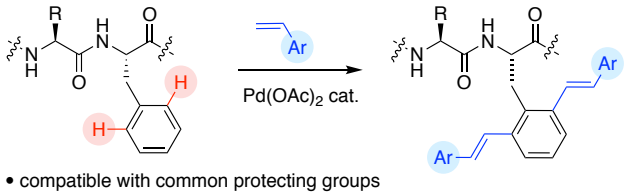
Post-synthetic modification of phenylalanine containing peptides by C-H functionalization.

Myles J. Terrey, 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, UK.

Supporting Information Placeholder



- compatible with common protecting groups
- broad scope of styrene reaction partner
- bidentate coordination of peptide to Pd appears crucial

ABSTRACT: New methods for peptide modification are in high demand in drug discovery, chemical biology and materials chemistry; methods that modify natural peptides are particularly attractive. A Pd-catalyzed, C-H functionalization protocol for the olefination of phenylalanine residues in peptides is reported, which is compatible with common amino acid protecting groups, and the scope of the styrene reaction partner is broad. Bidentate coordination of the peptide to the catalyst appears crucial for the success of the reaction.

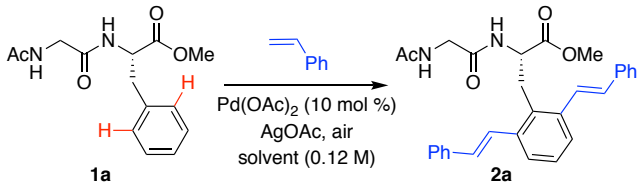
The chemical modification of peptides is critical to research in chemical biology and biomedicine, finding applications in advancing the understanding of biological function, the diagnosis and treatment of disease, and the manufacture of new materials.[1,2] For example, peptides have been modified with tags for the imaging of biological systems, and with reporters for the study of biological mechanism.[3-7] In drug discovery, peptide therapeutics are an attractive alternative to small molecule drugs, often possessing higher target specificity.[8,9] Native peptides usually have poor pharmacological properties, but chemical modification can greatly improve binding affinity to the target, stability of the peptide and cell penetration.[10-13]

Although a large number of methods have been developed for the site-selective modification of peptides,[2,14] these methods commonly rely upon the reactions of heteroatoms within a side-chain of the peptide, which are often important for biological function,[15,16] or upon the incorporation of non-natural amino acids to facilitate the subsequent modification.[17,18] Hence, new methods for peptide modification that operate on the natural peptide, and at sites other than heteroatoms, are highly desirable. The post-synthetic modification of natural peptides and peptidomimetics has been recently achieved through the C-H functionalization of hydrophobic residues:[19] reactions have been reported that directly modify either tryptophan or alanine resides.[20-27] However, methods for the direct modification of phenylalanine (Phe) residues in peptides are lacking.[2,28] Herein, we report a C-H olefination protocol for the direct post-synthetic modification of Phe containing peptides; very recently, a complementary method for the C-H olefination of peptides has been communicated.[29]

Inspired by previous research on the palladium-catalyzed Fujiwara-Moritani (oxidative-Heck) reaction,[28a,30] we began by investigating the olefination of the model phenylalanine containing dipeptide Ac-Gly-Phe-OMe (**1a**), Table 1. Treating dipeptide **1a** with an equimolar amount of styrene, $Pd(OAc)_2$ (10 mol %) and AgOAc (2.5 equiv.) at 130 °C in a mixture of 1,2-dichloroethane / DMF (15:1), gave the di-olefinated peptide **2a** in 19% isolated yield, entry 1. In the ^{1}H NMR spectrum for **2a**, the coupling constants for the alkene signals revealed that the alkene was exclusively the *trans* geometry. Increasing the number of equivalents of styrene increased the isolated yield of **2a** to 35%, entry 2; however, there was little benefit in using more than 4 equivalents of styrene in the reaction, see Supporting Information. Next, we investigated the effect of the reaction solvent on the yield of **2**: conducting the reaction in toluene, DMF or acetonitrile resulted in very similar yields (23-26%, entries 3-5). Hexafluoroisopropanol (HFIP), a solvent previously used to good effect in C-H functionalizations, including peptides,[24,31] gave only a trace yield of **2a,** entry 6. The best solvent for the reaction proved to be *tert*-amyl alcohol; the use of this solvent increased the yield of **2a** to 67%, entry 7. Alternative oxidants were investigated for the reaction, including benzoquinone, $Cu(OAc)_2$ and other silver salts, but none proved to be as competent as AgOAc. However, the best yield of **2a** was achieved by increasing the amount of AgOAc to 5 equivalents (81%, entry 8), when the

