JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Histidine-Directed Arylation/Alkenylation of Backbone N−H Bonds Mediated by Copper(II)

Jun Ohata, Matthew B. Minus, Morgan E. Abernathy, and Zachary T. Ball*

Department of Chemistry, Rice University, Houston, Texas 77005, United States

Supporting Information

ABSTRACT: Chemical modification of proteins and peptides represents a challenge of reaction design as well as an important biological tool. In contrast to side-chain modification, synthetic methods to alter backbone structure are extremely limited. In this communication, copper-mediated backbone *N*-alkenylation or *N*-arylation of peptides and proteins by direct modification of natural sequences is described. Histidine residues direct oxidative coupling of boronic acids at the backbone NH of a neighboring amino acid. The mild reaction conditions in common physiological buffers, at ambient temperature, are compatible with proteins and biological systems. This simple reaction demonstrates the potential for directed reactions in complex systems to allow modification of N−H bonds that directly affect polypeptide structure, stability, and function.

Chemical modification of polypeptide structures is important for drug development, biomaterials design, and biological probe construction. Protein modification methods currently available target the side chain functional group of amino acid(s) with unique reactivity.[1,2] Direct alteration of the backbone structure of as-produced peptides and proteins, rather than conjugation to a side chain, is relatively little explored,[3,4] despite the important consequences of backbone amide modification.[5,6] Nonribosomal peptides use backbone *N*-methylation and other modifications to modulate folding, stability, and function, and backbone modification methods could open new vistas for molecular design. For chemists, backbone *N*-alkylation or *N*-arylation disrupts the hydrogen bonding networks required for common folding motifs, including α-helices and β-sheets, allowing for simple ways to perturb folding, ligand bindings, and supramolecular assembly.[5,6]

Accessing *N*-alkyl or *N*-aryl derivatives of complex peptides or proteins remains a challenging problem. Though unnatural side chain structure can be incorporated in biological polypeptide synthesis through the use of unnatural amino-acyl-tRNA synthetases,[7] the use of amino acids with *N*-substitution is rare.[8] Solid-phase chemical synthesis is sometimes appropriate, but *N*-alkyl amino acids are sluggish coupling partners, many interesting targets (e.g., *N*-alkenyl amino acids) are incompatible with traditional peptide synthesis, and larger sequences become increasingly difficult or impossible.[9−12] Methods for direct backbone amide modification of polypeptides are extremely limited, such as oxidative scission with RuO_4.[13] General tools to alter backbone N−H bonds of as-produced peptides or proteins are sorely lacking.

As part of a program examining metal-catalyzed reactions of proteins,[17−21] we examined reactions of boronic acids with histidine-containing sequences in the presence of transition-metals. Readily available thyrotropin-releasing hormone (TRH, **1**) served as a convenient minimalist protein model. Treating the tripeptide at ambient temperature in air with arylboronic acid **2** (1 mM), in *N*-methylmorpholine (NMM) buffer (pH 7.4) in the presence of copper(II) salts (330 μM), led to the incorporation of the aryl moiety into the peptide, as judged by mass spectrometry (Figure 1c). Copper(II) was necessary for this transformation; no reaction was observed in the absence of copper nor in the presence of a variety of other metals.

Purification of the product (RP-HPLC) allowed characterization of the product **3** and identification of the modification site: arylation of the pyroglutamic acid (Glp) backbone NH, the residue immediately preceding histidine. A NOESY spectrum displays clear crosspeaks for interaction of the pyroglutamic H_α with both *ortho* protons on the aryl group (Figure 1b). In addition, a COSY spectrum identified only a single NH−H_α coupling (for histidine), confirming the disappearance of the pyroglutamate NH. Finally, fragmentation of the product by MS/MS confirmed this structural assignment (Supporting Information).

