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C−H Olefination of Tryptophan Residues in Peptides: Control of Residue Selectivity and Peptide−Amino Acid Cross-linking

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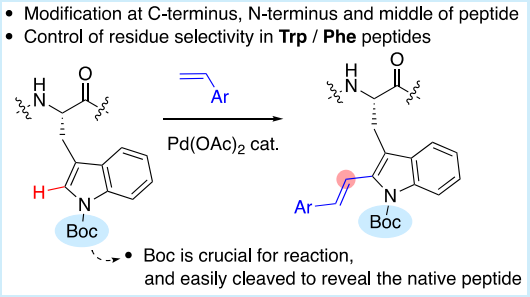
Supporting Information

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.



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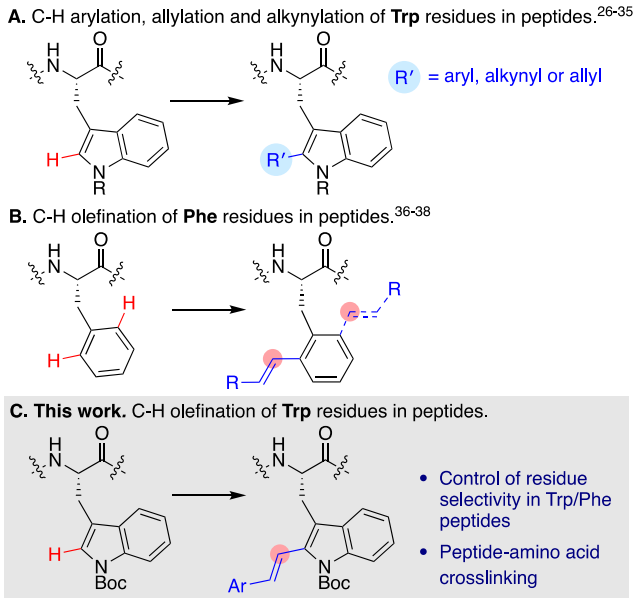
Peptide modification has a critical role to play in drug discovery, the diagnosis of disease, and the understanding of biological mechanism and function.[1,2] As examples, peptide-fluorophore conjugates,[3−5] which enable the imaging of biological systems, have been used to diagnose infection,[6,7] and 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.[23−38] 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 focused 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]

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 cross-linking; moreover, for peptides containing both Phe and Trp residues, we describe methods for the control of residue selectivity.

Scheme 1. C−H Functionalization of Aromatic Amino Acids in Peptides



Our investigation began by employing Fujiwara−Moritani reaction conditions that we had previously optimized for the olefination of phenylalanine-containing peptides.[37] Hence, the

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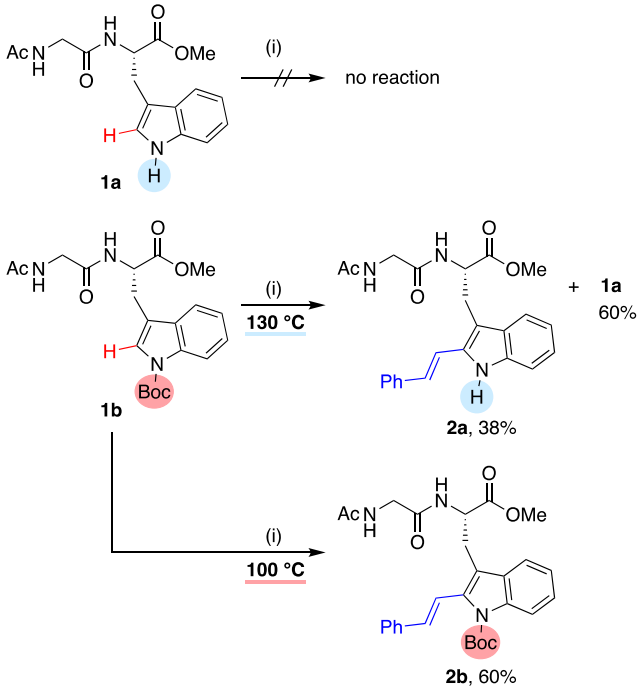
Org. Lett. XXXX, XXX, XXX−XXX

model dipeptide Ac-Gly-Trp-OMe (**1a**) was treated with 4 equiv of styrene in the presence of $Pd(OAc)_2$ (10 mol %) and AgOAc (2.5 equiv) at 130 °C in *tert*-amyl alcohol, Scheme 2.

Scheme 2. Initial Investigation of the C−H Olefination of Model Peptides 1a and 1b[a]

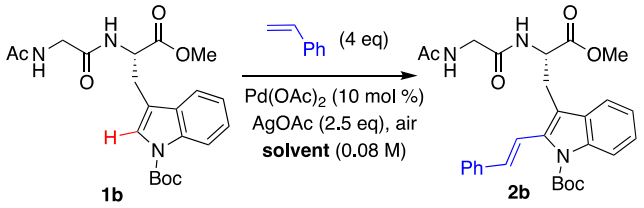


[a]Reaction conditions: (i) Styrene (4 equiv), $Pd(OAc)_2$ (10 mol %), Ag(OAc) (2.5 equiv), air, *t*-amylOH (0.08 M).

