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Selective editing of a peptide skeleton *via* C–N bond formation at N-terminal aliphatic side chains†

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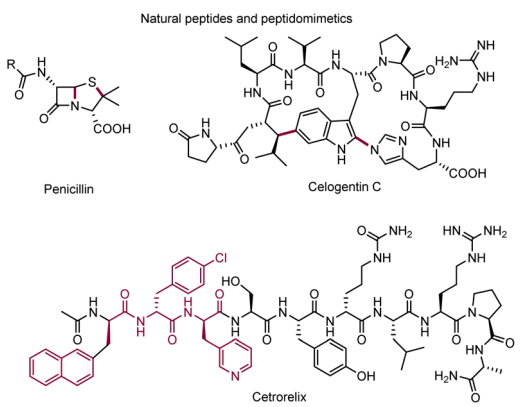
The applications of peptides and peptidomimetics have been demonstrated in the fields of therapeutics, diagnostics, and chemical biology. Strategies for the direct late-stage modification of peptides and peptidomimetics are highly desirable in modern drug discovery. Transition-metal-catalyzed C–H functionalization is emerging as a powerful strategy for late-stage peptide modification that is able to construct functional groups or increase skeletal diversity. However, the installation of directing groups is necessary to control the site selectivity. In this work, we describe a transition metal-free strategy for late-stage peptide modification. In this strategy, a linear aliphatic side chain at the peptide N-terminus is cyclized to deliver a proline skeleton *via* site-selective δ-C(sp^3)–H functionalization under visible light. Natural and unnatural amino acids are demonstrated as suitable substrates with the transformations proceeding with excellent regio- and stereo-selectivity.

Introduction

Late-stage modification of peptides and peptidomimetics exhibited its potential utility in the field of therapeutic and diagnostic agents, drug candidates, and biological tool compounds (Scheme 1).[1–6] Although peptides are abundant in nature, non-natural peptides often demonstrate improved biological and pharmaceutical properties compared with their parent analogs. Therefore, straightforward and efficient methods for the late-stage modification of diverse peptides are highly desirable. To date, various methodologies,[7–17] including methylation,[12] phosphorylation,[13] S-alkylation/arylation,[14,15] and glycosylation[16] of polar functional groups, have been revealed for the successful chemical modification of natural peptides. Inspired by the development of direct C–H bond functionalization methods, site-specific C–H functionalization has recently emerged as a valuable tool for the late-stage modification of peptides and proteins.[18–38] For examples, electrophilic aromatic substitution,[18,19] Rh-catalyzed carbenoid insertion,[21] Pd-catalyzed arylation/alkenylation,[22–26] and photo-induced His-alkylation[27] have all been used in this capacity.

The modification of aliphatic side chains is a greater challenge than the modification of aromatic residues. In pioneering work by Li and coworkers, copper-catalyzed alkynylation of the α-C–H bond of *N*-aryl substituted glycine was used to selectively modify short peptides in a racemic fashion.[39] Wang and Xu reported the alkylation of *N*-phenyl glycine residues in peptides *via* the decarboxylation of *N*-hydroxyphthalimide esters using a copper photocatalyst.[40] Shi and coworkers reported a chemoselective peptide modification strategy involving a photocatalytic tryptophan β-position conjugation;[41] oxidation of indole nitrogen initiates a single-electron transfer from the tryptophan β-position to achieve the conjugation. However, these methods are mainly limited to weak C–H bonds, such as benzylic C–H bonds or those adjacent to a heteroatom (Scheme 2a).

Recently, directing group assisted transition-metal catalyzed functionalization of inert C(sp^3)–H bonds within aliphatic residues of peptides has shown enormous potential, with applications in biochemistry and drug discovery.[44–59] Innovative contributions have been made by the groups of Yu,[42,43] Ackermann,[44–46] and Shi.[47,48] Both radical-induced and directing group assisted transition-metal catalyzed modifications functionalize the peptide at a single site (*i.e.*, point modifications) (Scheme 2b). To improve the skeletal diversity of peptides (Scheme 2c), Noisier/Albericio[49]



Scheme 1 Natural peptides/peptidomimetics.

