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Postsynthetic Modification of Peptides: Chemoselective C-Arylation of Tryptophan Residues

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In recent years the model on which the pharmaceutical industry is based has undergone dramatic changes. Every year, more biological entities are being accepted by the FDA and other regulatory agencies. Furthermore, the most recent biological drugs contain non-natural building blocks, as it has been shown in peptides, but also in antibodies and proteins.[1] Thus, the development of new methodologies for the selective and straightforward chemical modification of biomolecules is a scientific challenge that has tremendous implications in drug discovery, as well as in chemical biology and proteomics. The chemical methods to perform these modifications in native peptides or proteins are normally restricted to a reduced set of reactions which nearly always involve the nucleophilic side chains of amino acids such as Lys, Cys, Asp or Glu.[2]

In this context, the use of transition-metal catalysts to modify biomolecules in a tailored manner through C–C bond formation in aromatic rings could provide an attractive way to target amino acids that display this structural motif.[3] Although some remarkable achievements have been disclosed recently in this field, their suitability is seriously compromised because of the requirements of having previously functionalized non-natural residues. Among the synthetic transformations capable of handling delicate peptides, the rhodium-catalyzed conjugate addition of aryl siloxanes and boronates to dehydroalanine residues,[4] the stepwise arylation of Phe and Tyr side chains in peptides through a iodination-Suzuki coupling sequence[5] or the site-selective *O*- and *N*-arylation of Tyr and Trp residue[6] are the most illustrative examples. Particularly demanding is the case of Trp, a key amino acid for activity—rarely present in proteins (around 1%)—together with its photo-electronic properties, structural and biological roles make it an ideal site for chemical modification. Several approaches have been explored and, although with restrictions, they show the feasibility of these processes. Francis used rhodium carbenoids for chemoselective tryptophan labelling,[7] Lindner employed dicarbonyl compounds, such as malondialdehyde, that react with the indole nitrogen in Trp residues as reversible tags[8] and Winn recently described the Pd-catalyzed *N*-aryl-amination of Trp residues in small-peptides.[9] However, a general and selective transformation of the indole ring in Trp residues is still not available in the synthetic arsenal. Furthermore, the structures of many natural products, bioactive substances and drugs display indole rings, and the arylation reaction of these moieties is crucial from a synthetic point of view.[10] Reactions leading to the formation of new C–C bonds are pivotal in synthetic organic chemistry, and metal-catalyzed cross-coupling reactions are the most useful method for C_{sp^2}–C_{sp^2} bond formation.[11] A remarkable breakthrough in the field is the "metal-catalyzed direct arylation", through C–H bond activation.[12] This approach offers the advantage over most aryl–aryl coupling methods in that it does not require prefunctionalized organometallic precursors (such as boronic acids, organostannanes, silanes, etc.).[13] In recent years, significant advances have been made in direct arylation involving catalysis by transition metals such as Pd, Rh, Ru, Ir, Cu or Fe.[14] In many of the protocols developed, extremely severe conditions (high temperatures around 100–150 °C, long reaction times up to 12–48 h, and strong bases) are needed,[15] thereby resulting in a reduced range of ap-

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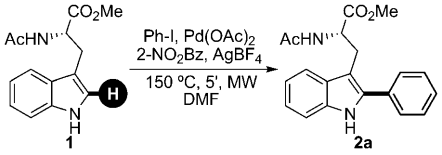
plicability, especially when dealing with multifunctionalized, complex substrates, such as peptides or proteins.

The direct *C*-functionalization of indoles has been the subject of extensive research with important consequences in organic synthesis.[16] The development of new transition-metal-catalyzed direct arylation methodologies have been extensively studied for the last decade.[17] Landmarks in the field include the first methodology that allows direct C2 arylation of indoles based on $Pd^{II/IV}$ catalytic cycle using hypervalent iodine reagents reported by Sanford.[18] Larrosa reported a remarkably mild related arylation on simple indoles using commercial aryl iodides.[19] Shi published a Pd^{II}-catalyzed direct arylation of heteroarenes with phenyl boronic acids using O_2 as the final oxidant in acidic media,[20] and Gaunt described the Cu^{II}-catalyzed, direct and site-selective arylation of indoles at C2 or C3 position.[21] These remarkable processes although synthetically useful on simple indoles, have the drawbacks of the limited availability of the arylation partners, substitution pattern and functional group incompatibilities and/or harsh conditions, which may compromise the stereochemical and connectivity patterns of delicate substrates, making them potentially problematic for the functionalization of peptides.

The introduction of aromatic rings into amino acids to modulate the structure and bioactivity of the ensuing peptides is the subject of active research and constitutes a challenging synthetic task.[22] Here we report our results on a general C–H arylation of unprotected indole rings in amino acids and peptides, at position 2 using distinct aryl iodides in aqueous media, under mild conditions, fully compatible with a variety of functional groups in a wide array of derivatives.

