Organic Process Research & Development

Article

pubs.acs.org/OPRD

Continuous Flow Synthesis of Thieno[2,3-*c*]isoquinolin-5(4*H*)-one Scaffold: A Valuable Source of PARP-1 Inhibitors

Paolo Filipponi,[†] Carmine Ostacolo,[‡] Ettore Novellino,[‡] Roberto Pellicciari,[†,§] and Antimo Gioiello*[,†]

[†]Dipartimento di Scienze Farmaceutiche, Università di Perugia, Via del Liceo 1, I-06123 Perugia, Italy

[‡]Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, Via D. Montesano 49, I-80131 Napoli, Italy

[§]TES Pharma S.r.l., Via Palmiro Togliatti 22bis, I-06073 Loc. Terrioli, Corciano (Perugia), Italy

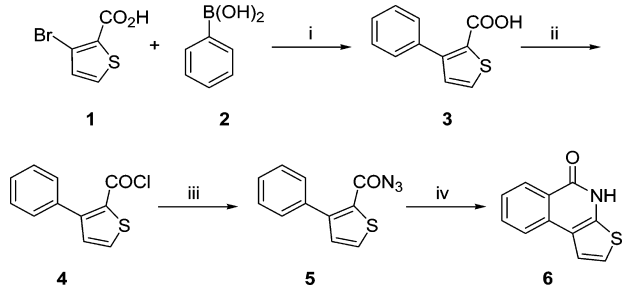
Supporting Information

ABSTRACT: An efficient multistep method for the continuous flow synthesis of thieno[2,3-*c*]isoquinolin-5(4*H*)-one-A (TIQ-A), an important pharmacological tool and building block for PARP-1 inhibitors, has been developed. The synthesis involves a Suzuki coupling reaction to generate 3-phenylthiophene-2-carboxylic acid which is transformed into the corresponding acyl azide and readily cyclized by a thermal Curtius rearrangement. A statistical design of experiments (DoE) was employed as a valuable support for decision-making of further experiments enabling the development of a robust and reliable protocol for large-scale preparation. As a result, the reactions are facile, safe, and easy to scale-up. The large-scale applicability of this improved flow method was tested by conducting the reactions on multigram scale to produce the desired product in high yield and quality for biopharmacological appraisals.

INTRODUCTION

PARP-1 inhibitors are an important class of compounds endowed with a wide range of potential therapeutic applications, including diabetes,[1] cancer,[2] cardiovascular,[3] inflammatory (inc. asthma/COPD),[4] and neurodegenerative disorders.[5] To date, the interest in PARP-1 inhibitors has been mainly focussed on ischemia and oncology, with a number of candidates in advanced clinical trials as standalone or in combination therapies.[6] In this framework, we have reported the design, synthesis, and structure−activity relationships (SAR) of a series of thieno[2,3-*c*]isoquinolin-5(4*H*)-one derivatives as potential anti-ischemic agents.[7] Among these, thieno[2,3-*c*]isoquinolin-5(4*H*)-one-A (TIQ-A, **6**) (Scheme 1) was able to inhibit the catalytic activity of PARP-1 with an IC_{50} of 0.9 μM and has been explored as a promising building block for further medicinal chemistry investigations.[8] Remarkably, *in vivo* appraisals demonstrated that TIQ-A (**6**) blocked PARP-1 action in the brain-reducing stroke volumes,[7] prevented allergic airway inflammation,[9] and induced the regression of atherosclerotic plaques in animal models of atherosclerosis.[10] Moreover, TIQ-A (**6**) selectively interfered with cell proliferation process causing G2/M arrest in human breast cancer cell lines MCF-7 and MDA231.[11] Altogether these results qualify TIQ-A (**6**) as a valuable pharmacological tool to validate PARP-1 as a therapeutic target, prompting different sellers to make it commercially available though at high costs.

Scheme 1. Current synthetic route of thieno[2,3-*c*]isoquinolin-5(4*H*)-one-A (TIQ-A, 6)[a]



[a]Reagents and conditions: (i) $Pd(PPh_3)_4$, $NaHCO_3$, DME, H_2O, reflux, 75%; (ii) $SOCl_2$, C_6H_6, reflux; (iii) NaN_3, THF, r.t.; (iv) $C_6H_4Cl_2$, reflux, 44% from **3**.

Despite the usefulness in the development of new potent, selective, and pharmacokinetically suitable derivatives as well as in supporting the preclinical and clinical development of thieno[2,3-*c*]isoquinolin-5(4*H*)-ones as PARP-1 inhibitors, no convenient process for the large preparation of this scaffold has been reported so far. The current batch synthesis of TIQ-A (**6**) (Scheme 1) is based on a Suzuki coupling reaction to give the intermediate 3-phenylthiophene-2-carboxylic acid (**3**), which is then transformed into the corresponding acyl azide (**5**) and cyclized by thermal Curtius rearrangement to afford the desired product, in 33% overall yield.[8] The method suffers from some limitations including a moderate overall yield, use of hazardous reagents, laborious protocols, and time-consuming work-ups and purifications.

Following our interest in PARP-1[7,8,12] and flow chemistry field,[13] in this paper, we report the continuous flow synthesis of TIQ-A (**6**) with the aim to devise a general methodology which would enable the multigram-scale preparation of **6** and related derivatives. In particular, the major goals of this study will focus on the synthetic efficiency and productivity, the product quality, and the cost of the process. To accomplish this, we explored

Special Issue: Continuous Processes 14

Received: March 3, 2014

   dx.doi.org/10.1021/op500074h | *Org. Process Res. Dev.* XXXX, XXX, XXX−XXX

Table 1. Suzuki coupling batch-screen reactions[a]

entry	solvent system	phase transfer agent	catalyst	base	yield[b]
1	DME		$Pd(PPh_3)_4$	Et_3N	no reaction
2	DME/H_2O/*t*-BuOH (5:4:1, v/v/v)		$Pd(PPh_3)_4$	$NaHCO_3$	no reaction
3	DME/H_2O/*t*-BuOH (5:4:1, v/v/v)		$Pd(PPh_3)_4$	AcONa	no reaction
4	DME/H_2O/*t*-BuOH (5:4:1, v/v/v)		$Pd(PPh_3)_4$	*t*-BuOK	no reaction
5	*t*-BuOH/H_2O (1:1, v/v)		$Pd(PPh_3)_4$	*t*-BuOK	16%
6	THF/H_2O (3:1, v/v)	aliquat	$Pd(PPh_3)_4$	NaOH	25%
7	THF/H_2O (3:1, v/v)	aliquat	$Pd(PPh_3)_4$; PPh_3	NaOH	34%
8	THF/PEG-400/H_2O (4.5:0.5:5, v/v/v)		$Pd(PPh_3)_4$	NaOH	52%
9	THF/PEG-400/H_2O (4.5:0.5:5, v/v/v)	TBAB	$Pd(PPh3)_4$	NaOH	67%
10	THF/PEG-400/H_2O (4.5:0.5:5, v/v/v)	TBAB	Pd EnCat	NaOH	no reaction