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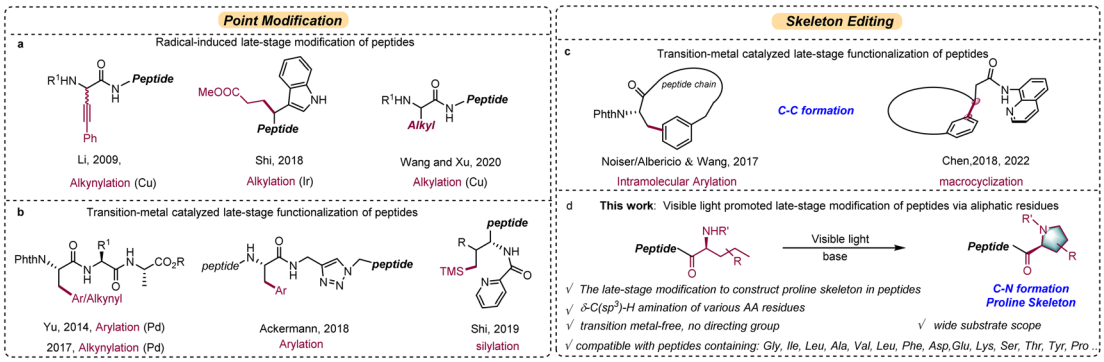
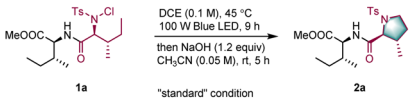


Scheme 2 The late-stage modification of peptides *via* C(sp^3)–H activation.

Table 1 Effect of reaction parameters[a]



Entry	Variations from "standard" conditions	Yield[b] (%)
1	None	89[c](85)
2	$CHCl_3$ instead of DCE	32
3	DCM instead of DCE	85
4	THF instead of DCE	43
5	CH_3CN instead of DCE	83
6	$PhCF_3$ instead of DCE	73
7	DMF instead of DCE	56
8	Added $Ru(bpy)_2Cl_2$	89
9	Added $Ir(ppy)_3$	88
10	Added $[Ir(ppy)_2dtbpy]PF_6$	88
11	Added eosin Y	89
12	Under dark	0

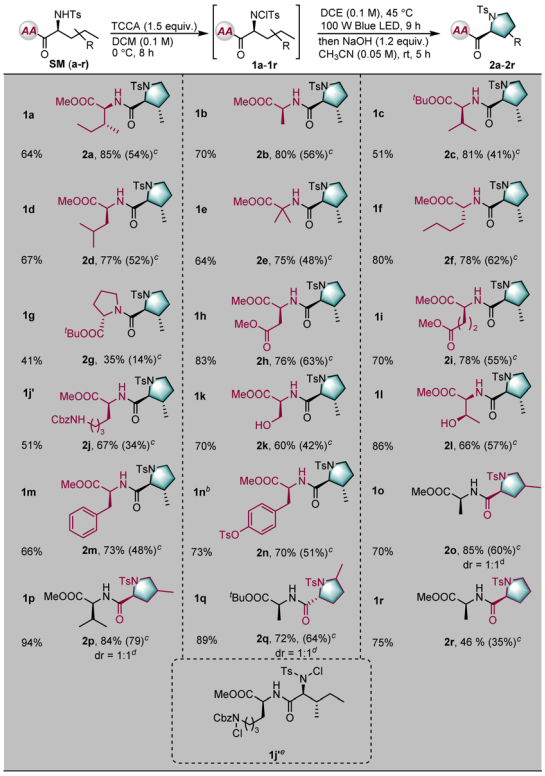
[a] Reaction was conducted on a 0.1 mmol scale. [b] Yield was determined by ^{1}H NMR. [c] Isolated yield in parentheses.

and Wang[50] independently reported intramolecular β-C(sp^3)–H arylations for peptide macrocyclization. In related work, Chen and coworkers[51] reported an elegant protocol for the preparation of constrained cyclic peptides *via* Pd-catalyzed intramolecular C(sp^3)–H arylation. Because these modifications are achieved *via* transition metal catalysis, residual metal impurities may affect the biocompatibility of the peptide product and may cause toxicity in pharmaceutical applications. In addition, the installation of a directing group is necessary to control the reactivity and selectivity. Furthermore, the reaction scope is restrictive, occurring predominantly at the β- or γ-C(sp^3)–H position of a small set of amino acid residues (mainly alanine). Skeletal modifications involving C–N bond formation are rarely reported. Continuing our studies on radical-transfer reactions,[52–55] here we describe a transition-metal free strategy for late-stage peptide modification (Scheme 2d). In our strategy, an N-terminal linear aliphatic side chain cyclizes to construct a proline skeleton *via* site-selective δ-C(sp^3)–H functionalization under visible light. The advantages of this protocol include: (1) transition metal-free conditions that cause skeletal modification *via* C–N bond formation; (2) compatibility with a broad range of amino acid residues; (3) no extra directing groups are required to promote reaction selectivity and activity.

