

Lithium Hexamethyldisilazane Transformation of Transiently Protected 4-Aza/Benzimidazole Nitriles to Amidines and their Dimethyl Sulfoxide Mediated Imidazole Ring Formation

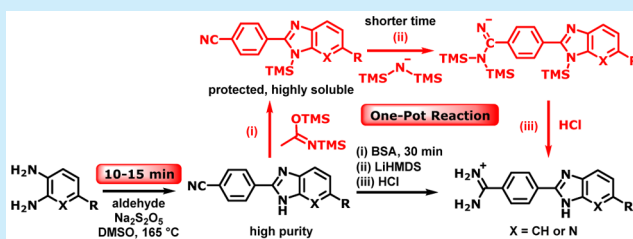
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S Supporting Information

ABSTRACT: Trimethylsilyl-transient protection successfully allowed the use of lithium hexamethyldisilazane to prepare benzimidazole (BI) and 4-azabenzimidazole (azaBI) amidines from nitriles in 58–88% yields. This strategy offers a much better choice to prepare BI/azaBI amidines than the lengthy, low-yielding Pinner reaction. Synthesis of aza/benzimidazole rings from aromatic diamines and aldehydes was affected in dimethyl sulfoxide in 10–15 min, while known procedures require long time and purification. These methods are important for the BI/azaBI-based drug industry and for developing specific DNA binders for expanded therapeutic applications.



Benzimidazole (BI) constitutes an important scaffold found in over a dozen approved pharmaceuticals.^{1,2} Additional BI derivatives show potent antiplasmodial,³ antiinflammatory,⁴ antiviral,⁵ and anticancer activities.⁶ Derivatives of the BI analogue 4-azabenzimidazole (azaBI) have recently been studied in animal models and identified as potent antitumor, anti-inflammatory,⁷ and antidiabetic agents⁸ (Figure 1).

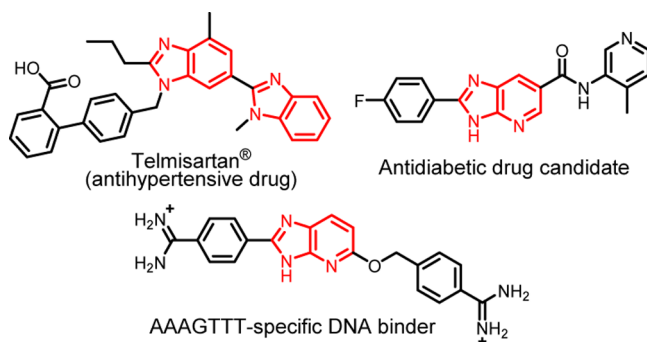


Figure 1. Representative, biologically active BI/azaBI structures.

Structures containing the BI nucleus can also be used as light-up adenine thymine base pair (AT) specific DNA binders⁹ and fluorescent probes in biological systems with potential for developing new biomarkers.^{10,11} Furthermore, rational design of azaBI-based DNA minor groove binders led to morphing traditional BI recognition of DNA from AT specific^{12–15} to guanine (G)-containing sequence specific (Figure 1) by introduction of the aza group.^{16–18} This represents a key milestone showing that small molecules can be structured to recognize any given sequence on DNA. Targeting DNA at

specific sequences augments a therapeutic strategy of controlling gene expression as a venue for treating many intractable diseases.^{19,20} DNA minor groove binders, typically, possess positively charged moieties for stronger interaction with the DNA backbone. Among known positively charged groups, amidines are appealing for their excellent cellular and nuclear uptake.^{12,21} Available methods to prepare amidino BI/azaBI and also to construct substituted BI/azaBI rings, in general, suffer from long reaction time and low efficiency.

