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Parallel Synthesis of 1*H*-Pyrazolo[3,4-*d*]pyrimidines via Condensation of *N*-Pyrazolylamides and Nitriles

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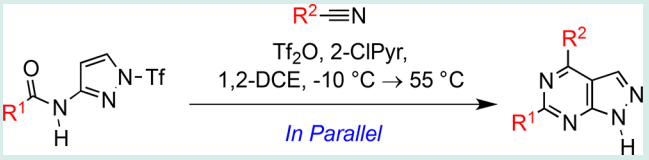
Supporting Information

ABSTRACT: A novel parallel medicinal chemistry (PMC)-enabled synthesis of 1*H*-pyrazolo[3,4-*d*]pyrimidines employing condensation of easily accessible *N*-pyrazolylamides and nitriles has been developed. The presented studies describe singleton and library enablements that allowed rapid generation of molecular diversity to examine C4 and C6 vectors. This chemistry enabled access to challenging alkyl substituents, expanding the overall chemical space beyond that available via typical C(sp^2)−C(sp^2) coupling and S_NAr transformations. Furthermore, monomer group interconversions allowing the use of larger and more diverse amides and carboxylic acids as precursors to nitriles are discussed.



KEYWORDS: *parallel medicinal chemistry (PMC), pyrazolopyrimidine, monomer interconversion, condensation chemistry, C(sp^3) substitutions*

Nitrogen-containing heterocycles are of significant importance for the design and development of pharmacologically active molecules. Among many such heterocycles, pyrazolo[3,4-*d*]pyrimidines have emerged as one of the highly sought chemical entities in pharmaceutical drug discovery.[1] Their unique structural features that allow opportunities for target engagement and rapid examination of structure−activity relationship (SAR) have facilitated the development of a number of small-molecule drug candidates. Specifically, much attention has been dedicated to development of pyrazolo[3,4-*d*]pyrimidine-based small-molecule kinase inhibitors.[2] Recently, the U.S. Food & Drug Administration approved the pyrazolo-[3,4-*d*]pyrimidine-based inhibitor ibrutinib **1** (trade name Imbruvica)[3] for the treatment of mantle cell lymphoma and chronic lymphocytic leukemia. A number of other derivatives have shown promising preclinical activities and are under investigation as inhibitors of kinase targets such as mTOR[2a] (**2**, see Figure 1A), PI3K,[2b] P38 MAPK,[2c] GSK-3,[2d] CDK9,[2e] Aurora kinase,[2f] Fyn,[2g] Src,[2h] Abl,[2i] and Sgk1.[2j] It is no surprise that a growing number of synthetic resources have been devoted to the development of new and efficient technologies for the synthesis of pyrazolo[3,4-*d*]pyrimidines.

In connection to an ongoing drug discovery program, we sought to develop pyrazolo[3,4-*d*]pyrimidine-based kinase inhibitors. High-throughput fragment screening and molecular modeling indicated the need for a comprehensive SAR study with respect to C4 and C6 vectors as illustrated in general structure **3** (Figure 1B). Our initial designs focused on examination of C4- and C6-alkyl- and -aryl-substituted pyrazolo[3,4-*d*]pyrimidines as a way to improve potency and selectivity and to achieve drug-like absorption, distribution, metabolism, and excretion (ADME) properties. A plethora of methods exist for the synthesis of substituted pyrazolo[3,4-*d*]pyrimidines that primarily employ two strategies: (1) condensation of appropriately substituted 5-aminopyrazoles **4** and formamide surrogates[4] and (2) condensation of pyrimidine carbaldehyde derivatives **6** and hydrazine,[5] as outlined in Figure 1B. These strategies are inherently more amenable to installation of heteroatom substituents at C4 and C6, although aryl and alkyl substituents can be introduced via metal-mediated cross-coupling chemistry.[6,7] Although significant advances in C(sp^2)−C(sp^2) and C(sp^2)−C(sp^3) coupling chemistry have been made, their scope is limited to commercially available alkyl and aryl coupling reagents. A survey of the literature indicated the need for the development of a new synthetic method that can expand the scope to include a wide range of alkyl substituents at C4 and C6 vectors of pyrazolo[3,4-*d*]pyrimidines.

In this communication, we report the development of a general method for the synthesis of C4- and C6-substituted pyrazolo[3,4-*d*]pyrimidines that allows the incorporation of a range of alkyl, aryl, and heteroaromatic substituents by means of a common synthetic technique. The methodology is well-suited for the generation of libraries which is a valuable feature in view of the growing interests in diversity-oriented library generation and high-throughput screening technologies.[8] Our method employs condensation of easily accessible *N*-pyrazolylamides **7** and readily available nitriles **8** to afford pyrazolo[3,4-*d*]pyrimidine analogues **3** (Figure 1C). This synthetic protocol is modified and optimized to facilitate the generation of molecular libraries in parallel wherein nitrile monomers can be reacted with chosen *N*-pyrazolylamide templates. Furthermore, monomer group interconversion that allows the transformation of amides and carboxylic acids into

