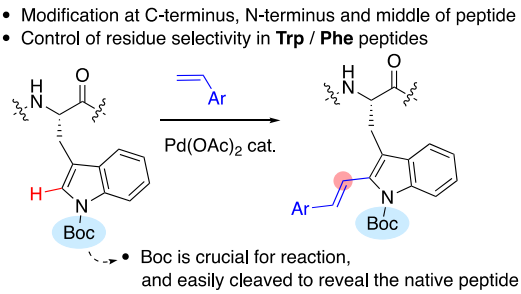
C-H olefination of tryptophan residues in peptides; control of residue selectivity and peptide–amino acid crosslinking

Myles J. Terrey, Ashley Holmes, Carole C. Perry, Warren B. Cross*

Department of Chemistry and Forensic Science, School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, U.K.

Supporting Information Placeholder



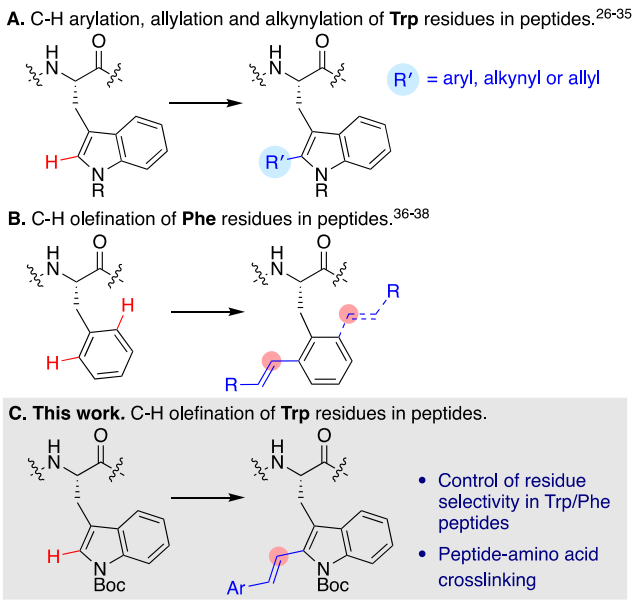
ABSTRACT: There is high-demand for new methods to modify peptides, for application in drug discovery and biomedicine. A C-H functionalization protocol for the olefination of tryptophan residues in peptides is described. The modification is successful for Trp residues at any position in the peptide, has broad scope in the styrene coupling partner, and offers opportunities for conjugating peptides with other biomolecules. For peptides containing both Trp and Phe, directing group manipulation enables full-control of residue selectivity.

Peptide modification has a critical role to play in drug discovery, the diagnosis of disease, and the understanding of biological mechanism and function.[1,2] As examples, peptide-fluorophore conjugates,[3-5] which enable the imaging of biological systems, have been used to diagnose infection,[6,7] and to aid surgery.[8,9] In drug discovery, where peptide therapeutics often have higher potency and target specificity than small molecule drugs,[10-12] synthetic peptide modification can provide necessary improvements to drug stability, cell penetration and oral bioavailability.[13-16]

Of the large number of methods that have been developed for the modification of peptides,[2,17] most rely upon the reactions of heteroatoms, which may themselves be crucial to structure and biological function.[18,19] Alternatively, peptides and proteins have been modified at carbon atoms by metal-catalyzed cross-coupling reactions, but these methods require the incorporation of non-natural amino acids.[20-22] Consequently, new methods of peptide modification that operate at carbon atoms and in native peptides are needed.

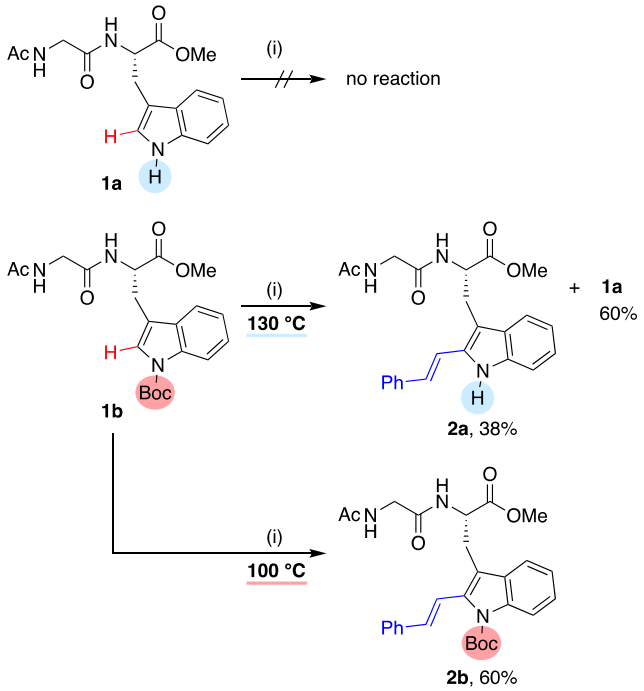
To achieve this goal, there has been rapid progress in developing methods for the C-H functionalization of peptides.[23-37] For example, C(sp^3)-H functionalization of alanine residues can give rise to phenylalanine derivates.[25] C(sp^2)-H functionalization of aromatic amino acids has focussed almost entirely on tryptophan (Trp) residues, that have been modified using arylation, alkynylation and allylation, Scheme 1A.[26-35] In addition, the modification of phenylalanine (Phe) residues in peptides has recently been achieved through C-H olefination, Scheme 1B.[36-38]

Scheme 1. C-H functionalization of aromatic amino acids in peptides.