reaction time could also be shortened to 12 h. Under these higher-yielding conditions, we were also able to isolate the mono-olefinated peptide **2a′** in 8% yield. It was also possible to carry out the reaction at lower temperatures (entries 9 and 10, 100 and 80 °C), but the yield was slightly reduced.

Table 1. Optimisation of the C-H olefination[a]

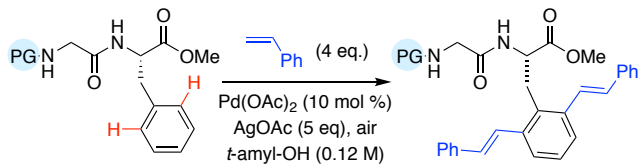


entry	equiv. styrene	equiv. AgOAc	solvent	T / °C	time / h	yield / %
1	1	2.5	DCE/DMF[b]	130	48	19
2	4	2.5	DCE/DMF[b]	130	48	35
3	4	2.5	PhMe	130	48	26
4	4	2.5	DMF	130	48	26
5	4	2.5	MeCN	130	48	23
6	4	2.5	HFIP	130	48	trace
7	4	2.5	*t*-amylOH	130	48	67
8	**4**	**5**	***t*-amylOH**	**130**	**12**	**81**[c]
9	4	5	*t*-amylOH	100	12	76
10	4	5	*t*-amylOH	80	12	68

[a]Full details of the optimisation study are provided in the Supporting Information; [b]DCE/DMF = 15:1; [c]The mono-olefinated peptide **2a′** was also isolated in 8% yield.

Having determined the optimum conditions for the olefination of dipeptide **1a**, we next investigated the effect of the nitrogen protecting group on the reaction, Scheme 1. This study demonstrated the compatibility of protecting groups commonly used in peptide synthesis. Although the original acetyl protecting group gave the best yield, the use of the carbamate protecting groups Boc, Fmoc and Cbz also provided the di-olefinated peptide in moderate to good yields (52-71%). There was no evidence for C-H functionalization of the aromatic C-H bonds in the Fmoc and CBz protecting groups. Intriguingly, protecting the glycine nitrogen as the phthalimide prevented any C-H functionalization of the peptide; in combination with other experiments, this outcome proved useful in delineating a mechanism for the reaction, *vide infra*.

Scheme 1. Scope of the *N*-protecting group for the C-H olefination of Gly-Phe dipeptides.

