Organic LETTERS

Letter

pubs.acs.org/OrgLett

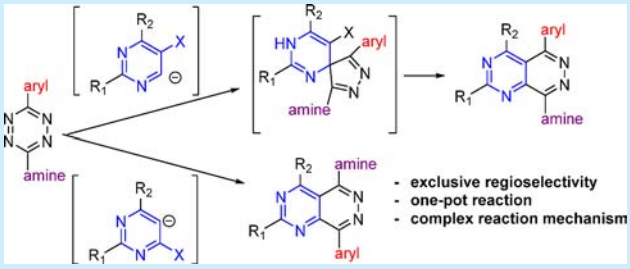
Single-Step Formation of Pyrimido[4,5-*d*]pyridazines by a Pyrimidine-Tetrazine Tandem Reaction

Juraj Galeta, Michal Šála, Martin Dračínský,* Milan Vrábel, Zdeněk Havlas, and Radim Nencka*

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague, Czech Republic

Supporting Information

ABSTRACT: A straightforward synthesis of pyrimido[4,5-*d*]pyridazines from pyrimidines and tetrazines under basic conditions is reported. Deprotonated, substituted 5-halopyrimidines readily react with variously substituted tetrazines in a highly regioselective manner via a complex reaction pathway, which was supported by DFT calculations. This mechanism leads to the empirically observed regioisomers without going through the conceivable hetaryne intermediate. These results on 5-halopyrimidines led to development of the methodology for preparation of opposite regioisomers based on 6-halopyrimidines.



Heterocycles are nearly ubiquitous in modern drug design as it is estimated that over 80% of all small-molecule remedies marketed in the USA contain a heterocyclic core.[1] Besides well-established procedures leading to heterocyclic compounds, e.g., Gutknecht pyrazine synthesis,[2] Pechmann condensation leading to coumarins,[3] Biginelli reaction,[4] Knorr quinoline synthesis,[5] Bischler−Napieralski reaction,[6] Traube purine synthesis,[7] and so forth[8] that predominate in the synthesis of heterocycles, the development of novel synthetic strategies and improvements are of considerable interest. These strategies are unfortunately limited, and therefore, a number of interesting heterocyclic scaffolds remain inaccessible. This is epitomized by the rapid recent developments in the field of kinase inhibitors[9] that demand novel synthetic protocols to provide previously underexplored bicyclic and tricyclic heterocycles.

In this study, we present the development of a novel transformation that provides efficient access to bicyclic heterocycles containing a pyrimidine ring in a straightforward manner. Because the pyrimidine core is found in a myriad of biologically active compounds[10] including nucleobases and many antiviral and anticancer drugs, the development of new synthetic strategies is of special importance. In particular, we show here that a class of medicinally highly relevant pyrimido[4,5-*d*]pyridazines, known as antiinflammatory Jak-2 and Syk inhibitors[11] or Wee-1 kinase inhibitors[12] with prospective application in anticancer therapy, can be formed in an unprecedented reaction cascade directly from readily accessible halopyrimidines and 1,2,4,5-tetrazines (Figure 1). Furthermore, we provide a detailed mechanistic study and support the data using DFT calculations.

We envisaged that expedient access to bicyclic derivatives containing the pyrimidine core could be gained via a precedented pyrimidyne intermediate, which when formed in situ, could be trapped by a second suitable reacting partner in a cycloaddition reaction to furnish the desired bicyclic product in a single step. A robust and reliable methodology to generate the pyrimidyne intermediate was to be the cornerstone of this study as there are scant examples that describe the generation of pyrimidyne and via only two processes.[13] One is based on dehydrohalogenation of the corresponding halopyrimidine in the presence of KNH_2 in liquid ammonia.[13a−d] The other exploits an oxidation step promoted by $Pb(OAc)_4$ of 1- or 3-aminotriazolo[4,5-*d*]pyrimidine.[13f,g] Although these works clearly show that such a hetaryne can be formed and trapped, the highly laborious preparation of the starting aminotriazolopyrimidines, the use of liquid ammonia, and low yield provide substantial barriers to broader application. An alternative approach of pyrimidyne generation from silyltriflate precursors was recently found to be unsuccessful.[14]

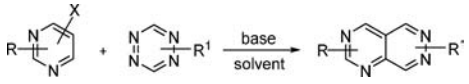


Figure 1. Straightforward access to pyrimido[4,5-*d*]pyridazines via a novel pyrimidine-tetrazine tandem reaction.

