Efficient Synthesis of Pyrazolopyrimidine Libraries

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The 1*H*-pyrazolo[3,4-d]pyrimidine ring system is an important structural template in anticancer research and in drug discovery as antibacterial[1] and histamine release agents.[2] There have been numerous recent literature reports on the synthesis of relatively small sets of compounds bearing the pyrazolopyrimidine core that have been shown to inhibit several oncogenic kinases including: GSK-3,[3] mTOR,[4−8] Src,[9] Abl,[10] EGF-R,[11] and PI3K.[12,13] Because of the prevalence of this template in drug discovery, several reports of pyrazolopyrimidine library synthesis have also appeared. For example, Tan and co-workers have recently reported traceless solid-phase syntheses of pyrazolo[3,4-*d*]-pyrimidines libraries to examine their efficacy against multidrug resistance protein 4.[14] Daniels et al. have reported conditions that have been used to construct pyrazolopyrimidine libraries from pyrazole precursors.[15] In addition, Revesz et al. have prepared pyrazolopyrimidine based libraries targeting novel p38α MAP kinase inhibitors.[16] While these previously published library protocols do generate interesting and useful compounds they have some limitations. These limitations include (1) only two diversity points stemming from the bicyclic core are diversified out of the three possible within this substructure,[14−16] (2) at least four synthetic steps are required,[15,16] (3) harsh reagents (e.g., sulfuric acid, phosphorus oxychloride, and chromium oxide) are utilized,[15,16] and (4) finally, some require aqueous workup of the final products,[15,16] which is often undesirable in high-throughput library production.

Considering this background, our programmatic interest in the identification of inhibitors of oncogenic kinase-driven pediatric cancers, for example, neuroblastoma[17,18] and anaplastic large cell lymphoma,[19,20] and the recent reports on a pyrrolopyrimidine series,[21,22] which exhibited activity against anaplastic lymphoma kinase (ALK),[23,24] we set out to develop a method that could provide both diverse and highly targeted pyrazolopyrimidine-based kinase libraries in a high throughput mode. Herein, we describe our rapid and productive process for the parallel synthesis of diverse 1*H*-pyrazolo[3,4-*d*]pyrimidines starting from readily prepared carbaldehydes **3**.

Starting from the commercially available 2,4,6-trichloropyrimidin-5-carbaldehyde[6,8] (**1**, Scheme 1) we investigated the stepwise synthesis of intermediates and found that the initial diversification by amino displacement with anilines **2**{*1*−*8*} (Figure 1) proceeded readily in moderate to high yield for these intermediates (56−98%, see Supporting Information) to give chemset **3**{*1*−*8*}, without the need for chromatography. After some experimentation, we found that if the intermediates in chemset **3** were treated with anilines **4**{*1*−*16*} (Figure 2) under mildly basic conditions, in the presence of catalytic amounts of phase transfer catalyst, the substituted diamino intermediates could be obtained, in a regioselective manner.[25] We then investigated conditions for the final high throughput diversification and cyclization of the resulting substituted 2,4-diamino-6-chloropyrimidin-5-carbaldehyde reaction mixture and eventually developed conditions that cleanly produced pyrazolopyrimidines **6** efficiently and directly upon treatment with hydrazines **5**{*1*−*4*} (Figure 3).

After this research and development on the stepwise protocol, we then proceeded to explore the possibility of a two-pot protocol *with no chromatographic purification of any intermediates*. This type of procedure would allow for the preparation of a large number of compounds in a high-throughput manner to explore the chemistry space surrounding this interesting substructure. Our goal was not the production of a few compounds in high yield, but rather the *high throughput* preparation of the largest and most diverse set of compounds possible. Since we routinely purify all library compounds by HPLC we did not require each crude reaction mixture to be pure, instead we only required that we could obtain sufficient material of high final purity. The overall yields of purified representative pyrazolopyrimidine examples using the optimized high-throughput protocol are provided in Table 1. Compounds **6**{*1*−*4*,*1*−*16*,*1*} were characterized by ^{1}H and ^{13}C NMR spectroscopy. All other members were confirmed by LC-MS, using UV and ELSD for additional purity criteria.

Generally, all anilines used for the second displacement worked rather well, regardless of the functionality present on the aromatic ring. In instances where (unsubstituted) hydrazine was used, we were able to successfully synthesize and purify greater than 1 mg of >85% pure material (purity based on a combination of UV and ELSD purities) 77% of the time with an average yield of 35%. As for the alkyl hydrazines, the success rate was not as high (58%) but the average yield was similar to the yields observed when hydrazine was used (32%). The major drawback in cases where alkyl hydrazines were used was lack of cyclization in the final step, presumably due to steric interactions. The uncyclized product tended to coelute with the desired product, which complicated purification and lowered the success rate or led to failures in some cases. Regardless, we were pleased with the scope and overall yield of this reaction sequence. Overall, it should be noted that a significant cause of failures or low yields was the

