OL | Organic Letters

Letter

Cite This: Org. Lett. 2019, 21, 6614−6618

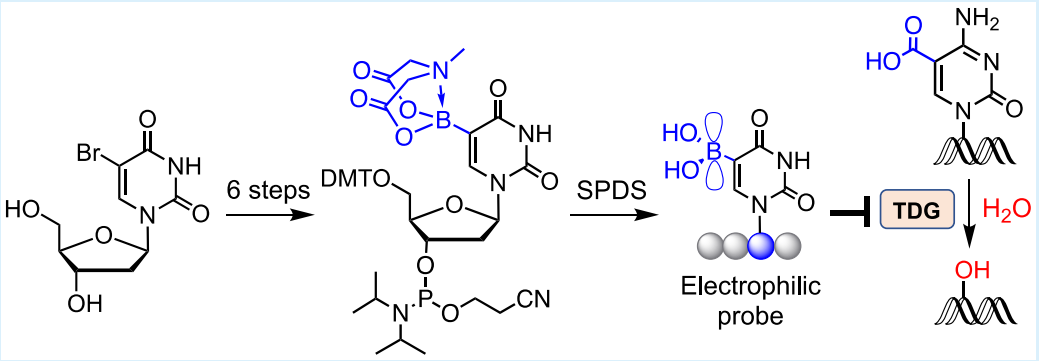
pubs.acs.org/OrgLett

Synthesis of 5-Dihydroxyboryluridine Phosphoramidite and Its Site-Specific Incorporation into Oligonucleotides for Probing Thymine DNA Glycosylase

Sam Kavoosi,[§] Debasis Dey,[§] and Kabirul Islam*

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States

Supporting Information



ABSTRACT: A concise synthetic strategy to 5-dihydroxyboryldexoyuridine (5boU) phosphoramidite has been developed. 5boU was introduced into short oligonucleotides in a site-specific manner, demonstrating compatibility of the boronic acid moiety with standard solid-phase DNA synthesis chemistry. Electrophilic 5boU DNAs inhibited thymine DNA glycosylase, a cancer-relevant DNA-modifying enzyme. We envisage diverse applications of 5boU in organic synthesis, medicinal chemistry, and chemical biology.

Enzyme-catalyzed chemical modifications on DNA are critical to controlling genome structure and integrity. A notable example includes cytosine methylation at carbon 5 (5mC) by DNA methyltransferases (DNMTs) (Figure 1A).[1] The methyl group undergoes iterative C−H oxidation by the 10−11 translocation (TET) enzymes to furnish 5-carboxycytosine (5caC).[2−4] In the active DNA demethylation pathway, 5caC is hydrolytically cleaved by thymine DNA glycosylase (TDG) to an abasic site, followed by base-excision repair (BER) to unmodified cytosine.[5,6] TDG also cleaves the glycolytic bond in thymine in T:G mismatch, resulting from enzymatic deamination of 5mC, to repair the ensuing abasic site (Figure 1A). Emerging evidence suggests that DNMT, TET, and TDG play fundamental roles during cellular differentiation and organismal development.[7,8] However, specific small-molecule inhibitors and functional probes targeting these enzymes, particularly TDG, are limited.[9,10]

Boronic acid containing small molecules have been extensively employed in designing irreversible inhibitors for hydrolytic enzymes.[11] The strong electrophilic character of boron due to its vacant p orbital favors transition from a neutral trigonal geometry to a stable anionic tetrahedral structure that effectively mimics the transition state of reactions catalyzed by hydrolytic enzymes.[12] Systematic studies have led to other modes of inhibition where an sp^3-hybrdized borate traps a substrate in the active site (e.g., tRNA synthetase[13]) or inactivates a nonhydrolytic enzyme (e.g., kinase[14]) by reacting with an activated nucleophilic amino acid in the active sites. We reasoned that oligonucleotides carrying a 5-dihydroxyborylpyrimidine nucleobase[15] could constitute covalent binders for DNA-modifying enzymes, particularly those acting on 5-carboxylpyrimidine, such as TDG. The hypothesis is based on the observation that boronic acid mimics carboxylic acid under physiological conditions due to its isosteric and isoelectronic nature (Figure 1B).[12]