With a view to modifying complex peptides that contain more than one aromatic amino acid, we sought to determine if C-H olefination could be applied selectively to Trp residues in peptides containing potentially competing Phe residues. Herein, we report a C-H olefination protocol for the postsynthetic modification of tryptophan containing peptides, and demonstrate peptide-amino acid crosslinking; moreover, for peptides containing both Phe and Trp residues, we describe methods for the control of residue-selectivity.

Scheme 2. Initial investigation of the C-H olefination of model peptides 1a and 1b[a]

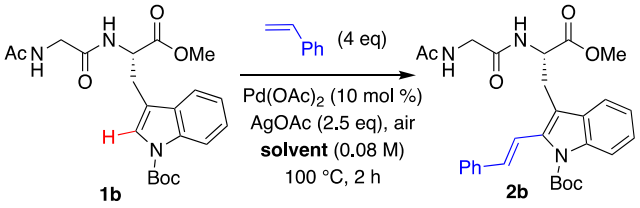


[a]Reaction conditions: (i) Styrene (4 equiv), $Pd(OAc)_2$ (10 mol %), Ag(OAc) (5 eq), air, *t*-amylOH (0.08 M).

Our investigation began by employing Fujiwara-Moritani reaction conditions that we had previously optimised for the olefination of phenylalanine containing peptides.[37] Hence, the model dipeptide Ac-Gly-Trp-OMe (**1a**) was treated with 4 equivalents of styrene in the presence of $Pd(OAc)_2$ (10 mol %) and AgOAc (2.5 equivalents) at 130 °C in *t*-amyl alcohol, Scheme 2. However, **1a** proved to be unreactive under these conditions. In contrast, when the indole nitrogen was protected with a Boc group, the Trp residue was reactive: reaction of the model peptide Ac-Gly-Trp(Boc)-OMe (**1b**), gave the olefinated peptide **2a** in 38% yield, in which the Boc group had also been cleaved. The unmodified and side-chain deprotected peptide **1a** accounted for the mass balance in this reaction. When the reaction temperature was lowered to 100 °C, the Boc group was not cleaved and the modified peptide **2b** was isolated in 60% yield. The use of a Boc group is a particularly attractive feature of this reaction, as Boc is the most commonly used tryptophan protecting group in Fmoc-based solid-phase peptide synthesis, and is readily cleaved to reveal the native Trp residue.[39]

To further optimize the reaction, the effect of the reaction solvent was studied, Table 1. Carrying out the reaction in 1,2-dichloroethane instead of *t*-amyl alcohol gave a similar yield of the modified peptide **2b** (60%, entry 2). Other polar aprotic solvents were also effective, but the yields of **2b** were lower (entries 3-5). Hexafluoroisopropanol (HFIP) is a commonly employed solvent in C-H functionalization and in peptide chemistry, yet it proved ineffective in the reaction (entry 6).[25,40] The best yield for the reaction was obtained using toluene (85%, entry 7); indeed, with toluene as the solvent, the reaction time could be significantly reduced to 2 h (82%, entry 8). Under these high-yielding conditions a second modified peptide was also isolated (**2b′**, 1% yield), in which the Trp residue had additionally been modified at C-4 of the indole ring, see Supporting Information. ICP analysis of **2b** indicated > 99.9% removal of Pd and Ag (Pd 6 ppm; Ag 50 ppm).

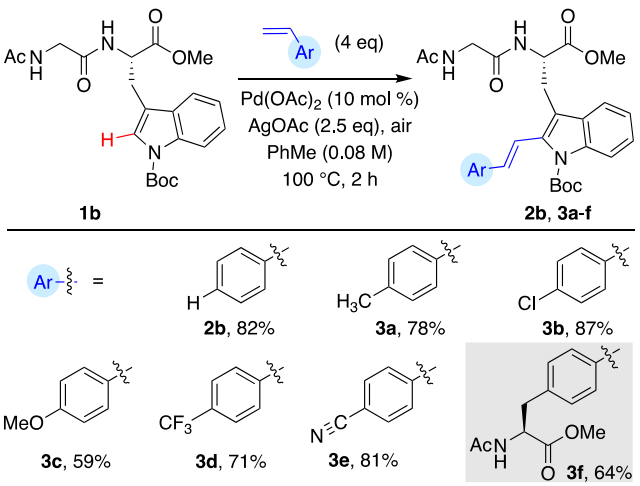
Table 1. Optimization of reaction solvent



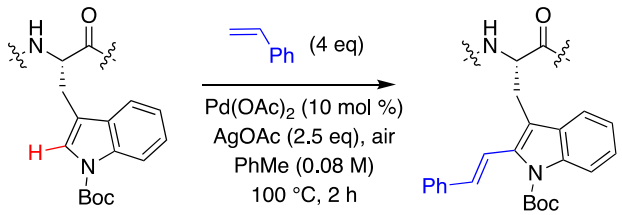
entry	solvent	*T* / °C	time / h	yield / %
1	*t*-amylOH	100	48	60
2	1,2-DCE	100	48	60
3	MeCN	100	48	42
4	1,4-dioxane	100	48	40
5	THF	100	48	36
6	HFIP	100	48	0
7	PhMe	100	48	85
8	**PhMe**	**100**	**2**	**82**