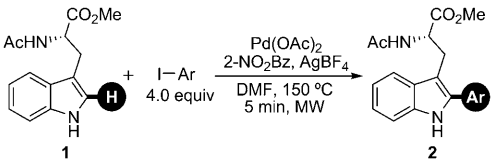
Inspired by Larrosa findings[19] and after screening several combinations of silver salts and carboxylic acids, it was found that the use of phenyl iodide, $Pd(OAc)_2$, $AgBF_4$, 2-nitrobenzoic acid in *N,N′*-dimethylformamide (DMF) at reflux for 24 h resulted in an efficient arylation of the *N*-acetyltryptophan methyl ester (Ac-Trp-OMe, **1**).[23] However, as high temperatures and long reaction times often compromise the stereochemical integrity of amino acids, we attempted microwave (MW) irradiation[24] as the heat source. In this way, the best conditions involved the use of $Pd(OAc)_2$ (5% mol), $AgBF_4$ (1 equiv) and 2-nitrobenzoic acid (1.5 equiv) at 150 °C for only 5 min to afford the C2 arylated compound **2a** in 89% yield in a scalable manner (Scheme 1). The NMR spectra of C2-phenylated Trp derivative **2a** match with the reported data for the racemic compound.[25] On the other hand, an *N*-arylated Trp derivative (**7**) was prepared using Buchwald conditions (see Supporting Information) and was used for comparison.[26] In order to test the stereogenic purity of the C2 aryltryptophans, the reaction was performed upon both isomers of the parent amino acid derivative. The resulting arylated products were purified and analyzed by HPLC on chiral stationary phase, which showed that racemization occurred in less than 4% in each case (see Supporting Information).



Scheme 1. Arylation of Ac-Trp-OMe (**1**) under microwave irradiation.

Excellent yields were obtained in the reaction of Ac-Trp-OMe (**1**) with a variety of differently substituted aryl iodides (Table 1). In this way, both electron-donor (entries 2–4) and electron-withdrawing (entries 5–7) substituents on the aryl iodide allowed the reaction, cleanly affording the arylated compounds in high yields. Remarkably, the reaction was chemoselective and allowed the differentiation of halogens, maintaining a C–Br bond untouched, as shown in entry 5. This result opens up interesting possibilities for further transformations, including new metal-catalyzed coupling processes. Heteroaryl iodides were less reactive, and 2-iodothiophene was coupled to the indole ring of the Trp although with low extension (entry 8), whereas 3-iodopyridine led to complex mixtures, where the expected compound could only be detected.

Table 1. Scope of C2 arylation of Ac-Trp-OMe (**1**).



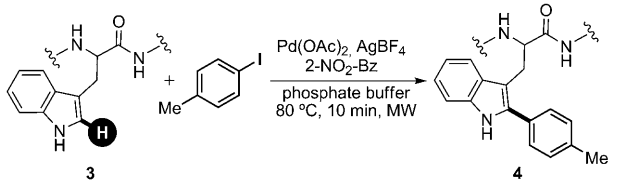
Entry	Peptide	Compound	Yield [%]
1	iodobenzene	**2a**	89
2	4-iodotoluene	**2b**	81
3	4-iodo-1,2-dimethylbenzene	**2c**	85
4	1-iodo-4-methoxybenzene	**2d**	76
5	1-bromo-4-iodobenzene	**2e**	92
6	1-iodo-4-(trifluoromethyl)benzene	**2f**	79
7	methyl 4-iodobenzoate	**2g**	86
8	2-iodothiophene	**2h**	23

The translation of this transformation to more fragile substrates such as peptides poses new challenges and requires a fine tuning of the reaction parameters. It was determined that an aqueous medium under milder conditions was convenient for peptide arylation. The catalysts and additives were maintained and the optimized conditions included the use of a tenfold excess of aryl iodide, in an aqueous phosphate buffer (pH 6.0) at 80 °C for 10 min under MW irradiation. The choice of amino acids in the model peptides covered the range of functionalities present in the side chains of the natural proteinogenic derivatives. These models allowed us to establish the practical range of functional groups that tolerate the arylation reaction under these conditions. All peptides were acetylated at the *N*-terminus,[27] while the carboxylic end was left unprotected.

The methodology for the direct arylation of peptides was fully compatible with aromatic (entries 2, 3, 5 and 9), acidic

(all entries) and basic amino acids (entries 4–6) (Table 2). Under these milder conditions, no noticeable racemization of the peptides was observed, as shown in their respective HPLC profiles.[28] Peptides that display sulphur-containing amino acids in their sequence (Met, entry 8)[29] were not suitably arylated presumably because of selective hydrolysis of the peptide bond, catalyzed by the palladium complexes formed in-situ.[30] Interestingly, this hydrolytic cleavage was also noticed when His was present in the sequence (entry 5),[31] although it took place at a very reduced extent, thus allowing the selective Trp arylation of His-containing peptides. The methodology does not rely on a specific situation of the Trp along the peptide sequence, as arylation worked equally well when the Trp residue is located at the *N*,*C*-termini or in inner positions.

Table 2. Pd-catalyzed direct arylation of tryptophan peptides in aqueous media.



