OL Organic Letters

pubs.acs.org/OrgLett Letter

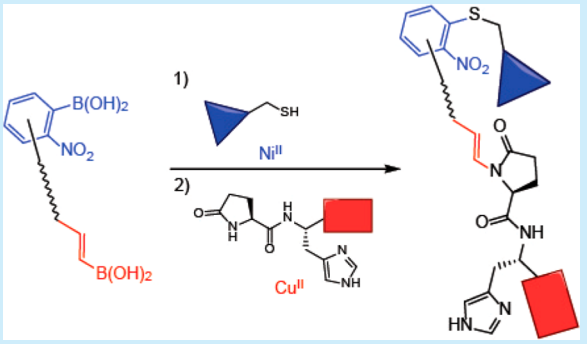
Boronic Acid Pairs for Sequential Bioconjugation

Mary K. Miller,[†] Michael J. Swierczynski,[†] Yuxuan Ding, and Zachary T. Ball*

Cite This: *Org. Lett.* 2021, 23, 5334–5338 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Boronic acids can play diverse roles when applied in biological environments, and employing boronic acid structures in tandem could provide new tools for multifunctional probes. This Letter describes a pair of boronic acid functional groups, 2-nitro-arylboronic acid (NAB) and (*E*)-alkenylboronic acid (EAB), that enable sequential cross-coupling through stepwise nickel- and copper-catalyzed processes. The selective coupling of NAB groups enables the preparation of stapled peptides, protein–protein conjugates, and other bioconjugates.



Chemical biology and biotechnology increasingly demand complex, polyfunctional biopolymers for diverse "smart" materials and molecules. Concepts such as theranostics[1] rely on multifunctional molecules with diverse, complementary, and often orthogonal reactivity. As a result, diverse biorthogonal chemistries facilitate the construction of complex bioconjugates by sequential couplings.

Organoborane reagents are useful chemical tools with remarkably diverse applications in chemical biology.[2−6] Their use as organometallic precursors in catalytic cross-coupling reactions is widely appreciated.[7] Organoboranes are also utilized in remarkably diverse applications in biological chemistry: recognition of poly hydroxy motifs,[8−10] enzyme inhibition,[11,12] reactive oxygen species (ROS) sensing,[13] bioconjugation[4,14,15] (including facilitation of oxime formation[16,17]), and other concepts.[18,19] These diverse possibilities raise questions about how multiple boronic acid functional groups might be used in tandem, playing complementary roles in multifunctional reagents. This goal requires boronic acid reagents that are mutually compatible so that the selective activation of one organoboronate group is possible while a second organoboronate remains inert. For small-molecule synthesis in an organic solvent, the development of specific boronic acid derivatives has enabled selective, sequential coupling reactions by masking boronic acid reactivity.[20−27] We envisioned an alternative strategy of boronic acids with inherently differential reactivity in coupling reactions, overcoming the limited hydrolytic stability of boronate esters. These concepts were motivated by our own studies of transition-metal-catalyzed bioconjugation with boronic acid substrates, promoted by copper,[28−31] rhodium,[32] or nickel salts.[33,34]

In the course of these efforts, we found that the precise substitution patterns of the boronic acid reagent had a profound effect on the reaction efficiency, kinetics, and chemoselectivity. For instance, catalytic cysteine arylation with arylboronic acids containing certain electron-withdrawing ortho substituents occurs efficiently within minutes,[33] whereas simple arylboronic acids without ortho substitution afford no product under identical conditions.[33] In contrast, such simple arylboronic acid reagents do readily participate in backbone N–H arylation catalyzed by Cu^{2+},[28,29] but the reactions are sensitive to steric demand, and ortho substitution of any kind is not tolerated. We therefore wondered if these methods with significantly different structure–reactivity frameworks could form the basis for sequential coupling partners for the construction of complex bioconjugate architectures. Previous efforts[33] identified arylboronic acids containing certain electron-withdrawing ortho substituents, such as 2-nitro-arylboronic acids (NABs), as displaying especially fast Chan–Lam product formation, indicating that they might serve as effective first coupling partners in a sequential coupling strategy (Figure 1).