The reactivity observed is similar to Chan−Lam coupling, the oxidative combination of boronate reagents with a variety of X−H bonds, using O_2 or other species as the stoichiometric oxidant.[22−24] Coupling of boronates with amide N−H bonds has been observed.[25,26] However, the reported reactions for simple small-molecule substrates require basic conditions in anhydrous solvent, sometimes at elevated temperature. Aqueous reaction conditions are rare since hydroxylation of the boronate is facile in the presence of water.[27] In our reactions with peptide substrates, further studies revealed that a histidine residue is absolutely required for the observed reactivity. For example, laminin 925−933, containing a cysteine residue in addition to a variety of backbone amides, did not give appreciable modification products. The specificity for modification of the backbone N−H bond in the $i-1$ position (i.e., one residue before histidine) has similarities to the known propensity of copper to bind histidine-containing sequences, such as the ATCUN motif[14−16,28] (Figure 1d), in which copper coordinates to the imidazole side chain and to deprotonated backbone nitrogen atoms at the i and $i-1$ positions. The

Received: April 1, 2016
Published: June 1, 2016

DOI: 10.1021/jacs.6b03390
J. Am. Chem. Soc. 2016, 138, 7472−7475

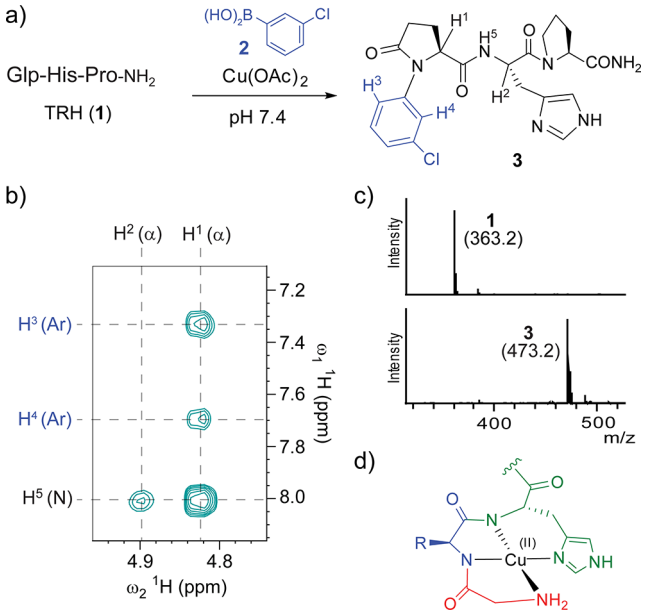


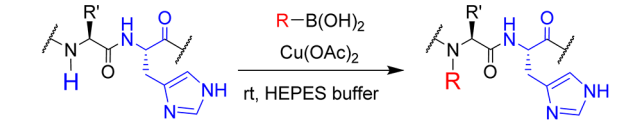
Figure 1. (a) Modification of TRH tripeptide **1** with boronate **2**. Conditions: peptide **1** (400 μM), boronic acid **2** (1 mM), and copper(II) acetate (330 μM) in *N*-methylmorpholine buffer (5 mM, pH 7.4) at rt overnight. For preparative synthesis of **3**, HEPES buffer/methanol (3:2) was used as solvent to increase solubility of the boronic acid. (b) Partial NOESY spectrum of modified product **3**. (c) ESI-mass spectrum of TRH (**1**, top) and the crude modification reaction (bottom). (d) Depiction of a copper-bound ATCUN (amino terminal copper and nickel) motif, illustrating activation of amide backbone NH neighboring a histidine residue.[14−16]

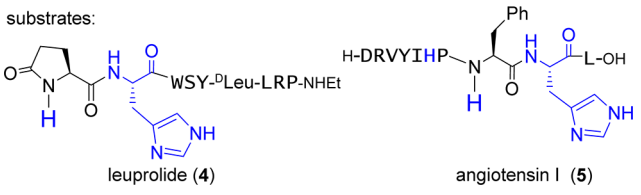
observed chemoselectivity may well be related to this histidine-directed activation of $i-1$ N−H bonds through copper amidate formation.