Having determined optimum conditions for the C-H olefination of dipeptide **1b**, we next investigated the scope of the alkene. In this study, the substituent in the *para* position of styrene was varied, Scheme 3. Pleasingly, both electron-withdrawing (Cl–, F_3C–, NC–) and electron-donating substituents (H_3C–, MeO–) were compatible with the C-H olefination reaction. With a view to future applications in biomolecule crosslinking, we also demonstrated the coupling of **1b** with the unnatural amino acid Ac-Phe(4-vinyl)-OMe (**SI-3**) to give the peptide–amino acid conjugate **3f**.

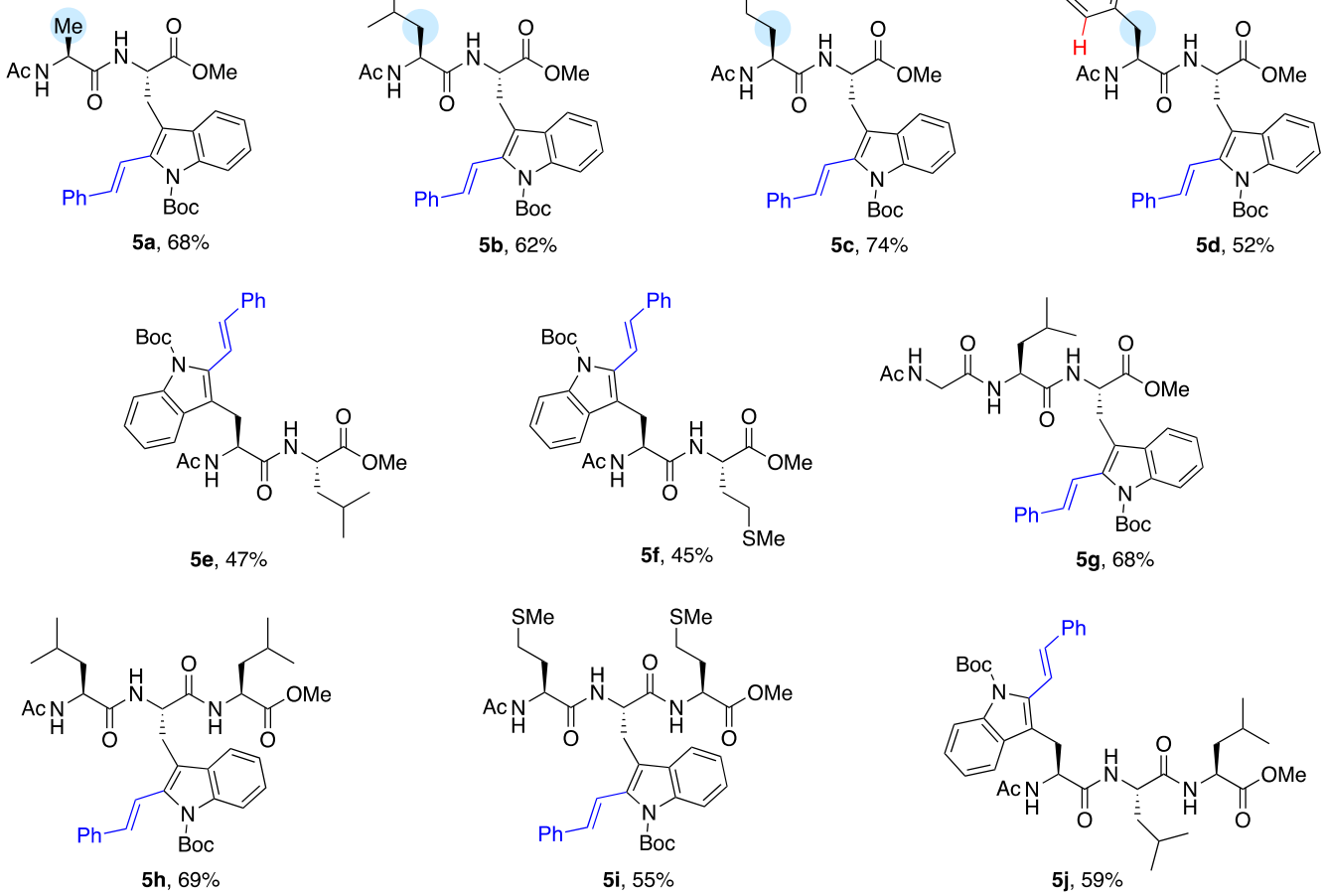
Scheme 3. Scope of the alkene for the modification of 1b



Scheme 4. C-H olefination of Trp residues in di- and tri-peptides



4a-j **5a-j**



5a, 68% **5b**, 62% **5c**, 74% **5d**, 52%

5e, 47% **5f**, 45% **5g**, 68%

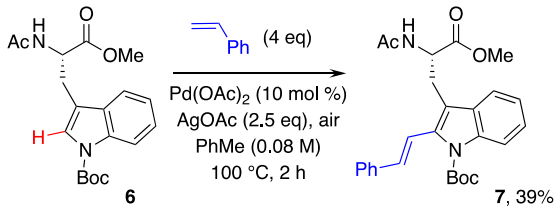
5h, 69% **5i**, 55% **5j**, 59%

In order to establish the general applicability of the C-H olefination, the reaction was applied to a range of di- and tri-peptides, Scheme 4. For dipeptides with the Trp residue at the C-terminus (general structure Ac-AA-Trp(Boc)-OMe), the adjacent aliphatic amino acids alanine and leucine proved amenable to the reaction (**5a-b**, 62-68% yields). Methionine, which contains a thioether group that is susceptible to oxidation, also proved to be a compatible neighbouring amino acid residue (**5c**, 74% yield).