The second sequential coupling would require boronic acid structures were are stable under the initial conditions. In addition to cross-coupling, boronic acids are prone to a variety of side reactions in water and under air, which can also be catalyzed by transition metals, including protodeborylation, oxidative hydroxylation, and C–C homocoupling.[30,33] Appropriate "second" boronic acids would need to avoid these

Received: May 12, 2021
Published: July 2, 2021

https://doi.org/10.1021/acs.orglett.1c01624
Org. Lett. 2021, 23, 5334–5338

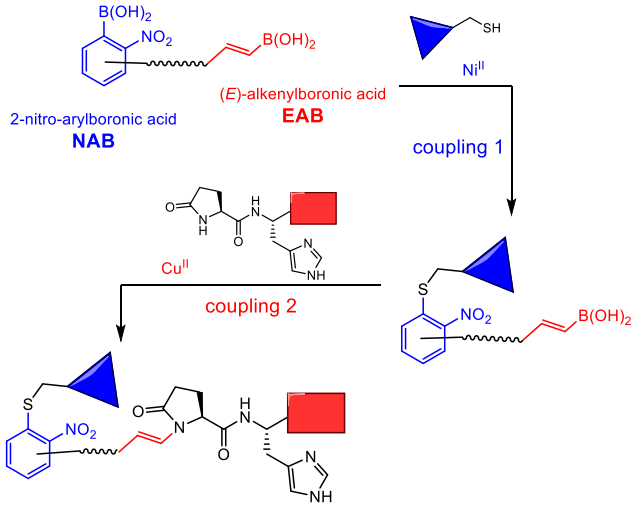


Figure 1. Schematic depiction of the sequential cross-coupling of NAB and EAB boronic acid pairs.

decomposition pathways. We compared the consumption of a variety of boronic acids under catalytic conditions. Boronic acids **2a–k** were subjected to a reaction with either copper(II) or nickel(II) and a coupling partner, *N*-acetylcysteine (Figure 2). No suitably stable boronic acids were discovered under copper(II) catalysis conditions; all boronic acid reagents were consumed to a significant extent (Figure 2, blue), even for compounds (e.g., **2f**, **2g**, **2i**, **2j**) for which previous studies[33] demonstrated no productive coupling to give products analogous to **3**. Conversely, there were profound differences in the reactivity with nickel(II) catalysis (Figure 2, red). Whereas 2-acetyl- (**2b**) and 2-nitro-phenylboronic (NAB, **2a**) are consumed quickly under these conditions, simple aryl- (**2h–2j**) and (*E*)-alkenylboronic acids (EABs) (**2k**) were substantially stable under these conditions, showing <1% conversion after 30 min. These results implied that boronic acid reagents reactive with copper could be utilized sequentially after a nickel-catalyzed process.

In a more stringent and relevant test, we next examined mixtures of putative "reactive" and "unreactive" boronic acids in the nickel(II)-mediated arylation of *N*-acetylcysteine. The stability of the "unreactive" boronic acids (**2k–m**) was monitored by ^{1}H NMR analysis, with dimethylformamide (DMF) as an internal standard (Figure 3a). Again, we found that an arylboronic acid (Figure 3b) and alkenylboronic acid

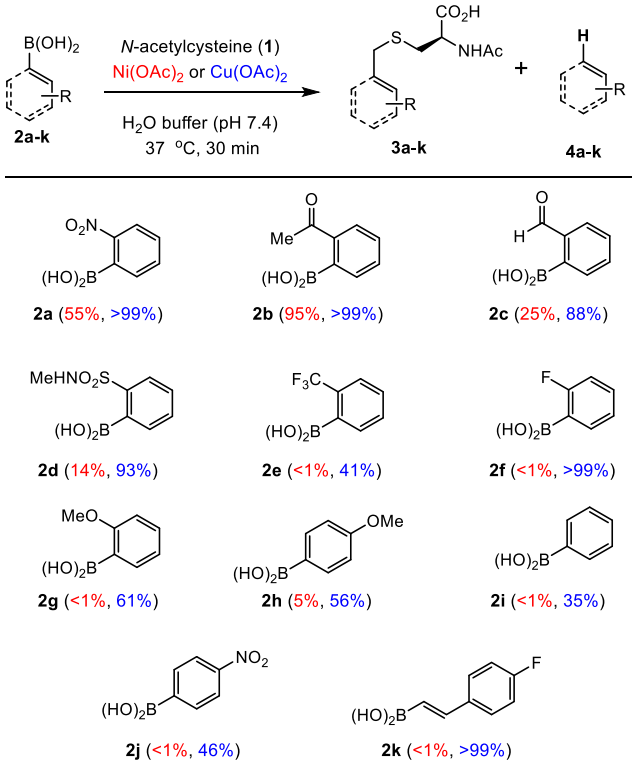


Figure 2. Conversion of boronic acid reagents **2a–k** in the presence of Ni^{2+} and Cu^{2+} salts. The total conversion to products **3** and **4** is shown for Ni^{2+} (red) and Cu^{2+} (blue). Conditions: boronic acid (2 mM), *N*-acetylcysteine (0.2 mM), and $M(OAc)_2$ (1 mM) in aqueous *N*-methylmorpholine (NMM) buffer (10 mM).