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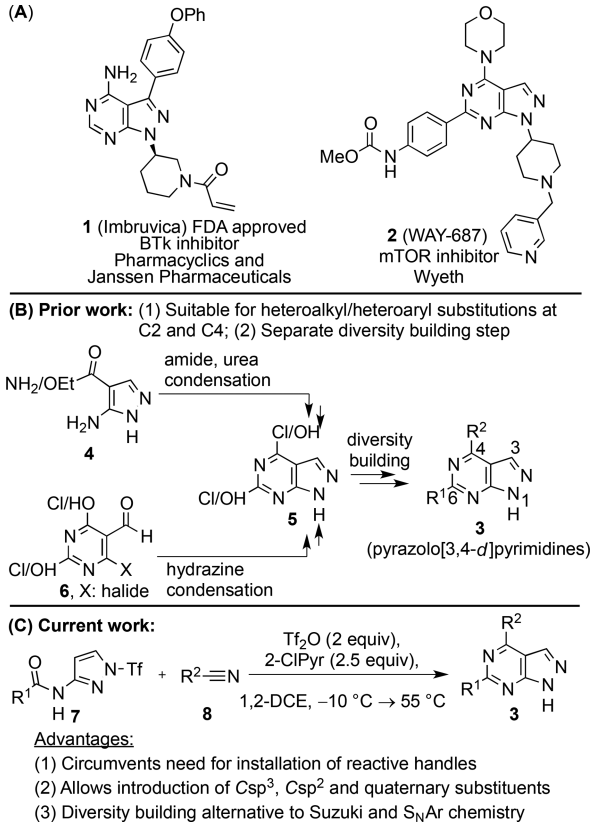
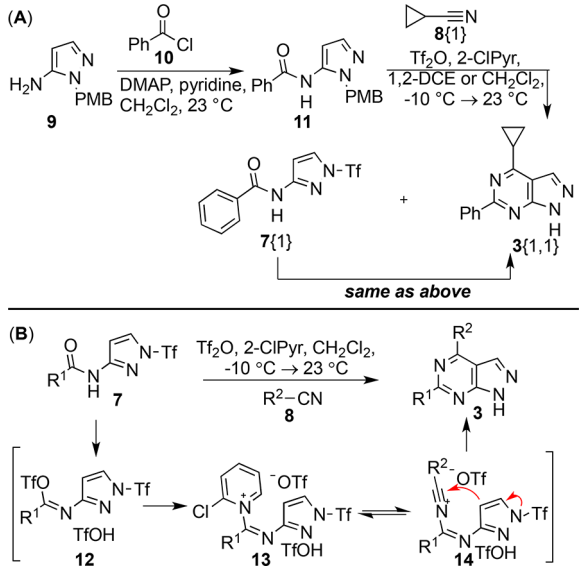


Figure 1. (A) Examples of pyrazolo[3,4-*d*]pyrimidine-based kinase inhibitors. (B) Prior strategies for the synthesis of pyrazolo[3,4-*d*]pyrimidines. (C) Current strategy for the synthesis of C4- and C6-substituted pyrazolo[3,4-*d*]pyrimidines.

nitriles in traditional batch and parallel synthetic workflows is described. The synthetic enablement and substrate scope are described in detail below.

In 2006, Movassaghi and Hill reported the one-step synthesis of pyrimidines and quinazolines via condensation of *N*-vinyl or *N*-aryl amides with nitriles.[9,10] This transformation leverages the unique reactivity of electrophilically activated amides,[11,12] in the presence of 2-chloropyridine (2-ClPyr) and trifluoromethanesulfonic anhydride (Tf_2O), to trap weakly nucleophilic nitriles. We sought to build upon this discovery to effect the formation of pyrazolo[3,4-*d*]pyrimidines via activation of *N*-pyrazolylamides toward nucleophilic addition and subsequent electrophilic aromatic substitution with nitriles. Preliminary studies focused on understanding the feasibility of our mechanistic framework to guide the synthesis of the desired pyrazolo[3,4-*d*]pyrimidines. Commercially available 1-*p*-methoxybenzyl-1*H*-pyrazol-5-amine (**9**) served as an entry point for the introduction of 1*H*-pyrazole. The choice of *p*-methoxybenzyl (PMB) as a masking agent was thought to provide favorable electronic activation of the pyrazole motif, as required for electrophilic aromatic substitution, and easily allow subsequent removal under mild reaction conditions. Amine **9** was readily converted to test substrate **11** (Scheme 1A). Preliminary experiments showed that when benzamide derivative **11** was reacted with cyclopropyl carbonitrile (**8**{*1*}) in the presence of Tf_2O and 2-ClPyr at −10 to 23 °C, only a ∼10% yield of the desired cyclization was observed, leading to pyrazolo[3,4-*d*]pyrimidine **3**{*1,1*} lacking the benzyl protecting group. Surprisingly, we also isolated *N*-(1-(trifluoromethylsulfonyl)-1*H*-pyrazol-3-yl)benzamide (**7**{*1*}) in ∼30% yield. Suitable crystalline material was obtained from residual NMR solvent ($CDCl_3$), and an X-ray diffraction study provided an unambiguous structural determination of **7**{*1*}.[13] Although unexpected, the formation of **7**{*1*} provided important mechanistic clues to decipher events underlying the reaction. These observed products indicated that **7**{*1*} likely comes from initial triflation of the protected pyrazole ring followed by hydrolytic cleavage of the PMB group and that further activation of **7**{*1*} in the presence of additional Tf_2O and 2-ClPyr may effect the intended pyrimidine ring formation to provide **3**{*1,1*}. To verify this, we independently subjected **7**{*1*} to the same reaction conditions and noted full conversion to the desired product, isolating **3**{*1,1*} in 50% yield. Although this result provided strong evidence for the reaction intermediacy of **7**{*1*}, it did not preclude the possibility of the direct formation of **3**{*1,1*} from **11** as intended initially. On the basis of mechanistic knowledge at the time, we attempted to increase the conversion of **11** to **3**{*1,1*} and minimize adduct **7**{*1*}. However, our efforts were met with limited success. A rational experimental design suggested the use of more robust protecting groups such as benzyl and tosyl instead of PMB, but such a change may jeopardize the final electrophilic aromatic substitution and elongate the reaction sequence by the addition of a deprotection step. The mechanistic knowledge proved to be key to our subsequent experimental designs in that we proposed the deliberate formation and isolation of interrupted adducts like **7**{*1*}, which then could be smoothly transformed into the desired pyrazolopyrimidine products as exemplified above. Our proposed working mechanistic model is outlined in Scheme 1B. The facile amide activation by triflic anhydride provides imidoyl triflate[11] **12**, which upon addition of 2-chloropyridine leads to activated electrophile **13**.[8] At this stage, the reversible addition of nitrile followed by electrophilic aromatic substitution provides the desired cyclization to afford pyrazolo[3,4-*d*]pyrimidine **3**.