Amidines are commonly prepared from nitriles by three methods: the Pinner reaction,²² amidoxime method,²³ and nucleophilic addition of lithium hexamethyldisilazane²⁴ (LHMDS). The latter approach is the most convenient, because it takes a single step to give the silylated amidines, which provides amidine salts upon HCl workup. It was also adapted to prepare *N*-substituted amidines²⁵ related to earlier derivatives.²⁶ However, for BI and azaBI systems, LHMDS deprotonates the ring NH, preventing the desired reaction due to a delocalized negative charge on the nitrile. Thus, amidines of such systems are usually prepared by one of the other two methods.^{27,28} The amidoxime method involves three steps, in which the nitrile is converted to the amidoxime, followed by acetylation to *O*-acetoxyamidoxime, and then hydrolysis to give the amidine. The drawbacks of this method are not only the multistep procedure but also the frequently sparingly soluble intermediates, leaving the Pinner method, by default, more popular. The Pinner reaction involves two steps: the formation of imidate ester by the action of anhyd HCl-saturated ethanol and conversion to the amidine by ammonia. Low

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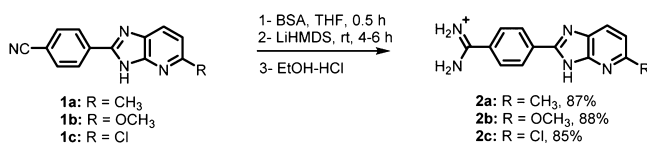
solubility of BI/azaBI molecules leads to long reaction times with the Pinner method, where reactions requiring 3–10 days for each step are commonly encountered.^{29–32} More problematic is that imidate esters readily hydrolyze to amides,²² which is difficult to avoid during their isolation to remove excess HCl. These facts result in poor yields and failure of the reaction in some cases.

Herein, we report a novel strategy that allows using LHMDS with BI/azaBI nitriles to make amidines via transient protection of BI/azaBI ring NH in a one-pot procedure. Given the significance of BI/azaBI structure in industry and as biologically active agents, we also present optimized conditions and suggest a mechanism for a facile formation of BI/azaBI rings from aldehydes and diamines in DMSO.

Transient Protection Strategy

The trimethylsilyl group (TMS) has extensively been used in nucleoside chemistry for the silyl Hilbert–Johnson synthesis³³ and for transient protection of 3'-OH groups.³⁴ We employed *N,O*-bis(trimethylsilyl)acetamide (BSA) as a mild and efficient reagent³⁵ to transiently protect BI and azaBI ring NH groups with TMS. Thus, suspensions of the azaBI nitrile derivatives **1a–c** in THF were treated with BSA for 0.5 h at rt (Scheme 1).

Scheme 1. One-Pot Synthesis of Simple azaBI Amidines



The silylation was visually indicated by the homogeneity of the mixture within 5–10 min. Subsequent addition of excess LHMDS led to the consumption of the silylated BI/azaBI nitrile intermediates in 4–6 h. On the other hand, our initial attempts to perform the reaction on Boc-protected **1a** resulted in deprotonation of the 5-methyl group as indicated by the maroon color, cleavage of the Boc group, and failure of the reaction. Thus, it is clear that the methyl on TMS-protected **1a** is stable. It is also worth noting that TMS intermediates enabled highly concentrated reaction solutions, leading to shorter reaction time. In contrast, the reaction of nitriles of Boc-protected indoles, as a suspension in THF, with LHMDS required up to 3 days.²⁷ This demonstrates that our strategy is successful and efficient.

Quenching the reaction with enough HCl to cause desilylation normally produces large amounts of NH₄Cl due to reaction with excess LHMDS. To avoid this, we first quenched with a stoichiometric amount of an acid to convert LHMDS to hexamethyldisilazane (HMDS), which was removed by evaporation. EtOH–HCl was then added to give the amidine salts and remove the TMS transient protection. The salts were precipitated by ether and mixed with water at pH > 9 to give the free bases and to remove inorganic salts. Conversion to the HCl salts and precipitation again gave **2a–c**. The resulting amidines were obtained in high yields (85–88%, Scheme 1) and were pure as judged by NMR and elemental analyses. This is highly remarkable because amidines prepared by other methods often require purification by crystallization³⁶ and chromatography on preparative HPLC,^{37,38} normal,³⁶ or C₁₈ reversed-phase³⁸ silica gel.

