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Rapid Access to Compound Libraries through Flow Technology: Fully Automated Synthesis of a 3-Aminoindolizine Library via Orthogonal Diversification

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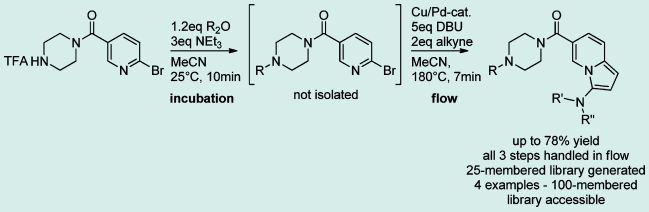
Supporting Information

ABSTRACT: A novel methodology for the synthesis of druglike heterocycle libraries has been developed through the use of flow reactor technology. The strategy employs orthogonal modification of a heterocyclic core, which is generated in situ, and was used to construct both a 25-membered library of druglike 3-aminoindolizines, and selected examples of a 100-member virtual library. This general protocol allows a broad range of acylation, alkylation and sulfonamidation reactions to be performed in conjunction with a tandem Sonogashira coupling/cycloisomerization sequence. All three synthetic steps were conducted under full automation in the flow reactor, with no handling or isolation of intermediates, to afford the desired products in good yields. This fully automated, multistep flow approach opens the way to highly efficient generation of druglike heterocyclic systems as part of a lead discovery strategy or within a lead optimization program.



KEYWORDS: *flow chemistry, library synthesis, indolizine, conjure, cross-coupling, enabling technology*

INTRODUCTION

The continued growth of unexplored molecular targets, coupled with widely available high-throughput screening (HTS) technology, provides continued impetus for the efficient generation of novel, druglike molecular structures that could potentially serve as starting points for drug-discovery programs.[1] To meet this demand for access to collections of novel molecules that already possess druglike structures and properties, new and efficient strategies for generating compound libraries, ideally in an automated fashion, are needed.

In this regard, flow chemistry in particular has received increased attention because of its intrinsic benefits over traditional batch techniques; these include excellent heat and mass transfer for better reaction control, safe operation at high temperatures/pressures, and when employing toxic or noxious reaction components, as well as automated reaction execution, purification and product isolation. Thus, increased efforts have been undertaken to incorporate flow technology into combinatorial chemistry and library synthesis.[2] Recent seminal reports on flow-based library synthesis include the combination of batch and flow techniques,[3] fully automated flow reactor systems,[4] stepwise synthesis of compounds under continuous[5] and segmented flow conditions,[6] and parallel synthesis of different compounds in multiple flow channels.[7] Although substantial improvement has been achieved through the incorporation of flow technology into library synthesis, current developments have primarily focused on one-step reactions in flow to afford the desired library members, thus limiting the potential size and diversity of the library. A flow methodology capable of performing multiple modifications on one molecule would be a significant advance for the field of combinatorial chemistry, since libraries with increased size and diversity could be generated efficiently, while benefitting from the advantages of flow technology.[8] To date, only two reports have appeared in the recent literature that describe a two-step modification to create library compounds in flow. A "catch and release" protocol was developed by researchers at GSK for the generation of a 48-membered library of secondary sulfonamides via deprotection and subsequent alkylation of the primary sulfonamide.[9] However, the protocol only allows two modifications of the substrate at one reactive site. Ulven et al. have shown that a series of reactors and scavenging cartridges can be used to create a small library of receptor ligands via subsequent modification of mono-Cbz-protected piperazine.[10] However, the methodology is limited to the formation of ureas generated in the first step, followed by Cbz-deprotection and *N*-alkylation in the second step. Yields for the final products are generally low. Thus, a true protocol for the orthogonal diversification of substrates to afford compound libraries with substantial size and diversity has yet to be developed.

In the course of our program to develop novel synthetic strategies for the construction of druglike molecules in batch[11] and flow,[12] we have recently reported a flow-based protocol for the expedient synthesis of 3-aminoindolizines **3** via a tandem

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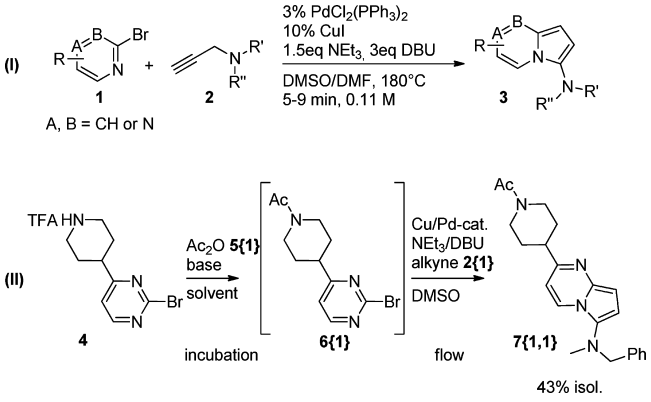
Sonogashira coupling/cycloisomerization reaction (Scheme 1, (I)).[13]