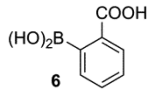
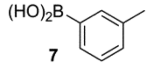
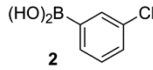
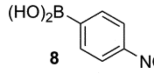
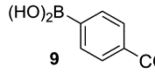
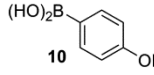
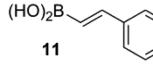
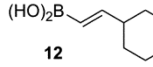
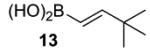
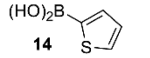
The modification reaction exhibits significant scope with respect to the boronate reagent (Table 1). Leuprolide (**4**) is a histidine-containing peptide containing an N-terminal pyroglutamate (Glp) residue. Modification of **4** provides a single modified product with efficiency similar to TRH (**1**) and couples effectively with a broad range of arylboronates and alkenylboronates as well. The success of a broad range of arylboronates suggests that electronic effects do not significantly affect the efficiency of the process (entry 1−6). Only 2-carboxyphenyl- and 2-thienyl-substituted boronates resulted in minimal coupling products (entry 1, 10). To distinguish among boronate reagents, we also considered reactions of angiotensin I (**5**), which we found to be a significantly more sluggish peptide. Though arylboronate reactivity is limited, alkenylboronates are significantly more reactive toward **5**, possibly due to the decreased steric demand of the alkenyl reagent relative to aryl reagents (entry 7−9). The increased efficiency of alkenylboronate reactivity also allows leuprolide modification at significantly lower concentrations (entry 8). Conversion of 99% was observed at significantly lower concentrations of all reagents (peptide, 20 μM; $Cu(OAc)_2$, 33 μM; boronic acid, 100 μM).

Analysis of modification reactions by MS/MS verifies that copper-mediated modification occurs consistently at $i-1$ residues neighboring histidine for both aryl- and alkenylboronates. For neuromedin B modification with alkenylboronate **12**, we observed clean modification to a singly modified product (Figure 2a). The secondary ion spectrum of the

Table 1. Scope of Boronate Reagents[a]





entry	boronate	convn **4** (%)[b]	convn **5** (%)[b]
1	 **6**	24	0
2	 **7**	quant	21
3	 **2**	quant	20
4	 **8**	quant	4
5	 **9**	quant	0
6	 **10**	95	2
7	 **11**	quant	38
8	 **12**	quant (99)[c]	44
9	 **13**	quant	23
10	 **14**	3	0

[a]Modification of peptides **4** and **5** with boronate reagents. Conditions: peptide (100 μM), boronic acid (1 mM), and copper(II) acetate (1 mM) in HEPES buffer (0.1 M, pH 7.4) at rt overnight. [b]Relative reactivity, as judged by conversion (MALDI−MS of the crude reaction). MS/MS analysis for leuprolide indicates modification at Glp1 (Supporting Information). For angiotensin I, the presence of two histidines (H6 and H9) complicates analysis; MS/MS supports F8 (neighboring H9) as a major site of modification, though a mixture of two products is possible (see Supporting Information for details). [c]Reaction conducted at decreased concentration: peptide (20 μM), $Cu(OAc)_2$ (33 μM), and boronic acid (100 μM).

modified peptide is shown in Figure 2c. A series of *y*- and *b*-ions confirms Gly7, which immediately precedes His8, as the site of modification. In the same manner, MS/MS data of leuprolide (**4**) modified with **2** displayed a similar series of *y*-ions and *b*-ions consistent with modification at Glp, together with a modified a_1 fragment ion. Modified fragments exhibit the telltale isotopic signature of the chlorine atom in the boronic acid (Supporting Information). Finally, luteinizing hormone-releasing hormone (LHRH) and the tripeptide *N*-Hippuryl-His-Leu were also modified (Figure 2d,e), and MS/MS spectra

