Copper-mediated peptide arylation selective for the N-terminus†

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Polypeptides present remarkable selectivity challenges for chemical methods. Amino groups are ubiquitous in polypeptide structure, yet few paradigms exist for reactivity and selectivity in arylation of amine groups. This communication describes the utilization of boronic acid reagents bearing certain o-electron withdrawing groups for copper-mediated amine arylation of the N-terminus under mild conditions and primarily aqueous solvent. The method adds to the toolkit of boronic acid reagents for polypeptide modification under mild conditions in water that shows complete selectivity for the N-terminus in the presence of lysine side chains.

Results and discussion

Remarkably, primary amine groups of lysine side chain were completely unreactive (Fig. 1d), and an N-terminally acetylated analog of a reactive sequence resulted in no modification, consistent with reaction at an amino group. MALDI-MS/MS (Fig. S27 and S28†) verified N-terminal reactivity in these cases. The structure of modified peptide 5a was confirmed by MALDI-MS/MS (Fig. 1f and h) and ²⁷N HSQC experiments (Fig. 1g), and MALDI-MS/MS also established N-terminal selectivity for peptides with multiple amino groups.

Encouraged by these observations, we set out to understand the scope of the reaction conditions using a model peptide, 5 (Table 1). Yields were determined by HPLC analysis with an internal standard (see ESI and Fig. S3† for details). For reactions...
with peptide 5, pH = 7.0 was best; increasing or decreasing pH resulted in decreased yields (entry 1, 3–4). Pleasantly, the reaction was scalable, and the arylated peptide (entry 2) was isolated in 68% yield, similar to that observed by HPLC on small scale under identical conditions.

While the reaction can be performed under strictly aqueous conditions (entry 5), the addition of an organic cosolvent improved yields (entry 7). The nature of the buffer impacts reaction efficiency. The yield increased to near-quantitative levels (97%) for reactions in HEPES buffer (entry 10), while Tris, which contains a primary amine, was a poor buffer choice (entry 9).

Examining the scope of boronic acids revealed a structure–reactivity relationship (Fig. 2). Arylation products with peptide 2 were observed only with select electron-withdrawing *ortho* substituents: sulfonamide group (1b–c), sulfone group (1d) and halogen groups (1e–i). This trend is quite different from that observed with metal-catalyzed reactions of boronic acids with backbone amide or cysteine side chains. Additional substitution at distal positions (1c, 1g, 1i) is tolerated. The successful coupling with 1i, for example, introduces an arylbromide handle for later elaboration. The reaction has a strict requirement for *ortho* substitution (1l). Surprisingly, no product was

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**Table 1** Scope of the reaction conditions

| Entry | pH  | Buffer | Cosolvent | Yield\(^a\) (%) |
|-------|-----|--------|-----------|-----------------|
| 1     | 6.0 | NMM    | 30% TFE   | 15              |
| 2     | 7.0 | NMM    | 30% TFE   | 66\(^b\) (68)\(^c\) |
| 3     | 8.0 | NMM    | 30% TFE   | 33              |
| 4     | 9.0 | NMM    | 30% TFE   | 18              |
| 5     | 7.0 | NMM    | None      | 53              |
| 6     | 7.0 | NMM    | 30% DMSO  | 70              |
| 7     | 7.0 | NMM    | 30% MeCN  | 87              |
| 8     | 7.0 | NMM    | 40% MeCN  | 44              |
| 9     | 7.0 | Tris   | 20% MeCN  | 11              |
| 10    | 7.0 | HEPES  | 20% MeCN  | 97              |

\(^a\) Yield calculated by RP-HPLC. \(^b\) 5 mg scale reaction. \(^c\) Isolated yield.
observed with an ortho-nitro group, despite the excellent reactivity of this compound in both cysteine[a] and amide N–H[+] arylation. These observations implicate a substantially different reactivity type in the present N-terminal reactivity.26,37

To determine the tolerance of the reaction for different N-terminal residues, we synthesized variants at the N-terminal residue (5–13, Table 2). The reaction tolerates a wide variety of N-terminal residues (entries 1–5, 7–11), including bulky residues (tryptophan, valine, and leucine), charged residues (arginine, aspartate) and glycine. Proline, which contains a secondary amine (entry 6), was not tolerated. In all cases, only a single product is observed. HPLC analysis shows no evidence of coupling at sites other than the N-terminus, or of any side products, even for peptides with potentially reactive N-terminal side chains (i.e. 4, 8) (see Fig. S6–S13†). The modest yields observed in a few cases (i.e. 8, 12) are the result of incomplete conversion or, more commonly, partial starting material decomposition into unknown species.