Results and discussion, experimental

To begin our investigation, we used intermediate **1a** as a model substrate (Table 1). After examining a range of reaction parameters, we were pleased to find that the intermediate **1a** can be easily

Table 2 Scope of amino acids at the C-terminus of dipeptides[a]



[a] Unless otherwise noted, the reaction conditions were as follows: **SM** (0.2 mmol), trichloroisocyanuric acid (TCCA, 1.5 equiv.), DCM 2.0 mL (c = 0.1 M), 0 °C, and 8 h; **1** (0.1 mmol), DCE 1.0 mL, blue LED (100 W), 45 °C, and 9 h; then NaOH (1.2 equiv.), CH_3CN (2.0 mL), rt, and 5 h. [b] 2.5 mmol of **SM** (n) was used, c = 0.1 M. [c] In parentheses was the calculated total yield. [d] dr was determined by crude ^{1}H NMR. [e] Dichloro-product **1j'** was obtained.

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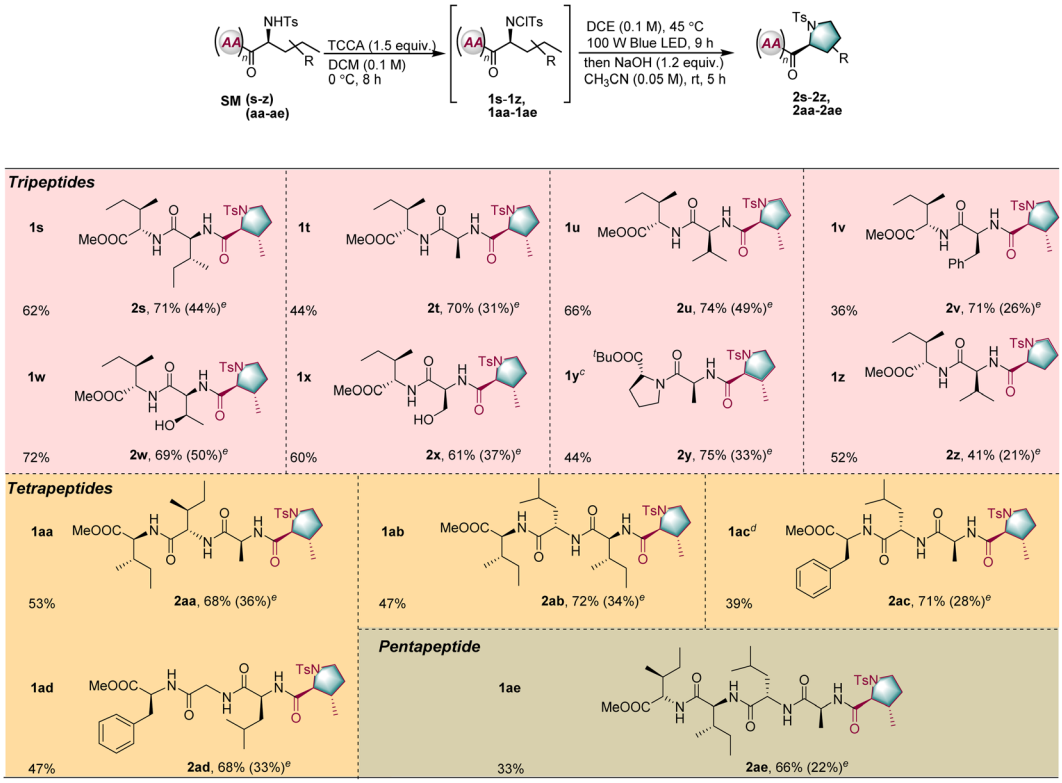
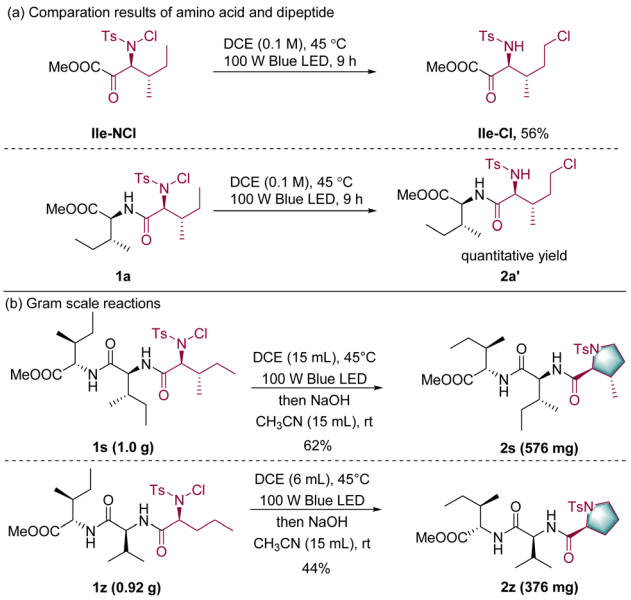
Table 3 Late-stage modification of polypeptides[a,b]