We made **2a** by the amidoxime method in a total yield of 21% (Scheme S1, Supporting Information), showing that the

one-pot LHMDS method is much more efficient in terms of the yield and reaction time.

DMSO-Mediated Cyclization

As mentioned above, we desired to optimize reaction conditions for efficient construction of substituted BI/azaBI rings. There are numerous conditions starting from aromatic aldehydes and *o*-phenylenediamine or 2,3-diaminopyridine to make the BI and azaBI, respectively, through an oxidative cyclization. Indeed, a 2012 review by Panda et al. listed 118 different conditions for this synthesis.³⁹ The first step in this cyclization is the formation of the imine, followed by the cyclic aminal, which is then oxidized to the imidazole ring. We examined the most common conditions to make **1a**. Reaction in DMF in the presence of Na₂S₂O₅ or NaHSO₃⁴⁰ at 120 or 165 °C took 48 h and produced side products which mandated lengthy purification. A recent method stirring the reactants in hot DMF/H₂O (9:1)⁴¹ gave incomplete reaction.

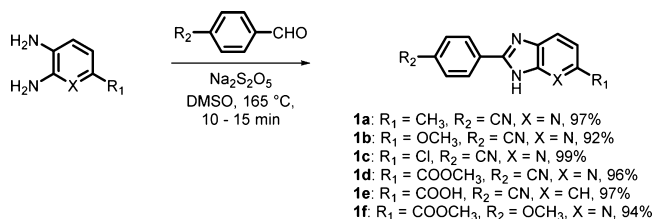
We speculated that catalyzing the oxidation would facilitate the cyclization. Using *p*-benzoquinone as an oxidant,³⁹ scandium triflate⁴² or ferric chloride⁴³ as a recyclable oxidant did not improve reaction time or yield of **1a**. Although DMSO is a known oxidant,⁴⁴ it does not appear to have been explored in this type of synthesis. Thus, we aimed to investigate the effect of DMSO in comparison to DMF for the synthesis of **1a** (entry 1, Table 1). Performing the reaction in DMSO under the

Table 1. Influence of Solvent on Cyclization of **1a at 165 °C**

entry	solvent	catalyst	atmosphere	time (h)	yield (%)
1	DMF	Na ₂ S ₂ O ₅	air	48	56
2	DMSO	Na ₂ S ₂ O ₅	air	0.25	97
3	DMSO	none	air	0.25–1	0
4	DMSO	Na ₂ S ₂ O ₅	argon	0.25	97

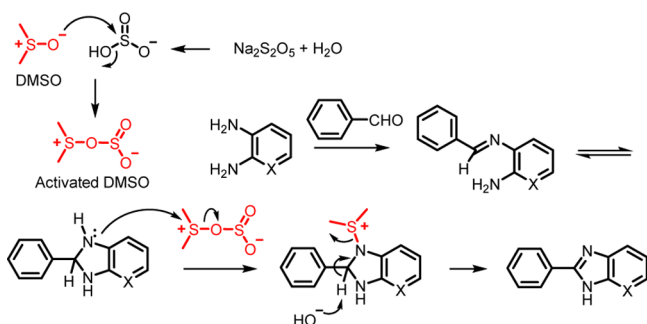
same conditions, surprisingly, resulted in a complete reaction in only 15 min (entry 2). Most importantly, the product was easily recovered by adding water and filtering the resulting precipitate, where no further purification was required as indicated by ¹H NMR. The cyclization of more derivatives (**1b–f**) under the same conditions was finished in 10–15 min, giving excellent yields of 92–99% (Scheme 2).