We intended to use dehydrohalogenation as an alternative method to generate synthetically useful pyrimidynes. We began the study by using commercially available 5-bromopyrimidine **1** and two types of trapping agents: furan as an electron-rich diene, a typical trapping agent in normal Diels−Alder reactions, and 3,6-diphenyl-1,2,4,5-tetrazine **5** as an electron-poor diene known[15] to participate in inverse electron-demand Diels−Alder (iEDDA) reactions. Moreover, we examined several bases to

Received: June 2, 2016
Published: July 8, 2016

DOI: 10.1021/acs.orglett.6b01601
Org. Lett. 2016, 18, 3594−3597

promote the initial dehydrohalogenation step that includes *n*-BuLi, *s*-BuLi, *t*-BuLi, LDA, PhMgBr, *i*-PrMgCl·LiCl, KHMDS, NaHMDS, LiHMDS, and $NaNH_2$. Our initial experiments showed that in the presence of KHMDS the reaction with furan produces only dimeric products **2**−**4** in less than 15% overall yield. All the other bases failed to give any isolatable products and led only to decomposition of the starting material. To our surprise, the reaction with tetrazine **5** under the same conditions afforded the desired pyrimido[4,5-*d*]pyridazine **6a** in 50% yield as also confirmed by crystallographic analysis[16] (Figure 2). Similar pyrimido[4,5-*d*]pyridazines were recently

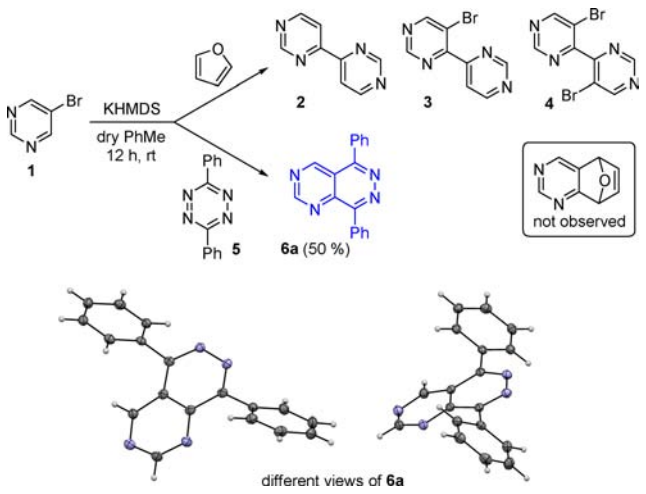


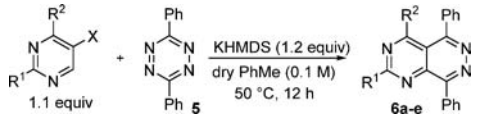
Figure 2. Comparative reactions of pyrimidine **1** with furan and tetrazine **5** in toluene. ORTEP representations of **6a** are shown at 50% probability level.

prepared by Knorr condensation.[17] Stimulated by these unexpected results, we optimized the reaction conditions. We found that the reaction strongly depended on the solvent (Table S1, Supporting Information (SI)). Gentle heating improved the yield, whereas increasing the amount of KHMDS and/or varying the molar ratios of the reagents did not have a significant effect.

We next performed a series of experiments to investigate the scope of the reaction by varying the substitution pattern on the pyrimidine scaffold. We found that only electron-rich or -neutral pyrimidines (Table 1, entries 4−10) gave the desired products. We noted the importance of the leaving halide on the result (entries 8−10) and fluorine was found to be the superior leaving group providing the best yield (entry 8). It is noteworthy that this fluoro derivative afforded much lower yields with weaker bases NaHMDS (18%) and LiHMDS (16%).