Scheme 1. (A) Early Attempt at Condensation of *N*-Pyrazolylamides and Nitriles; (B) Working Mechanistic Model



Although this transformation leverages the unique reactivity of electrophilically activated amides,[11,12] in the presence of 2-chloropyridine (2-ClPyr) and trifluoromethanesulfonic anhydride (Tf_2O), to trap weakly nucleophilic nitriles. We sought to build upon this discovery to effect the formation of pyrazolo[3,4-*d*]pyrimidines via activation of *N*-pyrazolylamides toward nucleophilic addition and subsequent electrophilic aromatic substitution with nitriles. Preliminary studies focused on understanding the feasibility of our mechanistic framework to guide the synthesis of the desired pyrazolo[3,4-*d*]pyrimidines. Commercially available 1-*p*-methoxybenzyl-1*H*-pyrazol-5-amine (**9**) served as an entry point for the introduction of 1*H*-pyrazole. The choice of *p*-methoxybenzyl (PMB) as a masking agent was thought to provide favorable electronic activation of the pyrazole motif, as required for electrophilic aromatic substitution, and easily allow subsequent removal under mild reaction conditions. Amine **9** was readily converted to test substrate **11** (Scheme 1A). Preliminary experiments showed that when benzamide derivative **11** was reacted with cyclopropyl carbonitrile (**8**{*1*}) in the presence of Tf_2O and 2-ClPyr at −10 to 23 °C, only a ∼10% yield of the desired cyclization was observed, leading to pyrazolo[3,4-*d*]pyrimidine **3**{*1,1*} lacking the benzyl protecting group. Surprisingly, we also isolated *N*-(1-(trifluoromethylsulfonyl)-1*H*-pyrazol-3-yl)benzamide (**7**{*1*}) in ∼30% yield. Suitable crystalline material was obtained from residual NMR solvent ($CDCl_3$), and an X-ray diffraction study provided an unambiguous structural determination of **7**{*1*}.[13] Although unexpected, the formation of **7**{*1*} provided important mechanistic clues to decipher events underlying the reaction. These observed products indicated that **7**{*1*} likely comes from initial triflation of the protected pyrazole ring followed by hydrolytic cleavage of the PMB group and that further activation of **7**{*1*} in the presence of additional Tf_2O and 2-ClPyr may effect the intended pyrimidine ring formation to provide **3**{*1,1*}. To verify this, we independently subjected **7**{*1*} to the same reaction conditions and noted full conversion to the desired product, isolating **3**{*1,1*} in 50% yield. Although this result provided strong evidence for the reaction intermediacy of **7**{*1*}, it did not preclude the possibility of the direct formation of **3**{*1,1*} from **11** as intended initially. On the basis of mechanistic knowledge at the time, we attempted to increase the conversion of **11** to **3**{*1,1*} and minimize adduct **7**{*1*}. However, our efforts were met with limited success. A rational experimental design suggested the use of more robust protecting groups such as benzyl and tosyl instead of PMB, but such a change may jeopardize the final electrophilic aromatic substitution and elongate the reaction sequence by the addition of a deprotection step. The mechanistic knowledge proved to be key to our subsequent experimental designs in that we proposed the deliberate formation and isolation of interrupted adducts like **7**{*1*}, which then could be smoothly transformed into the desired pyrazolopyrimidine products as exemplified above. Our proposed working mechanistic model is outlined in Scheme 1B. The facile amide activation by triflic anhydride provides imidoyl triflate[11] **12**, which upon addition of 2-chloropyridine leads to activated electrophile **13**.[8] At this stage, the reversible addition of nitrile followed by electrophilic aromatic substitution provides the desired cyclization to afford pyrazolo[3,4-*d*]pyrimidine **3**.

With the mechanistic model laid out, we then optimized the reaction conditions and examined the generality of the method, initially via the use of standard batch-style laboratory