Scheme 2. Efficient Cyclization of BI and azaBI Rings



In order to evaluate the role of DMSO, we ran the reaction in the absence of Na₂S₂O₅ and air (entries 3 and 4). The results indicated that both DMSO and Na₂S₂O₅ are crucial. The reactants were freely soluble in both DMF and DMSO; hence, the role of DMSO was beyond solvation.⁴⁵ Thus, we propose the mechanism shown in Scheme 3, suggesting that DMSO reacts with bisulfite to form an activated intermediate that takes part in the oxidation. This hypothesis is supported by related formation of an activated DMSO in the Swern, Moffatt–Pfitzner, and Parikh–Doering oxidations of alcohols.^{44,46}

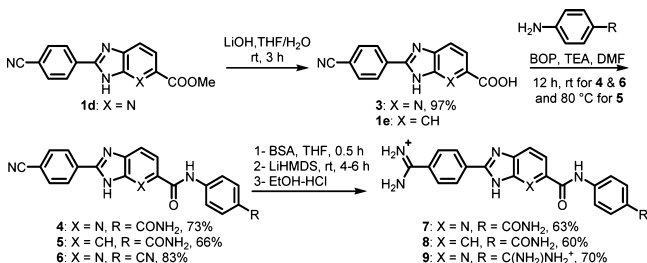
Scheme 3. Plausible Mechanism for DMSO Reaction



Application on More Complex Molecules

We tested our transient protection strategy for amidine synthesis on more complex BI/azaBI structures of six different amidines, which were designed as minor groove binders for specific recognition of DNA sequences. The synthesis of amide-containing mono- and diamidino derivatives 7–9 is shown in Scheme 4. Compound 1d was hydrolyzed to give acid 3, which

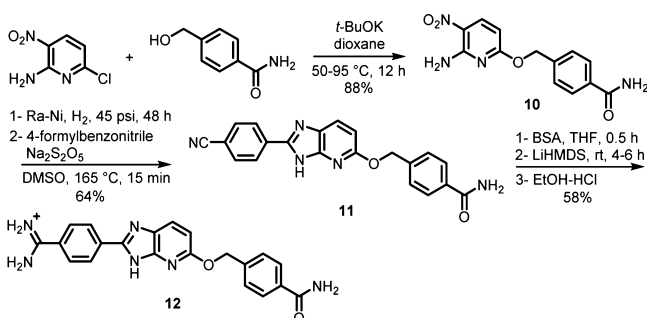
Scheme 4. Synthesis of Amidino-BI/azaBI-Carrying Amides



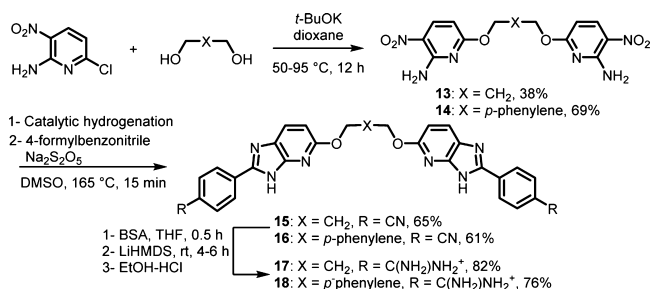
was coupled with both 4-aminobenzamide and 4-aminobenzonitrile to give the azaBI mononitrile derivative containing two amide groups (4) and the dinitrile with one amide (6), respectively. Similarly, 1e was reacted to give the BI mononitrile derivative (5) with one amide group. While transiently protecting 4–6 for the one-pot preparation of amidines 7–9, BSA was also expected to silylate the amide groups.^{35,47} Hence, a molar ratio of BSA was used to fully silylate all of the NHs. The amidines 7–9 were obtained in good yields (Scheme 4) and high purity.

Schemes 5 and 6 represent the preparation of the monoamidino 11 and the symmetric diamidines 17 and 18. 4-(Hydroxymethyl)benzamide⁴⁸ was coupled with 2-amino-6-chloro-3-nitropyridine in the presence of *t*-BuOK⁴⁹ to form the

Scheme 5. Synthesis of Amidino-azaBI-Carrying Benzyl Ether and Amide



Scheme 6. Synthesis of Diamidino-azaBI-Carrying Ether and Benzyl Ether Linkages



nitro derivative 10, which was reduced to the diamine and immediately used in the DMSO-mediated cyclization described above to give mononitrile 11 (Scheme 5). The dinitrile intermediates 15 and 16 (Scheme 6) were prepared in a similar fashion. The one-pot amidine synthesis gave 12, 17, and 18 (Schemes 5 and 6). All of the nitrile intermediates of Schemes 4–6 were highly soluble upon TMS protection. The cyclization reactions for 11, 15, and 16 were again finished in 15 min, but crystallization was required to afford pure azaBI products in yields of 61–65%.