Reactivity at N-terminal amino groups in the presence of lysine side-chain amines is an interesting chemoselectivity. We hypothesized that the origin of chemoselectivity could be either pKₐ differences* or the intermediacy of a chelation complex (Table 2, at right) of the N-terminal amine, not possible at lysine side chains. To probe this, we examined peptides with unnatural amino acids that would require larger ring-chelate structures: β-alanine (bAla) (entry 10) and γ-aminobutyric acid (4Abu) (entry 11). Relative to glycine, these peptide variants have a higher pKₐ (Table 2).‡ We found that the bAla peptide, capable of forming a 6-membered ring chelate, retained reactivity, while the 4Abu variant—which would require at least a 7-membered ring intermediate—was unreactive. The chelation-driven selectivity model seems most in accord with these results, although additional study is warranted. In this context, it is worth noting that the reaction does not exhibit the hallmark increasing reactivity with increasing pH that is typically observed in amine functionalization governed by pKₐ.

Reactivity studies also shed light on the nature of a putative κ² copper binding. While copper binding to N-terminal sequences is well studied,19–21 canonical structures typically adopt N-bound amide structures (Table 2, B). In N-terminal arylation reported here, peptides with proline as the second amino acid, which have no amide N–H and thus cannot adopt ATCUN-like amide structures, nonetheless are competent reaction partners (Table 3, entries 1–2). This observation would

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Table 2 Scope N-terminal residues

| Entry | R₁         | Yield (a) (%) |
|-------|------------|--------------|
| 1     | Val (5)    | 97           |
| 2     | Leu (6)    | 80           |
| 3     | Phe (7)    | 50           |
| 4     | Trp (8)    | 44           |
| 5     | Arg (9)    | 96           |
| 6     | Pro (10)   | <5           |
| 7     | Ser (11)   | 93           |
| 8     | Asp (12)   | 64           |
| 9     | Gly (13)   | 96           |
| 10    | bAla (14)  | 64           |
| 11    | 4Abu (15)  | <5           |

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Table 3 Additional peptide substrate examples

| Entry | Peptide                      | Yield (b) (%) |
|-------|------------------------------|---------------|
| 1     | H–RPKPQQWFWLL–NH₂(16)        | 45            |
| 2     | H–RPQGSPFR–OH (2)           | 36            |
| 3     | H–DRVYIHPFHL–OH (17)        | 20            |
| 4     | H–MEGVYBSPRSRVHYRNGK–OH (18)| 18            |

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*a Yield calculated by RP-HPLC. b 10 mg scale

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Fig. 3 (a) Modification of peptide 5 with boronate ester 1o and 1p. Yield calculated by RP-HPLC. (b) RP-HPLC analysis of the enzymatic degradation of angiotensin IV (5) and arylated angiotensin IV (5a) with aminopeptidase I. Conditions: 100 μM 5, 40 μM 5a, 0.08 μg mL⁻¹ aminopeptidase I, and 20 μM Co(OAc)₂ in NaOAc buffer (5 mM, pH = 6.0) at 95 °C. Internal standard 19 for quantification. (c) Peptidase activity: time course for aminopeptidase I cleavage of arylated angiotensin IV (5a, orange) and angiotensin IV (5, blue).
seem to indicate that neutral, O-bound proximal amide groups (Table 2, A) are competent species in catalysis.

We next examined reactivity of other peptide sequences (Table 3). A number of naturally occurring peptide sequences were amenable to this reaction. A 21-mer peptide indicates that the reaction tolerates quite lengthy sequences (entry 4), hinting at potential use in more demanding bioconjugation challenges.