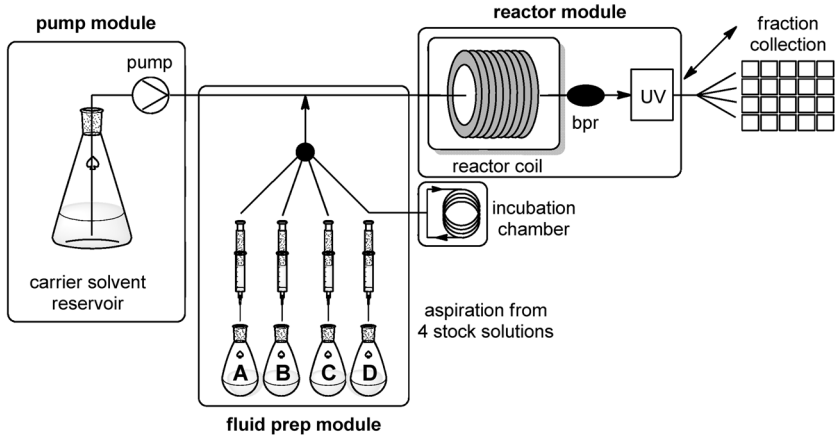
Scheme 1. Indolizine and Aza-indolizine Synthesis in Flow



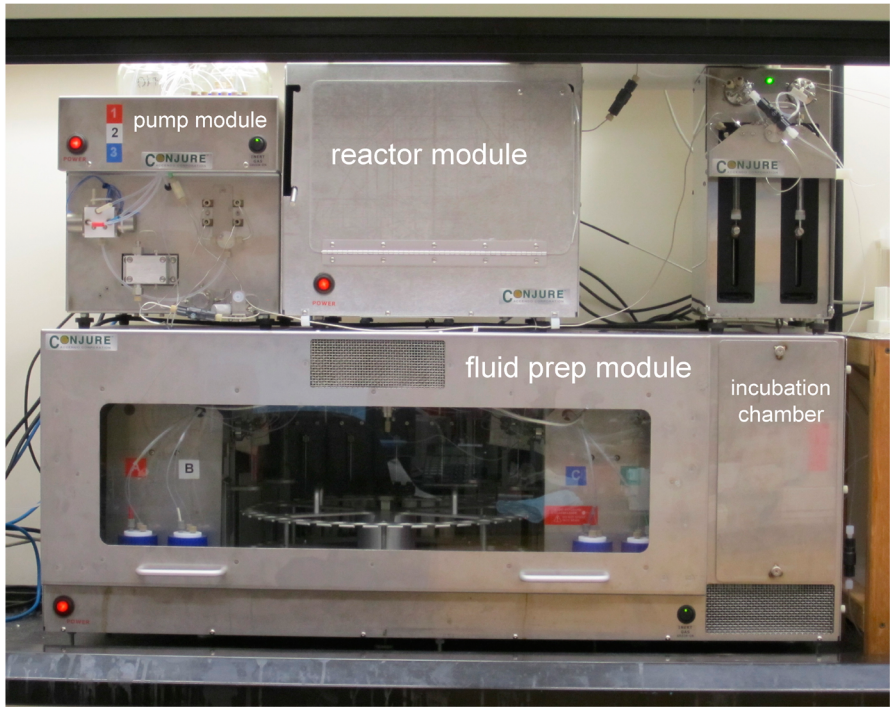
In that report, we described a flow-based protocol that offers distinct advantages in comparison to analogous batch procedures,[14] and highlights a substantially increased substrate scope, increased speed of reaction and relative ease and safety of scale-up. Moreover, we alluded to the potential of this new methodology, in conjunction with the flow reactor system employed, to access libraries of druglike molecules via orthogonal diversification of a suitable heterocyclic core (Scheme 1, (II)). On the basis of these initial findings, we herein present the development of the first fully automated, flow-based synthesis of a diverse 25-membered library of druglike 3-aminoindolizines. By employing the Conjure flow reactor system,[15] a protocol has been developed that allows the in situ construction and orthogonal diversification of an aminoindolizine in a multistep sequence to afford the desired library compounds in good yields. All modifications are performed successively within the flow reactor, thereby avoiding the need to handle or isolate of any intermediates.