In conclusion, transient protection with TMS using BSA is a convenient and efficient strategy, which allowed the use of LiHMDS to make BI and azaBI amidines from nitriles in a one-pot procedure. This strategy is advantageous in two ways: (1) it renders the nitrile intermediates highly soluble in THF leading to shorter reaction times, (2) it results in pure products, avoiding the need for extensive purification. This method is applicable on amide-containing nitriles and is a much needed alternative for the Pinner reaction. The amidines prepared by our new strategy were obtained in high yields of up to 88%. The transient protection should work in general where active OH/NH protons obstruct the LiHMDS reaction,³⁵ and the strategy is applicable for a wide range of cases where strong bases are used or increased solubility is needed to enhance the kinetics of a reaction.

The synthesis of the BI and azaBI imidazole rings from the aldehyde and diamine in the presence of metabisulfite has been optimized by using DMSO as a solvent. This condition led to significantly shorter reaction time than known conditions, where reactions were finished in only 10–15 min. The products were highly pure in most cases. Activated DMSO oxidation, presumably, plays an essential role in facilitating the imidazole ring formation.

These methods present significant advances, important for the facile synthesis of BI/azaBI derivatives industrially, for new drug synthesis and for the design of ligands with specific binding and recognition of DNA bases.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02359.

Experimental procedures, characterization, and ¹H and ¹³C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Bansal, Y.; Silakari, O. *Bioorg. Med. Chem.* **2012**, *20*, 6208.
- (2) Vitaku, E.; Smith, D. T.; Njardarson, J. T. *J. Med. Chem.* **2014**, *57*, 10257.
- (3) Ferreira, R. S.; Dessoy, M. A.; Pauli, I.; Souza, M. L.; Krogh, R.; Sales, A. I. L.; Oliva, G.; Dias, L. C.; Andricopulo, A. D. *J. Med. Chem.* **2014**, *57*, 2380.
- (4) Gaba, M.; Singh, S.; Mohan, C. *Eur. J. Med. Chem.* **2014**, *76*, 494.
- (5) LaPlante, S. R.; Boes, M.; Brochu, C.; Chabot, C.; Coulombe, R.; Gillard, J. R.; Jakalian, A.; Poirier, M.; Rancourt, J.; Stammers, T.; Thavonekham, B.; Beaulieu, P. L.; Kukolj, G.; Tsantrizos, Y. S. *J. Med. Chem.* **2014**, *57*, 1845.
- (6) Garuti, L.; Roberti, M.; Bottegoni, G. *Curr. Med. Chem.* **2014**, *21*, 2284.
- (7) Vasbinder, M. M.; Alimzhanov, M.; Augustin, M.; Bebernitz, G.; Bell, K.; Chuaqui, C.; Deegan, T.; Ferguson, A. D.; Goodwin, K.; Huszar, D.; Kawatkar, A.; Kawatkar, S.; Read, J.; Shi, J.; Steinbacher, S.; Steuber, H.; Su, Q.; Toader, D.; Wang, H.; Woessner, R.; Wu, A.; Ye, M.; Zinda, M. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 60.