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 *tert*-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

Table 1. Optimization of Reaction Solvent

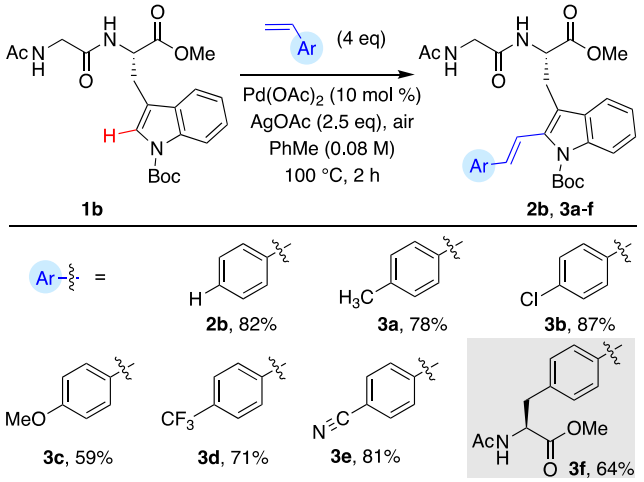


entry	solvent	T/°C	time/h	yield/%
1	*t*-amylOH	100	48	60
2	1,2-DCE	100	48	60
3	MeCN	100	48	42
4	1,4-dioxane	100	48	40
5	THF	100	48	36
6	HFIP	100	48	0
7	PhMe	100	48	85
8	**PhMe**	**100**	**2**	**82**

ring; see Supporting Information. ICP analysis of **2b** indicated >99.9% removal of Pd and Ag (Pd 6 ppm; Ag 50 ppm).

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-

Scheme 3. Scope of the Alkene for the Modification of 1b



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 cross-linking, 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**.

In order to establish the general applicability of the C−H olefination, the reaction was applied to a range of di- and tripeptides, 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 neighboring 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 rationalize this finding, we

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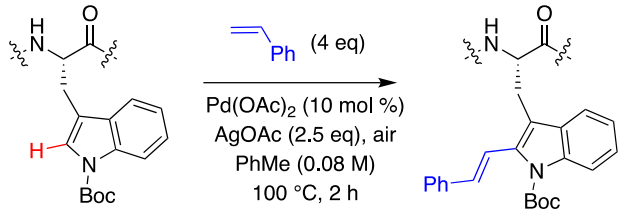
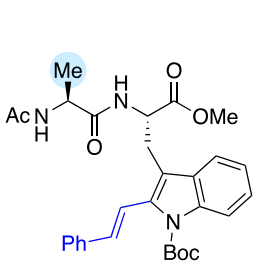
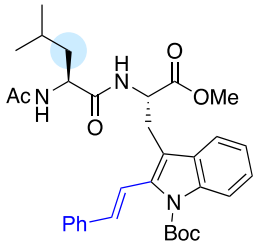
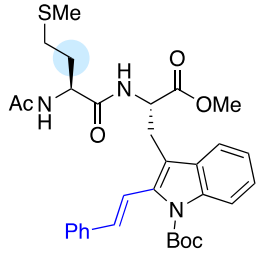
Org. Lett. XXXX, XXX, XXX−XXX

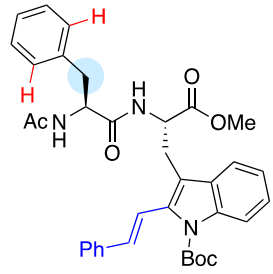
Scheme 4. C−H Olefination of Trp Residues in Di- and Tripeptides