Scheme 2. Schematic (a) and Actual (b) Set-up of the Conjure Flow System

a)



b)



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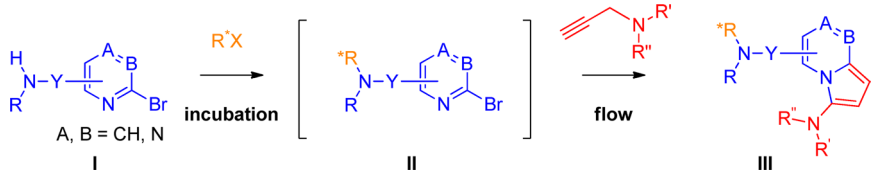
Scheme 3. Library Design Strategy



RESULTS AND DISCUSSION

We began our investigations employing the commercially available Accendo Conjure flow reactor system, in light of its ability both to undertake rapid reaction parameter screening and the automatic execution of two-step reaction sequences.

The Flow Reactor. The Conjure flow system consists of three units, the pump module, reactor module and fluid prep module, which will all be explained in further detail, to emphasize our choice to employ this particular flow device. All three modules can be individually controlled and settings adjusted by the user with the Conjure Software. The whole system is connected to a back pressure regulator (bpr), an Agilent UV-detector and a Gilson fraction collector (Scheme 2, UV-detector and fraction collector not shown in 2b).

Reactions are conducted in individual segments, which allow highly time-efficient screening of reaction parameters with minimal quantities of reagents, excellent reproducibility for scale-up and rapid generation of our 25-membered library.

The Pump Module. The pump module is directly connected to both the reactor and fluid prep module and allows the selective aspiration of three different system solvents (pump speeds 0.1−3.5 mL·min^{-1}).

The Reactor Module. The reactor coil used in the Conjure flow reactor is constructed from Hastelloy tubing (0.75 mm i. d., 4.5 m length, 2.0 mL i. v.) and enclosed in a reactor diskette (see ESI for details). Within the heating unit of the reactor module, the diskette can be heated to a maximum temperature of 300 °C. The inlet of the reactor is connected to a valve directing flow of the system solvent either directly from the solvent reservoir or via the fluid prep module. The outlet of the reactor is connected to a back pressure regulator immediately followed by the UV-detector and fraction collector. As soon as a reaction segment is detected by UV, it is automatically collected in an individual vial.

The Fluid Prep Module. The most intricate and unique part of the Conjure reactor system is the fluid prep module, which contains a rotating 40-vial carousel, an incubation chamber and four syringe pumps to aspirate up to four different reagent stock solutions (25−300 μL each, see ESI for details). Segments are prepared in a user-predetermined order and composition from the respective source vials containing the reagent stock solutions. Each segment is preceded and followed by an immiscible liquid spacer that prevents segment diffusion and reaction trailing.[16] The segment preparation is fully automated once the stock solutions are in place and the carousel is loaded. Segments can be prepared from up to four different reagent solutions simultaneously, drawing from a contingent of 36 vials held on the carousel.[17] Alternatively, as in this project and exemplified in our initial protocol,[11] a first step reaction segment can be prepared and positioned in one of the sample loops housed in the incubation chamber.[18] The mixture can be heated up to (incubated at) 100 °C for the desired reaction time and is then reaspirated, mixed with up to three different reagents[19] and subjected to the flow stream to process the second reaction step. The reaction segment is detected by UV upon exiting the reactor and subsequently collected.

With these unique features and design, the Conjure flow synthesis system is ideally suited for our development of a methodology for library synthesis of druglike heterocycles. In particular, we chose to exploit the fact that two-step sequences can be conducted automatically and that our previously described methodology could be incorporated as the second step in this approach.

Library Design. Our strategy for constructing the library involved introduction of three elements of diversity, as illustrated in Scheme 3. The first point of diversification is in the selection of a heteroaryl bromide core **I**, which is capable of both undergoing a derivatization reaction via a free amino substituent, and a Sonogashira reaction (followed by a cycliosomerization to generate a 3-aminoindolizine) via the bromo substituent. The second point of diversification is derivatization of the pendant amino substituent via, for example, an acylation reaction, to yield intermediate **II**. The final point of diversification is through the incorporation of a range of substituted propargyl amines in the Sonogashira coupling step to yield the 3-aminoindolizine product **III**.

We chose to exemplify the approach by initially employing one heteroaryl bromide core and a 5 × 5 matrix of amine substituents and propargyl amines, as the basis of a 25-membered library. We also went on to illustrate the applicability of the approach to three other heteroaryl core fragments, as well as a further variant of the protocol based upon derivatization of the propargylamine component. Thus, we have demonstrated that a corresponding virtual library of 100 molecules (4 × 5 × 5) could potentially be accessed using our approach.

Library Protocol Optimization. We chose heterocyclic building block **10**, which is easily synthesized in gram-quantities from commercially available starting materials (Scheme 4), as our initial core fragment to optimize the multistep flow protocol.