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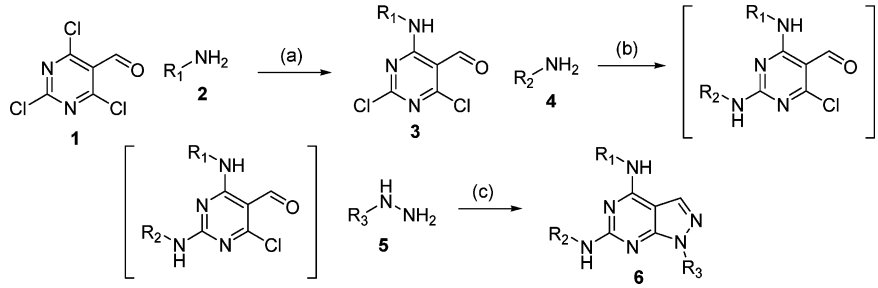
Scheme 1. Synthesis of the Pyrazolopyrimidine Core[a]



[a] Reagents and conditions: (a) R_1NH_2, $KHCO_3$, TBAI, CH_2Cl_2, or THF/H_2O (1:1) rt; (b) R_2NH_2, $KHCO_3$, TBAI, CH_2Cl_2/H_2O (1:1) rt; (c) R_3NHNH_2, THF reflux (where R_3 = H) or Et_3N, EtOH reflux (where R_3 = alkyl).

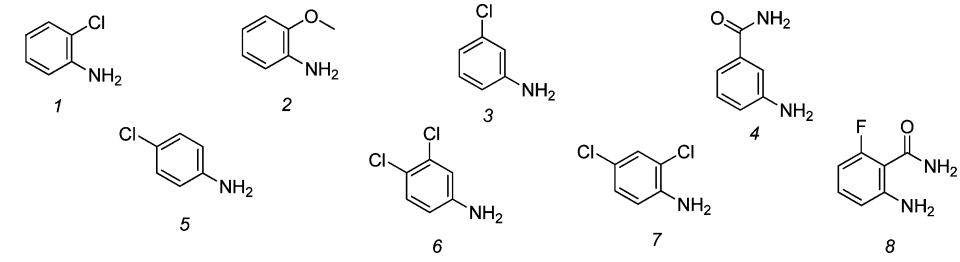


Figure 1. Anilines **2**{*1*−*8*} used as building blocks.

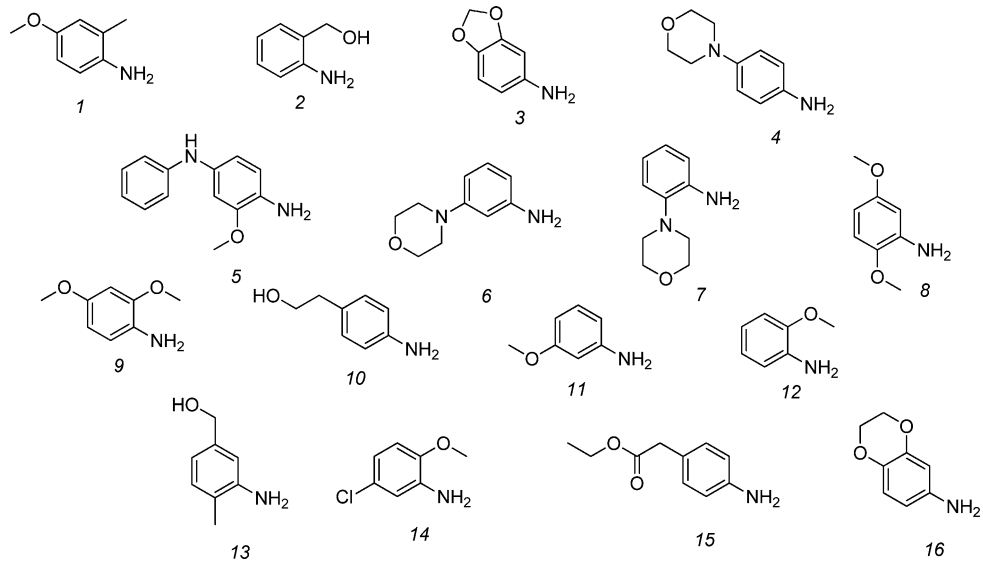


Figure 2. Anilines **4**{*1*−*16*} used as building blocks.

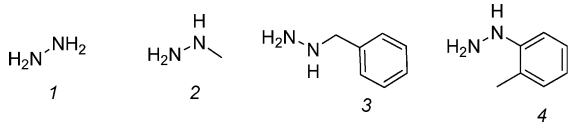


Figure 3. Hydrazines **5**{*1*−*4*} used as building blocks.

presence of impurities that overlapped with the product peak, but since we were interested in rapid high throughput preparation and purification, we did not make any attempts at developing special purification protocols for these cases, and so we present a procedure that is suitable for the routine and rapid production of libraries.

We have developed a rapid method for the generation of 1*H*-pyrazolo[3,4-*d*]pyrimidine libraries with three points of diversity in moderate yields and with a relatively high success rate. This process occurs in two pots without particularly toxic reagents and requires no protecting group manipulation to provide a source of diversification. The scope of the chemistry is well-defined and allows for ample diversification. In addition, this chemistry can easily be tailored to prepare individual compounds on a larger scale. Currently the size of this library is >1000 compounds, which could readily be expanded. We envision these libraries will be used in our HTS process for hit identification for current and future institutional research projects.