[a]Reactions were performed according to Scheme 1. [b]Determined by ^{1}H NMR analysis of the crude reaction mixtures.

various synthetic conditions applying the principle of design of experiments (DoE),[14] and more specifically the central composite design (CCD), in conjugation with automated flow synthesizers in order to come up with a rationalized, carefully chosen set of exploratory experiments. The experiments were performed, and the resulting data were fitted into a regression equation defining a model that was used to find the optimum reaction conditions in flow modality. Thus, we have studied and optimized each single synthetic step of the current procedure by consequential and integrated phases. These include: (a) selection of a model reaction and preliminary evaluation of the experimental conditions in batch mode; (b) design of a convenient flow setup, (c) execution and analysis of the DoE experiments for the reaction optimization under flow conditions, and (d) scale-up synthesis.

RESULTS AND DISCUSSION

DoE Optimization of Suzuki Coupling Reaction in the Homogeneous Phase. The Suzuki reaction is by far the most versatile and straightforward synthetic method for the generation of unsymmetrical biaryl compounds[15] though the use of inherent expensive and potential toxic palladium catalysts has often limited its applications especially upon scale-up operations. To overcome these limitations, several reports have been focused on the development of improved flow methods using either homogeneous[16] or heterogeneous catalysis.[17] In our case, we commenced with our experience on the preparation of thieno[2,3-*c*]isoquinolin-5(4*H*)-ones directing the initial investigations upon Suzuki coupling batch-screens to define the appropriate model reaction for the DoE-assisted flow optimization (Table 1). Thus, phenylboronic acid (**2**) was employed in a slight excess (1.1 equiv) with respect to the thiophenecarboxylic acid (**1**), while the palladium catalyst and the base required to promote the transmetalation were used at 0.03 equiv and 2.0 equiv, respectively.[18] A series of aqueous/organic green media was then evaluated (Table 1) to define the appropriate solvent system which would ensure the complete solubilization of all of the reaction components and a good yield. With the aim to minimize the environmental impact and costs, two impactful choices were considered with regard to solvent selection: (a) run reactions at a more concentrated molarity to reduce solvent usage and (b) select benign solvents. Accordingly, the solvents for the Suzuki reaction were chosen considering (a) previously reported studies,[16d] (b) solubility of the substrate, reagents, catalyst, and product in the solvent of choice, and (c) eco-compatibility. Moreover, the reaction components to be injected through loop A and B were employed at the maximum concentration by dissolving them in the minimum volume of solvent that guaranteed their complete solubilization. After screening a set of experimental conditions, we found the best outcome by using a solvent system constituted by H_2O−THF−PEG-400 (5:4.5:0.5, v/v/v), $Pd(PPh_3)_4$ as palladium catalyst and NaOH as the base (Table 1, entry 8, 9). The use of PEG-400 as a cosolvent was particularly profitable to increase the solubility of polar reagents as the thiophenecarboxylic acid **1**.[19]

Importantly, no significant benefit was obtained modifying the reagent stoichiometry (data not shown), while the addition of a phase transfer agent as tetrabutylammonium bromide (TBAB) was profitable to increase the reaction yield (Table 1, entry 9). Notably, the precipitation of colloidal palladium was noticed with a higher number of NaOH equivalents, whereas the use of triphenylphosphine to regenerate the active catalyst in situ and of Pd EnCat was unsuccessful (Table 1, entries 7, 10).

Having established the reagents and relative stoichiometry and concentration in the solvent of choice (Table 2), we next planned a DoE designed to investigate the effect of both temperature (A) and flow rate (B), variables initially suspected to be important in the process (Table 3).[16,20] By use of the DoE software package, a CCD was constructed and composed by eight experiments plus four replicates at the central point (Table 4).

Table 2. Reagents, stoichiometry, and concentration used for the Suzuki step optimization

reagent	equivalents	concentration[a]
3-bromothiophene-2-carboxylic acid	1.00	0.24 M
phenylboronic acid	1.10	0.27 M
palladium tetrakis	0.03	0.007 M
TBAB	0.50	0.12 M
NaOH	2.00	0.48 M

[a]Fixed considering the minimum volume of solvent ensuring the complete solubilization of all the reaction components.

The reactions were performed by loop injection of two stock solutions: the first one contained 3-bromothiophene-2-carboxylic acid (**1**, 0.48 mmol, 0.24 M) and $Pd(PPh_3)_4$ (0.014 mmol, 0.007 M) in THF−PEG-400 (9:1, v/v), and

Table 3. Variable settings for Suzuki coupling optimization

variable name	variable units	range
temperature (*A*)	°C	80−200
flow rate (*B*)	mL/min	0.2−2.0

dx.doi.org/10.1021/op500074h | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Table 4. CCD: experimental matrix and measured responses[a]

run	type	A (°C)	B (mL/min)	yield (%)[b]
1	axial	140	2.00	89
2	center	140	1.10	90
3	factorial	182	0.46	92
4	axial	200	1.10	68
5	factorial	98	0.46	46
6	factorial	98	1.74	28
7	axial	80	1.10	9
8	center	140	1.10	94
9	center	140	1.10	91
10	factorial	182	1.74	98
11	center	140	1.10	93
12	axial	140	0.20	98

[a]All reactions were conducted according to Figure 1 processing 100 mg (0.48 mmol) of **1**. [b]Determined by ^{1}H NMR analysis of the crude reaction mixture.

the second a solution of NaOH (0.96 mmol, 0.48 M), phenylboronic acid (**2**, 0.53 mmol, 0.27 M), and TBAB (0.24 mmol, 0.12 M) in water (Figure 1). The flow stream was generated pumping a reservoir of THF−PEG-400 (9:1, v/v) and water.

After the injection and the switching of the valves through the loops, the solutions were mixed in a T-piece and flowed into a 10 mL reaction coil heated at the selected temperature. The output was monitored by UV detector, collected, and washed with petroleum ether. The water phase was acidified with HCl 3 M, and the desired product **3** was extracted with ethyl acetate. The reaction yield was determined by ^{1}H NMR analysis of the crude reaction mixture.[21] A complete summary of experimental conditions and results is shown in Table 4.

The data acquired were fitted into a quadratic equation (eq 1 in the figure) defining a mathematical model and the corresponding response−surface as illustrated in Figure 2.

Exploring the design space, a set of optimal responses were found. Among those with a high desirability, two experimental points were chosen and tested to validate the model (Table 5). Notably, a good degree of correlation was observed between the predicted and the experimental yields.