Scheme 4. Straightforward Synthesis of the Common Building Block 10

We began our investigations with the optimization of the reaction conditions for the first step modification on the piperazine substituent. We reasoned that more thoroughly optimized conditions would allow a multitude of viable transformations under flow conditions and lead to much better yields for the final library compounds. We focused on two major issues regarding the first step: (1) The conditions required to effect a rapid and versatile N-modification must not inhibit the follow-up cross-coupling/cycloisomerization se-

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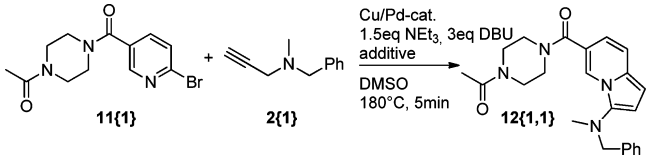
quence and (2) all desired N-modifications must be amenable to flow chemistry. At first, we analyzed whether any by-products inevitably formed during a first step N-acylation interfered with the follow-up indolizine synthesis. The coupling of acylated heteroaryl bromide **11{1}** and propargyl amine **2{1}** served as our model reaction for this analysis (Table 1).

Table 1. Analysis of Potential Factors Inhibiting the Indolizine Synthesis[a]



entry	additive[b]	12{1,1} (%)[c]
1		87
2	DMF	85
3	MeCN	84
4	$CHCl_3$	0
5	NEt_3·HOAc/DMF (1.0)	83
6	NEt_3·HCl/MeCN (1.0)	76
7	NEt_3·TFA/DMF (1.0)	84
8	Ac_2O/DMF (0.2)	87

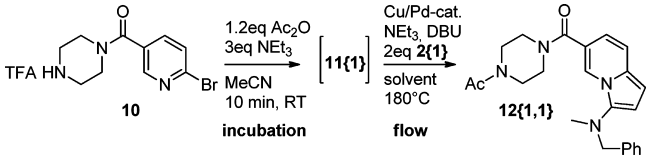
[a]Reaction conditions: Accendo Conjure Flow Reactor, Hastelloy tubing (0.75 mm i. d., 2.0 mL internal vol.), 400 μL segment: 0.05 mmol heteroaryl bromide **11{1}**, 0.10 mmol alkyne **2{1}**, 3 mol % $PdCl_2(PPh_3)_2$, 10 mol % CuI. [b]200 μL of solution added to the segment. Number in parentheses corresponds to equivalents of additive. [c]Percent UV from LC-MS analysis.

Indolizine **12{1,1}** was formed in good yield when using the developed protocol in the absence of any additives (entry 1). The addition of DMF or MeCN solvent had no negative effect on the outcome of the reaction (entries 2 and 3). However, chloroform, a standard solvent for acylation reactions, completely inhibited the catalyst system and only heteroaryl bromide **11{1}** along with a complex reaction mixture was observed (entry 4). As a result, we ruled out conducting the first step modification in this solvent. We also investigated the effect of stoichiometric amounts of ammonium salts that would be formed in the first step when using a typical base, such as triethylamine. Only triethylammonium chloride, which was added in MeCN solution due to its insolubility in DMF, had a slightly detrimental influence on the yield (entries 5–7). Moreover, the chloride salt is a highly crystalline solid that was found to easily precipitate from concentrated reaction solutions and subsequently block the fluidics of the reactor system. Thus, we investigated the effect of substoichiometric amounts of acetic anhydride, instead of acetyl chloride, as the potential acylating agent, and were delighted to find no diminution in yield (entry 8).

With these encouraging results in hand, we proceeded with the optimization of the amide formation. In preliminary batch experiments, we found that in the presence of a slight excess of acetic anhydride (1.2 equiv.) and triethylamine (3.0 equiv.) quantitative acylation of TFA salt **10** was observed at room temperature within only 10 min. DMF and MeCN were equally suitable solvents for this reaction, and no precipitation was observed in either case (see ESI for details). We then transferred this approach into the flow reactor by preparing a segment (200 μL, 0.25 M, MeCN or DMF) containing TFA salt **10**, along with the required amounts of base and acetic anhydride. The prepared mixture was positioned in the incubation chamber at 25 °C for 10 min, then quenched with MeOH (200 μL) upon reaspiration, and passed through the reactor coil at room temperature. LC-MS analysis of the collected segment showed full conversion of the amine to the amide without any notable side reactions in either DMF or MeCN solvent. For our further investigations, we chose to conduct all further *N*-modifications in MeCN because of its more environmentally benign properties, thermal stability in comparison to DMF, and higher volatility, thus simplifying purification and isolation of the final product.