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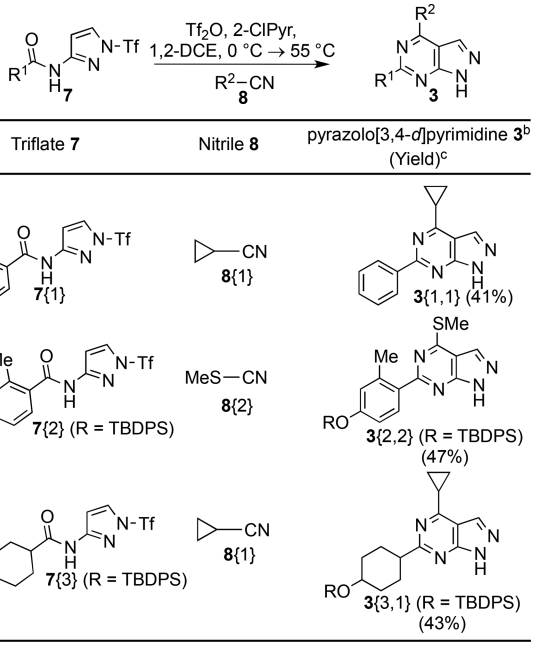
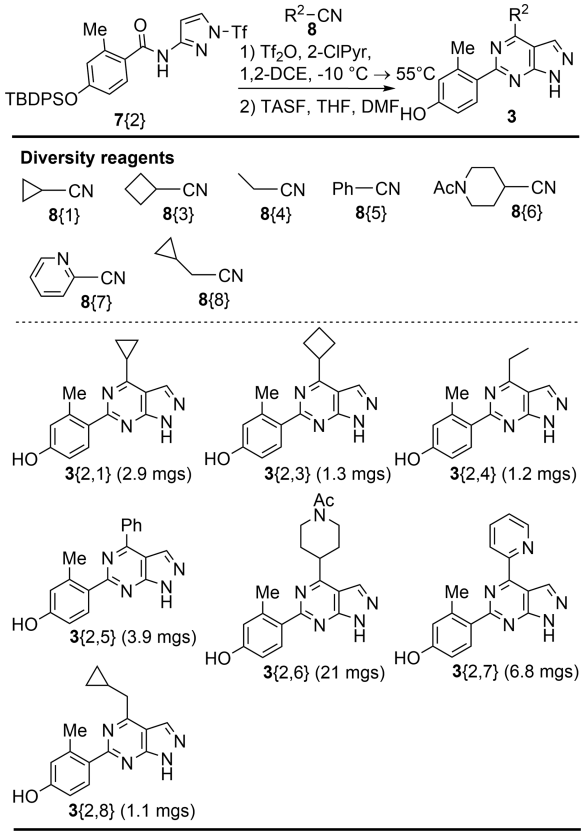
techniques. *N*-(1-(Trifluoromethylsulfonyl)-1*H*-pyrazol-3-yl)-amide derivatives 7{*1*}, 7{*2*}, and 7{*3*} (shown in Table 1)

Table 1. Pyrazolopyrimidine Synthesis[a]



[a]General reaction procedure: To amide 7 (1 equiv) in 1,2-DCE were added Tf_2O (2 equiv) and 2-ClPyr (2.1−2.5 equiv) followed by nitrile 8 (3 equiv) at 0 °C, and the mixture was stirred for 1−2 h at 55 °C. The reaction mixtures were then subjected to aqueous workup and purified via flash column chromatography. [b]NOESY studies for selected compounds noted correlations between the N−H and C3−H hydrogens and may suggest a majority of the tautomeric population in $CHCl_3$-*d*. [c]Isolated yields upon purification are reported.

Table 2. Pyrazolopyrimidine Synthesis via a Two-Step Parallel Method[a,b]



[a]For the reaction procedure, see the Supporting Information. Reactions were run on a 0.1 mmol scale. [b]Isolated amounts over two steps after final purification are reported.

were synthesized via a two-step protocol: coupling of **9** with appropriate carboxylic acids followed by treatment with triflic anhydride and 2-chloropyridine (see the Supporting Information for detailed reaction procedures and spectral data). The pyrimidine ring formation was optimized with respect to temperature, solvent, and reagent stoichiometry. We replaced dichloromethane with safer and environmentally friendlier 1,2-dichloroethane. Heating the reaction mixture to 55 °C provided complete conversions and greater yields. The reagent stoichiometry was optimized at 2 equiv of Tf_2O and 2.5 equiv of 2-ClPyr with respect to 7. Table 1 illustrates the synthesis of representative pyrazolo[3,4-*d*]pyrimidines 3{*1*−*3*,*1*−*2*} that incorporates sp^3 hydrocarbons and heteroalkyls at C4 and alkyl and aryl substituents at C6. Pyrazolo[3,4-*d*]pyrimidines 3{*1*−*3*,*1*−*2*} were isolated in moderate yields. The ease of reaction setup, fast in situ reaction monitoring techniques, and good conversions of the transformations involved suggested the suitability of these reactions in parallel mode. To demonstrate this, a two-step library was created using *N*-(1-(trifluoromethylsulfonyl)-1*H*-pyrazol-3-yl)amide 7{*2*} as a template and 11 structurally diverse nitriles according to a protocol identical to the standard procedure developed previously. The results are summarized in Table 2. The two-step protocol comprising initial pyrimidine ring formation and subsequent *tert*-butyldiphenylsilyl (TBDPS) ether deprotection was executed without purification of the intermediate products, and the final products were separated using a mass-triggered semiautomated HPLC purification system.[14] The goal of the parallel medicinal chemistry (PMC) was to access more compounds in quick fashion and in sufficient quantities to facilitate biological testing[15] as opposed to maximizing chemical yields. Hence, the success was measured in terms of the overall efficiency expressed as the "success rate" and not in terms of individual chemical yields. The data presented in Table 2 demonstrated a success rate of 64%, defined as the number of final test compounds isolated divided by the number of monomers submitted (see the Supporting Information for complete list of monomers submitted). A library of seven test compounds was quickly generated, allowing SAR studies with respect to the C4 vector. The examples in Table 2 illustrate the range of alkyls (Table 2, examples 3{*2*,*1*}, 3{*2*,*3*}, 3{*2*,*4*}, 3{*2*,*6*}, and 3{*2*,*8*}) and aryls (Table 2, examples 3{*2*,*5*} and 3{*2*,*7*}) that can be introduced at the C4 vector. Comparison with existing literature methods underscored the unique and diverse sp^3 chemical space that can be accessed via our method.[16]