Boronic acid containing compounds targeting DNA-modifying enzymes are largely unexplored in comparison to their counterparts developed to inhibit enzymes acting on polypeptide substrates.[12,16,17] This is likely due to the limited synthetic methods available for accessing nucleotide analogues carrying boronic acid at specific positions. More importantly, there is no precedence for the synthesis of the 5-dihydroxyborylpyrimidine phosphoramidite building block for site-specific introduction of the nucleotide analogue into DNA. Herein, we describe a novel synthesis of 5-dihydroxyboryldexoyuridine (5boU) phosphoramidite **1** via a short strategy (Scheme 1). We then incorporate this uniquely modified base on varied DNA sequences in a site-specific manner to demonstrate its applicability in standard solid-phase DNA

Received: June 13, 2019
Published: August 26, 2019

DOI: 10.1021/acs.orglett.9b02042
Org. Lett. 2019, 21, 6614−6618

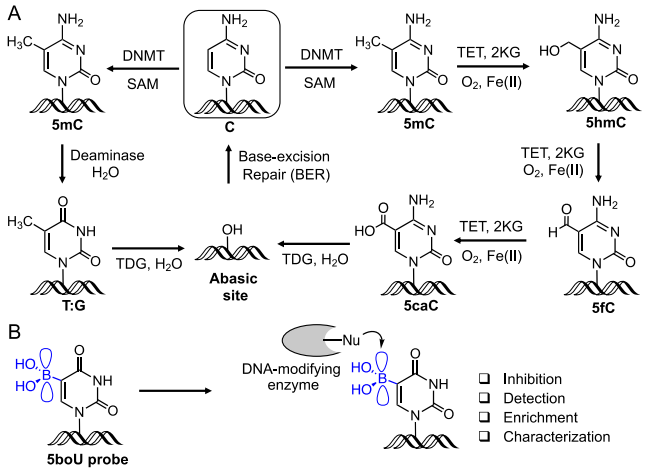
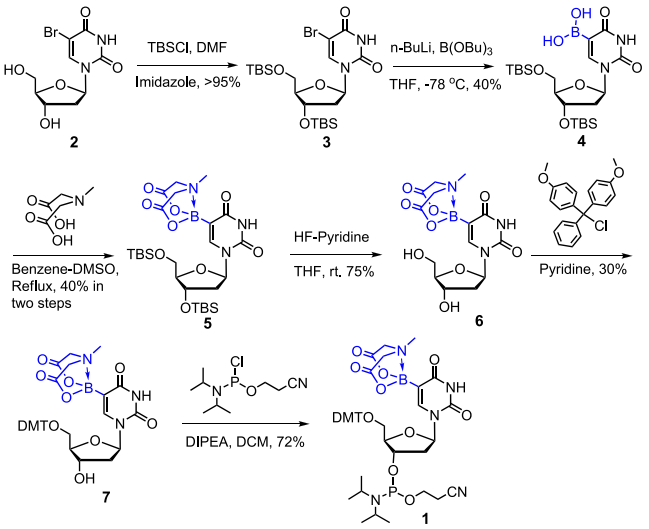


Figure 1. DNA-modifying enzymes. (A) Unmodified cytosine is methylated by DNMT to 5mC which undergoes a myriad of enzymatic modifications, such as C−H oxidations and base excision, by TET and TDG enzymes, respectively, as a part of DNA repair and active demethylation. (B) Short DNA strand carrying electrophilic 5boU nucleotide for covalent capture and inhibition of DNA-modifying enzymes.

Scheme 1. Synthesis of 5boU Phosphoramidite 1



synthesis. Finally, we examine the ability of 5boU-containing short oligonucleotides to modulate the hydrolytic activity of TDG. We show that a tetranucleotide bearing 5boU, but not the unmodified nucleobase, could robustly inhibit base-excision potential of TDG, thus providing novel chemical probes to study functions of TDG and likely of other DNA-modifying enzymes.