In our previous study of the C-H olefination of phenylalanine residues, we noted that Phe residues were not modified at the N-terminus of a peptide; to rationalise this finding, we proposed that for Phe modification, bidentate coordination of the peptide to the Pd catalyst was required, through two amide groups of the peptide backbone.[37,41] Likewise here, the peptide Ac-Phe-Trp(Boc)-OMe (**4d**) was only modified at the Trp residue and not at the N-terminal Phe residue (**5d**, 52% yield). We then explored if N-terminal Trp residues could be modified, and in contrast to N-terminal Phe, Trp residues at the N-terminus did react: C-H olefination of the peptides Ac-Trp(Boc)-Leu-OMe (**4e**) and Ac-Trp(Boc)-Met-OMe (**4f**) gave the modified peptides **5e** and **5f** with isolated yields of 47 and 45% respectively. The C-H olefination was also applied to three model tripeptides: these reactions proved successful with the Trp residue at the C-terminus, in the middle of the peptide and at the N-terminus (**5g-j**, 55-69% yield).

There is a clear difference between the lack of reaction of N-terminal Phe residues and the successful modification of N-terminal Trp. For the C-H olefination of Trp residues, it appears that bidentate coordination of the peptide backbone is not essential, and that the Boc group plays a directing group role. Further support was obtained from the reaction of the

protected amino acid Ac-Trp(Boc)-OMe (**6**), which gave the modified amino acid **7** in 39% yield, eq 1. The importance of the Boc group is a noteworthy finding, as carbamate directing groups are uncommon in C-H functionalization.[42] Indeed, for the C-H functionalization of indoles, Boc has been reported to be a poor directing group.[43,44] It may be that in this case the amide group of the Trp residue acts as a primary directing group, and Boc is acting as an ancillary directing group.[45]



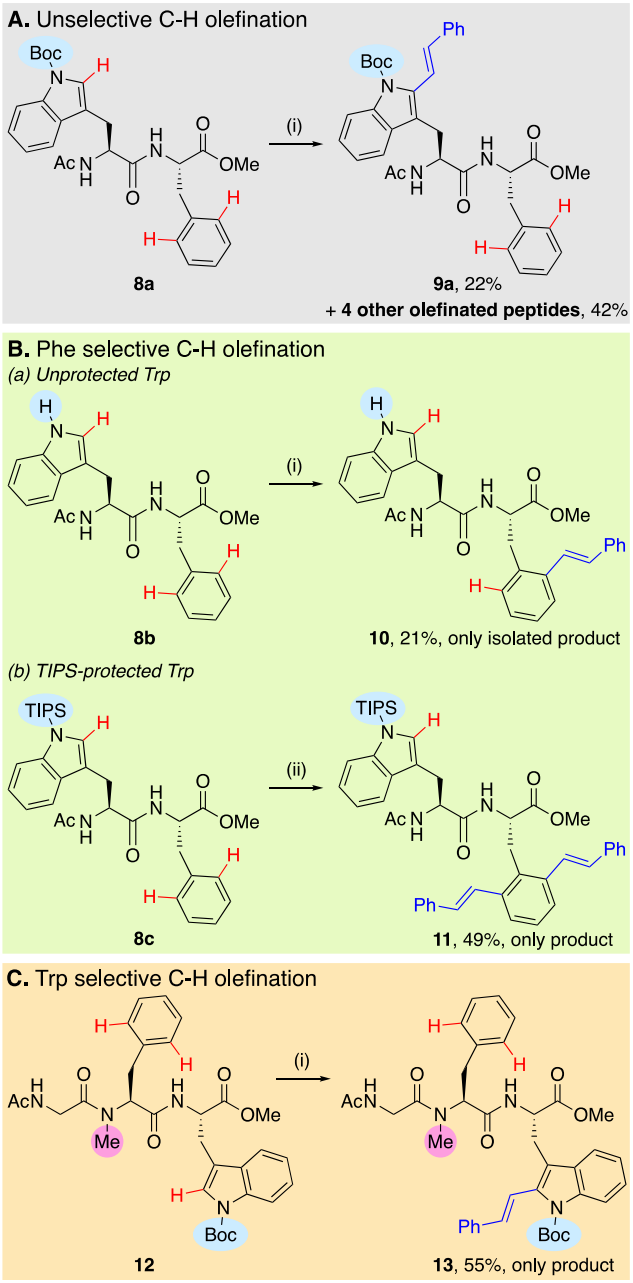
(Eq. 1)