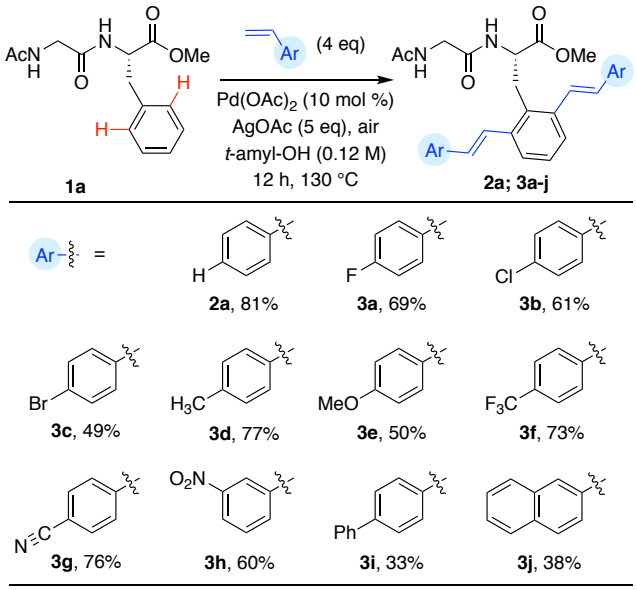


1a: PG = Ac
1b: PG = Boc
1c: PG = Fmoc
1d: PG = Cbz

2a: PG = Ac 81%
2b: PG = Boc 71%
2c: PG = Fmoc 52%
2d: PG = Cbz 70%

With a view to future applications in chemical biology, we next investigated the scope of the alkene. For the C-H olefination of dipeptide **1a**, we studied the reaction with a range of styrene derivatives, Scheme 2. Electron-withdrawing (F–, Cl–, Br–, F_3C–, NC–, O_2N–) and electron-donating substituents (H_3C–, MeO–) were compatible with the reaction; moreover, the incorporation of halogen atoms potentially enables further functionalization of the peptide.[32] Alkenes possessing more extensive conjugation were also tolerated (4-phenylstyrene and 2-vinylnaphthalene); tailoring the extent of π-conjugation can be important when modifying peptides for fluorescence imaging.[33]

Scheme 2. Scope of the alkene for the C-H olefination of model dipeptide 1a.



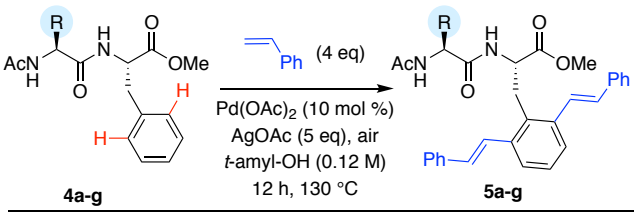
Next, we examined the scope of the amino acid at the N-terminus of the dipeptide, Scheme 3. The aliphatic amino acids Ala, Leu, Ile, Val all proved amenable to the reaction (**5a-d**), as was methionine (Met, **5e**), which possesses a thioether side-chain;[34] isolated yields ranged from 60-76% for the modification of these dipeptides. NMR and HPLC data confirmed that the peptide was not racemized during the reaction, see Supporting Information. The reaction did also proceed with proline (Pro) as the adjacent residue, but the yield was much lower (24%). The reduced yield in the synthesis of **5f** could be attributed to the tertiary amide group of proline, which may be a less capable ligand for the Pd catalyst, or to the conformational constraint imparted by the Pro residue.

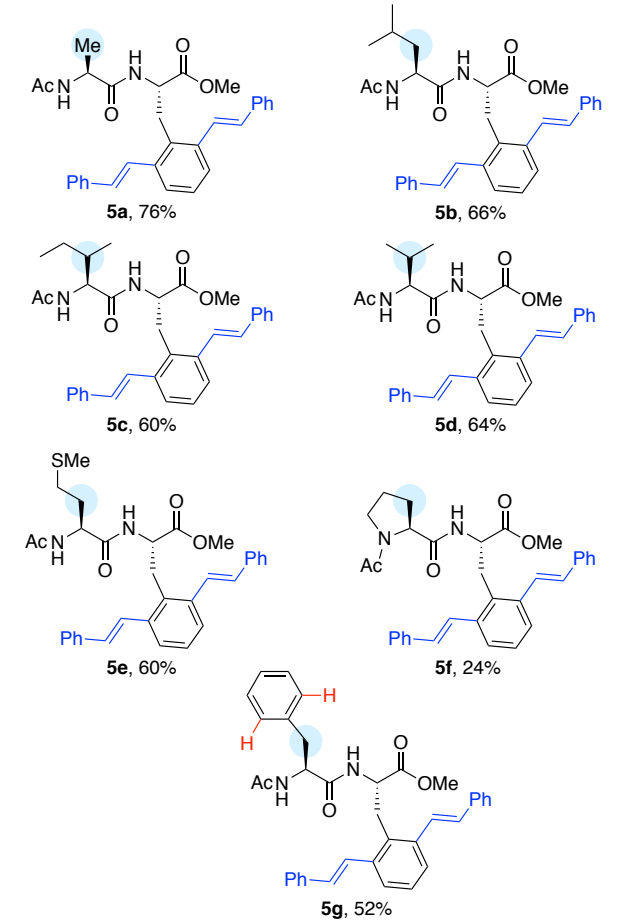
For the reaction of the peptide Ac-Phe-Phe-OMe (**4g**), we were interested to see if olefination would take place on both phenylalanine residues. From this reaction of **4g**, the modified peptide **5g** was obtained, in which only the phenylalanine residue at the C-terminus had undergone C-H olefination. Consequently, we prepared the dipeptide Ac-Phe-Gly-OMe (**4h**) to determine if modification of Phe at the N-terminus was possible in the absence of phenylalanine at the C-terminus, Eq. 1: dipeptide **4h** proved to be unaltered under the conditions of the C-H olefination reaction.[35]