"One-Pot Two-Step" Acyl Azide Synthesis and Thermal Cyclization. The instability and difficult handling of the acyl chloride intermediate **4** combined with the use of hazardous and toxic solvents and reactants as benzene and $SOCl_2$ (Scheme 1) require protocol improvement to make the preparation of the corresponding acyl azide **5** suitable for a large-scale production. To this aim, we decided to replace NaN_3 with diphenylphosphoryl azide (DPPA) being able to react with the carboxylic moiety and endowed with a wider solubility in the majority of organic solvents including 1,2-dichlorobenzene, the solvent used for the following Curtius rearrangement and cyclization step. Thus, when a solution of **3** (0.2 M) and triethylamine in 1,2-dichlorobenzene were pumped and reacted at room temperature with a solution of DPPA (0.26 M) in 1,2-dichlorobenzene with a total flow rate of 0.2 mL/min, the desired acyl azide **5** was obtained in 84% yield (Figure 3). Based on this positive outcome, we reasoned to focused our efforts in the fine-tune of the following cyclization step.

The poor reproducibility and low yield of the thermal cyclization of **5** represent the main drawback of the current route to TIQ-A (**6**). Indeed, the high temperature required to promote the reaction and the nonhomogenous heating of the reaction mixture in batch mode are potential cause of byproducts formation. In this respect, flow chemistry is well-known to be the ideal solution to overcome this issue offering an optimal temperature exchange and, at meantime, supercritical conditions. The acceptable environmental profile[22] combined with the high boiling point convinced us to confirm 1,2-dichlorobenzene as well-suited reaction media for the DoE reaction optimization of this step. A CCD matrix constituted by 12 experiments were then performed to evaluate the effect of temperature (A) and flow rate (B) on the reaction outcome[20] (Table 6) and to determine the experimental conditions that would ensure the maximum conversion yield. Flow experiments were conducted by pumping a reservoir of acyl azide (**5**) (0.1 M, 0.2 mmol) in 1,2-dichlorobenzene through the 10 mL stainless steel reactor heater and two back pressure regulators (2 × 100 psi) (Figure 4). The output of the single reaction was collected, and the relative yield was quantified by HPLC analysis of the crude reaction mixture (Table 7).[23]

The regression analysis of the experimental points led to a logarithmic quadratic mathematical model (eq 2 in the figure) and to the relative response−surface (Figure 5). As expected, the analysis of the results showed that a better yield would be obtained increasing both temperature and resident time of the substrate within the reactor heater.[24]

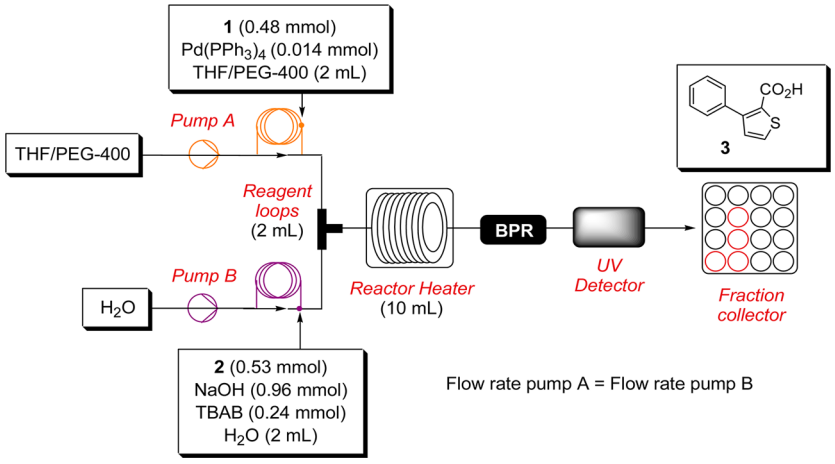


Figure 1. Flow setup used for the Suzuki coupling optimization.

dx.doi.org/10.1021/op500074h | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

$$\text{Equation 1: } (\text{Yield})^{1/2} = 9.66 + 1.86A - 0.23B + 0.45AB - 1.95A^2$$

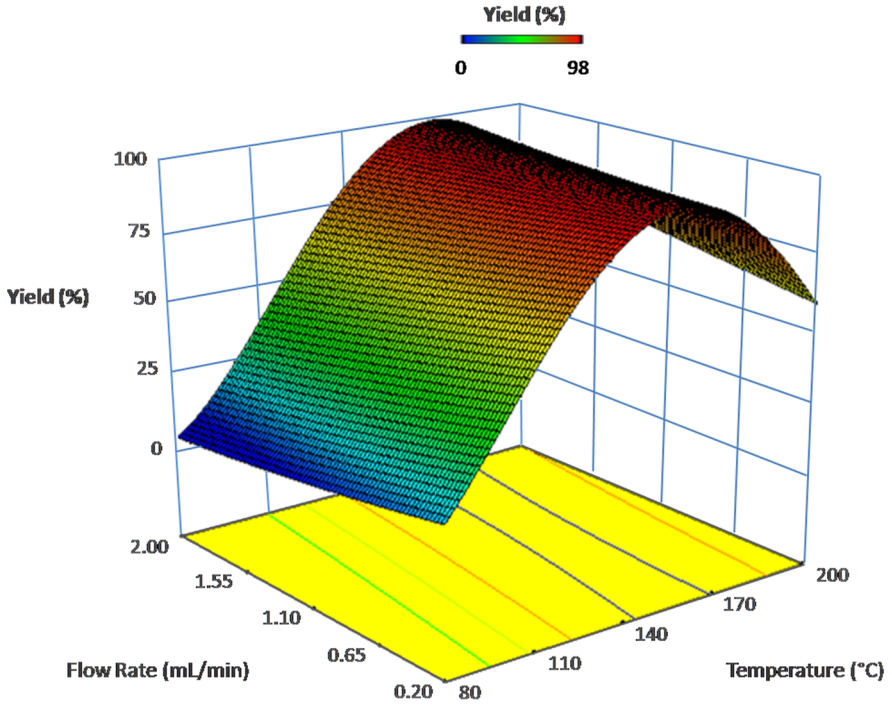


Figure 2. Response−surface plot for Suzuki coupling optimization under flow conditions.

Table 5. Comparison of predicted and experimental yields[a]

		yield (%)	
targets	conditions suggested	predicted	experimental[b]
maximize the yield	*A*: 146 °C	100	91
minimize *A*	*B*: 0.50 mL/min		
B in range			
maximize the yield	*A*: 173 °C	100	92
A in range	*B*: 1.50 mL/min		
maximize *B*			

[a]Reactions were conducted according to Figure 1 processing 100 mg (0.48 mmol) of **1**. [b]Determined by ^{1}H NMR analysis of the crude reaction mixture.