Having found conditions for the first reaction step that met all the aforementioned criteria, we combined this first step with the previously developed indolizine flow protocol and examined a range of base, solvent and reaction time variants in order to determine the optimal conditions for the multistep protocol (Table 2).

Table 2. Optimization of the Two-Step Protocol for Library Synthesis[a]



entry	base step 2[b]	solvent step 2	time min	12{1,1} (%)[c]
1	NEt_3 (1.5), DBU (3.0)	DMSO	5	52
2			7	55
3			10	62
4	NEt_3 (1.5), DBU (5.0)		5	75
5			7	92
6	DBU (5.0)			93
7		MeCN		92 (59)[d]

[a]Reaction conditions: Accendo Conjure Flow Reactor; step 1 200 μL segment: 0.05 mmol **10**, 0.06 mmol Ac_2O **5{1}**, 0.15 mmol NEt_3, PFA tubing (0.75 mm i. d., 0.7 mL internal vol.); step 2 400 μL segment: 0.05 mmol **11{1}**, 0.10 mmol alkyne **2{1}**, 3 mol % $PdCl_2(PPh_3)_2$, 10 mol % CuI, Hastelloy tubing (0.75 mm i. d., 2.0 mL internal vol.). [b]Number in parentheses corresponds to equivalents of base. [c]Percent UV from LC-MS analysis. [d]Isolated yield.

We were delighted to find that our first attempt to combine both steps did indeed lead to the formation of indolizine **12{1,1}** in 52% yield (entry 1). In this case, we observed full conversion of the aryl bromide intermediate **11{1}** to a 1:1 mixture of the desired indolizine and Sonogashira coupling product. Further optimization showed that an increased residence time did not affect this ratio (entries 2 and 3). However, increasing the amount of DBU base from three to five equivalents showed an improved yield of 75% for the desired indolizine (entry 4). Prolonging the residence time under these conditions had a profound effect on the intramolecular cycloisomerization and a 92% yield was obtained (entry 5). In the presence of a larger excess of DBU base we found that triethylamine, previously added to solubilize the catalyst precursors, was no longer required, and identical results were obtained in its absence (entry 6). Furthermore, due to the nondetrimental presence of MeCN in the reaction segment from the incubation step, we explored conducting the entire reaction sequence in this preferred solvent and were pleased to find that similar results were obtained when no DMSO was

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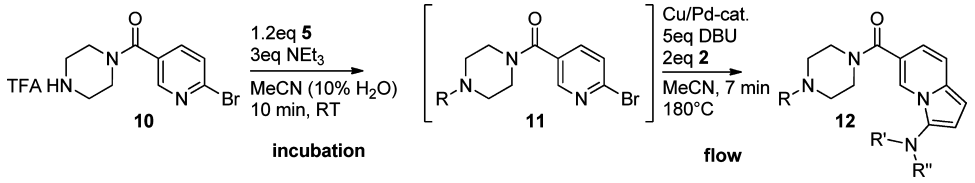
Table 3. Synthesized 25-Membered 3-Aminiondolizine Library[a]



	2{1}	2{2}	2{3}	2{4}	2{5}
5{1}	12{1,1}, 59%	12{1,2}, 78%	12{1,3}, 61%	12{1,4}, 62%	12{1,5}, 65%
5{2}	12{2,1}, 54%	12{2,2}, 59%	12{2,3}, 52%	12{2,4}, 76%	12{2,5}, 56%
5{3}	12{3,1}, 56%	12{3,2}, 60%	12{3,3}, 51%	12{3,4}, 62%	12{3,5}, 65%
5{4}	12{4,1}, 24%[b] 33%[c]	12{4,2}, 47%[b]	12{4,3}, 28%[b]	12{4,4}, 41%[b]	12{4,5}, 37%[b]
5{5}	12{5,1}, 42%[b] 49%[c]	12{5,2}, 53%[b]	12{5,3}, 31%[b]	12{5,4}, 54%[b]	12{5,5}, 40%[b]

[a]Reaction conditions: Accendo Conjure Flow Reactor; step 1 200 μL segment: 0.05 mmol **10**, 0.06 mmol **2**, 0.15 mmol NEt_3, PFA tubing (0.75 mm i. d., 0.7 mL internal vol.); step 2 400 μL segment: 0.05 mmol **11**, 0.10 mmol alkyne **5**, 3 mol % $PdCl_2(PPh_3)_2$, 10 mol % CuI, Hastelloy tubing (0.75 mm i. d., 2.0 mL internal vol.). Isolated yields. [b]Step 1 conducted in MeCN/H_2O (9:1). [c]7 equiv of DBU.

used for the cross-coupling step (entry 7), leading to a much more environmentally benign process. Moreover, the absence of DMSO facilitated a much faster and more reliable isolation of the final product. The volatile components, mainly MeCN solvent and polyfluorinated spacer, were removed under reduced pressure and the residue was purified by preparative TLC to afford the desired indolizine **12{1,1}** in 59% isolated yield.