For broad application, which includes the modification of peptides containing more than one aromatic amino acid, we were aware that control of residue-selectivity is important. Having established methods for the C-H olefination of Trp containing peptides and Phe containing peptides,[37] we next investigated the C-H functionalization of peptides containing both Trp and Phe, where modification of both aromatic residues was possible. This investigation began with the dipeptide Ac-Trp(Boc)-Phe-OMe (**8a**), which had the potential to react at the Trp residue *via* Boc directed C-H activation, and at the Phe residue *via* bidentate coordination of the peptide backbone. Peptide **8a** was treated under the optimised conditions described in Table 1, and the major product resulted from olefination of just the Trp residue (**9a**, 22% isolated yield, Scheme 5A). However, five modified peptides were isolated from the reaction, involving all combinations of Trp and Phe olefination. Modification of the tripeptide Ac-Gly-Trp(Boc)-Phe-OMe (**SI-17**) gave a similar product distribution.[46]

We anticipated that manipulation of the directing groups in a peptide would enable control of the residue-selectivity. To supress reaction at the Trp residue, alternatives to the Boc group were studied. First, the peptide Ac-Trp-Phe-OMe (**8b**), that lacked a protecting group on the Trp residue, was exposed to the same reaction conditions as peptide **8a**: reaction of **8b** gave peptide **10** in 21% yield, in which the Trp residue was not modified, but the Phe residue had undergone mono-olefination, Scheme 5B. In an attempt to increase the yield of the modified peptide, **8b** was treated using conditions we had previously optimized for Phe modification (heating at a higher temperature of 130 °C for 12 h in *t*-amylOH);[37] this harsher reaction, however, gave an intractable mixture of products. Next we studied the peptide Ac-Trp(TIPS)-Phe-OMe (**8c**), which contained a tri-*iso*-propylsilyl protecting group on the Trp residue. Peptide **8c** also underwent exclusive modification of the Phe residue: the reaction could be performed at 130 °C, to give the di-olefinated peptide **11** as the only product in 49% yield.

To suppress the reactivity of the Phe residue, bidentate coordination of the peptide to palladium needs to be prevented. Our previous study discovered that *N*-alkylation of the Phe residue rendered it unreactive in the C-H olefination reaction.[37] Consequently, we prepared the peptide Ac-Gly-(*N*-Me)-Phe-Trp-OMe (**12**), containing a Boc-protected tryptophan residue and an *N*-methyl phenylalanine residue.[47] When exposed to the optimised conditions for tryptophan C-H olefination, peptide **12** was modified exclusively at the Trp residue, giving the olefinated peptide **13** in 55% yield, Scheme 5C.

Scheme 5. Control of residue-selectivity in the C-H olefination of Trp-Phe peptides[a]



[a]Reaction conditions: (i) Styrene (4 equiv), $Pd(OAc)_2$ (10 mol %), Ag(OAc) (2.5 eq), air, PhMe (0.08 M), 100 °C, 2 h. (ii) Styrene (4 equiv), $Pd(OAc)_2$ (10 mol %), Ag(OAc) (5 eq), air, *t*-amylOH (0.12 M), 130 °C, 12 h.

The C-H olefination is successful for Trp residues at the C-terminus, the N-terminus or in the middle of peptides. Crucially, the reaction requires the Trp residue to be protected with a Boc group, which most likely acts as a directing group for the C-H activation. The Boc group may have been overlooked previously for the C-H functionalization of peptides, yet it facilitates the desired reactivity, is the most common Trp protecting group, and is readily cleaved to reveal the native peptide. Further, we have demonstrated that manipulation of the directing groups within the peptide enables full control of residue selectivity in Trp/Phe peptides.

The C-H olefination methodology reported here is complementary to prior methods for the modification of tryptophan residues by C-H arylation, alkynylation and allylation. This now broad combination of C-H functionalization methods offers significant new opportunities in chemical biology, in peptide therapeutics and in molecular imaging. By coupling peptide **1b** with the vinyl-phenylalanine compound **SI-3**, we have demonstrated how the C-H olefination can be used for peptide–biomolecule conjugation.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.
Detailed experimental procedures, reaction development, mechanistic studies, and characterization data for all compounds (PDF).

AUTHOR INFORMATION

Corresponding Author

* warren.cross@ntu.ac.uk

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by Nottingham Trent University (NTU) through studentships for M.J.T. and A.H. We thank Oliver Bolland and Mahrukh Mukhtar (both NTU) for carrying out preliminary investigations, Nigel Mould (NTU) for carrying out the HPLC experiments, Dr Graham Hickman (NTU) for assisting with the ICP analysis, and the National Mass Spectrometry Facility at Swansea University for high resolution mass spectrometry analysis.

REFERENCES

(1) Schumacher, D.; Hackenberger, C. P. R. More Than Add-on: Chemoselective Reactions for the Synthesis of Functional Peptides and Proteins. *Curr. Opin. Chem. Biol.* **2014**, *22*, 62–69.

(2) deGruyter, J. N.; Malins, L. R.; Baran, P. S. Residue-Specific Peptide Modification: a Chemist's Guide. *Biochemistry* **2017**, *56* (30), 3863–3873.

(3) Sinkeldam, R. W.; Greco, N. J.; Tor, Y. Fluorescent Analogs of Biomolecular Building Blocks: Design, Properties, and Applications. *Chem. Rev.* **2010**, *110* (5), 2579–2619.