With the optimized 5-fluoropyrimidine in hand, we focused on analyzing the scope of the second reacting partner by synthesizing a series of tetrazines bearing various substituents (Table 2). The reaction of symmetrical tetrazines proceeded smoothly to furnish the expected pyrimido[4,5-*d*]pyridazine products in good yields (Table 2, entries 1 and 2). Most importantly, the reaction with unsymmetrical tetrazines (Table 2, entries 3−10) yielded in all cases the desired products as single regioisomers. To irrefutably determine the structure of the regioisomers, we substituted the SEt group with a hydrogen by Pd-catalyzed desulfurization (SI). Using ROESY-NMR spectra, we observed the expected NOE interaction between

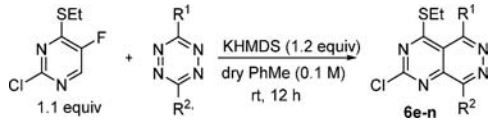
Table 1. Reactions of Various Pyrimidines with Tetrazine 5



entry	R^1	R^2	X	yield (%)	product
1	$MeSO_2$	H	Br	NR[a]	
2	CN	H	Br	NR[a]	
3	Cl	Cl	Br, Cl, F	decomp	
4	H	H	Br	50	**6a**
5	SMe	H	Br	43	**6b**
6	Cl	OMe	Br	46	**6c**
7	Cl	NMe_2	Br	33	**6d**
8	Cl	SEt	F	44, 73[b]	**6e**
9	Cl	SEt	Cl	34, 62[b]	**6e**
10	Cl	SEt	Br	25, 34[b]	**6e**

[a]NR = no reaction. [b]Carried out at room temperature.

Table 2. Reactions of 5-Fluoropyrimidine with Various Tetrazines



entry	R^1	R^2	yield (%)	product
1	Ph	Ph	73	**6e**
2	*i*-Pr	*i*-Pr	61	**6f**
3	*i*-Pr	Ph	55	**6g**
4	4-MeO-Ph	NEt_2	53	**6h**
5	4-MeO-Ph	piperidine	64	**6i**
6	4-MeO-Ph	morpholine	50	**6j**
7	Ph	morpholine	49	**6k**
8	4-CN-Ph	morpholine	49	**6l**
9	2-thiophenyl	morpholine	50	**6m**
10	2-furyl	morpholine	51	**6n**

the H atom at C4 and the ortho phenolic hydrogens, confirming the structure of the regioisomers.

In the course of the synthetic work, we started to have doubts about the hetaryne mechanism because the structure of some of the side products (SI) suggested that hetarynes, if formed at all, are not the only fate of the 5-halopyrimidines in basic environment. Therefore, we had recourse to use DFT calculations to find support for the hetaryne mechanism or to propose an alternative reaction pathway that would be compatible with the experimental observations. The heat of hydrogenation is a well-established means to explore the strains and relative stabilities of alkynes, and it has been used recently to predict the likelihood that a given hetaryne can be generated. Our pyrimidyne derivatives have an energy of dehydrogenation of ∼100 kcal/mol (SI), which is close to that of all experimentally confirmed hetarynes.[18] The second step in the supposed hetaryne mechanism is the formation of a product with a new cycle by iEDDA. The transition structure for the process was found, and the calculated reaction barriers were close to 20 kcal/mol. So far, the DFT results seemed to support the hetaryne mechanism, but then we turned our attention to the regioselectivity of the cyclization reaction and found the opposite regioselectivity in the simulations then observed experimentally. For example, the optimized transition state structures for the reaction leading to experimentally observed