[a] Unless otherwise noted, the reaction conditions were as follows: for tripeptides **SM(s–z)** 0.4 mmol, trichloroisocyanuric acid (TCCA, 1.5 equiv.), DCM 4.0 mL ($c = 0.1$ M), 0 °C, and 8 h; **1** (0.1 mmol), DCE 1.0 mL, blue LED (100 W), 45 °C, and 9 h; then NaOH (1.2 equiv.), CH_3CN (2.0 mL), rt, and 5 h. For tetrapeptides **SM(aa–ad)** or pentapeptide **SM(ae)** 0.1 mmol, trichloroisocyanuric acid (TCCA, 1.5 equiv.), DCM 20.0 mL ($c = 0.005$ M), 0 °C, and 8 h; **1** (0.1 mmol), DCE 1.0 mL, blue LED (100 W), 45 °C, and 9 h; then NaOH (1.2 equiv.), CH_3CN (2.0 mL), rt, and 5 h. [b] Isolated yield. [c] 0.4 mmol of tripeptide **SM(y)** was used. [d] 1 mmol of tetrapeptide **SM(ac)** was used. [e] In parentheses was the calculated total yield.

transferred to the desired product **2a** in 85% isolated yield using only visible light irradiation and basic conditions. Comparative experiments were conducted to test the efficiency under different conditions. Compared with other solvents (Table 1, entries 2–7), DCE proved to be the best choice for this transformation. Next, the effect of the photocatalyst was investigated. Ru-complexes, Ir-complexes, and the eosin Y organophotocatalyst gave similar results under the standard conditions (entries 8–11). Finally, we found that the transformation was inhibited in the dark; the starting material was recovered in almost quantitative yield (entry 12, 99%).

With optimized reaction conditions in hand, the tolerance of the reaction to various peptide substrates was examined. The peptide substrates were generally prepared through the condensation of amino acids and/or short peptides under the solvent conditions. First, to test the selectivity and efficiency of the transformation, various amino acid derivatives were installed in the C-terminal position of a dipeptide substrate (Table 2). Amino acid derivatives of isoleucine, alanine, valine, leucine, 2-amino-isobutyric acid, norleucine, proline, aspartate, glutamic acid, lysine, serine, threonine, phenylalanine, and tyrosine were well-tolerated, giving the desired modified peptides in excellent diastereoselectivities and good yields (**SMa–SMn**). Notably, when



Scheme 3 (a) Comparation results of amino acids and dipeptides; (b) gram scale reactions.

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dipeptide **SMj** was used as a substrate, the cyclization reaction occurred exclusively at the N-terminal position; the lysine side chain was untouched by the reaction, thereby demonstrating the unique selectivity of this transformation (**2j**). Furthermore, the aliphatic residues at the N–terminal positions were also examined (**SMo–SMq**). Cyclization occurred at both primary and secondary C–H bonds, selectively yielding the corresponding proline skeleton derivatives. Significantly, the linear unnatural amino acid 2-aminopentanoic acid was converted into a natural proline derivative, albeit in moderate yield, indicating the power of this method (**2r**).