One of the issues often encountered in a PMC-enabled synthesis is the limited size of the available monomer sets. The larger the available monomer set is, the greater is the chemical space accessible via the method. To extend and diversify the accessible chemical space, we sought to expand the methodology to include amide and carboxylic acid monomer sets. This strategy would not only increase the accessible chemical space but also allow the use of relatively abundant and inexpensive alternative feedstock materials at both exploratory and preparative scales. In view of these considerations, we

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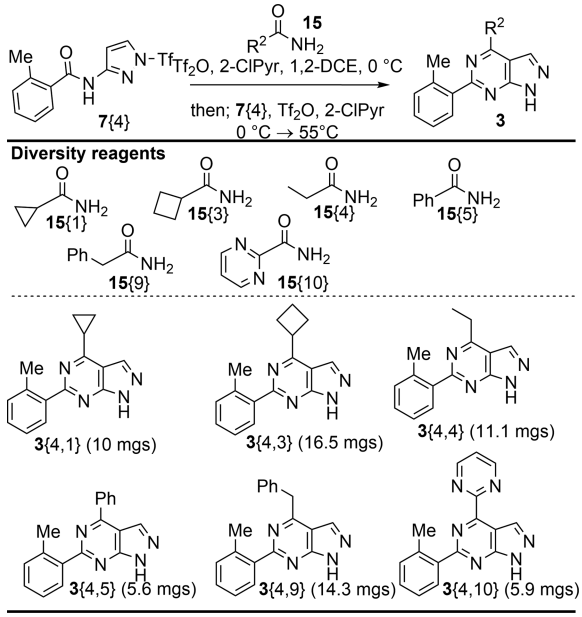
developed protocols that could use amide as well as carboxylic acid monomer sets to access C4- and C6-substituted pyrazolo[3,4-*d*]pyrimidines. Importantly, we were able to integrate monomer group interconversions with the key ring formation while avoiding intermediate purification steps. Table 3 outlines

Table 3. Pyrazolopyrimidine Synthesis Utilizing Primary Amide Monomers in a Parallel Workflow[a,b]

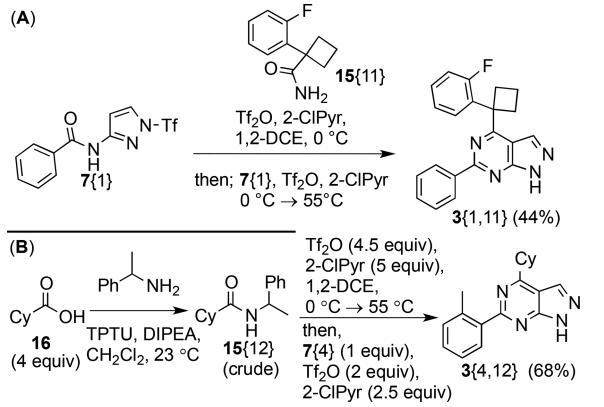


[a]For the reaction procedure, see the Supporting Information. Reactions were run on a 0.1 mmol scale. [b]Isolated amounts after purification are reported.

a two-step library that used primary amide monomers **15** and involved initial conversion to nitriles[17] followed by condensation with triflated pyrazolylamide template 7{*4*}. As displayed in Table 3, a success rate of 60% (see the Supporting Information for a complete list of monomers submitted) was achieved, and the test compounds were isolated in moderate to good yields. As illustrated in Table 3, the C4 substituents installed in earlier examples shown in Table 2 (compare examples 3{*2,1*}, 3{*2,3*}, 3{*2,4*}, and 3{*2,5*} with examples 3{*4,1*}, 3{*4,3*}, 3{*4,4*}, and 3{*4,5*}, respectively) were now accessed from amides. Such monomer group interconversion adds tremendous value by providing choices in feedstock selection. We also note the ability to easily introduce all of the substituted carbon substituents at C4, as exemplified in 3{*1,11*} (Scheme 2A). The ease of introduction of such quaternary carbon substituents opens the door for the design of challenging analogues that were virtually inaccessible before.

Further enablements led to the application of carboxylic acid monomers to generate diversity at the C4 vector of pyrazolopyrimidines. Scheme 2B describes the reaction scheme that employed cyclohexanecarboxylic acid (**16**) as the C4 diversity introduction element leading to product 3{*4,12*}. The protocol involved the formation of amide intermediate **15**{*12*}, which facilitated the smooth conversion to the corresponding nitrile under the same reaction conditions as for the pyrimidine ring formation. Attempts to transfer the protocol in Scheme 2B to the library platform met with limited success, indicating complexity associated with multicomponent and multistep procedures. Despite PMC limitations, the strategy shown in

Scheme 2. (A) Use of a Primary Amide for Diversity Building at the C4 Vector; (B) Use of a Carboxylic Acid for Diversity Building at the C6 Vector (Cy = Cyclohexyl)



Scheme 2B successfully demonstrated the use of a larger and more diverse nitrile surrogate in the form of carboxylic acid, which is of significant value.