DOI: 10.1021/acs.orglett.6b01601
Org. Lett. 2016, 18, 3594−3597

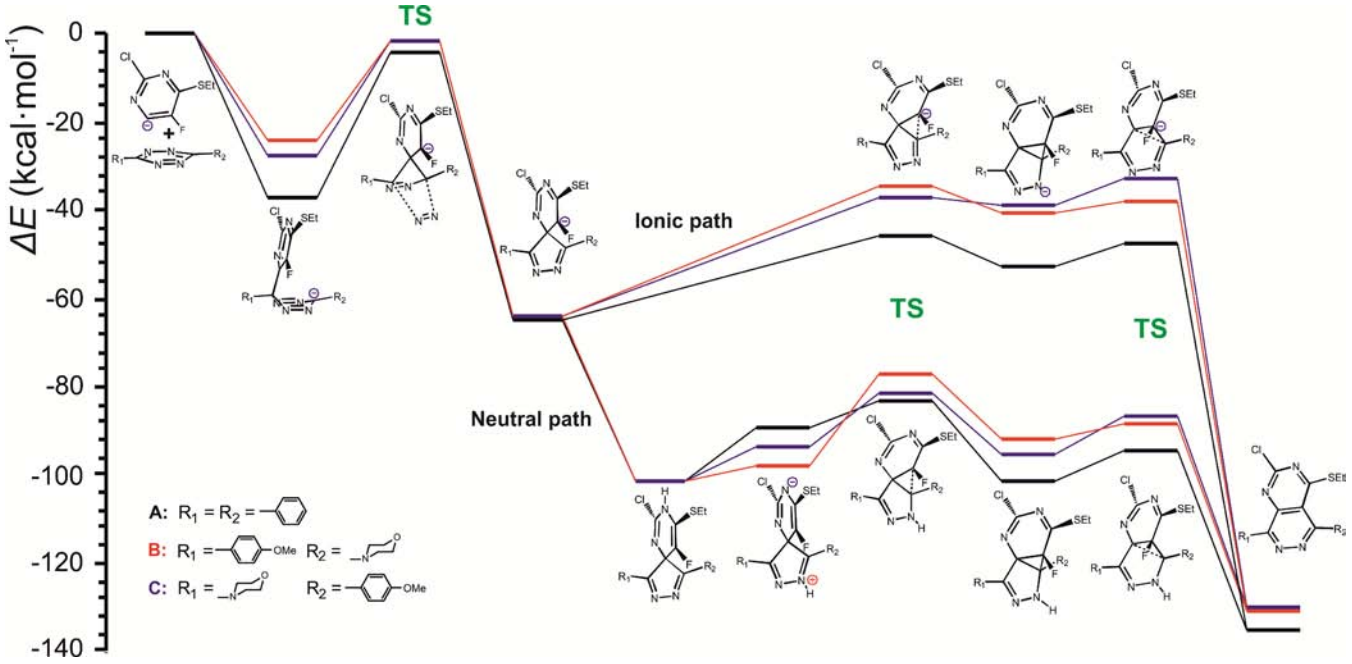


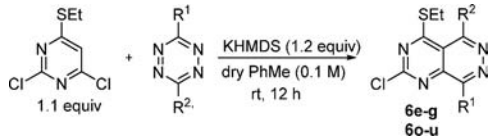
Figure 3. Simplified mechanism proposed for the reaction of 5-fluoropyrimidines with tetrazines.

compound **6j** had higher energy by 4 kcal/mol than that leading to the opposite regioisomer.

Because of the discrepancies described above, we abandoned the hetaryne mechanism as unreliable and proposed an alternative mechanism based on reaction of deprotonated 5-halopyrimidines with the tetrazines. A one-dimensional DFT energy scan revealed that reaction of deprotonated pyrimidine with tetrazine is a downhill reaction and thus preferred over dehalogenation. Additionally, we investigated the mechanism by HPLC-MS measurements of aliquots from the reaction mixture and by NMR analysis of the reaction mixture at various time points (SI), and the experimental observations were complemented with DFT calculations. An unusual spiro compound, the structure of which was determined by NMR spectroscopy, was observed as an intermediate of the reaction with aryl/amino-substituted tetrazines (e.g., Table 2, entry 6), and the conversion of this intermediate to the final product required the addition of water. On the other hand, the reaction with diphenyl tetrazine (Table 2, entry 1) proceeded smoothly in the anhydrous environment. DFT calculations fully confirmed the experimental observations. A simplified reaction pathway is depicted in Figure 3, and full details are in the SI. The reaction begins by nucleophilic attack of the deprotonated pyrimidine on the tetrazine, followed by intramolecular cyclization and nitrogen loss to form the spiro intermediate. In the case of the diphenyl tetrazine derivative, the following rearrangement of the spiro compound anion is a low-barrier process, whereas the barrier is significantly higher for the aryl/amino derivative. However, this rearrangement barrier is reduced after protonation (neutralization) of the intermediate.