Encouraged by the successful results obtained for dipeptides, the application of the reaction to oligopeptides consisting of three to five amino acid units was explored (Table 3, **SMs–z**, and **SMaa–ae**). Because of the larger number of peptide bonds, selective modification of longer peptides is more challenging. However, for all peptides, the transformation proceeded in moderate to good yield with excellent diastereoselectivity under the standard conditions (**2s–2z** and **2aa–2ae**). Unprotected serine or threonine moieties adjacent to the N-terminal position were not detrimental to the reaction (**2w** and **2x**). Furthermore, the phenylalanine moiety, with its highly active benzylic C–H bonds, was untouched during the transformation (**2v**).

To further understand the reactivity of this catalytic system, the reactivity of an isoleucine derivative **Ile-NCl** and the isoleucine-derived dipeptide **1a** was compared. After irradiation with visible light, dipeptide **1a** was successfully converted to the corresponding product **2a′** in quantitative yield, whereas the amino acid only yielded the corresponding product **Ile-Cl** in 56% yield; this highlights the superior reactivity of peptide substrates over single amino acids (Scheme 3a, details in the ESI†).[58] Such a superior reactivity of peptides may have resulted from the hydrogen bonding between amide and chloride in dipeptides, which could assist the C–Cl bond formation.[59] Finally, gram-scale experiments were conducted to evaluate the scalability of the transformation. Within two tripeptide substrates, the N-terminal amino acid moiety was transformed into the corresponding cyclic proline derivatives in acceptable yields (Scheme 3b).

Based on our previous studies,[52–55] and other reported research,[60] we considered that such transformation proceeded through a 1,5-hydrogen atom transfer (1,5-HAT) process as the Hofmann–Löffler reaction reported.[61,62] Under the irradiation of visible light, the homolytic cleavage of the N–Cl bond occurred to generate a nitrogen radial and chloride radical (trapped by TEMPO and detected by ESI-MS, see the ESI†). Subsequently, 1,5-HAT takes place yielding the carbon-centered radical, which could be trapped by the chloride radical to form a C–Cl bond. Then, an intramolecular nucleophilic substitution occurred under the base conditions to form the target product.

Conclusions

In summary, we have developed a visible light-promoted selective editing of peptide skeletons *via* C–N bond formation at N-terminal aliphatic side chains. A proline skeleton was constructed in peptides under such transition metal free conditions. The excellent diastereoselectivity and broad substrate scope of the reaction highlight its potential utility in biochemistry, medicinal chemistry, and/or drug discovery.

Author contributions

Y. H. and S. L. performed the experiments and analyzed the data. J. S., T. D. and W. Y. assisted the purification of compounds and analysis of data. M. Y. designed and directed the whole project and wrote the manuscript.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 K. L. Dunbar, D. H. Scharf, A. Litomska and C. Hertweck, *Chem. Rev.*, 2017, **117**, 5521–5577.
2 S. Sato and H. Nakamura, *Molecules*, 2019, **24**, 3980–3996.
3 J. C. Lewis, P. S. Coelho and F. H. Arnold, *Chem. Soc. Rev.*, 2011, **40**, 2003–2021.
4 A. Henninot, J. C. Collins and J. M. Nuss, *Med. Chem.*, 2018, **61**, 1382–1414.
5 J. Lu, Y. Li, Z. Bai, H. Lv and H. Wang, *Nat. Prod. Rep.*, 2021, **38**, 981–992.
6 S. Xu, Z. Zhao and J. Zhao, *Chin. Chem. Lett.*, 2018, **29**, 1009–1016.
7 T. Morioka, A. Nishizawa, K. Nakamura, M. Tobisu and N. Chatani, *Chem. Lett.*, 2015, **44**, 1729–1731.
8 J. N. deGruyter, L. R. Malins and P. S. Baran, *Biochemistry*, 2017, **56**, 3863–3873.
9 C. D. Spicer and B. G. Davis, *Nat. Commun.*, 2014, **5**, 4740.
10 L. R. Malins, *Curr. Opin. Chem. Biol.*, 2018, **46**, 25–32.
11 O. Boutureira and G. J. L. Bernardes, *Chem. Rev.*, 2015, **115**, 2174–2195.
12 K. K. Biggar and S. S. Li, *Nat. Rev. Mol. Cell Biol.*, 2015, **16**, 5–17.
13 R. Klingberg, J. O. Jost, M. Schümann, K. A. Gelato, W. Fischle, E. Krause and D. Schwarzer, *ACS Chem. Biol.*, 2015, **10**, 138–145.
14 D. Wang, M. Yu, N. Liu, C. Lian, Z. Hou, R. Wang, R. Zhao, W. Li, Y. Jiang, X. Shi, S. Li, F. Yin and Z. Li, *Chem. Sci.*, 2019, **10**, 4966–4972.
15 E. V. Vinogradova, C. Zhang, A. M. Spokoyny, B. L. Pentelute and S. L. Buchwald, *Nature*, 2015, **526**, 687–691.
16 D. P. Gamblin, E. M. Scanlan and B. G. Davis, *Chem. Rev.*, 2009, **109**, 131–163.
17 M. Yang, X. Wang and J. Zhao, *ACS Catal.*, 2020, **10**, 5230–5235.