To assess the full potential of our methodology in terms of building diversity, we carried out an analysis to determine the size of the virtual library utilizing the various viable starting materials as determined via our functional group interconversion strategy. The virtual library is defined as the total accessible chemical diversity based on available and usable monomers present in Pfizer's corporate chemical store. The analysis was performed using proprietary software that allowed identification of usable monomers via application of a number of filters such as material availability (>25 mg), range of molecular weight, reactivity and compatibility of functional groups, etc. This analysis enumerated 2461 usable nitriles, 2896 usable amides, and 11 965 usable carboxylic acids, thus representing a large virtual library and significant promise for our methodology to drive SAR around the C4 and C6 vectors of pyrazolo[3,4-*d*]pyrimidines in our efforts to identify compounds of pharmacological value.

In conclusion, we have developed a novel PMC-enabled methodology for the preparation of pyrazolo[3,4-*d*]pyrimidines via condensation of easily accessible *N*-pyrazolylamides and nitriles. The power of this method lies in the ability to access challenging chemical space, including that with greater C(sp^3) character, and was demonstrated effectively in both batch and library modes. Further enablements have established protocols that use abundant and inexpensive amide and carboxylic acid monomers, allowing rapid access to a range of substituents. The mechanistic underpinnings suggest promise for syntheses of other related heteroaromatics that are of value to drug discovery programs. Efforts are currently underway to define this scope and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscombsci.7b00116.

Complete description of reactions, characterization data (^{1}H and ^{13}C NMR, IR, and HRMS) of all new compounds, and copies of ^{1}H and ^{13}C NMR spectra (PDF)

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Author Contributions

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

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) For reviews, see: (a) Schenone, S.; Radi, M.; Musumeci, F.; Brullo, C.; Botta, M. Biologically Driven Synthesis of Pyrazolo[3,4-*d*]pyrimidines As Protein Kinase Inhibitors: An Old Scaffold As a New Tool for Medicinal Chemistry and Chemical Biology Studies. *Chem. Rev.* **2014**, *114*, 7189−7238. (b) Ferrari, S. M.; LaMotta, C.; Sartini, S.; Baldini, E.; Materazzi, G.; Politti, U.; Ruffilli, I.; Ulisse, S.; Miccoli, P.; Antonelli, A.; Fallahi, P. Pyrazolopyrimidine Derivatives as Antineoplastic Agents: with a Special Focus on Thyroid Cancer. *Mini-Rev. Med. Chem.* **2016**, *16*, 86−93.