(4) Mendive-Tapia, L.; Zhao, C.; Akram, A. R.; Preciado, S.; Albericio, F.; Lee, M.; Serrels, A.; Kielland, N.; Read, N. D.; Lavilla, R.; Vendrell, M. Spacer-Free BODIPY Fluorogens in Antimicrobial Peptides for Direct Imaging of Fungal Infection in Human Tissue. *Nat. Commun.* **2016**, *7*, 10940.

(5) Jadhav, P. D.; Shen, J.; Sammynaiken, R.; Reaney, M. J. T. Site Covalent Modification of Methionyl Peptides for the Production of FRET Complexes. *Chem.-Eur. J.* **2015**, *21* (47), 17023–17034.

(6) Akram, A. R.; Avlonitis, N.; Lilienkampf, A.; Perez-Lopez, A. M.; Mcdonald, N.; Chankeshwara, S. V.; Scholefield, E.; Haslett, C.; Bradley, M.; Dhaliwal, K. A Labelled-Ubiquicidin Antimicrobial Peptide for Immediate in Situ Optical Detection of Live Bacteria in Human Alveolar Lung Tissue. *Chem. Sci.* **2015**, *6* (12), 6971–6979.

(7) van Oosten, M.; Schäfer, T.; Gazendam, J. A. C.; Ohlsen, K.; Tsompanidou, E.; de Goffau, M. C.; Harmsen, H. J. M.; Crane, L. M. A.; Lim, E.; Francis, K. P.; Cheung, L.; Olive, M.; Ntziachristos, V.; van Dijl, J. M.; van Dam, G. M. Real-Time in Vivo Imaging of Invasive- and Biomaterial-Associated Bacterial Infections Using Fluorescently Labelled Vancomycin. *Nat. Commun.* **2013**, *4* (1), 2584.

(8) Staderini, M.; Megia-Fernandez, A.; Dhaliwal, K.; Bradley, M. Peptides for Optical Medical Imaging and Steps Towards Therapy. *Bioorg. Med. Chem.* **2018**, *26* (10), 2816–2826.

(9) van Dam, G. M.; Themelis, G.; Crane, L. M. A.; Harlaar, N. J.; Pleijhuis, R. G.; Kelder, W.; Sarantopoulos, A.; de Jong, J. S.; Arts, H. J. G.; van der Zee, A. G. J.; Bart, J.; Low, P. S.; Ntziachristos, V. Intraoperative Tumor-Specific Fluorescence Imaging in Ovarian Cancer by Folate Receptor-A Targeting: First in-Human Results. *Nat. Med.* **2011**, *17* (10), 1315–1319.

(10) Lau, J. L.; Dunn, M. K. Therapeutic Peptides: Historical Perspectives, Current Development Trends, and Future Directions. *Bioorg. Med. Chem.* **2018**, *26* (10), 2700–2707.

(11) Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D. The Future of Peptide-Based Drugs. *Chem. Biol. Drug Des.* **2012**, *81* (1), 136–147.

(12) Fosgerau, K.; Hoffmann, T. Peptide Therapeutics: Current Status and Future Directions. *Drug Discov. Today* **2015**, *20* (1), 122–128.

(13) Grison, C. M.; Burslem, G. M.; Miles, J. A.; Pilsl, L. K. A.; Yeo, D. J.; Imani, Z.; Warriner, S. L.; Webb, M. E.; Wilson, A. J. Double Quick, Double Click Reversible Peptide "Stapling." *Chem. Sci.* **2017**, *8* (7), 5166–5171.

(14) Lau, Y. H.; de Andrade, P.; Wu, Y.; Spring, D. R. Peptide Stapling Techniques Based on Different Macrocyclisation Chemistries. *Chem. Soc. Rev.* **2015**, *44* (1), 91–102.

(15) Chang, Y. S.; Graves, B.; Guerlavais, V.; Tovar, C.; Packman, K.; To, K.-H.; Olson, K. A.; Kesavan, K.; Gangurde, P.; Mukherjee, A.; Baker, T.; Darlak, K.; Elkin, C.; Filipovic, Z.; Qureshi, F. Z.; Cai, H.; Berry, P.; Feyfant, E.; Shi, X. E.; Horstick, J.; Annis, D. A.; Manning, A. M.; Fotouhi, N.; Nash, H.; Vassilev, L. T.; Sawyer, T. K. Stapled α-Helical Peptide Drug Development: a Potent Dual Inhibitor of MDM2 and MDMX for P53-Dependent Cancer Therapy. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110* (36), E3445–E3454.

(16) White, C. J.; Yudin, A. K. Contemporary Strategies for Peptide Macrocyclization. *Nat. Chem.* **2011**, *3* (7), 509–524.

(17) Stephanopoulos, N.; Francis, M. B. Choosing an Effective Protein Bioconjugation Strategy. *Nat. Chem. Biol.* **2011**, *7* (12), 876–884.

(18) Lee, H. G.; Lautrette, G.; Pentelute, B. L.; Buchwald, S. L. Palladium-Mediated Arylation of Lysine in Unprotected Peptides. *Angew. Chem. Int. Ed.* **2017**, *56* (12), 3177–3181.