Encouraged by these findings, we speculated that the opposite regioisomers might be accessible from the corresponding 6-halo-substituted pyrimidines. To explore this possibility, we reacted the same series of tetrazines with 2,4-dichloro-6-(ethylthio)pyrimidine. Indeed, in all cases except isopropyl-phenyl tetrazine, we observed the formation of the opposite regioisomers in good yields (Table 3). These experiments clearly show that it is possible to control the regioselectivity of

Table 3. Reactions of 6-Chloropyrimidine with Various Tetrazines



entry	R^1	R^2	yield (%)	product
1	Ph	Ph	22, 58[a]	**6e**
2	*i*-Pr	*i*-Pr	16, 57[a]	**6f**
3	Ph	*i*-Pr	56	**6g**
4	4-MeO-Ph	NEt_2	*b*	**6o**
5	4-MeO-Ph	piperidine	56	**6p**
6	4-MeO-Ph	morpholine	58	**6q**
7	Ph	morpholine	42	**6r**
8	4-CN-Ph	morpholine	39	**6s**
9	2-thiophenyl	morpholine	46	**6t**
10	2-furyl	morpholine	40	**6u**

[a]Carried out at 50 °C. [b]Only traces observed.

the reaction by simply switching the position of the leaving halogen atom. The ultimate observation of both regioisomers also unambiguously discarded the hetaryne mechanism, where only one regioisomer should be obtained regardless of the position of the halogen atom on the starting halopyrimidine (Figure 4).

In the case of 6-chloropyrimidines, we propose a simpler reaction mechanism based on these steps (details in SI): deprotonation of the pyrimidine, spontaneous downhill reaction of the anion with tetrazine (this is the step that governs the regioselectivity of the reaction), and cyclization of the intermediate with simultaneous loss of the chloride anion and nitrogen molecule. The transition state for this cyclization with a modest energy barrier of 10 kcal/mol was found, and the observation of single regioisomer **6g** formed from both 5-fluoro- and 6-chloropyrimidine was also supported by DFT calculations (details in SI). Note that an analogous transition

DOI: 10.1021/acs.orglett.6b01601
Org. Lett. 2016, 18, 3594−3597

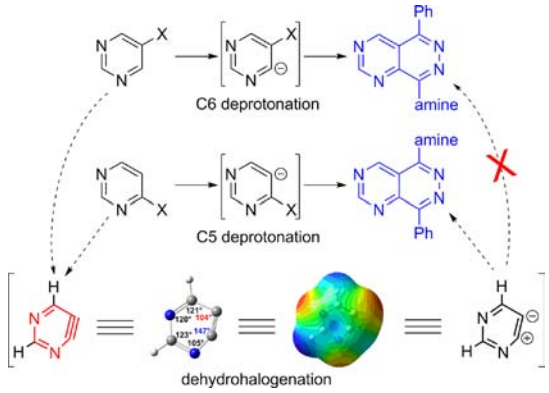


Figure 4. General scheme showing the formation of obtained products including hypothetical pyrimidyne formation.

state for the cyclization reaction was found also for the 5-fluoropyrimidines, but the energy barrier was higher than that leading to the spiro compound.