The alkyne group is one of the most useful and general handles for manipulation of biomolecules, and we were gratified to find that boronic acids containing an alkyne handle engendered peptide stability towards enzymatic degradation.

Using aminopeptidase I, an enzyme that liberates the N-terminal residue from peptides and proteins, we followed the reaction tolerates quite lengthy sequences (entry 4), hinting that boronic acids containing an alkyne handle were amenable to this reaction. A 21-mer peptide indicates that the product aniline is expected to be uncharged under physiological conditions. We decided to investigate whether the reaction significantly alters the N-terminal charge state, since the product aniline is expected to be uncharged under physiological conditions. We decided to investigate whether the reaction alters the N-terminal charge state.

Using aminopeptidase I, an enzyme that liberates the N-terminal residue from peptides and proteins, we followed the reaction of both angiotensin IV 5 and its arylated analog 5a with Pfu aminopeptidase I and found that after 10 minutes 5 was completely consumed (Fig. 3b) while the 5a remained stable even after 2 h incubation (Fig. 3c).

Arylation significantly alters the N-terminal charge state, since the product aniline is expected to be uncharged under physiological conditions. We decided to investigate whether the charge and structural perturbation afforded by the N-arylation engendered peptide stability towards enzymatic degradation. Using aminopeptidase I, an enzyme that liberates the N-terminal residue from peptides and proteins, we followed the reaction of both angiotensin IV 5 and its arylated analog 5a with Pfu aminopeptidase I and found that after 10 minutes 5 was completely consumed (Fig. 3b) while the 5a remained stable even after 2 h incubation (Fig. 3c).

Conclusion

In conclusion, copper(n) salts together with boronic acids bearing ortho-sulfonamide groups induce N–H arylation that is specific for the N-terminus. The reaction proceeds under neutral conditions in water and allows arylation of a wide variety of N-terminal residues. The reactivity is indicative of a new selectivity paradigm for copper-catalyzed amine functionalization that relies on local structure.

Conflicts of interest

There are no conflicts to declare.