(2) For selected examples of pyrazolo[3,4-*d*]pyrimidine-based kinase inhibitors, see: (a) Curran, K. J.; Verheijen, J. C.; Kaplan, J.; Richard, D. J.; Toral-Barza, L.; Hollander, I.; Lucas, J.; Ayral-Kaloustian, S.; Yu, K.; Zask, A. Pyrazolopyrimidines as highly potent and selective, ATP-competitive inhibitors of the mammalian target of rapamycin (mTOR): Optimization of the 1-substituent. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1440−1444. (b) Staben, S. T.; Heffron, T. P.; Sutherlin, D. P.; Bhat, S. R.; Castanedo, G. M.; Chuckowree, I. S.; Dotson, J.; Folkes, A. J.; Friedman, L. S.; Lee, L.; Lesnick, J.; Lewis, C.; Murray, J. M.; Nonomiya, J.; Olivero, A. G.; Plise, E.; Pang, J.; Prior, W. W.; Salphati, L.; Rouge, L.; Sampath, D.; Tsui, V.; Wan, N. C.; Wang, S.; Weismann, C.; Wu, P.; Zhu, B. Y. Structure-based optimization of pyrazolo-pyrimidine and -pyridine inhibitors of PI3-kinase. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6048−6051. (c) Revesz, L.; Blum, E.; Di Padova, F. E.; Buhl, T.; Feifel, R.; Gram, H.; Hiestand, P.; Manning, U.; Neumann, U.; Rucklin, G. Pyrazoloheteroaryls: Novel p38α MAP kinase inhibiting scaffolds with oral activity. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 262−266. (d) Peat, A. J.; Garrido, D.; Boucheron, J. A.; Schweiker, S. L.; Dickerson, S. H.; Wilson, J. R.; Wang, T. Y.; Thomson, S. A. Novel GSK-3 inhibitors with improved cellular activity. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2127−2130. (e) Bahceci, S.; Chan, B.; Chan, D. S.; Chen, J.; Forsyth, T. P.; Franzini, M.; Jammalamadaka, V.; Jeong, J. W.; Jones, L. R.; Kelley, R. M.; Kim, M. H.; Leahy, J. W.; Mac, M. B.; Noguchi, R. T.; Rao, P.; Ridgway, B. H.; Xu, W.; Wang, Y. Cdk Modulators. WO Patent WO2010003133, 2010. (f) Le Brazidec, J.-Y.; Pasis, A.; Tam, B.; Boykin, C.; Black, C.; Wang, D.; Claassen, G.; Chong, J.-H.; Chao, J.; Fan, J.; Nguyen, K.; Silvian, L.; Ling, L.; Zhang, L.; Choi, M.; Teng, M.; Pathan, N.; Zhao, S.; Li, T.; Taveras, A. Synthesis, SAR and biological evaluation of 1,6-disubstituted-1*H*-pyrazolo[3,4-*d*]pyrimidines as dual inhibitors of Aurora kinases and CDK1. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2070−2074. (g) Tintori, C.; La Sala, G.; Vignaroli, G.; Botta, L.; Fallacara, A. L.; Falchi, F.; Radi, M.; Zamperini, C.; Dreassi, E.; Dello Iacono, L.; Orioli, D.; Biamonti, G.; Garbelli, M.; Lossani, A.; Gasparrini, F.; Tuccinardi, T.; Laurenzana, I.; Angelucci, A.; Maga, G.; Schenone, S.; Brullo, C.; Musumeci, F.; Desogus, A.; Crespan, E.; Botta, M. Studies on the ATP Binding Site of Fyn Kinase for the Identification of New Inhibitors and Their Evaluation as Potential Agents against Tauopathies and Tumors. *J. Med. Chem.* **2015**, *58*, 4590−4609. (h) Vignaroli, G.; Iovenitti, G.; Zamperini, C.; Coniglio, F.; Calandro, P.; Molinari, A.; Fallacara, A. L.; Sartucci, A.; Calgani, A.; Colecchia, D.; Mancini, A.; Festuccia, C.; Dreassi, E.; Valoti, M.; Musumeci, F.; Chiariello, M.; Angelucci, A.; Botta, M.; Schenone, S. Prodrugs of Pyrazolo[3,4-*d*]pyrimidines: From Library Synthesis to Evaluation as Potential Anticancer Agents in an Orthotopic Glioblastoma Model. *J. Med. Chem.* **2017**, *60*, 6305−6320. (i) Manetti, F.; Brullo, C.; Magnani, M.; Mosci, F.; Chelli, B.; Crespan, E.; Schenone, S.; Naldini, A.; Bruno, O.; Trincavelli, M. L.; Maga, G.; Carraro, F.; Martini, C.; Bondavalli, F.; Botta, M. Structure-Based Optimization of Pyrazolo[3,4-*d*]pyrimidines as Abl Inhibitors and Antiproliferative Agents toward Human Leukemia Cell Lines. *J. Med. Chem.* **2008**, *51*, 1252−1259. (j) D'Antona, L.; Amato, R.; Talarico, C.; Ortuso, F.; Menniti, M.; Dattilo, V.; Iuliano, R.; Gigliotti, F.; Artese, A.; Costa, G.; Schenone, S.; Musumeci, F.; Abbruzzese, C.; Botta, L.; Trapasso, F.; Alcaro, S.; Paggi, M. G.; Perrotti, N. SI113, a specific inhibitor of the Sgk1 kinase activity that counteracts cancer cell proliferation. *Cell. Physiol. Biochem.* **2015**, *35*, 2006−2018. (k) Heine, K.; Eickmeier, C.; Ferrara, M.; Giovannini, R.; Rosenbrock, H.; Schaenzle, G. 6-Cycloalkyl-pyrazolopyrimidinones for the treatment of CNS disorders. U.S. Patent 9,328,120, Oct 10, 2014. (l) Hendrix, M.; Bärfacker, L.; Erb, C.; Hafner, F.-T.; Heckroth, H.; Karthaus, D.; Tersteegen, A.; Staay, F.-J.; Kampen, M. V. 6-Arylmethylpyrazolo[3,4-*d*]pyrimidines. U.S. Patent 8,809,348, Oct 16, 2009. (m) Hendrix, M.; Bärfacker, L.; Erb, C.; Hafner, F.-T.; Heckroth, H.; Karthaus, D.; Tersteegen, A.; Staay, F.-J.; Kampen, M. V. 6-Cyclylmethyl- and 6-alkylmethyl-substituted pyrazolepyrimidines. U.S. Patent 8,642,605, Sept 15, 2011.

(3) Honigberg, L. A.; Smith, A. M.; Sirisawad, M.; Verner, E.; Loury, D.; Chang, B.; Li, S.; Pan, Z.; Thamm, D. H.; Miller, R. A.; Buggy, J. J. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 13075−13080.

(4) (a) Robins, R. K. Potential Purine Antagonists. I. Synthesis of Some 4,6-Substituted Pyrazolo [3,4-*d*] pyrimidines. *J. Am. Chem. Soc.* **1956**, *78*, 784−790. (b) Schmidt, P.; Druey, J. Heilmittelchemische Untersuchungen in der heterocyclischen Reihe. Mitteilung. Pyrazolo-[3,4-d]-pyrimidine. *Helv. Chim. Acta* **1956**, *39*, 986−991.

(5) (a) Seela, F.; Steker, H. Synthesis of 2′-Deoxyribofuranosides of 8-Aza-7-deazaguanine and Related Pyrazolo[3,4-*d*]pyrimidines. *Helv. Chim. Acta* **1986**, *69*, 1602−1613. (b) Quiroga, J.; Trilleras, J.; Insuasty, B.; Abonía, R.; Nogueras, M.; Marchal, A.; Cobo, J. Microwave-assisted synthesis of pyrazolo[3,4-*d*]pyrimidines from 2-amino-4,6-dichloropyrimidine-5-carbaldehyde under solvent-free conditions. *Tetrahedron Lett.* **2008**, *49*, 3257−3259. (c) Slavish, P. J.; Price, J. E.; Hanumesh, P.; Webb, T. R. Efficient Synthesis of Pyrazolopyrimidine Libraries. *J. Comb. Chem.* **2010**, *12*, 807−809.

(6) For examples of $C(sp^2)-C(sp^2)$ coupling, see: Nowak, P.; Cole, D. C.; Brooijmans, N.; Bursavich, M. G.; Curran, K. J.; Ellingboe, J. W.; Gibbons, J. J.; Hollander, I.; Hu, Y.; Kaplan, J.; Malwitz, D. J.; Toral-Barza, L.; Verheijen, J. C.; Zask, A.; Zhang, W.-G.; Yu, K. Discovery of Potent and Selective Inhibitors of the Mammalian Target of Rapamycin (mTOR) Kinase. *J. Med. Chem.* **2009**, *52*, 7081−7089.