(19) Cheng, W.-M.; Lu, X.; Shi, J.; Liu, L. Selective Modification of Natural Nucleophilic Residues in Peptides and Proteins Using Arylpalladium Complexes. *Org. Chem. Front.* **2018**, *5* (21), 3186–3193.

(20) Lang, K.; Chin, J. W. Cellular Incorporation of Unnatural Amino Acids and Bioorthogonal Labeling of Proteins. *Chem. Rev.* **2014**, *114* (9), 4764–4806.

(21) 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* (45), 16346–16347.

(22) Spicer, C. D.; Triemer, T.; Davis, B. G. Palladium-Mediated Cell-Surface Labeling. *J. Am. Chem. Soc.* **2012**, *134* (2), 800–803.

(23) Noisier, A. F. M.; Brimble, M. A. C-H Functionalization in the Synthesis of Amino Acids and Peptides. *Chem. Rev.* **2014**, *114* (18), 8775–8806.

(24) Wang, W.; Lorion, M. M.; Shah, J.; Kapdi, A. R.; Ackermann, L. Late-Stage Peptide Diversification by Position-Selective C-H Activation. *Angew. Chem. Int. Ed.* **2018**, *57* (45), 14700–14717.

(25) Gong, W.; Zhang, G.; Liu, T.; Giri, R.; Yu, J.-Q. Site-Selective C(sp^3)–H Functionalization of Di-, Tri-, and Tetrapeptides at the N-Terminus. *J. Am. Chem. Soc.* **2014**, *136* (48), 16940–16946.

(26) (a) Williams, T. J.; Reay, A. J.; Whitwood, A. C.; Fairlamb, I. J. S. A Mild and Selective Pd-Mediated Methodology for the Synthesis of Highly Fluorescent 2-Arylated Tryptophans and Tryptophan-Containing Peptides: a Catalytic Role for Pd^0 Nanoparticles? *Chem. Commun.* **2014**, *50* (23), 3052–3054; (b) Reay, A. J.; Williams, T. J.; Fairlamb, I. J. S. Unified Mild Reaction Conditions for C2-Selective Pd-Catalysed Tryptophan Arylation, Including Tryptophan-Containing Peptides. *Org. Biomol. Chem.* **2015**, *13* (30), 8298−8309.

(27) Reay, A. J.; Hammarback, L. A.; Bray, J. T. W.; Sheridan, T.; Turnbull, D.; Whitwood, A. C.; Fairlamb, I. J. S. Mild and Regiose-

lective Pd(OAc)$_2$-Catalyzed C-H Arylation of Tryptophans by [ArN$_2$]X, Promoted by Tosic Acid. *ACS Catal.* **2017**, *7* (8), 5174–5179.

(28) Noisier, A. F. M.; García, J.; Ionuț, I. A.; Albericio, F. Stapled Peptides by Late-Stage C(sp^3)-H Activation. *Angew. Chem. Int. Ed.* **2017**, *56* (1), 314–318.

(29) Mendive-Tapia, L.; Preciado, S.; García, J.; Ramón, R.; Kielland, N.; Albericio, F.; Lavilla, R. New Peptide Architectures Through C-H Activation Stapling Between Tryptophan-Phenylalanine/Tyrosine Residues. *Nat. Commun.* **2015**, *6*, 7160.

(30) Liu, T.; Qiao, J. X.; Poss, M. A.; Yu, J.-Q. Palladium(II)-Catalyzed Site-Selective C(sp^3)−H Alkynylation of Oligopeptides: a Linchpin Approach for Oligopeptide–Drug Conjugation. *Angew. Chem. Int. Ed.* **2017**, *56* (36), 10924–10927.

(31) Wang, W.; Lorion, M. M.; Martinazzoli, O.; Ackermann, L. BODIPY Peptide Labeling by Late-Stage C(sp^3)−H Activation. *Angew. Chem. Int. Ed.* **2018**, *57* (33), 10554–10558.

(32) Zhu, Y.; Bauer, M.; Ackermann, L. Late-Stage Peptide Diversification by Bioorthogonal Catalytic C-H Arylation at 23 °C in H_2O. *Chem.-Eur. J.* **2015**, *21* (28), 9980–9983.

(33) Schischko, A.; Ren, H.; Kaplaneris, N.; Ackermann, L. Bioorthogonal Diversification of Peptides Through Selective Ruthenium(II)-Catalyzed C-H Activation. *Angew. Chem. Int. Ed.* **2017**, *56* (6), 1576–1580.

(34) Lorion, M. M.; Kaplaneris, N.; Son, J.; Kuniyil, R.; Ackermann, L. Late-Stage Peptide Diversification Through Cobalt-Catalyzed C−H Activation: Sequential Multicatalysis for Stapled Peptides. *Angew. Chem. Int. Ed.* **2019**, *20*, 122–126.

(35) Kaplaneris, N.; Rogge, T.; Yin, R.; Wang, H.; Sirvinskaite, G.; Ackermann, L. Late-Stage Diversification by Manganese-Catalyzed C-H Activation: Access to Acyclic, Hybrid and Stapled Peptides. *Angew. Chem. Int. Ed.* **2019**, *58*, 3476–3480.

(36) Bai, Z.; Cai, C.; Yu, Z.; Wang, H. Backbone-Enabled Directional Peptide Macrocyclization Through Late-Stage Palladium-Catalyzed δ-C(sp^2)−H Olefination. *Angew. Chem. Int. Ed.* **2018**, *57* (42), 13912–13916.