We adopted a modular approach to access **1** from commercially available 5-bromodeoxyuridine **2**. Key to our synthetic strategy toward 5boU phosphoramidite **1** was metal−halogen exchange mediated installation of the boronic acid moiety into suitably protected deoxyuridine (Figures S1−S7, Scheme 1). It has been reported that boronic acid could be introduced into TMS-protected 5-bromodeoxyuridine but only with 12% isolated yield.[18] To circumvent the issues related to labile TMS groups, we generated an organolithium species using hydrolytically stable TBDMS-protected uridine **3** and allowed it to react with tributyl boronate ester for an extended period of time. The desired boronic acid **4** was indeed obtained in high yield (~40% **4**, 20% **3** recovered), a significant improvement on the earlier approach.

We next protected the free boronic acid in **4** as the *N*-methyliminodiacetic acid (MIDA) ester to access **5** in good yield (Scheme 1).[19] The rationale to employ a base-sensitive MIDA protecting group instead of an acid-labile pinacol ester is that the former is expected to remain intact during trichloroacetic acid mediated cleavage of the 5′-dimethoxytrityl (DMT) group during solid-phase DNA synthesis. Furthermore, MIDA can be removed by ammonia while performing global deprotection of the synthesized oligonucleotide and its cleavage from the solid support. Removal of the TBDMS groups by HF−pyridine buffer led to diol **6** in 75% yield, and selective DMT protection on 5′-hydroxyl group proceeded smoothly to furnish **7**. The final 5boU phosphoramidite **1** was obtained in good yield by exposing **7** to 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite and was fully characterized by analytical methods (Figures S1−S7).

After securing the 5boU phosphoramidite, we intended to incorporate it into a short DNA sequence using standard solid-phase procedure. We first introduced 5boU at 5′-end of the palindromic sequence ^{5bo}UCGA (DNA **8**). Ammonia-mediated global deprotection and cleavage from solid support followed by HPLC purification yielded analytically pure DNA **8** carrying the 5boU moiety, as evident from LC−MS data (Figure 2A, Figure S8, Table 1). Given that solid-phase DNA

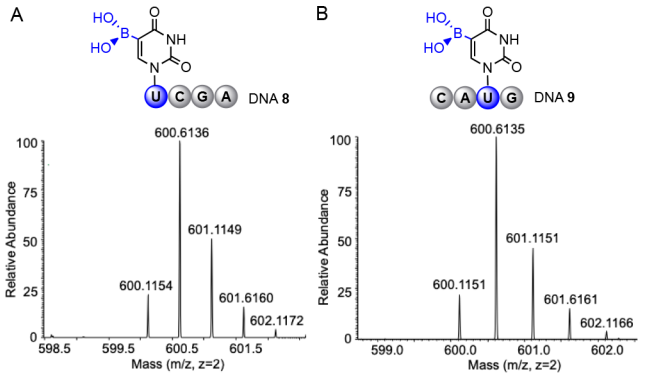


Figure 2. Solid-phase synthesis of 5boU-containing DNAs. (A) ESI-HRMS of DNA **8** carrying a terminal 5boU unit in 4-mer palindromic sequence. (B) ESI-HRMS of DNA **9** carrying an internal 5boU unit in 4-mer palindromic sequence.

synthesis starts from 3′-end, 5boU was introduced at the last step (5′-end) during assembly of DNA **8**. To examine if the MIDA-protected uridine boronate could be placed at an internal position in a sequence, we designed two more palindromic sequences CA^{5bo}UG (DNA **9**) and CG^{5bo}UCG (DNA **10**). After the solid-phase assembly and postsynthetic ammonia treatment, we obtained the required DNAs bearing intact 5boU unit as confirmed by LC−MS (Figure 2B, Figures S9 and S10, Table 1). These efforts constitute the first synthesis of 5boU phosphoramidite and introduction of this novel nucleotide into DNA strands in a site-specific manner.