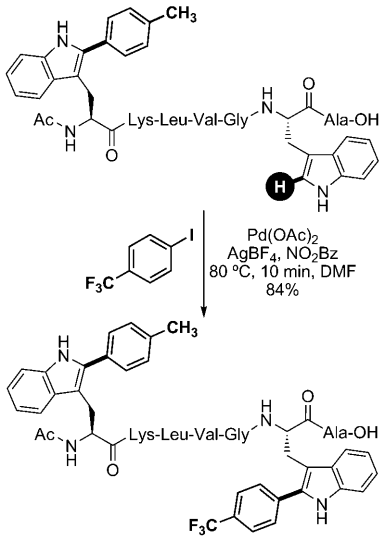
Entry	Peptide	Compound/Yield [%][a]
1	Ac-Ala-**Trp**-Ala-OH (**3a**)	**4a**/57
2	Ac-**Trp**-Leu-Asp-Phe-OH (**3b**)	**4b**/68
3	Ac-Tyr-Pro-**Trp**-Phe-OH (**3c**)	**4c**/75
4	Ac-Arg-Gly-**Trp**-Ala-OH (**3d**)	**4d**/94
5	Ac-His-Gly-**Trp**-Ala-OH (**3e**)	**4e**/51
6	Ac-Lys-Gly-**Trp**-Ala-OH (**3f**)	**4f**/95
7	Ac-Ser-Gly-**Trp**-Ala-OH (**3g**)	**4g**/62
8	Ac-Met-Gly-**Trp**-Ala-OH (**3h**)	–
9	Ac-Gln-Phe-Ala-**Trp**-OH (**3i**)	**4i**/88

[a] Calculated by integration of HPLC peaks.

In a further experiment, the arylation reaction was successfully performed on peptide (**5**), which contains two Trp units (one arylated, the other unmodified). This compound was prepared as described above (Table 1) by introducing the arylated amino acid through standard solid-phase synthesis using general peptide coupling agents (Scheme 2).

This result demonstrates the feasibility of the protocol, the synthetic usefulness of the modified amino acids as building blocks to be introduced in designed sequences, and the possibility of combining both approaches to prepare chemically modified peptide sequences simultaneously displaying distinct aryl groups on Trp units. In addition, this approach may overcome the limitation encountered in the direct arylation of peptide sequences, including Met or Cys amino acids.

In summary, here we describe a new route for C2 arylation of unprotected indoles in Trp derivatives and peptides through a Pd-catalyzed C–H activation protocol with a wide range of reactivity regarding both the peptide/amino acid substrates as well as the aryl iodides. Interestingly, the process takes place under milder conditions than those needed for simple indoles, suggesting an active role of the amide groups in the activation mechanism, presumably by stabilizing palladium intermediates. Also important for success is the use of MW irradiation, which allows the process to proceed rapidly without epimerisation. This protocol guarantees a convenient and general access to new amino acid derivates and modified Trp peptides that provide novel and useful applications in organic synthesis, drug discovery and medicinal chemistry. In addition it opens up the possibility of preparing new chemically modified peptides that are potentially useful in chemical biology (for instance as new fluorescent probes[32] or substrates for studying electron-transfer events in complex peptides).[33]



Scheme 2. Selective arylation of a peptide carrying a modified tryptophan unit.

Experimental Section

General procedure for the synthesis of 2-aryltryptophans (2): (*S*)-*N*-Acetyltryptophan methyl ester (**1**; 0.38 mmol), aryl iodide (4 equiv), $AgBF_4$ (1 equiv), 2-nitrobenzoic acid (1.5 equiv) and $Pd(OAc)_2$ (5% mol) were placed in a microwave reactor vessel and dry DMF (300 μL) was added. The mixture was stirred for 30 min at room temperature and heated under microwave irradiation (150 W) at 150 °C for 5 min. Ethyl acetate (20 mL) was added and the resulting suspension was filtered through Celite. The filtrate was successively washed with aqueous saturated solutions of NH_4Cl (3×10 mL), $NaHCO_3$ (3×10 mL) and brine (3×10 mL), then the organic phase was dried over Na_2SO_4, filtered and the solvent was removed under vacuum. The crude extract was purified by flash chromatography on silica gel to obtain **2** as a pure product.

General procedure for the C2 arylation of tryptophan residues in peptides (4): Peptide **3** (100 mg), aryl iodide (10 equiv), $AgBF_4$ (1 equiv), 2-nitrobenzoic acid (1.5 equiv) and $Pd(OAc)_2$ (5% mol) were placed in a microwave reactor vessel and phosphate buffer at pH 6.0 (300 μL) was added. The mixture was stirred for 30 min at room temperature and heated under microwave irradiation (80 W) at 80 °C for 10 min. Water (10 mL) was added and the resulting suspension was filtered through Celite. The filtrate was washed with Et_2O (3×10 mL) for remove the

excess of aryl iodide and the aqueous phase was lyophilized. The crude residue was purified by semi-preparative RP-HPLC to obtain **4** as a pure product.

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Keywords: arylation · C–H activation · indoles · palladium · peptides

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