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18 A. K. Mishra, R. Tessier, D. P. Hari and J. Waser, *Angew. Chem., Int. Ed.*, 2021, **60**, 17963–17968.
19 M. W. Jones, G. Mantovani, C. A. Blindauer, S. M. Ryan, X. Wang, D. J. Brayden and D. M. Haddleton, *J. Am. Chem. Soc.*, 2012, **134**, 7406–7413.
20 A. E. Mangubat-Medina, S. C. Martin, K. Hanaya and Z. T. Ball, *J. Am. Chem. Soc.*, 2018, **140**, 8401–8404.
21 J. M. Antos and M. B. Francis, *J. Am. Chem. Soc.*, 2004, **126**, 10256–10257.
22 Q. Bai, Z. Bai and H. Wang, *Org. Lett.*, 2019, **21**, 8225–8228.
23 X. Li, L. Qi, B. Li, Z. Zhao, G. He and G. Chen, *Org. Lett.*, 2020, **22**, 6209–6213.
24 J. Tan, J. Wu, S. Liu, H. Yao and H. Wang, *Sci. Adv.*, 2019, **5**, eaaw0323.
25 J. Tang, H. Chen, Y. He, W. Sheng, Q. Bai and H. Wang, *Nat. Commun.*, 2018, **9**, 3383.
26 Z. Bai and H. Wang, *Synlett*, 2020, **31**, 199–204.
27 X. Chen, F. Ye, X. Luo, X. Liu, J. Zhao, S. Wang, Q. Zhou, G. Chen and P. Wang, *J. Am. Chem. Soc.*, 2019, **141**, 18230–18237.
28 J. Ruiz-Rodríguez, F. Albericio and R. Lavilla, *Chem.–Eur. J.*, 2010, **16**, 1124–1127.
29 L. Mendive-Tapia, S. Preciado, J. García, R. Ramón, N. Kielland, F. Albericio and R. Lavilla, *Nat. Commun.*, 2015, **6**, 7160.
30 W. Gong, G. Zhang, T. Liu, R. Giri and J. Q. Yu, *J. Am. Chem. Soc.*, 2014, **136**, 16940–16946.
31 B.-B. Zhan, Y. Li, J.-W. Xu, X.-L. Nie, J. Fan, L. Jin and B.-F. Shi, *Angew. Chem., Int. Ed.*, 2018, **57**, 5858–5862.
32 Y. Zhu, M. Bauer, J. Ploog and L. Ackermann, *Chem.–Eur. J.*, 2014, **20**, 13099–13102.
33 J. Tang, Y. He, H. Chen, W. Sheng and H. Wang, *Chem. Sci.*, 2017, **78**, 4565–4570.
34 Y. Zheng and W. Song, *Org. Lett.*, 2019, **21**, 3257–3260.
35 M. Yang, X. Jiang and Z.-J. Shi, *Org. Chem. Front.*, 2015, **2**, 51–54.
36 E. Hernando, J. Villalva, Á. M. Martínez, I. Alonso, N. Rodriguez, R. G. Arrayás and J. C. Carretero, *ACS Catal.*, 2016, **6**, 6868–6882.
37 B. Li, X. Li, B. Han, Z. Chen, X. Zhang, G. He and G. Chen, *J. Am. Chem. Soc.*, 2019, **141**, 9401–9407.
38 T. J. Osberger, D. C. Rogness, J. T. Kohrt, A. F. Stepan and M. C. White, *Nature*, 2016, **537**, 214–219.
39 L. Zhao, O. Baslé and C. J. Li, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 4106–4111.
40 C. Wang, R. Qi, H. Xue, Y. Shen, M. Chang, Y. Chen, R. Wang and Z. Xu, *Angew. Chem., Int. Ed.*, 2020, **59**, 7461–7466.