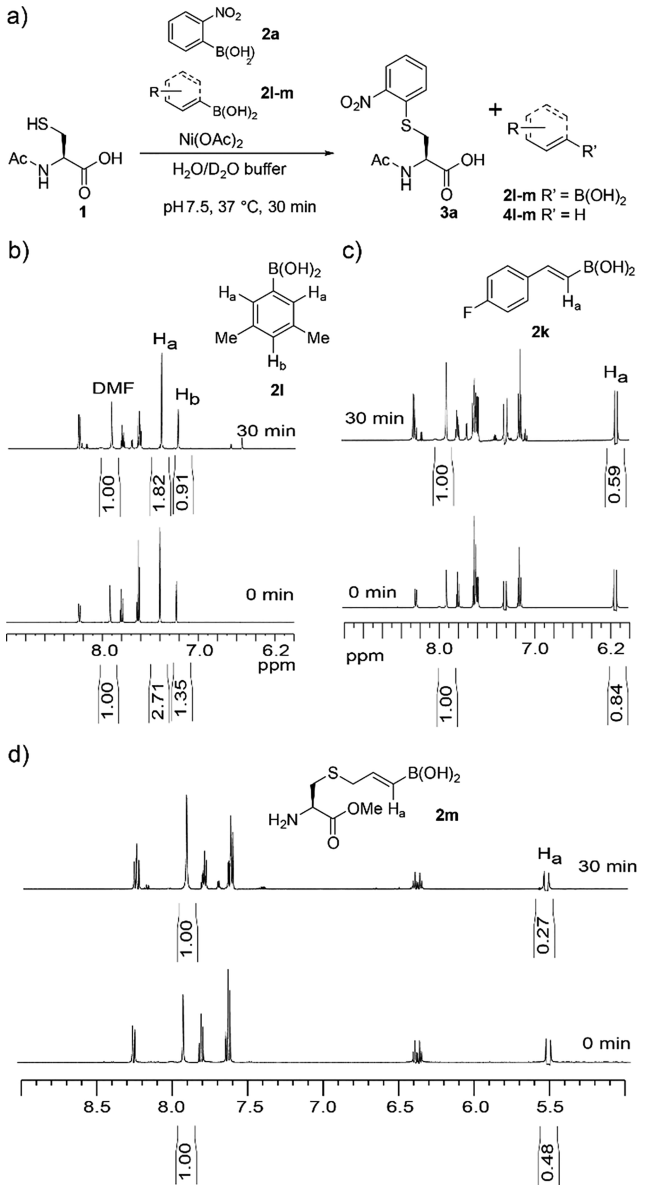


Figure 3. (a) Stability of aryl- and alkenyl-boronic acid reagents in the nickel(II)-mediated reaction. (b,c) NMR monitoring of the stability of "bystander" boronic acids **2l** and **2m** in an NAB-type Ni^{2+} coupling with boronic acid **2a**.

https://doi.org/10.1021/acs.orglett.1c01624
Org. Lett. 2021, 23, 5334–5338

(Figure 3c) showed minimal conversion after 30 min. An alkenylboronic acid reagent that could be utilized to design a diboronic acid scaffold was also tested (Figure 3d) and was likewise found to survive the reaction conditions.

We next applied this sequential coupling concept to the preparation of a stapled peptide with a nonsymmetrical linkage of defined orientation (Figure 4). Stapled peptides can confer