We then sought to assess the potential of short DNA sequences carrying the electrophilic 5boU moiety to modulate the hydrolytic activity of TDG. Overexpression of TDG is implicated in multiple cancers, and thus targeting TDG may represent a novel therapeutic approach to human neoplasia.[20] As noted above, TDG recognizes a T:G mismatch in double-

DOI: 10.1021/acs.orglett.9b02042
Org. Lett. 2019, 21, 6614−6618

Table 1. Sequences and Expected and Observed Monoisotopic Masses of the Synthetic Oligonucleotides

DNA sequence (5′-3′)	expected mass	observed mass
DNA **8**: 5boUCGA[a]	600.6107	600.6136
DNA **9**: CA5boUG[a]	600.6107	600.6136
DNA **10**: CGA5boUCG[a]	909.6601	909.6640
DNA **11**: FAM-TCGGATGTTGTGGGTCAGTGCATGATAGTGTA	10513.7845	10513.8415
DNA **12**: TACACTATCATGCGCTGACCCACAACATCCGA	9677.6630	9677.6863
DNA **13**: FAM-TCGGATGTTGTGGGTCAG5boUGCATGATAGTGTA	10543.7758	10543.8080
DNA **14**: TCGA[a]	585.6150	585.6134

[a]Indicates expected and observed masses are for $z = 2$.

stranded DNA, flips out the thymine base in its active site, and catalytically cleaves the N-glycosidic bond in thymine to generate an abasic site. Based on this activity, we developed a base-excision assay that involves (i) formation of duplexed DNA carrying T:G mismatch and a fluorescent unit 5′-carboxyfluorescein (FAM), (ii) TDG-mediated base-excision of the duplexed DNA, and (iii) alkaline strand cleavage resulting in a fluorescently labeled short DNA fragment that can be visualized on a denaturing gel (Figure 3A). To examine

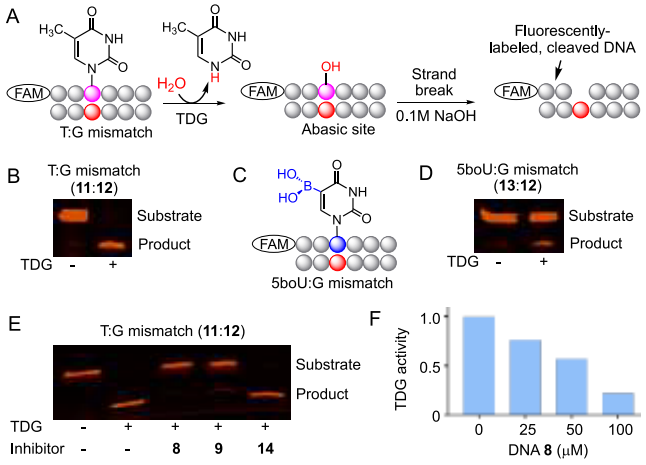


Figure 3. Activity of 5boU-containing DNAs toward TDG. (A) Schematic showing assay involving base excision of FAM-labeled DNA by TDG followed by alkaline strand cleavage and visualization using in-gel fluorescence. (B) TDG can excise duplexed DNAs **11** and **12** carrying a T:G mismatch as confirmed by in-gel fluorescence. (C) Duplexed DNAs **13** and **11** carrying 5boU:T mismatch. TDG can excise **13**, albeit to a smaller degree. (D) Inhibition of TDG activity on T:G mismatched DNAs (**11**:**12**) by 5boU-contianing DNAs **8** (^{5bo}UCGA) and **9** (CA^{5bo}UG), but not by nonboron DNA **14** (TCGA), each at 100 μM concentration. (E) Dose-dependent inhibition of TDG by DNA **8**.

TDG activity using this assay, we first synthesized a 32-nt substrate DNA **11** bearing a FAM unit and duplexed it with a complementary strand **12** to generate T:G mismatch (Table 1).[21] Both DNAs were confirmed by LC−HRMS (Figures S11 and S12). We expressed recombinant TDG protein carrying the catalytic domain and examined its activity on the above-mentioned duplexed DNA.[6,22] TDG was indeed able to recognize and excise the T:G mismatch as evident from a shorter DNA segment on the gel upon alkaline strand cleavage (Figure 3B). Consistently, in the negative control lacking TDG, fragmented DNA product was not observed (Figure 3B).