In summary, we discovered an efficient approach to variously substituted pyrimido[4,5-*d*]pyridazines from easily accessible pyrimidine and tetrazine derivatives. Our methodology opens new possibilities in the design and synthesis of pyrimidine containing bicyclic products with potential utilization as druglike molecules. The reaction with unsymmetrical tetrazine precursors proceeds with exclusive regioselectivity and can be easily controlled by the structure of the starting pyrimidine. Our careful mechanistic investigations showed that this reaction proceeds by an unprecedented stepwise tandem mechanism rather than via hetaryne intermediate, and we support this observation by DFT calculations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01601.

Experimental and computational methods, spectral data, and supplemental data and figures (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: dracinsky@uochb.cas.cz.
*E-mail: nencka@uochb.cas.cz.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the Czech Science Foundation (Grants 15-09310S, 15-11223S, 15-06020Y, and P208/12/G016) and Gilead Sciences, Inc. (Foster City, CA, USA). We are grateful to Veronika Holečková for the preparation of starting materials and to Michal Babiak for X-ray diffraction studies with the support of the X-ray Diffraction and Bio-SAXS Core Facility of CEITEC, Brno, Czech Republic. We are grateful to A. Michael Downey, IOCB, Prague, for critical reading of our manuscript. Subvention for the development of research organization (RVO: 61388963) is also acknowledged.