DOI: 10.1021/jacs.6b03390
J. Am. Chem. Soc. 2016, 138, 7472−7475

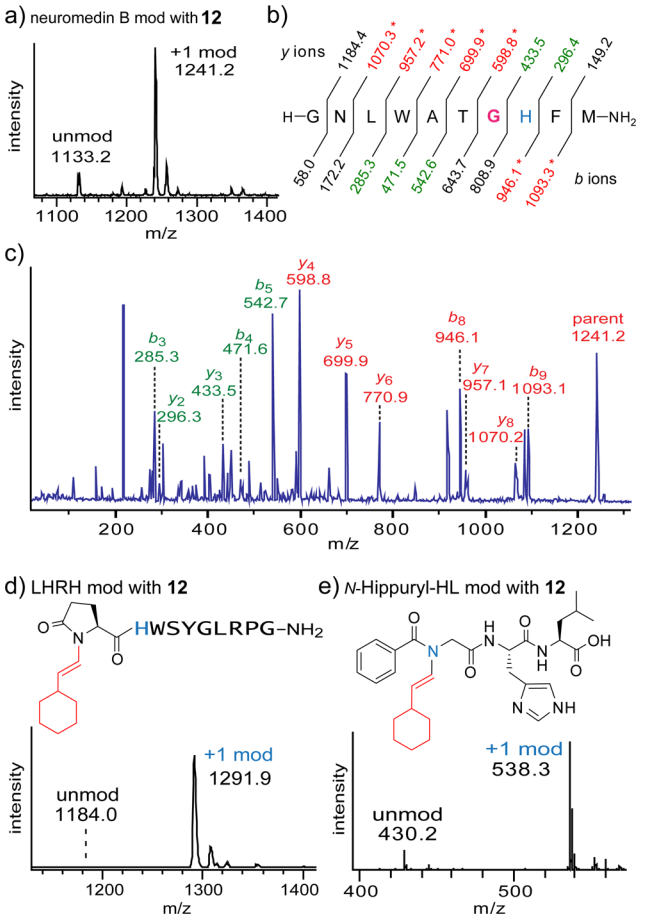


Figure 2. (a) MALDI-MS spectrum of crude modification reaction with neuromedin B and **12**. Conditions: neuromedin B (100 μM), boronic acid **13** (1 mM), and copper(II) acetate (400 μM) in NMM buffer (5 mM, pH 7.4) at rt overnight. (b) Sequence and fragmentation ladder of neuromedin B modified with **12** at Gly7. Observed *b* ions and *y* ions are shown in color. Fragments with a modification are noted with "*". (c) MALDI–MS/MS spectrum of neuromedin B modified with **12** with selected peaks annotated. (d) MALDI–MS of crude modification reaction with luteinizing hormone-releasing hormone (LHRH) and **12**. Conditions: LHRH (100 μM), boronic acid **12** (1 mM), and copper(II) acetate (1 mM) in HEPES buffer (0.1 M, pH 7.4) at rt overnight. (e) ESI–MS of crude modification reaction with *N*-Hippuryl-His-Leu and boronate **12**. Conditions: *N*-Hippuryl-His-Leu (100 μM), boronic acid **12** (1 mM), and copper(II) acetate (330 μM) in NMM buffer (5 mM, pH 7.4) at rt overnight.