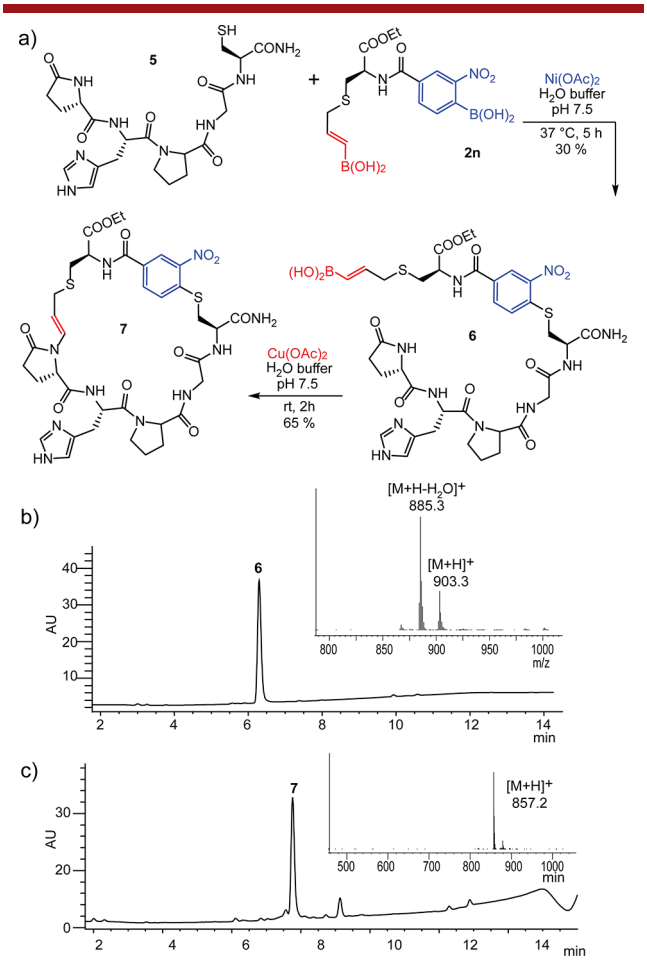
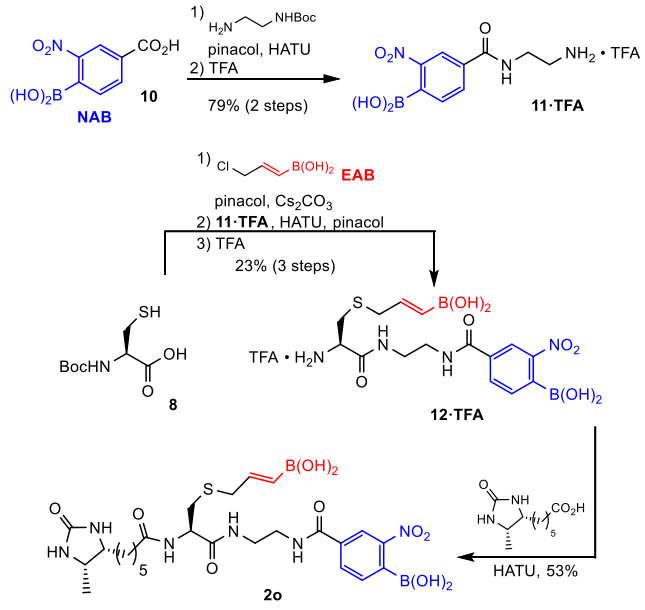


Figure 4. Peptide stapling with sequential boronic acid coupling. (a) Synthetic scheme for the preparation of the peptide staple **7**. Conditions: cysteine modification: **5** (0.2 mM), **2n** (2 mM), and $Ni(OAc)_2$ (1 mM) in NMM buffer (10 mM, pH 7.5), TCEP (1 mM), 37 °C, 5 h; pEH alkenylation: **6** (0.2 mM) and $Cu(OAc)_2$ (1 mM) in NMM buffer pH 7.5 for 2 h. (b) LCMS chromatogram of **6**. Insert: mass chromatogram of **6**. (c) LCMS of the crude reaction mixture for the conversion of **6** to **7**. Insert: mass chromatogram of the new peak **7**.

functional and stability advantages in vivo, and we have previously demonstrated the stability of these arylation linkages in serum and proteolytic stability assays.[33] A peptide with a cysteine site for S−H coupling with an NAB and a "second" pyroglutamate−histidine (pEH) dipeptide sequence for N−H coupling with EAB was prepared and tested (**5**). The diboronic acid reagent **2n** was prepared with both NAB and EAB moieties. Nickel-catalyzed coupling of the peptide **5** with the diboronic acid reagent **2n** afforded the S-arylation product **6**. Matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS/MS) confirmed cysteine as the site of reactivity, and the material could be purified by reverse-phase high-performance liquid chromatography (RP-HPLC). Cyclization of the linear intermediate **6** was then accomplished by copper-catalyzed N−H alkenylation, providing the cyclic **7** (crude reaction analysis: Figure 4c). This result was particularly enlightening, as it demonstrated the success of aqueous copper-mediated N−H alkenylation where the intramolecular nature of the cyclization necessitates 1:1 stoichiometry.