To examine if TDG could act on 5boU:G mismatch, we synthesized DNA **13** carrying 5boU in a sequence identical to **11** (Figure 3C, Table 1). Following the protocol similar to that used for DNAs **8**−**10**, we introduced the 5boU moiety into the 32-mer sequence **13** that also includes a FAM unit at the 5′ end (Table S1). The integrity of **13** was established by ESI-HRMS and ^{11}B NMR (Figures S13 and S14), demonstrating the utility of our approach to access long-chain oligonucleotide with 5boU moiety. In the base-excision assay, TDG was indeed able to flip 5boU out into its adaptable active site and cleave the glycosidic bond, reminiscent of its activity toward 5caC (Figure 3D). The hydrolytic activity toward 5boU:G mismatch was, however, significantly lower compared to T:G (3B vs 3D). Given that a stepwise $D_N{}^*A_N$ (*d*eparture of the *n*ucleobase leaving group and *a*ddition of the *n*ucleophile) pathway predominates in TDG-mediated catalysis,[23] the leaving group aptitude guides the reaction rate significantly. A key marker of leaving group quality is the acidity of the glycosidic nitrogen N1 in uridine; increased acidity ensures greater stability of the conjugate nucleobase and hence better leaving group aptitude. Although an electron-withdrawing dihydroxyboryl moiety is expected to increase N1 acidity of 5boU and enhance TDG activity, the contrary observation that we made in regard to 5boU excision is indicative of a putative tetracoordinated boronate anion in the active site that could potentially destabilize the corresponding conjugate nucleobase. This boronate species could form via nucleophilic addition of an amino acid or an activated water molecule to 5boU in the active site.

We next evaluated the binding of 5boU DNA **13** to TDG. In the electrophoretic mobility shift assay (EMSA), both duplexed DNAs (**11**:**12** and **13**:**12**) showed strong binding toward TDG (Figure S15). Encouraged by the results that 5boU DNA could bind to TDG, we proceeded to examine if 5boU could act as an inhibitor of the enzyme. Toward this end, we incubated DNAs **8** and **9**, one at a time, with TDG prior to adding the duplexed substrate **11**:**12** in the base-excision assay described above. DNAs **8** and **9** are self-complementary due to their palindromic nature and form non T:G duplexes. We observed that TDG was indeed inhibited by both the electrophilic DNAs (Figure 3E). Furthermore, both the DNAs exhibited dose-dependent inhibition of the enzyme activity (Figure 3F, Figure S16). To examine if 5boU is crucial to the inhibition of TDG, we synthesized DNA **14**, which is same as DNA **8** but lacking the 5boU unit (Table 1, Figure S17). This DNA failed to inhibit glycosylase activity of TDG (Figure 3E). To examine if only the monomer 5boU **15** could inhibit TDG, we synthesized the compound from **7** in two steps (Scheme S1, Figure S7). However, the compound failed to inhibit glycosylase activity of TDG (Figure S18), suggesting a DNA fragment is required for optimal binding and inhibition.

DOI: 10.1021/acs.orglett.9b02042
Org. Lett. 2019, 21, 6614−6618

Although the exact mechanism of inhibition by DNAs **8** and **9** is not defined, a tetrahedral boronate species could form, as discussed above, through nucleophilic addition of an activated amino acid in the catalytic site, likely resulting in covalent inhibition. It would be of future interest to explore the mode of inhibition using mutational and biochemical studies. Taken together, our results provide strong evidence that 5boU moiety could be employed to perturb the enzymatic activity of TDG. To the best of our knowledge, this is the first demonstration that short synthetic DNAs with site-specific boronic acid-containing nucleotide could modulate DNA glycosylases.

In summary, we report the first synthesis of 5boU phosphoramidite building block employing a metal−halogen exchange reaction as key step and its site-specific incorporation into synthetic oligonucleotides. In further biochemical experiments, we demonstrate the potential of electrophilic 5boU DNAs in inhibiting developmentally essential and cancer-relevant TDG. We further envisage developing activity-based probes carrying 5boU to capture novel enzymes particularly those implicated in active DNA demethylation pathway. Furthermore, given the importance of boronic acid functionality in metal-catalyzed C−C bond formation, sensor and inhibitor development,[24−26] we postulate that our efforts delineated here would find widespread applications in synthetic organic chemistry, DNA-based materials, mechanistic enzymology, and pharmacology.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02042.