- (8) Kim, K. M.; Lee, K.-S.; Lee, G. Y.; Jin, H.; Durrance, E. S.; Park, H. S.; Choi, S. H.; Park, K. S.; Kim, Y.-B.; Jang, H. C.; Lim, S. *Mol. Cell. Endocrinol.* **2015**, *409*, 1.
- (9) Dumat, B.; Bordeaux, G.; Faurel-Paul, E.; Mahuteau-Betzer, F.; Saettel, N.; Metge, G.; Fiorini-Debuisschert, C.; Charra, F.; Teulade-Fichou, M. P. *J. Am. Chem. Soc.* **2013**, *135*, 12697.
- (10) Kim, H. J.; Heo, C. H.; Kim, H. M. *J. Am. Chem. Soc.* **2013**, *135*, 17969.
- (11) Zhang, N.; Tian, X.; Zheng, J.; Zhang, X.; Zhu, W.; Tian, Y.; Zhu, Q.; Zhou, H. *Dyes Pigm.* **2016**, *124*, 174.
- (12) Munde, M.; Wang, S.; Kumar, A.; Stephens, C. E.; Farahat, A. A.; Boykin, D. W.; Wilson, W. D.; Poon, G. M. K. *Nucleic Acids Res.* **2014**, *42*, 1379.
- (13) Liu, Y.; Kumar, A.; Depauw, S.; Nhili, R.; David-Cordonnier, M.-H.; Lee, M. P.; Ismail, M. A.; Farahat, A. A.; Say, M.; Chackal-Catoen, S.; Batista-Parra, A.; Neidle, S.; Boykin, D. W.; Wilson, W. D. *J. Am. Chem. Soc.* **2011**, *133*, 10171.
- (14) Rahimian, M.; Kumar, A.; Say, M.; Bakunov, S. A.; Boykin, D. W.; Tidwell, R. R.; Wilson, W. D. *Biochemistry* **2009**, *48*, 1573.
- (15) Fairley, T. A.; Tidwell, R. R.; Donkor, I.; Naiman, N. A.; Ohemeng, K. A.; Lombardy, R. J.; Bentley, J. A.; Cory, M. J. *Med. Chem.* **1993**, *36*, 1746.
- (16) Harika, N. K.; Paul, A.; Stroeve, E.; Chai, Y.; Boykin, D. W.; Germann, M. W.; Wilson, W. D. *Nucleic Acids Res.* **2016**, *44*, 4519.
- (17) Paul, A.; Chai, Y.; Boykin, D. W.; Wilson, W. D. *Biochemistry* **2015**, *54*, 577.
- (18) Chai, Y.; Paul, A.; Rettig, M.; Wilson, W. D.; Boykin, D. W. *J. Org. Chem.* **2014**, *79*, 852.
- (19) Darnell, J. E. *Nat. Rev. Cancer* **2002**, *2*, 740.
- (20) Koehler, A. N. *Curr. Opin. Chem. Biol.* **2010**, *14*, 331.
- (21) Wilson, W. D.; Tanious, F. A.; Mathis, A.; Tevis, D.; Hall, J. E.; Boykin, D. W. *Biochimie* **2008**, *90*, 999.
- (22) Dox, A. W.; Whitmore, F. C. Acetamidine Hydrochloride. In *Organic Syntheses*, 2nd ed.; Blatt, A. H., Ed.; John Wiley and Sons: New York, 1941; Vol. 1, p 5.
- (23) Judkins, B. D.; Allen, D. G.; Cook, T. A.; Evans, B.; Sardharwala, T. E. *Synth. Commun.* **1996**, *26*, 4351.
- (24) Thurkauf, A.; Hutchison, A.; Peterson, J.; Cornfield, L.; Meade, R.; Huston, K.; Harris, K.; Ross, P. C.; Gerber, K.; Ramabhadran, T. V. *J. Med. Chem.* **1995**, *38*, 2251.
- (25) Khalifa, M. M.; Bodner, M. J.; Berglund, J. A.; Haley, M. M. *Tetrahedron Lett.* **2015**, *56*, 4109.
- (26) Zhang, Y.; DeKorver, K. A.; Lohse, A. G.; Zhang, Y.-S.; Huang, J.; Hsung, R. P. *Org. Lett.* **2009**, *11*, 899.
- (27) Laughlin, S.; Wang, S.; Kumar, A.; Farahat, A. A.; Boykin, D. W.; Wilson, W. D. *Chem. - Eur. J.* **2015**, *21*, 5528.
- (28) Ismail, M. A.; Batista-Parra, A.; Miao, Y.; Wilson, W. D.; Wenzler, T.; Brun, R.; Boykin, D. W. *Bioorg. Med. Chem.* **2005**, *13*, 6718.