■ REFERENCES

(1) (a) Pitt, W. R.; Parry, D. M.; Perry, B. G.; Groom, C. R. *J. Med. Chem.* **2009**, *52*, 2952−2963. (b) Gomtsyan, A. *Chem. Heterocycl. Compd.* **2012**, *48*, 7−10.
(2) Gutknecht, H. *Ber. Dtsch. Chem. Ges.* **1879**, *12*, 2290−2292.
(3) (a) von Pechmann, H. *Ber. Dtsch. Chem. Ges.* **1884**, *17*, 929−936. (b) Tyndall, S.; Wong, K. F.; VanAlstine-Parris, M. A. *J. Org. Chem.* **2015**, *80*, 8951−8953.
(4) (a) Biginelli, P. *Ber. Dtsch. Chem. Ges.* **1891**, *24*, 1317−1319. (b) Li, N.; Chen, X.-H.; Song, J.; Luo, S.-W.; Fan, W.; Gong, L.-Z. *J. Am. Chem. Soc.* **2009**, *131*, 15301−15310.
(5) (a) Knorr, L. *Justus Liebigs Ann. Chem.* **1886**, *236*, 69−115. (b) Sai, K. K. S.; Gilbert, T. M.; Klumpp, D. A. *J. Org. Chem.* **2007**, *72*, 9761−9764.
(6) (a) Bischler, A.; Napieralski, B. *Ber. Dtsch. Chem. Ges.* **1893**, *26*, 1903−1908. (b) White, K. L.; Mewald, M.; Movassaghi, M. *J. Org. Chem.* **2015**, *80*, 7403−7411.
(7) Traube, W. *Ber. Dtsch. Chem. Ges.* **1900**, *33*, 1371−1383.
(8) For recent examples of heterocyclic chemistry reviews, see: (a) Deiters, A.; Martin, S. F. *Chem. Rev.* **2004**, *104*, 2199−2238. (b) Quin, L. D.; Tyrell, J. In *Fundamentals of Heterocyclic Chemistry: Importance in Nature and in the Synthesis of Pharmaceuticals*, 1st ed.; John Wiley & Sons, 2010. (c) Joule, J. A.; Mills, K. In *Heterocyclic Chemistry*, 5th ed.; John Wiley & Sons, 2010. (d) Alvarez-Builla, J.; Vaquero, J. J.; Barluenga, J. In *Modern Heterocyclic Chemistry*, 4 volume set; Wiley-VCH: Weinheim, 2011. (e) Yamamoto, Y. *Chem. Soc. Rev.* **2014**, *43*, 1575−1600. (f) Guo, X.-X.; Gu, D.-W.; Wu, Z.; Zhang, W. *Chem. Rev.* **2015**, *115*, 1622−1651 and references therein.
(9) (a) Bharate, S. B.; Sawant, S. D.; Singh, P. P.; Vishwakarma, R. A. *Chem. Rev.* **2013**, *113*, 6761−6815. (b) Schenone, S.; Radi, M.; Musumeci, F.; Brullo, C.; Botta, M. *Chem. Rev.* **2014**, *114*, 7189−7238. (c) Mejdrova, I.; Chalupska, D.; Koegler, M.; Sala, M.; Plackova, P.; Baumlova, A.; Hrebabecky, H.; Prochazkova, E.; Dejmek, M.; Guillon, R.; Strunin, D.; Weber, J.; Lee, G.; Birkus, G.; Mertlikova-Kaiserova, H.; Boura, E.; Nencka, R. *J. Med. Chem.* **2015**, *58*, 3767−3793.
(10) (a) Lagoja, I. M. *Chem. Biodiversity* **2005**, *2*, 1−150. (b) Brown, D. J.; Evans, R. F.; Cowden, W. B.; Fenn, M. D. In *The Pyrimidines*; John Wiley & Sons: New York, 1994.
(11) Venkatesan, A.; Smith, R. A.; Hosahalli, S.; Potluri, V.; Panigrahi, S. K.; Basetti, V.; Kuntu, K. Pyrimido-pyridazinone compounds and use thereof. WO 2013/028818 A1, 28.02.2013.
(12) Zhu, G.; Gong, J.; Judd, A. S.; Ghandi, V. B.; Shoemaker, A. R.; Penning, T. D.; Michaelides, M. R.; Lai, Ch.; Woods, K. W. Pyridazino[4,5-*d*]pyrimidin-(6*H*)-one inhibitors of wee-1 kinase. WO 2013/013031 A1, 24.01.2013.
(13) (a) Schwan, T. J.; Tieckelmann, H. *J. Org. Chem.* **1964**, *29*, 941−942. (b) van der Plas, H. C.; Guertsen, G. *Tetrahedron Lett.* **1964**, *5*, 2093−2098. (c) van der Plas, H. C. *Tetrahedron Lett.* **1965**, *6*, 555−559. (d) van der Plas, H. C.; Smit, P.; Koudijs, A. *Tetrahedron Lett.* **1968**, *9*, 9−13. (e) Kauffmann, T.; Nürnberg, R.; Udluft, K. *Chem. Ber.* **1969**, *102*, 1177−1190. (f) Christophe, D.; Promel, R.; Maeck, M. *Tetrahedron Lett.* **1978**, *19*, 4435−4438. (g) Tielemans, M.; Promel, R.; Geerlings, P. *Tetrahedron Lett.* **1988**, *29*, 1687−1690.
(14) Medina, J. M.; Jackl, M. K.; Susick, R. B.; Garg, N. K. *Tetrahedron* **2016**, *72*, 3629−3634.
(15) (a) Bachmann, W. E.; Deno, N. C. *J. Am. Chem. Soc.* **1949**, *71*, 3062−3072. (b) Carboni, R. A.; Lindsey, R. V., Jr. *J. Am. Chem. Soc.* **1959**, *81*, 4342−4346. (c) Sauer, J.; Wiest, H. *Angew. Chem., Int. Ed. Engl.* **1962**, *1*, 269. (d) Jiang, X.; Wang, R. *Chem. Rev.* **2013**, *113*, 5515−5546. (e) Knall, A.-C.; Slugovc, C. *Chem. Soc. Rev.* **2013**, *42*, 5131−5142.
(16) The crystal structure of compound **6a** has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 1406413.
(17) Karami, B.; Akrami, S.; Khodabakhshi, S.; Rahmatzadeh, S. S. *ARKIVOC* **2013**, 323−333.
(18) Goetz, A. E.; Bronner, S. M.; Cisneros, J. D.; Melamed, J. M.; Paton, R. S.; Houk, K. N.; Garg, N. K. *Angew. Chem., Int. Ed.* **2012**, *51*, 2758−2762.

DOI: 10.1021/acs.orglett.6b01601
Org. Lett. 2016, 18, 3594−3597