Synthetic procedure and characterization of all relevant compounds, methods for protein expression and purification and biochemical assay, supplementary figures and tables, and NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: kai27@pitt.edu.

ORCID

Debasis Dey: 0000-0002-3612-860X

Kabirul Islam: 0000-0002-8680-6130

Author Contributions

§S.K. and D.D. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the University of Pittsburgh, the NIH (R01GM123234), and the National Science Foundation (MCB-1817692) for financial support. We also thank Profs. A. Drohat and C. He for the expression constructs used in the current study, S. Sappa for TDG expression, and Dr. D. Chakraborty and members of our laboratory for critical reading and editing of the manuscript.

■ REFERENCES

(1) Li, E.; Zhang, Y. DNA methylation in mammals. *Cold Spring Harbor Perspect. Biol.* **2014**, *6*, a019133.

(2) Tahiliani, M.; Koh, K. P.; Shen, Y.; Pastor, W. A.; Bandukwala, H.; Brudno, Y.; Agarwal, S.; Iyer, L. M.; Liu, D. R.; Aravind, L.; Rao, A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* **2009**, *324*, 930−5.

(3) Ito, S.; Shen, L.; Dai, Q.; Wu, S. C.; Collins, L. B.; Swenberg, J. A.; He, C.; Zhang, Y. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science* **2011**, *333*, 1300−3.

(4) Kriaucionis, S.; Heintz, N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science* **2009**, *324*, 929−30.

(5) He, Y. F.; Li, B. Z.; Li, Z.; Liu, P.; Wang, Y.; Tang, Q.; Ding, J.; Jia, Y.; Chen, Z.; Li, L.; Sun, Y.; Li, X.; Dai, Q.; Song, C. X.; Zhang, K.; He, C.; Xu, G. L. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science* **2011**, *333*, 1303−7.

(6) Maiti, A.; Drohat, A. C. Thymine DNA glycosylase can rapidly excise 5-formylcytosine and 5-carboxylcytosine: potential implications for active demethylation of CpG sites. *J. Biol. Chem.* **2011**, *286*, 35334−8.

(7) Song, C. X.; He, C. Potential functional roles of DNA demethylation intermediates. *Trends Biochem. Sci.* **2013**, *38*, 480−4.

(8) Pastor, W. A.; Aravind, L.; Rao, A. TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 341−56.

(9) Dai, Q.; Lu, X.; Zhang, L.; He, C. Synthesis of DNA oligos containing 2'-deoxy-2'-fluoro-D-arabinofuranosyl-5-carboxylcytosine as hTDG inhibitor. *Tetrahedron* **2012**, *68*, 5145−5151.

(10) Bennett, M. T.; Rodgers, M. T.; Hebert, A. S.; Ruslander, L. E.; Eisele, L.; Drohat, A. C. Specificity of human thymine DNA glycosylase depends on N-glycosidic bond stability. *J. Am. Chem. Soc.* **2006**, *128*, 12510−9.

(11) Lesnikowski, Z. J. Recent developments with boron as a platform for novel drug design. *Expert Opin. Drug Discovery* **2016**, *11*, 569−78.

(12) Yang, W.; Gao, X.; Wang, B. Boronic acid compounds as potential pharmaceutical agents. *Med. Res. Rev.* **2003**, *23*, 346−68.

(13) Rock, F. L.; Mao, W.; Yaremchuk, A.; Tukalo, M.; Crepin, T.; Zhou, H.; Zhang, Y. K.; Hernandez, V.; Akama, T.; Baker, S. J.; Plattner, J. J.; Shapiro, L.; Martinis, S. A.; Benkovic, S. J.; Cusack, S.; Alley, M. R. An antifungal agent inhibits an aminoacyl-tRNA synthetase by trapping tRNA in the editing site. *Science* **2007**, *316*, 1759−61.

(14) Ban, H. S.; Usui, T.; Nabeyama, W.; Morita, H.; Fukuzawa, K.; Nakamura, H. Discovery of boron-conjugated 4-anilinoquinazoline as a prolonged inhibitor of EGFR tyrosine kinase. *Org. Biomol. Chem.* **2009**, *7*, 4415−27.