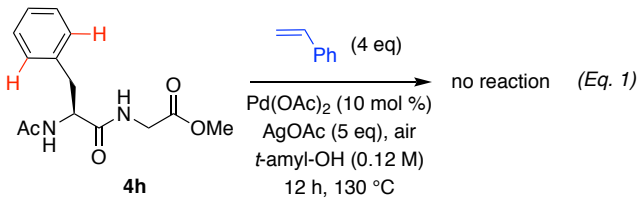
To evaluate the potential of the olefination reaction for the modification of longer peptides, we surveyed the C-H olefination of a range of tri- and tetra-peptides, Scheme 4. For the

tripeptides, di-olefination occurred for phenylalanine residues in the middle of the peptide chain or at the C-terminus (**8a**, **8b**), but not at the N-terminus (**8c** was not obtained). Tetrapeptides with a Phe residue were also successfully modified by the reaction (**9a-c**).

Scheme 3. Scope of the amino acid at the N-terminus of dipeptides.

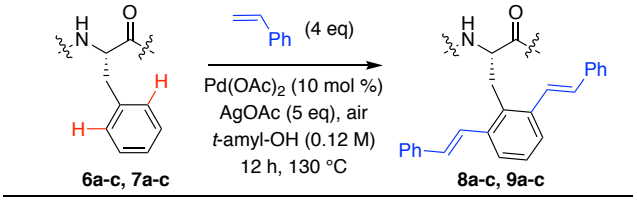


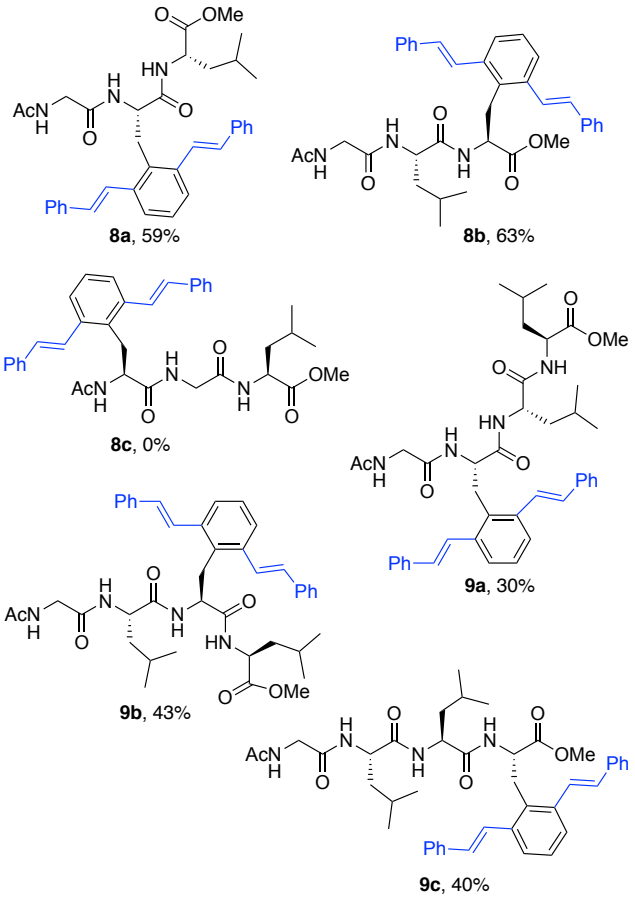




Why does the reaction not proceed when the phenylalanine residue is at the N-terminus of the peptide? A key feature of the peptide is that it can coordinate to the Pd-catalyst through amide groups of the peptide backbone, and hence facilitate the C-H functionalization. As there is more than one amide group in the peptide, several distinct coordination modes are possible. Prior calculations have demonstrated that a bidentate directing group can stabilize intermediates and transition states in C-H activation.[36] Here we propose that the peptide coordinates to Pd through two nitrogen atoms, and when the Phe residue is not at the N-terminus of the peptide, this coordination mode offers a low energy pathway for C-H activation, Scheme 5(a).[37]

Scheme 4. C-H olefination of tri- and tetra-peptides.





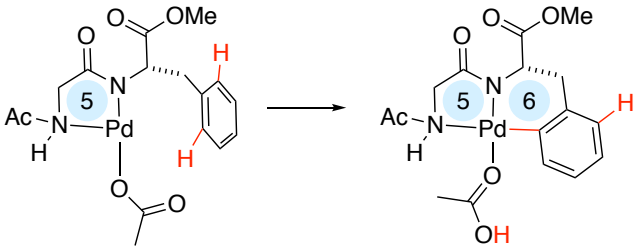
In contrast, if the Phe residue is situated at the N-terminus, bidentate coordination of the peptide means that C-H activation is geometrically unfeasible at square planar Pd(II), Scheme 5(b). In this latter case, C-H activation can only proceed through a monodentate intermediate. Presumably this higher energy pathway is inaccessible under the reaction conditions.