Moreover, although the ANOVA analysis indicated that the mathematical model was suitable to explore efficiently the chemical reaction space (Table S7, Supporting Information), a lack of predictivity was observed nearby the optimal region (Table 8, entry 1) in line with the high curve slope of the response-surface plot (Figure 5). On the contrary, a good correlation among calculated and experimental yields was obtained at suboptimal reaction conditions (Table 8, entries 2−5).

Table 6. Factors and ranges of choice for the DoE-assisted optimization of the cyclization step

factor	units	range
temperature (*A*)	°C	175−245
flow rate (*B*)	mL/min	0.1−1.0

As an additional consideration, the investigated parameter ranges appear too wide for an optimal multivariate analysis, being the majority of predicted responses below 10% yield. Noteworthy, the maximum level of the temperature and the minimum flow rate (Figure 6) were dictated by instrumental, safety, and procedural limitations.[24]

Moreover, a reduction of the experimental space in terms of variable ranges is likely to give a linear response, as evidence by the ANOVA analysis (Table 7S, Supporting Information) that clearly reveals the nonsignificance of the interaction term *AB* (*p*

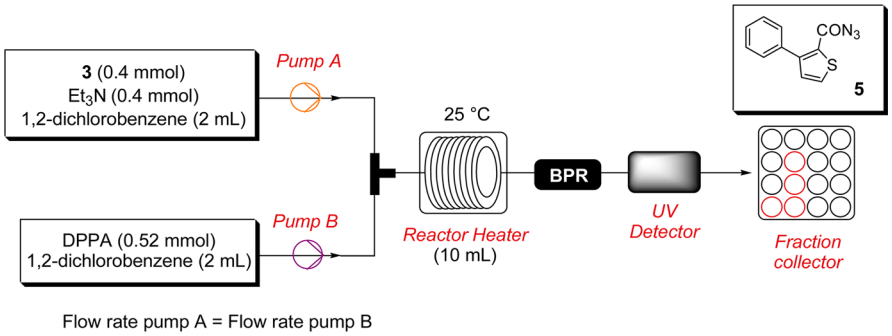


Figure 3. Flow setup for the synthesis of the acyl azide **5**.

dx.doi.org/10.1021/op500074h | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

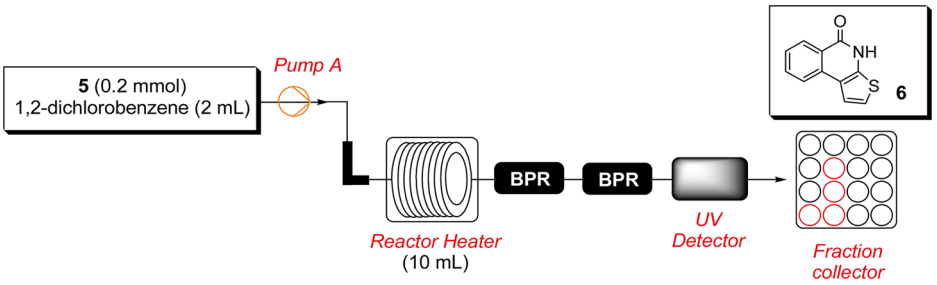


Figure 4. Flow setup for the DoE optimization of the cyclization step.

Table 7. CCD experimental matrix and measured response[a]

run	type	A (°C)	B (mL/min)	yield (%)[b]
1	center	210	0.55	0.8
2	center	210	0.55	0.6
3	axial	210	1	0.5
4	factorial	185	0.87	0.1
5	axial	210	0.1	50
6	factorial	185	0.23	4
7	center	210	0.55	0.7
8	factorial	235	0.87	2.4
9	axial	175	0.55	0.3
10	factorial	235	0.23	69
11	axial	245	0.55	44
12	center	210	0.55	0.4

[a]Reactions were conducted according to Figure 4 processing 0.2 mmol of **5**. [b]Determined by HPLC analysis of the crude reaction mixture.

Table 8. Comparison of predicted and experimental yields[a]

			yield (%)	
entry	A (°C)	B (mL/min)	predicted	experimental[b]
1	235	0.23	100	69
2	235	0.87	3.6	2.5
3	185	0.23	4	4
4	210	0.55	0.6	0.6
5	210	0.1	48	50

[a]Reactions were conducted according to Figure 4 processing 0.2 mmol of **5**. [b]Determined by HPLC analysis of the crude reaction mixture.

value = 0.6592) and the absence of a joint factor effect. In other words, the effect of temperature on the response (yield) results to be independent from the flow rate suggesting the possibility to refine the model by an OVAT approach without a significant loss of information. Accordingly, an additional set of experiments was performed at the maximum temperature that can be reached with our apparatus (245 °C), using two different flow rates (0.1 and 0.2 mL/min) (Table 9). Surprisingly, the results clearly indicated that an increase of both temperature and

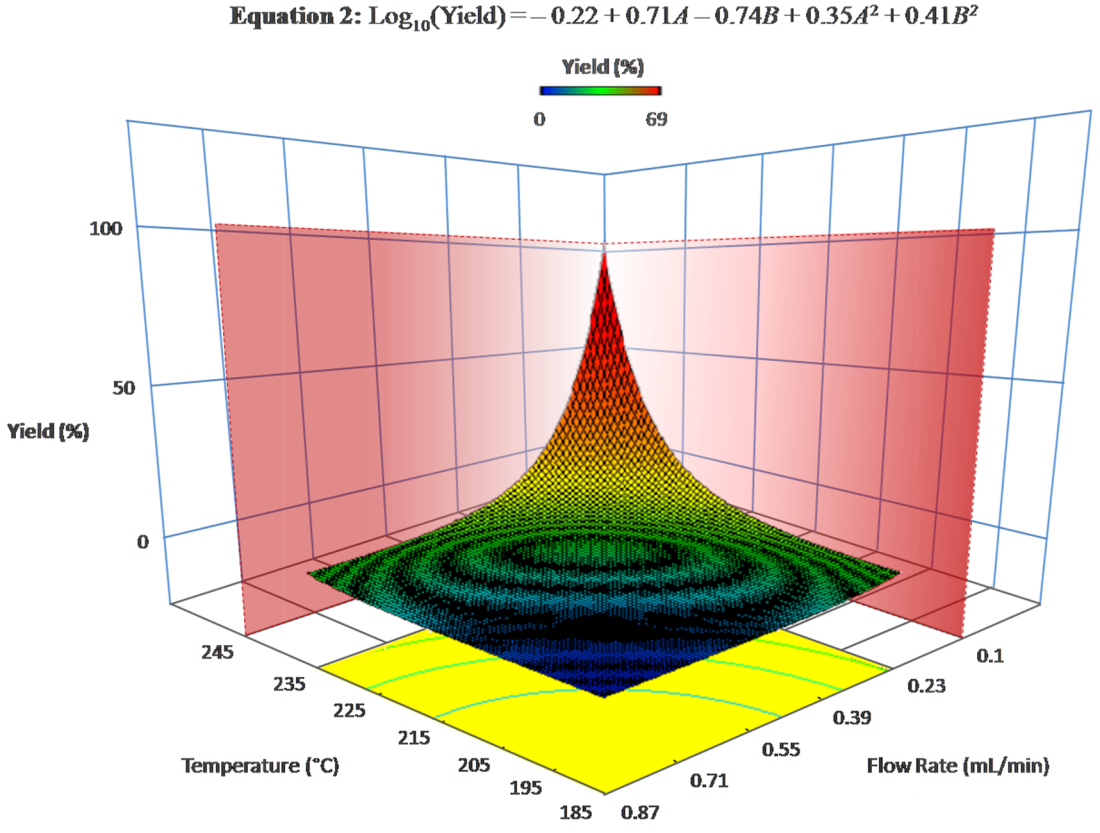


Figure 5. Response−surface plot for continuous thermal cyclization.