(15) Liao, T. K.; Podrebarac, E. G.; Cheng, C. C. Boron-Substituted Pyrimidines. *J. Am. Chem. Soc.* **1964**, *86*, 1869−1870.

(16) Lin, N.; Yan, J.; Huang, Z.; Altier, C.; Li, M.; Carrasco, N.; Suyemoto, M.; Johnston, L.; Wang, S.; Wang, Q.; Fang, H.; Caton-Williams, J.; Wang, B. Design and synthesis of boronic-acid-labeled thymidine triphosphate for incorporation into DNA. *Nucleic Acids Res.* **2007**, *35*, 1222−9.

(17) El Amri, C.; Martin, A. R.; Vasseur, J. J.; Smietana, M. Bononucleotides as substrates/binders for human NMP kinases: enzymatic and spectroscopic evaluation. *ChemBioChem* **2012**, *13*, 1605−12.

(18) Schinazi, R. F.; Prusoff, W. H. Synthesis of 5-(dihydroxyboryl)-2'-deoxyuridine and related boron-containing pyrimidines. *J. Org. Chem.* **1985**, *50*, 841−847.

(19) Gillis, E. P.; Burke, M. D. Multistep synthesis of complex boronic acids from simple MIDA boronates. *J. Am. Chem. Soc.* **2008**, *130*, 14084−5.

(20) Mancuso, P.; Tricarico, R.; Bhattacharjee, V.; Cosentino, L.; Kadariya, Y.; Jelinek, J.; Nicolas, E.; Einarson, M.; Beeharry, N.; Devarajan, K.; Katz, R. A.; Dorjsuren, D. G.; Sun, H.; Simeonov, A.; Giordano, A.; Testa, J. R.; Davidson, G.; Davidson, I.; Larue, L.; Sobol, R. W.; Yen, T. J.; Bellacosa, A. Thymine DNA glycosylase as a novel target for melanoma. *Oncogene* **2019**, *38*, 3710−3728.

DOI: 10.1021/acs.orglett.9b02042
Org. Lett. 2019, 21, 6614−6618

(21) Nabel, C. S.; Jia, H.; Ye, Y.; Shen, L.; Goldschmidt, H. L.; Stivers, J. T.; Zhang, Y.; Kohli, R. M. AID/APOBEC deaminases disfavor modified cytosines implicated in DNA demethylation. *Nat. Chem. Biol.* **2012**, *8*, 751−8.
(22) Zhang, L.; Lu, X.; Lu, J.; Liang, H.; Dai, Q.; Xu, G. L.; Luo, C.; Jiang, H.; He, C. Thymine DNA glycosylase specifically recognizes 5-carboxylcytosine-modified DNA. *Nat. Chem. Biol.* **2012**, *8*, 328−30.
(23) Drohat, A. C.; Maiti, A. Mechanisms for enzymatic cleavage of the N-glycosidic bond in DNA. *Org. Biomol. Chem.* **2014**, *12*, 8367−78.
(24) Bull, S. D.; Davidson, M. G.; van den Elsen, J. M.; Fossey, J. S.; Jenkins, A. T.; Jiang, Y. B.; Kubo, Y.; Marken, F.; Sakurai, K.; Zhao, J.; James, T. D. Exploiting the reversible covalent bonding of boronic acids: recognition, sensing, and assembly. *Acc. Chem. Res.* **2013**, *46*, 312−26.
(25) Whyte, G. F.; Vilar, R.; Woscholski, R. Molecular recognition with boronic acids-applications in chemical biology. *J. Chem. Biol.* **2013**, *6*, 161−74.
(26) Ono, T.; Wang, S.; Koo, C. K.; Engstrom, L.; David, S. S.; Kool, E. T. Direct fluorescence monitoring of DNA base excision repair. *Angew. Chem., Int. Ed.* **2012**, *51*, 1689−92.

NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on August 26, 2019, with errors in the TOC and abstract graphics. This was corrected in the version published on August 27, 2019.

DOI: 10.1021/acs.orglett.9b02042
Org. Lett. 2019, 21, 6614−6618