Library Construction. Using these optimized conditions, we undertook the construction of a library of indolizine derivatives based upon the core fragment **10** (Table 3). We chose to employ a series of propargyl amines (reagent chemset **2**) for the generation of our library, including aromatic (**2{1−2}**) and aliphatic (**2{3−4}**) substituents of different polarity, as well as the *N*-Boc-protected propargylamine **2{5}**.

Diversity Through Amine Acylation. Building upon our pilot studies using an acetylation reaction as the amine diversification step, we generated the first wave of the library using a series of acylation reactions. Thus, starting from TFA salt **10** the desired compounds were formed in good isolated yields (**12{1,1−1,5}**, 59−78%, 65% average yield) after undergoing three sequential modifications (amidation, Sonogashira coupling, cycloisomerization). We were able to quickly expand our library with two other commercially available

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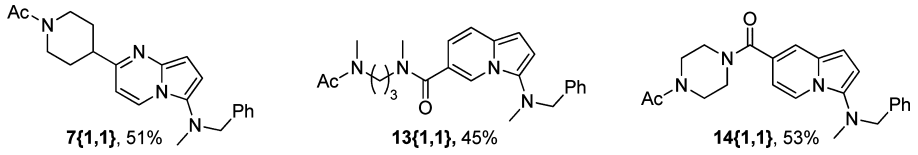

Figure 1. Library members featuring alternative heterocyclic cores.

anhydrides, benzoic anhydride **5{2}** and butyric anhydride **5{3}** using this general protocol. In these cases, all desired library compounds were obtained in good yields and excellent purity after preparative TLC purification (52−76%, 59% average yield for chemset **12{2,1−2,5}**; 51−65%, 59% average yield for chemset **12{3,1−3,5}**). Thus, we were able to rapidly generate 15 members of the library, covering a range of physical properties, illustrating how the properties of the library can readily be tuned through the choice of substituents at the two points of diversity on the core heteroaryl bromide fragment. Notably, *N*-Boc-protected amines are very well tolerated under the reaction conditions (**12{1,5−5,5}**), allowing the future possibility of subsequent deprotection and further derivatization of the resultant amine.

The required laboratory work for the synthesis of these first 15 library members involved preparation of the corresponding ten stock solutions (TFA salt **10** and NEt_3, catalyst, reagent chemsets **2{1−3}** and **5{1−5}**) and programming of the Conjure software to perform the correct loading of source vials and aspiration of stock solutions. Each of the library compounds in Table 3 was isolated from two identical segments, allowing straightforward purification and characterization of each compound. For the entire sequence involved in executing these two experiments/segments, the reactor requires approximately 30 min from loading the first vial to completion of collection.[20] As a result, the first 15 library compounds **12{1,1−3,5}** were prepared and separately collected within 7.5 h in fully automated fashion. Final isolation and purification was achieved by preparative TLC. For lead discovery purposes however, single segment runs would be sufficient to generate the necessary material due to the high reaction concentrations (0.125 M for step 2), thus decreasing the overall reactor time to approximately 5 h.[21] Furthermore, in principle, the reactor could be combined with a preparative HPLC-MS device for automatic purification of the desired compounds, as was recently disclosed by researchers at Abbott Laboratories.[22]

Diversity Through Other Amine Derivatizations. At this point we investigated whether our optimized protocol was suitable for N-modifications other than amide formation. A variety of electrophilic reagent chemsets were therefore screened for their suitability. Electrophiles such as alkyl halides and sulfonyl chlorides readily afforded the desired tertiary amines and sulfonamides respectively, within 10 min in MeCN. However, both reactions were routinely accompanied by rapid formation of voluminous precipitates (the corresponding ammonium halide salts), thus making them unsuitable for a flow chemistry sequence. Since the reactivity was acceptable in each case, we re-evaluated the solvent system in the hope of avoiding the precipitation issue. We reasoned that the addition of minimal amounts of water might suffice to efficiently solubilize the ammonium salts while having no adverse effect on the first reaction and, more importantly, on the subsequent Sonogashira coupling.[23] We were delighted to find that a mixture of MeCN and water (9:1) resulted in full conversion for both the alkylation using benzyl bromide **5{4}** and the sulfonamidation with tosyl chloride **5{5}** without precipitation of the corresponding ammonium salts.[24] Moreover, we found that these conditions were compatible with the overall indolizine formation protocol, thereby opening the way to yet further diversification of our library. The presence of water (20 μL of 400 μL for the cross-coupling step) had a slightly detrimental effect on the cycloisomerization to the indolizine, and thus yields were generally lower. However, in case of very low yields, a greater excess of DBU base (7 equiv) may be employed to increase the isolated yields (**12{4,1}** and **12{5,1}**).