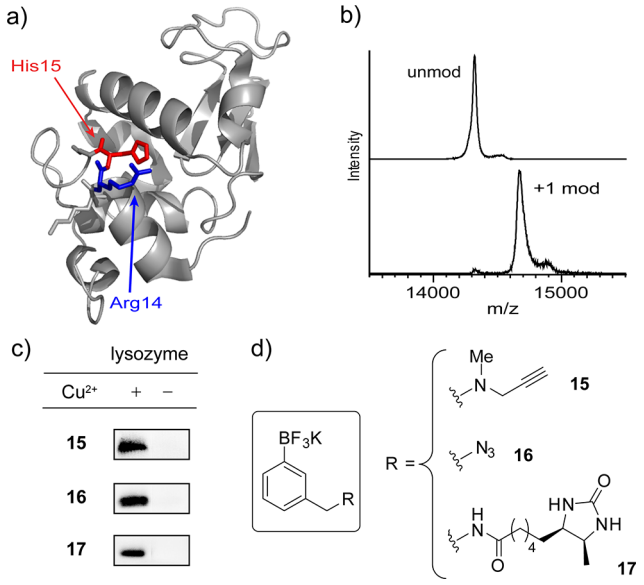


Figure 3. Modification of lysozyme with borates **15**–**17**. Conditions: lysozyme (20 μM), boronic acid **15**–**17** (1 mM), and copper(II) acetate (1 mM) in HEPES buffer (0.1 M, pH 7.4) at rt overnight. (a) Structure of lysozyme (PDB code: 1HEL) with its solitary histidine residue (His15) and neighboring Arg14 depicted explicitly. (b) MALDI–MS of lysozyme (top) and lysozyme modified with **17** after dialysis and affinity purification to remove unmodified lysozyme (bottom). (c) Blotting analysis of lysozyme modification with borate reagents **15**–**17**. Reaction mixture was analyzed by chemical blotting[29] (**15** and **16**) or Western blotting (**17**). (d) Structure of borate reagents with affinity handles.

for these peptides are also consistent with modification adjacent to histidine, at Glp and glycine, respectively.

Finally, we turned our attention to lysozyme (~14 kDa) to assess the potential for modification of proteins. In this case, modification was conducted with trifluoroborate salts **15**, **16**, or **17**, which enabled subsequent elaboration or affinity-based separation (Figure 3). Copper-dependent modification with all three reagents **15**, **16**, or **17** was readily visualized on a blot membrane (Figure 3c).[29] Modification proceeded with partial conversion (~25%), and pure lysozyme modified with desthiobiotin-BF_3K **17** could be obtained after dialysis and affinity purification using the introduced desthiobiotin handle (Figure 3b). Mass spectrometry confirmed modification and verified that a single modification had taken place, consistent with the lysozyme sequence that has only a single histidine residue (His15, Figure 3a).

These studies demonstrate selective modification of backbone N–H bonds with aryl- or alkenyl-boronate reagents, directed by a neighboring histidine residue. The chemistry provides a reactivity site unique from side-chain-based conjugation techniques. The product structures, most notably *N*-alkenyl derivatives, have unique reactivity, but would be difficult or impossible to obtain by other methods. The reaction conditions, aqueous solutions at neutral pH and ambient temperature in common physiological buffers, are noteworthy. These studies indicate that modification is selective, at least some sequences are highly efficient, and even a large protein is a suitable substrate. As histidine often plays a crucial role in the active sites of enzymes and the metal-binding motifs of metalloproteins, this reactivity could prove useful as a tool to understand, perturb, or inactivate biological function of these proteins.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b03390.

Experimental procedures, characterization, and additional MS/MS data; additional reaction screening tables (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*zb1@rice.edu

DOI: 10.1021/jacs.6b03390
J. Am. Chem. Soc. 2016, 138, 7472–7475

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

M.B.M. was supported by a Ruth L. Kirchstein National Service Award (NIH F31CA180696). We acknowledge support from the Robert A. Welch Foundation Research Grant C-1680 (to Z.T.B.), from the National Science Foundation under grant number CHE-1055569 (to Z.T.B.).