Sequential reactivity of diboronic acid reagents with protein substrates could also be realized. We have previously shown that the cysteine arylation chemistry could be used to obtain protein−polymer conjugates,[35] and we therefore wondered if sequential conjugations could be used to create large biopolymer structures. Peptide and protein heterodimers find use in vaccine development and targeted drug delivery.[36,37] Both cysteine and pyroglutamate-histidine tags[38] are readily incorporated into the protein substrate from *E. coli* expression systems. To explore these possibilities, a trifunctional reagent (**2o**) containing a desthiobiotin affinity purification handle was synthesized (Scheme 1). A model protein, T4 lysozyme V131C (T4L), bearing a single cysteine residue, reacted with **2o** under nickel conditions to afford an alkenylboronic-acid-labeled protein (Figure 5).

Scheme 1. Synthesis of Di-boronic Acid Reagent 2o



This boronic-acid-labeled T4L protein could then be directly coupled to afford protein−peptide conjugates. Using an excess of pEH-containing peptides leuprolide (LE, **13**, pEHWSY-LLRP) or LHRH (LH, **14**, pEHWSYGLRPG) in the presence of copper(II), efficient conjugation was achieved (Figure 5b). This observation demonstrates the robust nature of individual boronic acid labels to survive nickel-mediated conjugation, purification, and routine protein manipulations and handling. It also highlights the remarkable efficiency of this process, in which the complex protein−boronic acid acts as the limiting reagent in an intermolecular coupling process.

Protein−protein conjugates were also constructed using this approach. When the T4L−boronic acid was treated with excess green fluorescent protein (GFP, 5 equiv) bearing a pyroglutamate-histidine tag, substantial conversion (46% by gel densitometry) was achieved in 30 min under copper-