- (29) Berger, O.; Kaniti, A.; van Ba, C. T.; Vial, H.; Ward, S. A.; Biagini, G. A.; Bray, P. G.; O'Neill, P. M. *ChemMedChem* **2011**, *6*, 2094.
- (30) Chackal-Catoen, S.; Miao, Y.; Wilson, W. D.; Wenzler, T.; Brun, R.; Boykin, D. W. *Bioorg. Med. Chem.* **2006**, *14*, 7434.
- (31) Chaires, J. B.; Ren, J.; Hamelberg, D.; Kumar, A.; Pandya, V.; Boykin, D. W.; Wilson, W. D. *J. Med. Chem.* **2004**, *47*, 5729.
- (32) Farahat, A. A.; Paliakov, E.; Kumar, A.; Barghash, A.-E. M.; Goda, F. E.; Eisa, H. M.; Wenzler, T.; Brun, R.; Liu, Y.; Wilson, W. D.; Boykin, D. W. *Bioorg. Med. Chem.* **2011**, *19*, 2156.
- (33) Vorbrüggen, H.; Ruh-Pohlenz, C. *Synthesis Of Nucleosides*. In *Organic Reactions*; John Wiley & Sons, 2004.
- (34) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316.
- (35) Klebe, J. F.; Finkbeiner, H.; White, D. M. *J. Am. Chem. Soc.* **1966**, *88*, 3390.
- (36) Goker, H.; Ozden, S.; Yildiz, S.; Boykin, D. W. *Eur. J. Med. Chem.* **2005**, *40*, 1062.
- (37) Wendt, M. D.; Rockway, T. W.; Geyer, A.; McClellan, W.; Weitzberg, M.; Zhao, X.; Mantei, R.; Nienaber, V. L.; Stewart, K.; Klinghofer, V.; Giranda, V. L. *J. Med. Chem.* **2004**, *47*, 303.
- (38) Bakunova, S. M.; Bakunov, S. A.; Wenzler, T.; Barszcz, T.; Werbovetz, K. A.; Brun, R.; Tidwell, R. R. *J. Med. Chem.* **2009**, *52*, 4657.
- (39) Panda, S. S.; Malik, R.; Jain, S. C. *Curr. Org. Chem.* **2012**, *16*, 1905.
- (40) Alp, M.; Goker, H.; Brun, R.; Yildiz, S. *Eur. J. Med. Chem.* **2009**, *44*, 2002.
- (41) Lee, Y.-S.; Cho, Y.-H.; Lee, S.; Bin, J.-K.; Yang, J.; Chae, G.; Cheon, C.-H. *Tetrahedron* **2015**, *71*, 532.
- (42) Ohsawa, A.; Itoh, T.; Nagata, K.; Ishikawa, H. *Heterocycles* **2004**, *63*, 2769.
- (43) Singh, M. P.; Sasmal, S.; Lu, W.; Chatterjee, M. N. *Synthesis* **2000**, *2000*, 1380.
- (44) Tidwell, T. T. *Synthesis* **1990**, *1990*, 857.
- (45) We thank a reviewer for suggesting evaluation of the importance of DMSO's solvation role in the enhanced cyclization reaction. We tested this using *o*-phenylenediamine and 4-cyanobenzaldehyde in DMF in the presence of 1.1 and 2.0 equiv of DMSO, under the same conditions. 70% conversion of the starting material was observed with 1.1 equiv of DMSO, while 100% conversion was attained using 2.0 equiv as detected by proton NMR.
- (46) Wu, X.-F.; Natte, K. *Adv. Synth. Catal.* **2016**, *358*, 336.
- (47) Li, N.-S.; Piccirilli, J. A. *Chem. Commun.* **2012**, *48*, 8754.
- (48) Kita, Y.; Nishii, Y.; Onoue, A.; Mashima, K. *Adv. Synth. Catal.* **2013**, *355*, 3391.
- (49) Tasker, S. Z.; Bosscher, M. A.; Shandro, C. A.; Lanni, E. L.; Ryu, K. A.; Snapper, G. S.; Utter, J. M.; Ellsworth, B. A.; Anderson, C. E. *J. Org. Chem.* **2012**, *77*, 8220.