dx.doi.org/10.1021/op500074h | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

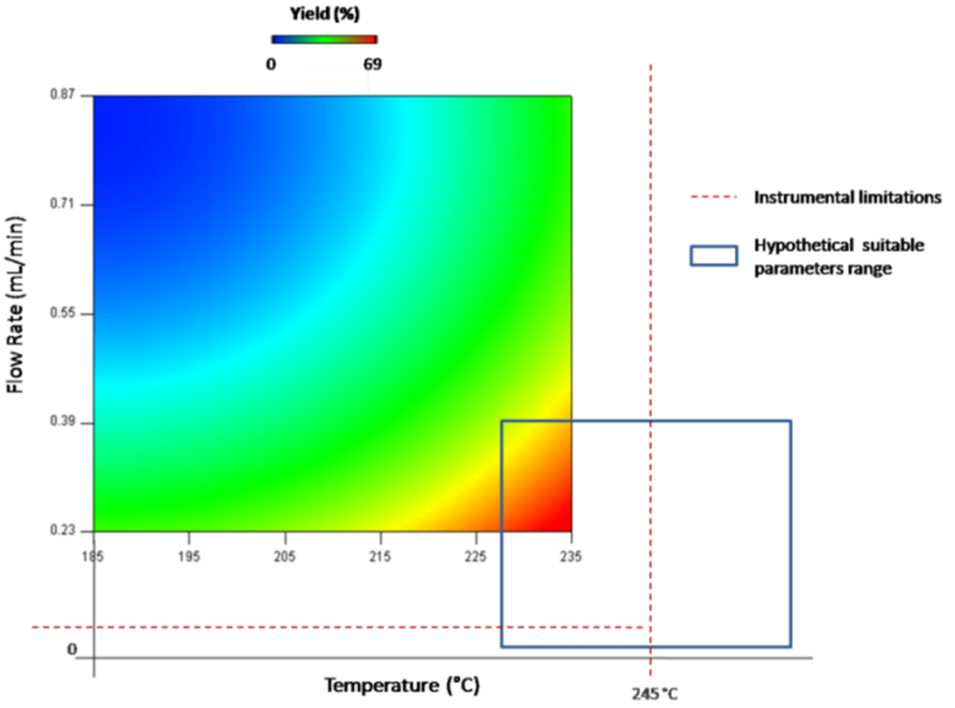


Figure 6. Revised contour plot for continuous thermal cyclization.

residence time does not significantly affect the reaction yield that remains below 70%.

Table 9. Re-evaluation of the optimal cyclization step conditions[a]

entry	*A* (°C)	*B* (mL/min)	yield (%)[b]
1	245	0.1	65
2	245	0.2	66

[a]Reactions were conducted according to Figure 4 processing 0.2 mmol of **5**. [b]Determined by the HPLC analysis of the crude reaction mixtures.

Next, in an effort to produce TIQ-A (**6**) in a continuous stream from the 3-phenylthiophene-2-carboxylic acid (**3**), we have evaluated the possibility and effectiveness of a two-step flow procedure as illustrated in Figure 7. Thus, a solution of the acid **3** (0.2 M) and triethylamine (0.2 M) in 1,2-dichlorobenzene was mixed in a T-piece with DPPA (0.26 M) and pumped at 0.1 mL/min in a 10 mL coil reactor to promote the formation of the acyl azide intermediate. The crude outflow was readily reacted at 235 °C through a second 10 mL reactor heater. The black mixture thus obtained was collected and purified by flash chromatography affording the desired TIQ-A (**6**) in 48% yield.

Scale-up. With optimal conditions established, the synthetic route was scaled-up and validated using the flow setup illustrated in Figure 8. Noteworthy, flow synthesis offers the advantage to easy scaling-up either by continuous running or by the numbering up of flow systems. Thus, 10 g (48.3 mmol) of 3-bromothiophene-2-carboxylic acid (**1**) were premixed with 1.67 g of $Pd(PPh_3)_4$ (1.45 mmol) in THF−PEG-400 (200 mL, 9:1, v/v) (pump A) and reacted with an aqueous solution (200 mL) of NaOH (3.86 g, 96.6 mmol), phenylboronic acid (**2**) (6.48 g, 53.1 mmol), and TBAB (7.77 g, 24.1 mmol) (pump B). The total flow rate and the temperature of the reactor coil were fixed at 1.5 mL/min and 173 °C, respectively. A petroleum ether stream was inserted at the output line of the reactor heater useful to wash out apolar impurities. The biphasic mixture was then directed toward an Omnifit PEEK column (150 mm × 6.6 mm) packed with sea sand capable to reduce the laminar circulation and increase the extraction theoretical stages. The outflow was finally collected into a separatory funnel, and the water layer was acidified with HCl

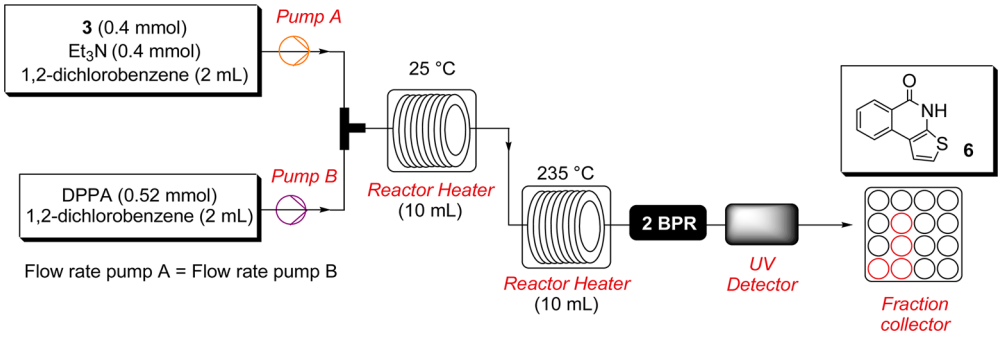


Figure 7. Multistep procedure for continuous flow synthesis of TIQ-A (**6**).