https://doi.org/10.1021/acs.orglett.1c01624
Org. Lett. 2021, 23, 5334−5338

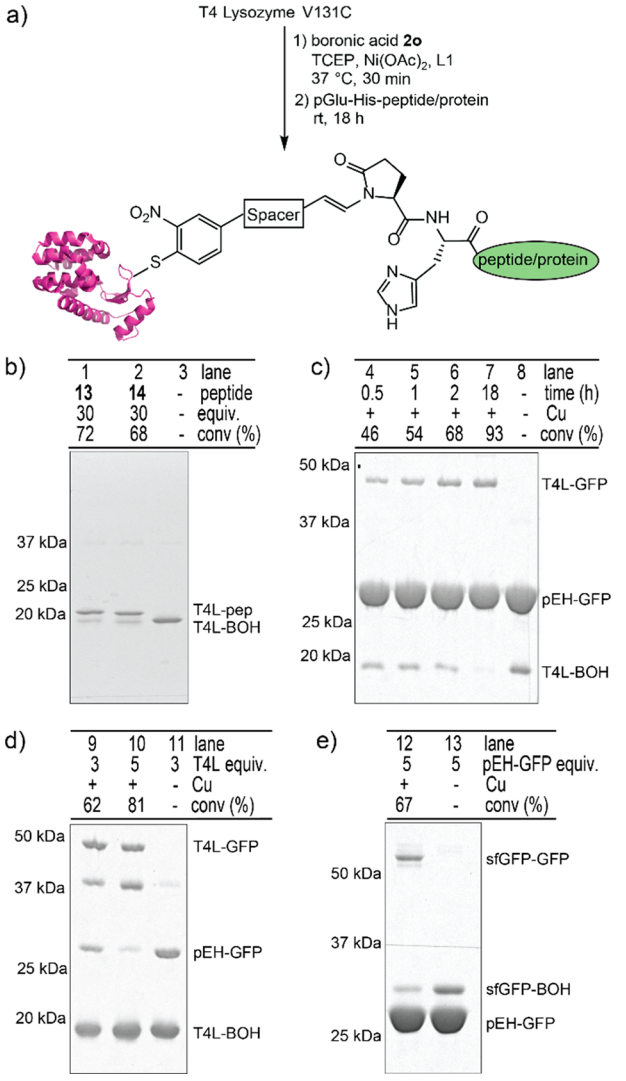


Figure 5. (a) Overview of the conjugation of T4L with pyroglutamate−histidine-tagged structures using diboronic acid **2o**. Conditions: (1) T4L (20 μM), **2o** (200 μM), tris(2-carboxyethyl)-phosphine (TCEP) (0.2 mM), $Ni(OAc)_2$ (0.4 mM), and 6,6′-dimethyl-2,2′-bipyridine (**L1**) (0.4 mM) in NMM buffer (50 mM, pH 7.5) at 37 °C for 30 min. (2) Boronic-acid-modified T4L (10 μM), pGlu-His-peptide/protein (50 μM), and $Cu(OAc)_2$ (0.25 mM) in NMM buffer (50 mM, 150 mM NaCl, pH 7.5) at rt for 18 h. (b−e) Gel images for the conjugation of T4L-boronic acid with (b) pGlu-His peptides and (c) pGlu-His GFP. (d) Conjugation of T4L-boronic acid to pGlu-His-GFP with excess T4L. (e) Conjugation of sfGFP-boronic acid with pGlu-His-GFP. Conversion was measured by gel densitometry (ImageJ) relative to the starting material and was uncalibrated.

catalyzed conditions (Figure 5c). The anticipated conjugate was further confirmed by MALDI-MS (Figure S17). After 18 h, 93% conversion was observed without significant side products. Conjugation was also performed with tagged GFP as the limiting reagent. In this case, 81% conversion was observed within 2 h (Figure 5d). A minor product was also observed, with a molecular weight consistent with homocoupled T4L. It is interesting to note that this minor homocoupled protein product does not appear when tagged GFP is used in excess (Figure 5c). Nonetheless, the reaction is compatible with either coupling partner in excess. This protein−protein conjugation protocol was further utilized to link a cysteine-bearing green fluorescent protein (sfGFP) with a pyroglutamate-histidine GFP (68% conversion, Figure 5e).

In conclusion, many simple boronic acids (including EAB) are stable and inert to aqueous nickel-catalyzed S−H arylation with NAB moieties. This observation allows sequential aqueous coupling reactions with peptides and proteins. The approach allows the simple two-step construction of diverse bioconjugates and indicates that NAB coupling reactions may be possible in the presence of a wide range of functional boronic acid groups, permitting the creative implementation of boronic-acid-based applications in protein chemistry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c01624.

General experimental details, methods for peptide and protein modification and product analysis, and characterization of new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Zachary T. Ball − *Department of Chemistry, Rice University, Houston, Texas 77005, United States;* orcid.org/0000-0002-8681-0789; Email: zb1@rice.edu

Authors

Mary K. Miller − *Department of Chemistry, Rice University, Houston, Texas 77005, United States*

Michael J. Swierczynski − *Department of Chemistry, Rice University, Houston, Texas 77005, United States*

Yuxuan Ding − *Department of Chemistry, Rice University, Houston, Texas 77005, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.1c01624

Author Contributions

†M.K.M. and M.J.S. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge support from the Robert A. Welch Foundation Research Grant C-1680 (Z.T.B.) and the National Science Foundation under grant number CHE-1904865. We thank Raphael Hofmann and Jeffrey Bode (ETH Zurich) for the T4L(V131C) and sfGFP(S14C).

■ REFERENCES

(1) Lim, E.-K.; Kim, T.; Paik, S.; Haam, S.; Huh, Y.-M.; Lee, K. Nanomaterials for Theranostics: Recent Advances and Future Challenges. *Chem. Rev.* **2015**, *115*, 327−394.

(2) António, J. P. M.; Russo, R.; Carvalho, C. P.; Cal, P. M. S. D.; Gois, P. M. P. Boronic acids as building blocks for the construction of therapeutically useful bioconjugates. *Chem. Soc. Rev.* **2019**, *48*, 3513−3536.

(3) Chatterjee, S.; Anslyn, E. V.; Bandyopadhyay, A. Boronic acid based dynamic click chemistry: recent advances and emergent applications. *Chem. Sci.* **2021**, *12*, 1585−1599.