(7) For examples of $C(sp^3)-C(sp^2)$ coupling, see: (a) Ye, X.; Yuan, J.; Zhou, Y.; Deng, Z.; Mao, X.; Peng, Y. Palladium-Catalyzed Cross-Coupling of 4-Tosyloxyquinazolines with Organoindium Reagents: An Efficient Route to 4-Substituted Quinazolines. *Synthesis* **2016**, *48*, 3976−3984. (b) Jorgensen, W. L.; Bollini, M.; Thakur, V. V.; Domaoal, R. A.; Spasov, K. A.; Anderson, K. S. Efficient Discovery of

DOI: 10.1021/acscombsci.7b00116
ACS Comb. Sci. 2017, 19, 675−680

Potent Anti-HIV Agents Targeting the Tyr181Cys Variant of HIV Reverse Transcriptase. *J. Am. Chem. Soc.* **2011**, *133*, 15686−15696.
(8) (a) Goodnow, R. A., Jr.; Guba, W.; Haap, W. Library Design Practices for Success in Lead Generation With Small Molecule Libraries. *Comb. Chem. High Throughput Screening* **2003**, *6*, 649−660. (b) Schuffenhauer, A.; Popov, M.; Schopfer, U.; Acklin, P.; Stanek, J.; Jacoby, E. Molecular Diversity Management Strategies for Building and Enhancement of Diverse and Focused Lead Discovery Compound Screening Collections. *Comb. Chem. High Throughput Screening* **2004**, *7*, 771−781.
(9) Movassaghi, M.; Hill, M. D. Single-Step Synthesis of Pyrimidine Derivatives. *J. Am. Chem. Soc.* **2006**, *128*, 14254−14255.
(10) Ahmad, O. K.; Hill, M. D.; Movassaghi, M. Synthesis of Densely Substituted Pyrimidine Derivatives. *J. Org. Chem.* **2009**, *74*, 8460−8463.
(11) For studies of amide activation in the presence of Tf_2O and pyridine, see: (a) Charette, A. B.; Grenon, M. Spectroscopic studies of the electrophilic activation of amides with triflic anhydride and pyridine. *Can. J. Chem.* **2001**, *79*, 1694−1703. (b) Charette, A. B.; Mathieu, S.; Martel, J. Electrophilic Activation of Lactams with Tf_2O and Pyridine: Expedient Synthesis of (±)-Tetraponerine T4. *Org. Lett.* **2005**, *7*, 5401−5404.
(12) For the application of electrophilic activation of amides in pyridine synthesis, see: Movassaghi, M.; Hill, M. D.; Ahmad, O. K. Direct Synthesis of Pyridine Derivatives. *J. Am. Chem. Soc.* **2007**, *129*, 10096−10097.
(13) CCDC 1561873 contains the supplementary crystallographic data for this paper (structure of **7**{*1*}). These data can be obtained free of charge from the The Cambridge Crystallographic Data Centre.
(14) For an example of a high-throughput purification workflow, see: Liu, M.; Chen, K.; Christian, D.; Fatima, T.; Pissarnitski, N.; Streckfuss, E.; Zhang, C.; Xia, L.; Borges, S.; Shi, Z.; Vachal, P.; Tata, J.; Athanasopoulos, J. High-Throughput Purification Platform in Support of Drug Discovery. *ACS Comb. Sci.* **2012**, *14*, 51−59.
(15) For an example of the effective application of PMC in drug discovery, see: Claffey, M. M.; Helal, C. J.; Verhoest, P. R.; Kang, Z.; Fors, K. S.; Jung, S.; Zhong, J.; Bundesmann, M. W.; Hou, X.; Lui, S.; Kleiman, R. J.; Vanase-Frawley, M.; Schmidt, A. W.; Menniti, F.; Schmidt, C. J.; Hoffman, W. E.; Hajos, M.; McDowell, L.; O'Connor, R. E.; MacDougall-Murphy, M.; Fonseca, K. R.; Becker, S. L.; Nelson, F. R.; Liras, S. Application of Structure-Based Drug Design and Parallel Chemistry to Identify Selective, Brain Penetrant, In Vivo Active Phosphodiesterase 9A Inhibitors. *J. Med. Chem.* **2012**, *55*, 9055−9068.
(16) For the importance of sp^3 topology in drug design and relevance to clinical success, see: (a) Waring, M. J.; Arrowsmith, J.; Leach, A. R.; Leeson, P. D.; Mandrell, S.; Owen, R. M.; Pairaudeau, G.; Pennie, W. D.; Pickett, S. D.; Wang, J.; Wallace, O.; Weir, A. An analysis of the attrition of drug candidates from four major pharmaceutical companies. *Nat. Rev. Drug Discovery* **2015**, *14*, 475−486. (b) Yang, Y.; Engkvist, O.; Llinàs, A.; Chen, H. Beyond Size, Ionization State, and Lipophilicity: Influence of Molecular Topology on Absorption, Distribution, Metabolism, Excretion, and Toxicity for Druglike Compounds. *J. Med. Chem.* **2012**, *55*, 3667−3677.
(17) Geng, H.; Huang, P.-Q. Versatile and chemoselective transformation of aliphatic and aromatic secondary amides to nitriles. *Tetrahedron* **2015**, *71*, 3795−3801.

DOI: 10.1021/acscombsci.7b00116
ACS Comb. Sci. 2017, 19, 675−680