In summary, we have developed an efficient peptide modification protocol that employs the Fujiwara-Moritani reaction to functionalize phenylalanine residues in peptides. Specifically, Phe containing peptides have been modified by reaction with styrene derivatives in the presence of $Pd(OAc)_2$ catalyst and Ag(OAc); good yields of the di-olefinated peptide were achieved at a temperature of 80 °C, but the best yields were obtained at 130 °C. This protocol is complementary to previously reported methods for the C-H functionalization of tryp-

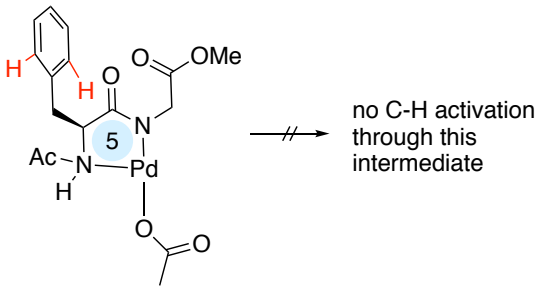
tophan and alanine residues. A range of styrene derivatives were used in the reaction, and several of these can enable further chemistry to be carried out on the modified peptide. The chemistry we describe offers new opportunities for the development of peptide pharmaceuticals or for application in chemical biology; we are currently pursuing applications in these areas.

Scheme 5. Proposed effect of peptide binding mode on C-H activation.

(a) C-H activation of Ac-Gly-**Phe**-OMe, involving proposed bidentate coordination of the peptide



(b) For bidentate coordination of Ac-**Phe**-Gly-OMe, C-H activation is geometrically unfeasible



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 new compounds (PDF).

AUTHOR INFORMATION

Corresponding Author

* warren.cross@ntu.ac.uk

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(37) Coordination *via* the oxygen atom of the amide group is also feasible, as are alternative protonation states of the peptide, but this proposal is consistent with experimental evidence: either *N*-methylation of the Phe residue or phthaloyl protection of the neighbouring residue render the reaction ineffective, see Supporting information for attempted modification of **4h** and **4i**.