(4) Akgun, B.; Hall, D. G. Boronic Acids as Bioorthogonal Probes for Site-Selective Labeling of Proteins. *Angew. Chem., Int. Ed.* **2018**, *57*, 13028−13044.
(5) Diaz, D. B.; Yudin, A. K. The versatility of boron in biological target engagement. *Nat. Chem.* **2017**, *9*, 731−742.
(6) Hall, D. G. *Boronic Acids: Preparation and Applications in Organic Synthesis, Medicine and Materials*, 2nd ed.; Wiley-VCH: Weinheim, Germany, 2011.
(7) Chalker, J. M.; Wood, C. S. C.; Davis, B. G. A Convenient Catalyst for Aqueous and Protein Suzuki−Miyaura Cross-Coupling. *J. Am. Chem. Soc.* **2009**, *131*, 16346−16347.
(8) Halo, T. L.; Appelbaum, J.; Hobert, E. M.; Balkin, D. M.; Schepartz, A. Selective Recognition of Protein Tetraserine Motifs with a Cell-Permeable, Pro-fluorescent Bis-boronic Acid. *J. Am. Chem. Soc.* **2009**, *131*, 438−439.
(9) Whyte, G. F.; Vilar, R.; Woscholski, R. Molecular recognition with boronic acids—applications in chemical biology. *J. Chem. Biol.* **2013**, *6*, 161−174.
(10) Zhang, X.; Liu, G.; Ning, Z.; Xing, G. Boronic acid-based chemical sensors for saccharides. *Carbohydr. Res.* **2017**, *452*, 129−148.
(11) Ban, H. S.; Nakamura, H. Boron-Based Drug Design. *Chem. Rec.* **2015**, *15*, 616−635.
(12) Smoum, R.; Rubinstein, A.; Dembitsky, V. M.; Srebnik, M. Boron Containing Compounds as Protease Inhibitors. *Chem. Rev.* **2012**, *112*, 4156−4220.
(13) Albers, A. E.; Okreglak, V. S.; Chang, C. J. A FRET-Based Approach to Ratiometric Fluorescence Detection of Hydrogen Peroxide. *J. Am. Chem. Soc.* **2006**, *128*, 9640−9641.
(14) Ohata, J.; Ball, Z. T. Ascorbate as a pro-oxidant: mild N-terminal modification with vinylboronic acids. *Chem. Commun.* **2017**, *53*, 1622−1625.
(15) Eising, S.; Lelivelt, F.; Bonger, K. M. Vinylboronic Acids as Fast Reacting, Synthetically Accessible, and Stable Bioorthogonal Reactants in the Carboni−Lindsey Reaction. *Angew. Chem., Int. Ed.* **2016**, *55*, 12243−12247.
(16) Cambray, S.; Gao, J. Versatile Bioconjugation Chemistries of *ortho*-Boronyl Aryl Ketones and Aldehydes. *Acc. Chem. Res.* **2018**, *51*, 2198−2206.
(17) Gillingham, D. The role of boronic acids in accelerating condensation reactions of α-effect amines with carbonyls. *Org. Biomol. Chem.* **2016**, *14*, 7606−7609.
(18) Brustad, E.; Bushey, M. L.; Lee, J. W.; Groff, D.; Liu, W.; Schultz, P. G. A Genetically Encoded Boronate-Containing Amino Acid. *Angew. Chem., Int. Ed.* **2008**, *47*, 8220−8223.
(19) Kita, M.; Yamamoto, J.; Morisaki, T.; Komiya, C.; Inokuma, T.; Miyamoto, L.; Tsuchiya, K.; Shigenaga, A.; Otaka, A. Design and synthesis of a hydrogen peroxide-responsive amino acid that induces peptide bond cleavage after exposure to hydrogen peroxide. *Tetrahedron Lett.* **2015**, *56*, 4228−4231.
(20) Kelly, A. M.; Chen, P.-J.; Klubnick, J.; Blair, D. J.; Burke, M. D. A Mild Method for Making MIDA Boronates. *Org. Lett.* **2020**, *22*, 9408−9414.
(21) Li, J.; Grillo, A. S.; Burke, M. D. From Synthesis to Function via Iterative Assembly of *N*-Methyliminodiacetic Acid Boronate Building Blocks. *Acc. Chem. Res.* **2015**, *48*, 2297−2307.
(22) Lee, S. J.; Anderson, T. M.; Burke, M. D. A Simple and General Platform for Generating Stereochemically Complex Polyene Frameworks by Iterative Cross-Coupling. *Angew. Chem., Int. Ed.* **2010**, *49*, 8860−8863.