The library members bearing a tertiary amine (resulting from an alkylation step) were mostly isolated in lower yields (chemset **12{4,1−4,5}**, 24−47%, 35% average yield). The reason for this is unclear, but we presume that the tertiary amine moiety might undergo decomposition under the basic aqueous reaction conditions, as LC-MS analysis usually showed a much more complex reaction mixture for these transformations. However, because of the high reaction concentrations of the developed protocol, 10−20 mg of pure material could be isolated from only 2 segments for each compound. In contrast, the sulfonamides were usually formed in good yields (chemset **12{5,1−5,5}**, 42−54%, 44% average yield).

Diversity Through Heteroaryl Bromide Core. To further exemplify the applicability of this protocol and the potential size the corresponding virtual library, we carried out additional experiments using several other amine substituted heterocyclic bromide cores (Figure 1). Compound **7{1,1}** represents our prototypical library member,[11] resulting from acetylation of core fragment **4** and coupling with propargylamine **2{1}**. This agent was resynthesized using our optimized multistep flow protocol, resulting in an improved yield. This indicates that an analogous 25-member library, employing the variations illustrated in Table 3, would be accessible from this core fragment. Similarly, **13{1,1}** could be prepared using the same acetylation/coupling protocol, again indicating access to yet another 25-member library featuring the substitution patterns illustrated in Table 3. Finally, **14{1,1}**, a regioisomer of **12{1,1}**, could be prepared, indicating its compatibility with generation of a matrix of analogs along the lines illustrated in Table 3. Thus, based on the already generated 25-membered library in Table 3, a virtual library of at least 100 compounds is now accessible in less than three days using our protocol.

Diversity Through Further Propargylamine Variants. Importantly, our optimized method is not limited to orthogonal modification of the heteroaryl bromide core. Thus, the readily synthesized TFA salt of *N*-propargylpiperazine can also serve as a building block for a further variant of the library. Again, N-modification is carried out according to the described procedure in the incubation chamber, but this time a secondary amine within the alkyne coupling partner was acylated and then coupled with methyl 6-bromonicotinate in the Sonogashira/cycloisomerization sequence to afford indolizine **15{1,1}** in good yield (Figure 2).

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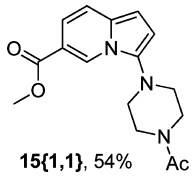

15{1,1}, 54%

Figure 2. Prototypical library member featuring diversification via the propargyl amine fragment.

As a result, the size and diversity of the alternative library generated using this approach would be determined by acylation, alkylation and sulfonamidation reactions of the propargylamine, and availablility of heteroaryl bromides for the second step cross-coupling. These can include a variety of 2-bromopyridines, -pyrimidines, -pyrazines, and -quinolines, as described in our earlier report.[11]

CONCLUSION

In summary, we have developed a novel flow methodology for the orthogonal modification of heterocyclic building blocks and its subsequent application for automated library synthesis of druglike molecules. All reaction steps are conducted within the flow reactor and products are isolated in good yields following three sequential transformations. We have exemplified this methodology through generation of a diverse 25-membered library of druglike 3-aminoindolizines. We were able to show that the optimized conditions allow a variety of quantitative transformations to take place on the secondary amine within 10 min, including acylations, alkylations and sulfonamidations. The latter two modifications require the addition of water (10%) to solubilize ammonium halide salts. Under optimized flow conditions the subsequent tandem Sonogashira/cycloisomerization reaction can be carried out with a variety of propargyl amines within 7 min at 180 °C in MeCN to afford the desired compounds in good yields. All products were isolated by preparative TLC and fully analyzed and characterized.

The size and diversity of the virtual library accessible via this protocol is substantial, since it allows a variety of *N*-modifications including acylations, alkylations and sulfonamidations to be carried out in short time. Moreover, the second step coupling can be conducted within minutes employing a broad range of propargyl amines and amides that bear aromatic and aliphatic groups of varying polarities. We have also demonstrated the ability to introduce further diversity through variation of the amino-substituted heteroaryl bromide core fragment, where the three further variants examined indicate that a total of 100 3-aminoindolizines could be prepared within several days.

Building upon the robust first step reaction conditions, expansion of the second step protocol to include other cross-coupling reactions appears viable, thus potentially expanding the accessible chemical space still further with one general protocol. The option of combining the flow reactor employed in this program with an automated purification system has the potential to achieve a highly efficient production of large libraries of druglike heterocycles. This will be the subject of future investigations.