(37) Terrey, M. J.; Perry, C. C.; Cross, W. B. Postsynthetic Modification of Phenylalanine Containing Peptides by C–H Functionalization. *Org. Lett.* **2019**, *21* (1), 104–108.

(38) Zheng, Y.; Song, W. Pd-Catalyzed Site-Selective C(sp^2)–H Olefination and Alkynylation of Phenylalanine Residues in Peptides *Org. Lett.* **2019**, *21* (9), 3257–3260.

(39) Isidro-Llobet, A.; Álvarez, M.; Albericio, F. Amino Acid-Protecting Groups. *Chem. Rev.* **2009**, *109* (6), 2455–2504.

(40) Dherbassy, Q.; Schwertz, G.; Chessé, M.; Hazra, C. K.; Wencel-Delord, J.; Colobert, F. 1,1,1,3,3,3-Hexafluoroisopropanol as a Remarkable Medium for Atroposelective Sulfoxide-Directed Fujiwara-Moritani Reaction with Acrylates and Styrenes. *Chem.-Eur. J.* **2015**, *22* (5), 1735–1743.

(41) For prior calculations on bidentate directing groups in C-H activation, see: (a) Cross, W. B.; Hope, E. G.; Lin, Y.-H.; Macgregor, S. A.; Singh, K.; Solan, G. A.; Yahya, N. *N,N*-Chelate-Control on the Regioselectivity in Acetate-Assisted C-H Activation. *Chem. Commun.* **2013**, *49* (19), 1918–1920. (b) Tang, H.; Huang, X.-R.; Yao, J.; Chen, H. Understanding the Effects of Bidentate Directing Groups: a Unified Rationale for sp(2) and sp(3) C-H Bond Activations. *J. Org. Chem.* **2015**, *80* (9), 4672–4682.

(42) For examples of Boc as a directing group in C-H functionalization, see: (a) Beck, E. M.; Grimster, N. P.; Hatley, R.; Gaunt, M. J. Mild Aerobic Oxidative Palladium (II) Catalyzed C−H Bond Functionalization: Regioselective and Switchable C−H Alkenylation and Annulation of Pyrroles. *J. Am. Chem. Soc.* **2006**, *128* (8), 2528–2529. (b) Morita, T.; Satoh, T.; Miura, M. Rhodium(III)-Catalyzed Ortho-Alkenylation of Anilines Directed by a Removable Boc-Protecting Group. *Org. Lett.* **2017** *19* (7), 1800–1803.

(43) For C-H functionalization of *N*-Boc indole that is completely unproductive or low yielding, see: (a) García-Rubia, A.; Urones, B.; Gómez Arrayás, R.; Carretero, J. Pd^{II}-Catalysed C-H Functionalisation of Indoles and Pyrroles Assisted by the Removable N-(2-Pyridyl)sulfonyl Group: C2-Alkenylation and Dehydrogenative Homocoupling. *Chem.-Eur. J.* **2010**, *16* (31), 9676–9685. (b) Lanke, V.; Prabhu, K. R. Highly regioselective C2-alkenylation of indoles using the N-benzoyl directing group: an efficient Ru-catalyzed coupling reaction. *Org. Lett.* **2013**, *15* (11), 2818–2821. (c) Gong, B.; Shi, J.; Wang, X.; Yan, Y.; Li, Q.; Meng, Y.; Xu, H. E.; Yi, W. Rhodium(III)-Catalyzed Regioselective Direct C-2 Alkenylation of Indoles Assisted by the Removable N-(2-Pyrimidyl) Group. *Adv. Synth. Cat.* **2014**, *356* (1), 137–143. (d) Yan, Z.; Chen, W.; Gao, Y.; Mao, S.; Zhang, Y.; Wang, Y. Palladium-Catalyzed Intermolecular C-2 Alkenylation of Indoles Using Oxygen as the Oxidant. *Adv. Synth. Cat.* **2014**, 356 (5), 1085–1092.

(44) For C-H borylation of *N*-Boc indole that is selective for C3 rather than C2, see: Kallepalli, V. A.; Shi, F.; Paul, S.; Onyeozili, E. N.; Maleczka, Jr., R. E.; Smith, III, R. E. Boc Groups as Protectors and Directors for Ir-Catalyzed C−H Borylation of Heterocycles. *J. Org. Chem.* **2009**, *74* (23), 9199–9201.

(45) Leitch, J. A.; McMullin, C. L.; Mahon, M. F.; Bhonoah, Y.; Frost, C. G. Remote C-6 Selective Ruthenium-Catalyzed C-H Alkylation of Indole Derivatives via σ-Activation. *ACS Catalysis* **2017**. *ACS Catal.* **2017**, *7* (4), 2616–2623.

(46) See Supporting Information for full details of the C-H olefination of **8a** and **SI-17**.

(47) We first attempted to prepare the peptide Ac-Trp(Boc)-(*N*-Me)-Phe-OMe, as a direct analogue of **8a-c**. However, the coupling of H-(*N*-Me)-Phe-OMe with Ac-Trp-OH proved difficult. Instead, peptide **12** was readily prepared and enabled us to investigate the protecting group strategy.