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Table 1. Isolated Overall Yields of the Displacement-Cyclization Reaction Sequence from **3**

Chemset	yield	Chemset	yield	Chemset	yield	Chemset	yield
6{*1,1,1*}	30	**6**{*3,1,1*}	38	**6**{*4,1,2*}	28	**6**{*5,5,3*}	40
6{*1,2,1*}	56	**6**{*3,2,1*}	17	**6**{*4,2,2*}	20	**6**{*5,9,3*}	42
6{*1,3,1*}	28	**6**{*3,3,1*}	27	**6**{*4,3,2*}	0	**6**{*5,12,3*}	40
6{*1,4,1*}	49	**6**{*3,4,1*}	50	**6**{*4,4,2*}	39	**6**{*6,1,3*}	57
6{*1,5,1*}	44	**6**{*3,5,1*}	38	**6**{*4,5,2*}	13	**6**{*6,2,3*}	50
6{*1,6,1*}	60	**6**{*3,6,1*}	45	**6**{*4,9,2*}	44	**6**{*6,3,3*}	74
6{*1,7,1*}	45	**6**{*3,7,1*}	33	**6**{*4,12,2*}	32	**6**{*6,4,3*}	49
6{*1,8,1*}	49	**6**{*3,8,1*}	46	**6**{*5,1,2*}	51	**6**{*6,5,3*}	54
6{*1,9,1*}	18	**6**{*3,9,1*}	25	**6**{*5,2,2*}	33	**6**{*6,9,3*}	52
6{*1,10,1*}	21	**6**{*3,10,1*}	19	**6**{*5,3,2*}	30	**6**{*6,12,3*}	46
6{*1,11,1*}	33	**6**{*3,11,1*}	38	**6**{*5,4,2*}	46	**6**{*5,1,4*}	24
6{*1,12,1*}	51	**6**{*3,12,1*}	44	**6**{*5,5,2*}	47	**6**{*5,2,4*}	17
6{*1,13,1*}	47	**6**{*3,13,1*}	47	**6**{*5,9,2*}	41	**6**{*5,3,4*}	20
6{*1,14,1*}	51	**6**{*3,14,1*}	36	**6**{*5,12,2*}	38	**6**{*5,4,4*}	10
6{*1,15,1*}	28	**6**{*3,15,1*}	28	**6**{*6,1,2*}	50	**6**{*5,5,4*}	12
6{*1,16,1*}	26	**6**{*3,16,1*}	25	**6**{*6,2,2*}	52	**6**{*5,9,4*}	28
6{*2,1,1*}	30	**6**{*4,1,1*}	45	**6**{*6,3,2*}	58	**6**{*5,12,4*}	24
6{*2,2,1*}	29	**6**{*4,2,1*}	34	**6**{*6,4,2*}	0	**6**{*7,1,4*}	9
6{*2,3,1*}	17	**6**{*4,3,1*}	17	**6**{*6,5,2*}	57	**6**{*7,2,4*}	0
6{*2,4,1*}	38	**6**{*4,4,1*}	58	**6**{*6,9,2*}	59	**6**{*7,3,4*}	24
6{*2,5,1*}	43	**6**{*4,5,1*}	53	**6**{*6,12,2*}	51	**6**{*7,4,4*}	7
6{*2,6,1*}	40	**6**{*4,6,1*}	56	**6**{*4,1,3*}	0	**6**{*7,5,4*}	25
6{*2,7,1*}	23	**6**{*4,7,1*}	46	**6**{*4,2,3*}	31	**6**{*7,9,4*}	27
6{*2,8,1*}	37	**6**{*4,8,1*}	48	**6**{*4,3,3*}	10	**6**{*7,12,4*}	26
6{*2,9,1*}	42	**6**{*4,9,1*}	29	**6**{*4,4,3*}	46	**6**{*8,1,4*}	9
6{*2,10,1*}	23	**6**{*4,10,1*}	39	**6**{*4,5,3*}	39	**6**{*8,2,4*}	8
6{*2,11,1*}	26	**6**{*4,11,1*}	29	**6**{*4,9,3*}	0	**6**{*8,3,4*}	7
6{*2,12,1*}	15	**6**{*4,12,1*}	15	**6**{*4,12,3*}	33	**6**{*8,4,4*}	27
6{*2,13,1*}	25	**6**{*4,13,1*}	30	**6**{*5,1,3*}	50	**6**{*8,5,4*}	14
6{*2,14,1*}	30	**6**{*4,14,1*}	27	**6**{*5,2,3*}	44	**6**{*8,9,4*}	10
6{*2,15,1*}	23	**6**{*4,15,1*}	46	**6**{*5,3,3*}	50	**6**{*8,12,4*}	60
6{*2,16,1*}	29	**6**{*4,16,1*}	16	**6**{*5,4,3*}	42		

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Supporting Information Available. General procedures for the preparation of all compounds and spectroscopic data (^{1}H, ^{13}C NMR spectra and HPLC chromatograms) for all products. This material is available free of charge via the Internet at http://pubs.acs.org.

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