dx.doi.org/10.1021/op500074h | *Org. Process Res. Dev.* XXXX, XXX, XXX−XXX

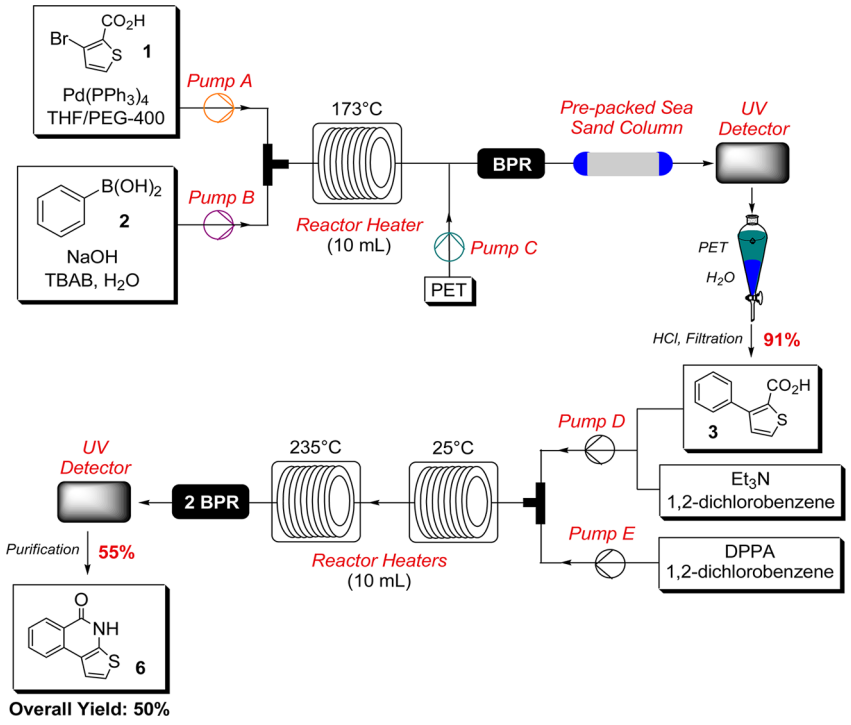


Figure 8. Multigram-scale continuous flow synthesis of TIQ-A (**6**).

37%. The resulting solid was filtered off affording the desired adduct **3** (91% yield) ready available for the following step (purity> 95%). 3-Phenylthiophene-2-carboxylic acid (**3**) (5 g, 24.5 mmol) was then dissolved in 1,2-dichlorobenzene (125 mL) in the presence of triethylamine (24.5 mmol) and processed along with DPPA (31.7 mmol) in the usual fashion (Figure 8). The formed acyl azide **5** was readily reacted within the second 10 mL coil reactor heated at 235 °C. Silica gel purification afforded 2.7 g of TIQ-A (**6**) in 50% overall yield.

CONCLUSIONS

The understanding of the physiological functions of PARP-1 has disclosed unprecedented therapeutic opportunities for this nuclear enzyme with an intense research activity aimed to facilitate the development of PARP-1 inhibitors. In this work, we have shown how the combination of flow technology and statistical DoE can be profitable for the continuous flow optimization and scale-up of TIQ-A (**6**), a crucial pharmacological tool and building block toward PARP-1 inhibitors. In particular, the effect of flow rate and temperature was evaluated allowing the definition of a robust experimental protocol for a large-scale production, increasing the yield while reducing hazardous handling and reactions, cost, and manpower. Accordingly, TIQ-A (**6**) was prepared in continuous flow manner in 50% overall yield (versus 33% of batch mode) using a single, final chromatographic purification. The method is facile, safe, and easy to scale-up and, hence, superior to the other methods that have been previously reported.[7,8] We foresee that this approach will be applicable to diverse isoquinolinone scaffold thus enabling the further extension of the structure−activity relationships for this class of compounds as PARP-1 inhibitors.

EXPERIMENTAL SECTION

General Methods. ^{1}H NMR spectra were recorded at 400 MHz, and ^{13}C NMR spectra were recorded at 100.6 MHz using the solvents indicated below. Chemical shifts are reported in parts per million. The abbreviations used are as follows: s, singlet; d, doublet; dd, double doublet; ddd, double double doublet, dddd, double double double doublet, t, triplet, dt, double triplet, qt, quartet triplet, bs, broad signal. TLC was performed on aluminum backed silica plates (silica gel 60 F254). All the reagents were of analytical grade. HPLC-grade water was obtained from a tandem Milli-Ro/Milli-Q apparatus. The analytical HPLC measurements were made on a Shimadzu LC-20A Prominence equipped with a CBM-20A communication bus module, two LC-20AD dual piston pumps, a SPD-M20A photodiode array detector, and a Rheodyne 7725i injector with a 20 μL stainless steel loop. A Luna RP18 column 250 × 4.6 mm i.d., 5 μm, 100 Å was used as the analytical column. The column temperature was controlled through a Grace heather/chiller thermostat. All flow experiments were performed using a commercially available Vapourtec R2+/R4 module equipped with two-loop injection system (2 mL each), two pumps, a 10 mL PTFE reactor heater, a back pressure regulator (BPR, 100 psi), an UV detector and a fraction collector. Statistical analysis and response-surface plots were determined by the use of Design Expert software (Stat ease software, Minneapolis, MN, USA).

DoE-Assisted Reaction Optimization. *Reactions for Sukuzi Coupling Optimization.* The reactions were performed by loop injection of two solutions: one containing the 3-bromothiophene-2-carboxylic acid (**1**, 0.48 mmol, 0.24 M) and $Pd(PPh_3)_4$ (0.014 mmol, 0.007 M) dissolved in THF−PEG-400 (9:1, v/v) and the second constituted by the phenyl boronic acid (**2**, 0.53 mmol, 0.27 M), NaOH (0.96 mmol, 0.48 M), and TBAB (0.24 mmol, 0.12 M) in water. After the injection and the switching of the solvents stream through the

dx.doi.org/10.1021/op500074h | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

loops the two sample solutions were pumped, at the desired flow rate, toward a T-piece and directed in the coil reactor heated at the selected temperature. The outflow was collected in a fraction collector, and the crude mixture was washed with petroleum ether (2 × 20 mL). The separated aqueous layer was thus acidified with HCl 3 M and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum to give a crude sample which is then analyzed by ^{1}H NMR.