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References

1 N. Stephanopoulos and M. B. Francis, Nat. Chem. Biol., 2011, 7, 876–884.
2 C. D. Spicer and B. G. Davis, Nat. Commun., 2014, 5, 4740.
3 J. Ohata, M. B. Minus, M. E. Abernathy and Z. T. Ball, J. Am. Chem. Soc., 2016, 138, 7472–7475.
4 J. Ohata, Y. Zeng, L. Segatori and Z. T. Ball, Angew. Chem., Int. Ed., 2018, 57, 4015–4019.
5 K. Hanaya, M. K. Miller and Z. T. Ball, Org. Lett., 2019, 21, 2445–2448.
6 J. Ohata, S. C. Martin and Z. T. Ball, Angew. Chem., Int. Ed., 2019, 58, 6176–6199.
7 J. Ruiz-Rodriguez, F. Albericio and R. Lavilla, Chem. Eur. J., 2010, 16, 1124–1127.
8 K. Hanaya, J. Ohata, M. K. Miller, A. E. Mangubat-Medina, M. J. Swierczynski, D. C. Yang, R. M. Rosenthal, B. V. Popp and Z. T. Ball, Chem. Commun., 2019, 55, 2841–2844.
9 K. K.-Y. Kung, H.-M. Ko, J.-F. Cui, H.-C. Chong, Y.-C. Leung and M.-K. Wong, Chem. Commun., 2014, 50, 11899–11902.
10 M. S. Messina, J. M. Stauber, M. A. Waddington, A. L. Rheingold, H. D. Maynard and A. M. Spokony, J. Am. Chem. Soc., 2018, 140, 7065–7069.
11 E. V. Vinogradova, C. Zhang, A. M. Spokony, B. L. Pentelute and S. L. Buchwald, Nature, 2015, 526, 687–691.
12 S. D. Tilley and M. B. Francis, J. Am. Chem. Soc., 2006, 128, 1080–1081.
13 H. G. Lee, G. Lautrette, B. L. Pentelute and S. L. Buchwald, Angew. Chem., Int. Ed., 2017, 56, 3177–3181.
14 D. Ma, Y. Zhang, J. Yao, S. Wu and F. Tao, J. Am. Chem. Soc., 1998, 120, 12459–12467.
15 Z. Lu and R. J. Twieg, Tetrahedron Lett., 2005, 46, 2997–3001.
16 N. Narendra and S. Velmathi, Tetrahedron Lett., 2009, 50, 5159–5161.
17 F. Ma, X. Xie, L. Ding, J. Gao and Z. Zhang, Tetrahedron, 2011, 67, 9405–9410.
18 S. M. King and S. L. Buchwald, Org. Lett., 2016, 18, 4128–4131.
19 Y. Jin, M. A. Lewis, N. H. Gokhale, E. C. Long and J. A. Cowan, J. Am. Chem. Soc., 2007, 129, 8353–8361.
20 Y.-A. Choi, J. O. Keem, C. Y. Kim, H. R. Yoon, W. D. Heo, B. H. Chung and Y. Jung, Chem. Sci., 2015, 6, 1301–1307.
21 L. W. Donaldson, N. R. Skrynnikov, W.-Y. Choy, D. R. Muhandiram, B. Sarkar, J. D. Forman-Kay and L. E. Kay, J. Am. Chem. Soc., 2001, 123, 9843–9847.
22 A. E. Mangubat-Medina, S. C. Martin, K. Hanaya and Z. T. Ball, J. Am. Chem. Soc., 2018, 140, 8401–8404.
23 H. B. F. Dixon, J. Protein Chem., 1984, 3, 99–108.
24 C. Cennamo, Naturwissenschaften, 1954, 41, 39.
25 J. M. Gilmore, R. A. Scheck, A. P. Esser-Kahn, N. S. Joshi and M. B. Francis, Angew. Chem., Int. Ed., 2006, 45, 5307–5311.
26 L. S. Witus, C. Neto, K. Palla, E. M. Muehl, H. Weng, A. T. Iavarone and M. B. Francis, J. Am. Chem. Soc., 2013, 135, 17223–17229.
27 M. Zhang, X. Zhang, J. Li, Q. Guo and Q. Xiao, Chin. J. Chem., 2011, 29, 1715–1720.
28 D. Chen, M. M. Disotuar, X. Xiong, Y. Wang and D. H.-C. Chou, Chem. Sci., 2017, 8, 2717–2722.
29 J. I. MacDonald, H. K. Munch, T. Moore and M. B. Francis, Nat. Chem. Biol., 2015, 11, 326–331.
30 E. Reid, Nature, 1951, 168, 955.
31 G. Gaudriault and J.-P. Vincent, Peptides, 1992, 13, 1187–1192.
32 I. Sélo, L. Négroni, C. Crémoin, J. Grassi and J. M. Wal, J. Immunol. Methods, 1996, 199, 127–138.
33 S. Schoffelen, M. B. van Eldijk, B. Rooijakkers, R. Raijmakers, A. J. R. Heck and J. C. M. van Hest, Chem. Sci., 2011, 2, 701–705.
34 A. C. Obermeyer, J. B. Jarman and M. B. Francis, *J. Am. Chem. Soc.*, 2014, 136, 9572–9579.

35 Y. E. Sim, O. Nwajiobi, S. Mahesh, R. D. Cohen, M. Y. Reibarkh and M. Raj, *Chem. Sci.*, 2019, 11, 53–61.

36 M. J. West, J. W. B. Fyfe, J. C. Vantourout and A. J. B. Watson, *Chem. Rev.*, 2019, 119, 12491–12523.

37 J. C. Vantourout, H. N. Miras, A. Isidro-Llobet, S. Sproules and A. J. B. Watson, *J. Am. Chem. Soc.*, 2017, 139, 4769–4779.

38 T. J. Sereda, C. T. Mant, A. M. Quinn and R. S. Hodges, *J. Chromatogr. A*, 1993, 646, 17–30.

39 H. K. Hall, *J. Am. Chem. Soc.*, 1957, 79, 5441–5444.