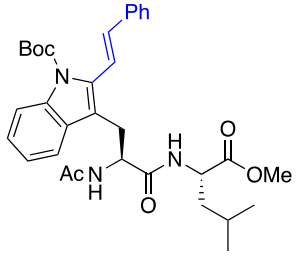


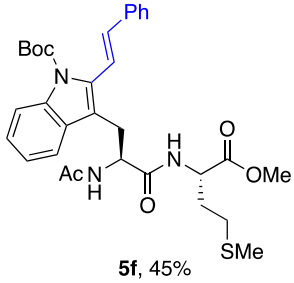

5a, 68%

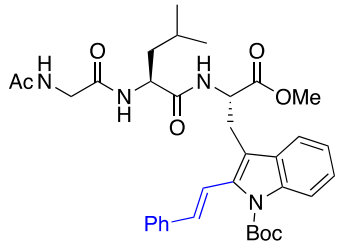

5b, 62%

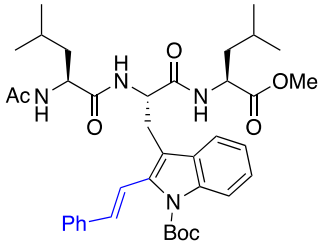

5c, 74%

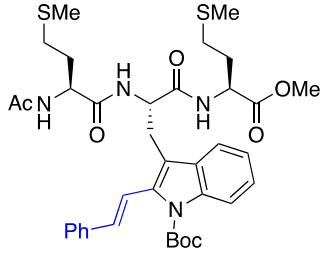

5d, 52%

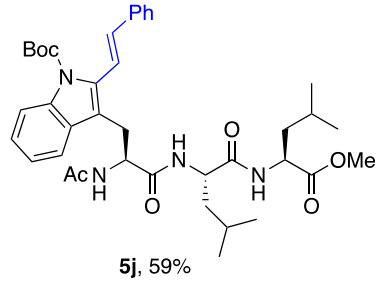

5e, 47%


5f, 45%


5g, 68%

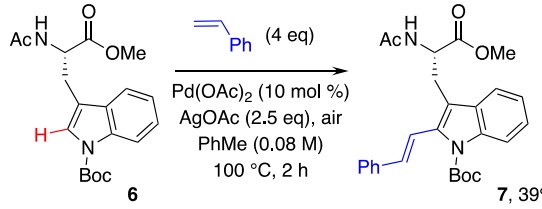

5h, 69%


5i, 55%


5j, 59%

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



(1)

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

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Org. Lett. XXXX, XXX, XXX−XXX

amide group of the Trp residue acts as a primary directing group, and Boc is acting as an ancillary directing group.[45]

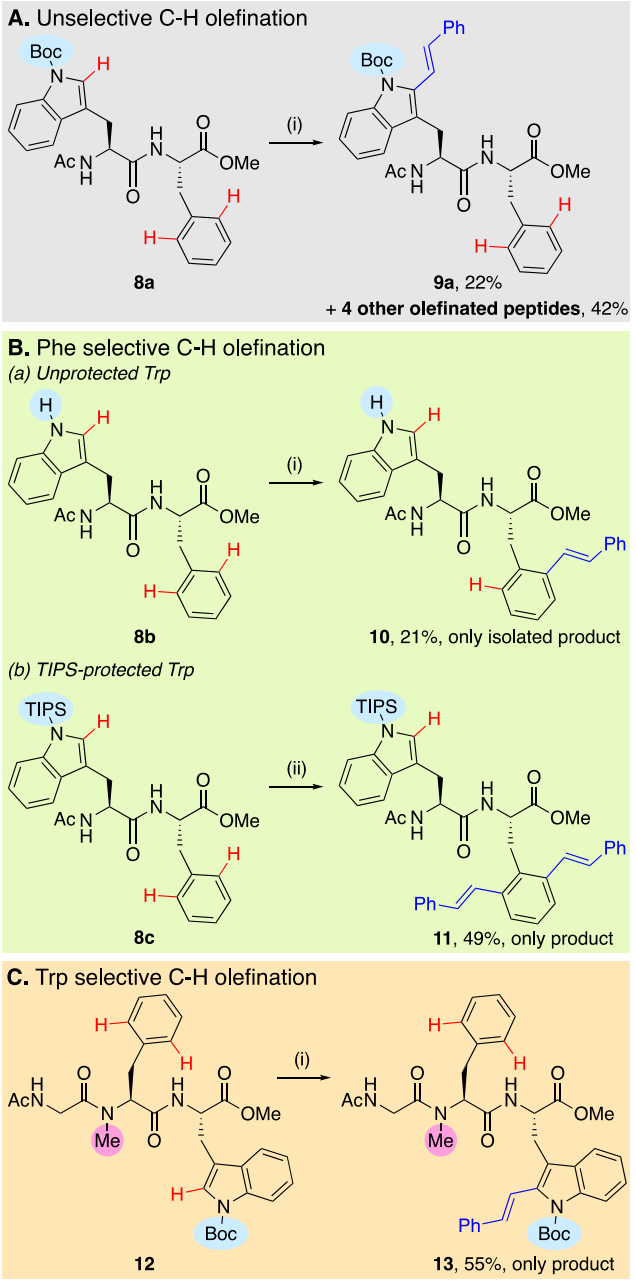
For broad application, which includes the modification of peptides containing more than one aromatic amino acid, we were aware that control of residue selectivity is important. Having established methods for the C−H olefination of Trp containing peptides and Phe containing peptides,[37] we next investigated 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 optimized 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 suppress reaction at the Trp residue, alternatives to the Boc group were studied. First, the peptide Ac-Trp-Phe-OMe (**8b**), which 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-isopropylsilyl 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 diolefinated 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(Boc)-OMe (**12**), containing a Boc-protected tryptophan residue and an *N*-methyl phenylalanine residue.[47] When exposed to the optimized 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.

In summary, the C−H olefination is successful for Trp residues at the C-terminus, at 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.

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 equiv), air, PhMe (0.08 M), 100 °C, 2 h. (ii) Styrene (4 equiv), $Pd(OAc)_2$ (10 mol %), Ag(OAc) (5 equiv), air, *t*-amylOH (0.12 M), 130 °C, 12 h.

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.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02894.

Detailed experimental procedures, reaction development, mechanistic studies, and characterization data for all compounds (PDF)

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Notes

The authors declare no competing financial interest.

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Org. Lett. XXXX, XXX, XXX−XXX