Acyl Azide Synthesis. A solution of 3-phenylthiophene-2-carboxylic acid (**3**, 0.4 mmol, 0.2 M) and triethylamine (0.4 mmol) in 1,2-dichlorobenzene was pumped and reacted at room temperature with a solution of DPPA (0.52 mmol, 0.26 M) in 1,2-dichlorobenzene with a total flow rate of 0.2 mL/min. The desired was acyl azide **5** was collected in a fraction collector, and the crude mixture was directly submitted to chromatographic purification to obtain the pure intermediate **5** (77 mg, 0.34 mmol) in 84% yield.

Reactions for Thermal Cyclization Optimization. A solution of 3-phenylthiophene-2-carbonyl azide (**5**, 0.2 mmol, 0.1 M) in 1,2-dichlorobenzene was pumped at the selected flow rate through a stainless steel coil reactor heated at the temperature of choice. The outflow was monitored by an UV detector collecting 10 mL of the crude reaction mixture. The HPLC sample was prepared dissolving 75 μL of the crude reaction mixture in 1 mL of methanol.

Scale Up. *Continuous Flow Synthesis of the 3-Phenylthiophene-2-carboxylic Acid (3).* A water suspension of the boronic acid **2** (53.1 mmol, 0.27 M), NaOH (96.6 mmol, 0.48 M), and TBAB (24.1 mmol, 0.12 M) was preheated at 50 °C until the complete reactant solubilization. The resulting solution was mixed in a T-piece with 3-bromothiophene-2-carboxylic acid (**1**, 48.3 mmol, 0.24 M) and $Pd(PPh_3)_4$ (1.45 mmol, 0.007 M) dissolved in THF−PEG-400 (9:1, v/v) and the mixture pumped at 1.5 mL/min in a coil reactor (10 mL) heated at 173 °C. The crude outflow met an additional stream of petroleum ether pumped at 1.5 mL/min. The flow stream was then directed toward an Omnifit PEEK column (150 mm × 6.6 mm) filled with sea sand (50−70 mash). The greenish biphasic mixture was collected in a separatory funnel, and the separated water layer was acidified with HCl 37% until complete precipitation. The white solid formed was filtered off and dried under vacuum obtaining the pure 3-phenylthiophene-2-carboxylic acid (**3**, 9 g, 44 mmol) (91% yield, purity > 95%) which was used for the next step without further purifications. Mp: 166−168 °C. ^{1}H NMR (DMSO-d_6) δ 7.25 (d, 1H, J = 5.1 Hz), 7.35−7.41 (m, 3H), 7.44−7.47 (m, 2H), 7.94 (d, 1H, J = 5.1 Hz), 12.85 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 127.67, 127.78 (2C), 128.11, 129.32 (2C), 131.07, 131.79, 135.55, 147.23, 162.91.

Continuous Flow Synthesis of Thieno[2,3-c]isoquinolin-5(4H)-one-A (TIQ-A, 6). A solution of 3-phenylthiophene-2-carboxylic acid (**3**) (24.5 mmol, 0.2 M) and Et_3N (24.5 mmol, 0.2 M) dissolved in 1,2-dichlorobenzene was mixed through a T-piece with a solution of DPPA (31.7 mmol, 0.26 M) dissolved in the same solvent. The main flow stream was pumped at 0.2 mL/min into a 10 mL coil reactor maintained at 25 °C. The outflow fed a second 10 mL stainless steel coil heated at 235 °C, and the black solution thus collected was submitted to flash chromatography to afford TIQ-A (**6**, 2.7 g, 13.5 mmol) in 55% yield as pure white solid. Mp: 252−254 °C. ^{1}H NMR (DMSO-d_6) δ 7.23 (d, J = 5.6 Hz, 1H), 7.51 (m, 1H), 7.70 (d, J = 5.3 Hz, 1H), 7.78 (m, 1H), 8.10 (dd, J = 7.4, 1.1 Hz, 1H), 8.25 (dd, J = 8.0, 0.9 Hz), 12.3 (s, 1H). ^{13}C NMR (DMSO-d6) δ 116.4, 119.2, 120.6, 122.6, 123.3, 126.2, 128.4, 133.1, 134.1, 139.9, 163.2. MS (ES+) m/z 224.4 (M + Na; 100%); (ES-) m/z 200.4 (M − H; 100%).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

CCD matrices, ANOVA tables, and HPLC analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: antimo.gioiello@unipg.it.

Author Contributions

P.F. and C.O. contributed equally to the work.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Pang, J.; Gong, H.; Xi, C.; Fan, W.; Dai, Y.; Zhang, T. M. *J. Cell Biochem.* **2011**, *112*, 299.

(2) Peralta-Leal, A.; Rodríguez-Vargas, J. M.; Aguilar-Quesada, R.; Rodríguez, M. I.; Linares, J. L.; de Almodóvar, M. R.; Oliver, F. *J. Free Radical Biol. Med.* **2009**, *47*, 13.

(3) Pacher, P.; Szabó, C. *Cardiovasc. Drug Rev.* **2007**, *25*, 235.

(4) Havranek, T.; Aujla, P. K.; Nickola, T. J.; Rose, M. C.; Scavo, L. M. *Exp. Lung Res.* **2010**, *36*, 381.

(5) (a) Haile, W. B.; Echeverry, R.; Wu, F.; Guzman, J.; An, J.; Wu, J.; Yepes, M. *Neuroscience* **2010**, *171*, 1256. (b) Alano, C. C.; Garnier, P.; Ying, W.; Higashi, Y.; Kauppinen, T. M.; Swanson, R. A. *J. Neurosci.* **2010**, *30*, 2967.

(6) (a) Garber, K. *Nat. Rev. Drug Discovery* **2013**, *12*, 725. (b) Usmani, H.; Hussain, S. A.; Sheikh, A. *Infect Agent Cancer* **2013**, *8*, 46. (c) Wielgos, M.; Yang, E. S. *Pharm. Pat. Anal.* **2013**, *2*, 755. (d) Singh, N.; Sharma, G.; Singh, N.; Hanif, K. *Int. J. Stroke* **2012**, DOI: 10.1111/j.1747-4949.2012.00916.x.

(7) (a) Pellicciari, R.; Camaioni, E.; Costantino, G.; Marinozzi, M.; Macchiarulo, A.; Moroni, F.; Natalini, B. *Farmaco* **2003**, *58*, 851. (b) Costantino, G.; Macchiarulo, A.; Camaioni, E.; Pellicciari, R. *J. Med. Chem.* **2001**, *44*, 3786.

(8) Pellicciari, R.; Camaioni, E.; Gilbert, A. M.; Macchiarulo, A.; Bikker, J. A.; Shah, F.; Bard, J.; Costantino, G.; Gioiello, A.; Robertson, G. M.; Sabbatini, P.; Venturoni, F.; Liscio, P.; Carotti, A.; Bellocchi, D.; Cozzi, A.; Wood, A.; Gonzales, C.; Zaleska, M. M.; Ellingboe, J. W.; Moroni, F. *Med. Chem. Commun.* **2011**, *2*, 559.