41 Y. Yu, L.-K. Zhang, A. V. Buevich, G. Li, H. Tang, P. Vachal, S. L. Colletti and Z.-C. Shi, *J. Am. Chem. Soc.*, 2018, **140**, 6797–6800.
42 W. Gong, G. Zhang, T. Liu, R. Giri and J.-Q. Yu, *J. Am. Chem. Soc.*, 2014, **136**, 16940–16946.
43 T. Liu, J.-X. Qiao, M. A. Poss and J.-Q. Yu, *Angew. Chem., Int. Ed.*, 2017, **56**, 10924–10927.
44 M. Bauer, W. Wang, M. M. Lorion, C. Dong and L. Ackermann, *Angew. Chem., Int. Ed.*, 2018, **57**, 203–207.
45 W. Wang, M. M. Lorion, O. Martinazzoli and L. Ackermann, *Angew. Chem., Int. Ed.*, 2018, **57**, 10554–10558.
46 J. Wu, N. Kaplaneris, S. Ni, F. Kaltenhäuser and L. Ackermann, *Chem. Sci.*, 2020, **11**, 6521–6526.
47 B.-B. Zhan, J. Fan, L. Jin and B.-F. Shi, *ACS Catal.*, 2019, **9**, 3298–3303.
48 J.-W. Xu, Z.-Z. Zhang, W.-H. Rao and B.-F. Shi, *J. Am. Chem. Soc.*, 2016, **138**, 10750–10753.
49 A. F. M. Noisier, J. García, I. A. Ionut and F. Albericio, *Angew. Chem., Int. Ed.*, 2017, **56**, 314–318.
50 J. Tang, Y. He, H. Chen, W. Sheng and H. Wang, *Chem. Sci.*, 2017, **8**, 4565–4570.
51 X. Zhang, G. Lu, M. Sun, M. Mahankali, Y. Ma, M. Zhang, W. Hua, Y. Hu, Q. Wang, J. Chen, G. He, X. Qi, W. Shen, P. Liu and G. Chen, *Nat. Chem.*, 2018, **10**, 540–548.
52 M. Yang, B. Su, Y. Wang, K. Chen, X. Jiang, Y.-F. Zhang, X.-S. Zhang, G. Chen, Y. Cheng, Z. Cao, Q.-Y. Guo, L. Wang and Z.-J. Shi, *Nat. Commun.*, 2014, **5**, 4707.
53 D. Meng, Y. Tang, J. Wei, X. Shi and M. Yang, *Chem. Commun.*, 2017, **53**, 5744–5747.
54 Y. Tang, Y. Qin, D. Meng, C. Li, J. Wei and M. Yang, *Chem. Sci.*, 2018, **9**, 6374–6378.
55 Y. Qin, Y. Han, Y. Tang, J. Wei and M. Yang, *Chem. Sci.*, 2020, **11**, 1276–1282.
56 C. Che, Y.-N. Li, X. Cheng, Y.-N. Lu and C.-J. Wang, *Angew. Chem., Int. Ed.*, 2021, **60**, 4698–4704.
57 W. Wang, J. Wu, R. Kuniyil, A. Kopp, R. N. Lima and L. Ackermann, *Chem*, 2020, **6**, 3428–3439.
58 Q. Qin and S. Yu, *Org. Lett.*, 2015, **17**, 1894–1897.
59 K. J. Winstanley and D. K. Smith, *J. Org. Chem.*, 2007, **72**, 2803–2815.
60 A. E. Bosnidou, T. Duhamel and K. Muñiz, *Eur. J. Org. Chem.*, 2020, **40**, 6361–6365.
61 A. W. Hofmann, *Chem. Ber.*, 1883, **16**, 558–560.
62 K. Löffler and C. Freytag, *Chem. Ber.*, 1909, **42**, 3427–3431.

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