■ REFERENCES

(1) Stephanopoulos, N.; Francis, M. B. *Nat. Chem. Biol.* **2011**, *7*, 876.
(2) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974.
(3) Datta, S.; Bayer, A.; Kazmaier, U. *Org. Biomol. Chem.* **2012**, *10*, 8268.
(4) Miller, S. A.; Griffiths, S. L.; Seebach, D. *Helv. Chim. Acta* **1993**, *76*, 563.
(5) Deska, J.; Kazmaier, U. *Curr. Org. Chem.* **2008**, *12*, 355.
(6) Chatterjee, J.; Rechenmacher, F.; Kessler, H. *Angew. Chem., Int. Ed.* **2013**, *52*, 254.
(7) Liu, C. C.; Schultz, P. G. *Annu. Rev. Biochem.* **2010**, *79*, 413.
(8) Subtelny, A. O.; Hartman, M. C. T.; Szostak, J. W. *J. Am. Chem. Soc.* **2008**, *130*, 6131.
(9) Aurelio, L.; Hughes, A. B. In *Amino Acids, Peptides and Proteins in Organic Chemistry: Origins and Synthesis of Amino Acids*; Hughes, A. B., Ed.; Wiley-VCH Verlag GmbH & Co: Weinheim, Germany, 2009; Vol. *1*.
(10) Kaljuste, K.; Undén, A. *Int. J. Pept. Protein Res.* **1993**, *42*, 118.
(11) Chatterjee, J.; Laufer, B.; Kessler, H. *Nat. Protoc.* **2012**, *7*, 432.
(12) Turner, R. A.; Hauksson, N. E.; Gipe, J. H.; Lokey, R. S. *Org. Lett.* **2013**, *15*, 5012.
(13) Ranganathan, D.; Vaish, N. K.; Shah, K. *J. Am. Chem. Soc.* **1994**, *116*, 6545.
(14) Jin, Y.; Lewis, M. A.; Gokhale, N. H.; Long, E. C.; Cowan, J. A. *J. Am. Chem. Soc.* **2007**, *129*, 8353.
(15) Choi, Y.-A.; Keem, J. O.; Kim, C. Y.; Yoon, H. R.; Heo, W. D.; Chung, B. H.; Jung, Y. *Chem. Sci.* **2015**, *6*, 1301.
(16) Donaldson, L. W.; Skrynnikov, N. R.; Choy, W.-Y.; Muhandiram, D. R.; Sarkar, B.; Forman-Kay, J. D.; Kay, L. E. *J. Am. Chem. Soc.* **2001**, *123*, 9843.
(17) Minus, M. B.; Liu, W.; Vohidov, F.; Kasembeli, M. M.; Long, X.; Krueger, M.; Stevens, A.; Kolosov, M. I.; Tweardy, D. J.; Redell, M. S.; Ball, Z. T. *Angew. Chem., Int. Ed.* **2015**, *54*, 13085.
(18) Chen, Z.; Vohidov, F.; Coughlin, J. M.; Stagg, L. J.; Arold, S. T.; Ladbury, J. E.; Ball, Z. T. *J. Am. Chem. Soc.* **2012**, *134*, 10138.
(19) Popp, B. V.; Ball, Z. T. *Chem. Sci.* **2011**, *2*, 690.
(20) Chen, Z.; Popp, B. V.; Bovet, C. L.; Ball, Z. T. *ACS Chem. Biol.* **2011**, *6*, 920.
(21) Popp, B. V.; Ball, Z. T. *J. Am. Chem. Soc.* **2010**, *132*, 6660.
(22) Xu, H.-J.; Zhao, Y.-Q.; Feng, T.; Feng, Y.-S. *J. Org. Chem.* **2012**, *77*, 2878.
(23) Combs, A. P.; Saubern, S.; Rafalski, M.; Lam, P. Y. S. *Tetrahedron Lett.* **1999**, *40*, 1623.
(24) Lam, P. Y. S.; Clark, C. G.; Saubern, S.; Adams, J.; Winters, M. P.; Chan, D. M. T.; Combs, A. *Tetrahedron Lett.* **1998**, *39*, 2941.
(25) Rossi, S. A.; Shimkin, K. W.; Xu, Q.; Mori-Quiroz, L. M.; Watson, D. A. *Org. Lett.* **2013**, *15*, 2314.
(26) Chan, D. M. T.; Monaco, K. L.; Wang, R.-P.; Winters, M. P. *Tetrahedron Lett.* **1998**, *39*, 2933.
(27) Xu, J.; Wang, X.; Shao, C.; Su, D.; Cheng, G.; Hu, Y. *Org. Lett.* **2010**, *12*, 1964.
(28) Gokhale, N. H.; Cowan, J. A. *Chem. Commun.* **2005**, 5916.
(29) Ohata, J.; Vohidov, F.; Ball, Z. T. *Mol. BioSyst.* **2015**, *11*, 2846.

DOI: 10.1021/jacs.6b03390
J. Am. Chem. Soc. 2016, 138, 7472−7475