(9) (a) Oumouna, M.; Datta, R.; Oumouna-Benachour, K.; Suzuki, Y.; Hans, C.; Matthews, K.; Fallon, K.; Boulares, H. *J. Immunol.* **2006**, *177*, 6489. (b) Naura, A. S.; Hans, C. P.; Zerfaoui, M.; You, D.; Cormier, S. A.; Oumouna, M.; Boulares, A. H. *Clin. Exp. Allergy* **2008**, *38*, 839.

(10) Hans, C. P.; Zerfaoui, M.; Naura, A. S.; Troxclair, D.; Strong, J. P.; Matrougui, K.; Boulares, A. H. *J. Pharmacol. Exp. Ther.* **2009**, *329*, 150.

(11) (a) Inbar-Rozensal, D.; Castiel, A.; Visochek, L.; Castel, D.; Dantzer, F.; Izraeli, S.; Cohen-Armon, M. *Breast Cancer Res.* **2009**, *11*, R78. (b) Castiel, A.; Visochek, L.; Mittelman, L.; Dantzer, F.; Izraeli, S.; Cohen-Armon, M. *BMC Cancer* **2011**, *11*, 412.

(12) Pellicciari, R.; Camaioni, E.; Costantino, G.; Formentini, L.; Sabbatini, P.; Venturoni, F.; Eren, G.; Bellocchi, D.; Chiarugi, A.; Moroni, F. *Chem. Med. Chem.* **2008**, *3*, 914.

(13) (a) Venturoni, F.; Gioiello, A.; Sardella, R.; Natalini, B.; Pellicciari, R. *Org. Biomol. Chem.* **2012**, *10*, 4109. (b) Gioiello, A.; Rosatelli, E.; Teofrasti, M.; Filipponi, P.; Pellicciari, R. *ACS Comb. Sci.* **2013**, *15*, 235.

(14) (a) Leardi, R. *Anal. Chim. Acta* **2009**, *652*, 161. (b) Tye, H. *Drug Discovery Today* **2004**, *9*, 485.
(15) For reviews on the Suzuki cross-coupling reaction, see: (a) Noel, T.; Buchwald, S. L. *Chem. Soc. Rev.* **2011**, *40*, 5010. (b) Miura, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 2201. (c) Bellina, F.; Carpita, A.; Rossi, R. *Synthesis* **2004**, 2419. (d) Pershichini, P. *J. Curr. Org. Chem.* **2003**, *7*, 1725. (e) Kotha, S.; Lahiri, S.; Kashinath, D. *Tetrahedron* **2002**, *58*, 9633.
(16) For recent examples in the use of homogeneous catalysts: (a) Shu, W.; Pellegatti, L.; Oberli, M. A.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2011**, *50*, 10665. (b) Noel, T.; Kuhn, S.; Musacchio, A. J.; Jensen, K. F.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2011**, *50*, 5943. (c) Noel, T.; Musacchio, A. J. *Org. Lett.* **2011**, *13*, 5180. (d) Glasnov, T. N.; Kappe, C. O. *Adv. Synth. Catal.* **2010**, *352*, 3089.
(17) (a) Mennecke, K.; Kirschning, A. *Beilstein J. Org. Chem.* **2009**, *5*, 21. (b) Baxendale, I. R.; Griffiths-Jones, C. M.; Ley, S. V.; Tranmer, G. K. *Chem.—Eur. J.* **2006**, *12*, 4407. (c) Phan, N. T. S.; Khan, J.; Styring, P. *Tetrahedron* **2005**, *61*, 12065. (d) Lee, C. K. Y.; Holmes, A. B.; Ley, S. V.; McConvey, I. F.; Al-Duri, B.; Leeke, G. A.; Santos, R. C. D.; Seville, J. P. K. *Chem. Commun.* **2005**, 2175. (e) Leeke, G. A.; Santos, R. C. D.; Al-Duri, B.; Seville, J. P. K.; Smith, C. J.; Lee, C. K. Y.; Holmes, A. B.; McConvey, I. F. *Org. Process Res. Dev.* **2007**, *11*, 144.
(18) Reagent stoichiometries including the equivalents of boronic acid, catalyst, and base were fixed at the minimum amount in order to minimize the number of experiments, reduce costs, and facilitate the purification of the Suzuki adduct.
(19) (a) Phan, N. T. S.; Khan, J.; Styring, P. *Tetraherdron* **2005**, *61*, 12065−12073. (b) Vasudevan, V. N.; Rajender, S. V. *Green Chem.* **2001**, *3*, 146−148.
(20) Reagent stoichiometry was not considered in the DoE for two main reasons: (a) the principles of atom economy and step economy are historically the underpinnings of efficiency critical to process development and manufacturing and have increasingly been moving to the forefront of strategic considerations for reaction optimization; (b) the use of fixed reagent stoichiometry at the minimum amount to promote reaction would reduce the timing and number of experiments to be performed, waste production, and costs while it provides material quality without tedious purifications to remove the excess of reagents.
(21) The yield of the Suzuki coupling reaction was assessed by the ratio between the peak area of the proton at the C5 position of the 3-bromothiophene-2-carboxylic acid and of the resulting Suzuki product, after workup of the crude reaction mixture.
(22) Henderson, R. K.; Jimenez-Gonzalez, C.; Constable, D. J. C.; Alston, S. R.; Inglis, G. G. A.; Fisher, G.; Sherwood, J.; Binksa, S. P.; Curzons, A. D. *Green Chem.* **2011**, *13*, 854.
(23) HPLC analysis was used to determine the yield of the cyclization step since the reaction is performed in 1,2-dichlorobenzene, a high boiling solvent which can be removed by silica gel filtration, as well as because the method is instrumental to establish the purity of the target compound TIQ-A. The calibration of the HPLC analysis was conducted according to a standard protocol. Thus, the mother (test) solution of thieno[2,3-*c*]isoquinolin-5(4*H*)-one (0.03 M) was prepared dissolving 60.4 mg of reference compound (purity > 99%) in 10 mL of methanol. Four solutions at different concentrations were then obtained by diluting the test solution in methanol as follows: (a) 50 μL in 10 mL of methanol (0.00015 M), (b) 150 μL in 10 mL of methanol (0.00045 M), (c) 250 μL in 10 mL of methanol (0.00075 M), and (d) 500 μL in 10 mL of methanol (0.0015 M). A portion of 20 μL of each solution were analyzed in triplicate according to the method described in the Supporting Information (see page S5). A calibration curve was obtained considering the peak area as a function of the analyte concentration.
(24) A potential improvement of this step may consist in the use of a different reactor heater with higher temperature limits to further explore the reaction space or in the employment of microwave heating in combination with the flow system.