(23) Lee, S. J.; Gray, K. C.; Paek, J. S.; Burke, M. D. Simple, Efficient, and Modular Syntheses of Polyene Natural Products via Iterative Cross-Coupling. *J. Am. Chem. Soc.* **2008**, *130*, 466−468.
(24) Li, J.; Ballmer, S. G.; Gillis, E. P.; Fujii, S.; Schmidt, M. J.; Palazzolo, A. M. E.; Lehmann, J. W.; Morehouse, G. F.; Burke, M. D. Synthesis of many different types of organic small molecules using one automated process. *Science* **2015**, *347*, 1221−1226.
(25) Noguchi, H.; Hojo, K.; Suginome, M. Boron-Masking Strategy for the Selective Synthesis of Oligoarenes via Iterative Suzuki−Miyaura Coupling. *J. Am. Chem. Soc.* **2007**, *129*, 758−759.
(26) Iwadate, N.; Suginome, M. Differentially Protected Diboron for Regioselective Diboration of Alkynes: Internal-Selective Cross-Coupling of 1-Alkene-1,2-diboronic Acid Derivatives. *J. Am. Chem. Soc.* **2010**, *132*, 2548−2549.
(27) Mao, L.; Li, C.; Xiang, L.; Fu, D.; Ma, W.; Ye, Q. Synthesis of Allenyl-Bdan via the Cu(I)-Catalyzed Borylation of Propargylic Alcohols with the Unsymmetric Diboron Reagent (Bpin-Bdan). *J. Org. Chem.* **2020**, *85*, 2823−2831.
(28) Ohata, J.; Zeng, Y.; Segatori, L.; Ball, Z. T. A Naturally Encoded Dipeptide Handle for Bioorthogonal Chan−Lam Coupling. *Angew. Chem., Int. Ed.* **2018**, *57*, 4015−4019.
(29) Ohata, J.; Minus, M. B.; Abernathy, M. E.; Ball, Z. T. Histidine-Directed Arylation/Alkenylation of Backbone N−H Bonds Mediated by Copper(II). *J. Am. Chem. Soc.* **2016**, *138*, 7472−7475.
(30) Miller, M. K.; Wang, H.; Hanaya, K.; Zhang, O.; Berlaga, A.; Ball, Z. T. Copper-mediated peptide arylation selective for the N-terminus. *Chem. Sci.* **2020**, *11*, 10501−10505.
(31) Mangubat-Medina, A. E.; Martin, S. C.; Hanaya, K.; Ball, Z. T. A Vinylogous Photocleavage Strategy Allows Direct Photocaging of Backbone Amide Structure. *J. Am. Chem. Soc.* **2018**, *140*, 8401.
(32) Ohata, J.; Miller, M. K.; Mountain, C. M.; Vohidov, F.; Ball, Z. T. A Three-Component Organometallic Tyrosine Bioconjugation. *Angew. Chem., Int. Ed.* **2018**, *57*, 2827−2830.
(33) Hanaya, K.; Ohata, J.; Miller, M. K.; Mangubat-Medina, A. E.; Swierczynski, M. J.; Yang, D. C.; Rosenthal, R. M.; Popp, B. V.; Ball, Z. T. Rapid nickel(II)-promoted cysteine S-arylation with arylboronic acids. *Chem. Commun.* **2019**, *55*, 2841−2844.
(34) Hanaya, K.; Miller, M. K.; Ball, Z. T. Nickel(II)-Promoted Amide N−H Arylation of Pyroglutamate−Histidine with Arylboronic Acid Reagents. *Org. Lett.* **2019**, *21*, 2445−2448.
(35) Swierczynski, M. J.; Ball, Z. T. One-Step Protein−Polymer Conjugates from Boronic-Acid-Functionalized Polymers. *Bioconjugate Chem.* **2020**, *31*, 2494−2498.
(36) Weiss, C.; Figueras, E.; Borbely, A. N.; Sewald, N. Cryptophycins: cytotoxic cyclodepsipeptides with potential for tumor targeting. *J. Pept. Sci.* **2017**, *23*, 514−531.
(37) Bolhassani, A.; Jafarzade, B. S.; Mardani, G. In vitro and in vivo delivery of therapeutic proteins using cell penetrating peptides. *Peptides* **2017**, *87*, 50−63.
(38) Shih, Y.-P.; Chou, C.-C.; Chen, Y.-L.; Huang, K.-F.; Wang, A. H.-J. Linked Production of Pyroglutamate-Modified Proteins via Self-Cleavage of Fusion Tags with TEV Protease and Autonomous N-Terminal Cyclization with Glutaminyl Cyclase In Vivo. *PLoS One* **2014**, *9*, e94812.

https://doi.org/10.1021/acs.orglett.1c01624
Org. Lett. 2021, 23, 5334−5338