of Additives, *Chem. Asian J.* **2013**, 8, 800-808.

(31) Grignard, B.; Schmeits, S.; Riva, R.; Detrembleur, C.; Lecomte, P.; Jérôme, C. First example of "click" copper(i) catalyzed azide-alkynecycloaddition in supercritical carbon dioxide: application to the functionalization of aliphatic polyesters, *Green Chem.* **2009**, 1, 1525-1529.

(32) Jiang, Y.; He, X.; Zhang, W.; Li, X.; Guo, N.; Zhao, Y.; Xu, G.; Li, W. Metallic copper wire: a simple, clear and reusable catalyst for the CuAAC reaction in supercritical carbon dioxide, *RSC Adv.* **2015**, 5, 73340-73345.

(33) Zhang, W.; He, X.; Ren, B.; Jiang, Y.; Hu, Z. $Cu(OAc)_2 \cdot H_2O$ An efficient catalyst for Huisgen-click reaction in supercritical carbon dioxide, Tetrahedron Lett. **2015**, 56, 2472-2475.

(34) Fuchs, M.; Goessler, W.; Pilger, C.; Kappe, C.O. Mechanistic Insights into Copper(I)-Catalyzed Azide- Alkyne Cycloadditions using Continuous Flow Conditions, *Adv. Synth. Catal.* **2010**, 352, 323-328.

(35) Rodionov, V. O.; Presolski, S. I.; Gardinier, S.; Lim, Y.-H.; Finn, M. G. Benzimidazole and Related Ligands for Cu-Catalyzed Azide-Alkyne Cycloaddition, *J. Am. Chem. Soc.* **2007**, 129, 42, 12696-12704.

(36) Kuang, G.-C.; Guha, P. M.; Brotherton, W. S.; Simmons, J. T.; Stankee, L. A.; Nguyen, B. T.; Clark, R. J.; Zhu, L. Experimental Investigation on the Mechanism of Chelation-Assisted, Copper(II) Acetate-Accelerated Azide-Alkyne Cycloaddition, *J. Am. Chem. Soc.* **2011**, 133, 13984-14001.

(37) Hassner, A.; Stern, M. Synthesis of Alkyl Azides with a Polymeric Reagent, *Angew. Chem. Int. Ed. Engl.* **1986**, 25, 478-479.

(38) Padmaja, R. D.; Chanda, K. A Short Review on Synthetic Advances toward the Synthesis of Rufinamide, an Antiepileptic Drug, *Org. Process Res. Dev.* **2018**, 22, 4, 457-466.

(39) Jessop, P. G.; Subramaniam, B. Gas-Expanded Liquids, *Chem. Rev*. **2007**, 107, 2666-2694.

(40) Akien, G. R.; Poliakoff, M. A critical look at reactions in class I and II gas-expanded liquids using CO_2 and other gases, *Green Chem*. **2009**, 11, 1083-1100.

(41) Harju, K; Vahermo, M; Mutikainen, I; Yli-Kauhaluoma, J. Solid-Phase Synthesis of 1,2,3-Triazoles via 1,3-Dipolar Cycloaddition, *J. Comb. Chem.* **2003**, 5, 6, 826-833.