EXPERIMENTAL SECTION

General Procedure for the Synthesis of Library Compounds Using a Conjure Flow Reactor. The corresponding TFA salt and base (0.33 M in MeCN, 150 μL, 0.05 mmol for the TFA salt, 0.15 mmol for NEt_3 or 0.25 mmol for pyridine) and electrophilic reagent (1.20 M in MeCN, 50 μL, 0.06 mmol) were aspirated from their respective source vials located in position C (TFA salt/base) and position D (electrophilic reagent), mixed through a PFA mixing tube (0.2 mm i. d.), and loaded into a sample loop in the incubation chamber to remain for 10 min at room temperature. The alkyne (2.00 M in MeCN, 50.0 μL, 0.10 mmol) and catalyst stock solution (1.5 μmol $PdCl_2(PPh_3)_2$, 5 μmol CuI, 0.25 mmol DBU in MeCN, 150 μL) were aspirated from their respective source vials located in position A (alkyne) and position B (catalyst), mixed with the segment containing the *N*-modified material in MeCN through a PFA mixing tube (0.2 mm inner diameter), and loaded into an injection loop. The reaction segment (400 μL) was injected into the flow reactor (Hastelloy coil, 0.75 mm i. d., 2.0 mL internal vol.) set at 180 °C and passed through the reactor at a flow-rate of 286 μL·min^{-1} (7 min residence time). A total of 2 reaction segments prepared in this manner were collected.

Upon complete collection, the volatile reaction components were removed from the reaction solution in a stream of nitrogen. The crude oily residue was purified by preparative TLC (silica gel, hexanes/ethyl acetate or dichloromethane/methanol) to afford the desired library compounds.

Compound 12{1,1}. Compound **12{1,1}** was synthesized according to the general flow procedure using 2 segments (400 μL each) containing TFA salt **10** and NEt_3 (0.33 M in MeCN, 150 μL, 0.05 mmol for the TFA salt, 0.15 mmol for NEt_3), acetic anhydride **5{1}** (1.20 M in MeCN, 50 μL, 0.06 mmol), *N*-methyl-*N*-propargylbenzylamine **2{1}** (2.00 M in MeCN, 50.0 μL, 0.10 mmol) and catalyst stock solution (in MeCN, 150 μL) and a flow-rate of 286 μL min^{-1} (7 min residence time). Upon completion of the reaction, the volatile reaction components (mainly MeCN, NEt_3, and fluorous spacer) were removed in a stream of nitrogen. The oily residue was purified by preparative TLC (silica gel, dichloromethane/methanol 19:1) to afford 23.1 mg (59%) of a yellow solid. ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 2.14 (s, 3 H) 2.69 (s, 3 H) 3.51 (br. s., 2 H) 3.63 (d, *J* = 8.44 Hz, 4 H) 3.69 (br. s., 2 H) 4.08 (s, 2 H) 6.42 (d, *J* = 4.04 Hz, 1 H) 6.52 (d, *J* = 4.03 Hz, 1 H) 6.58 (d, *J* = 9.17 Hz, 1 H) 7.24 - 7.28 (m, 1 H) 7.31 (d, *J* = 4.40 Hz, 5 H) 8.21 (s, 1 H); ^{13}C NMR (126 MHz, chloroform-*d*) δ ppm 21.3, 41.4, 41.5, 46.1, 60.6, 98.4, 104.8, 114.0, 117.4, 118.9, 122.3, 127.3, 128.3, 128.5, 131.9, 135.8, 137.7, 169.1, 169.4; HRMS (ESI-TOF): $C_{23}H_{26}N_4O_2$ $[M + H]^+$ calculated 391.2128, found 391.2138.

All compounds in Table 3 and Figures 1 and 2 were prepared accordingly. For full experimental procedures and analytical data, see the ESI.

ASSOCIATED CONTENT

Supporting Information

Conjure flow reactor information, additional screening experiments, and analytical data for all compounds is available online. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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(16) Every segment that is prepared in the fluid prep module of the Conjure reactor is preceded and followed by an immiscible liquid spacer (perfluoro(methyldecalin), 50 μL) and is then introduced into the flow stream and passed through the reactor as an isolated reaction.

(17) The 40-vial carousel is designed to hold 36 reagent solutions and 4 rinse vials.

(18) The incubation chamber contains five individual sample loops allowing the preparation of five different first step segments.

(19) The number of additional reagents is limited to three for the second step, as the fourth syringe is used to reaspirate the first step reaction segment.

(20) Once the first segment is subjected to the flow stream to process the second reaction step, the fluid prep module immediately begins the required operations to prepare the first step of the second segment.
(21) The overall reaction time is 17 min (10 min for the first step and 7 min residence time in the flow reactor coil). An additional 3 min is required for operations of the fluid prep module including loading of vials and aspirating and mixing of stock solutions.
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