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# Arylation of Phe and Tyr Side Chains of Unprotected Peptides by a Suzuki−Miyaura Reaction in Water

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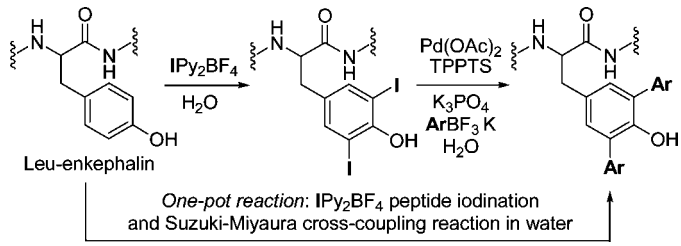
## ABSTRACT




**An efficient arylation in water of tyrosine and phenylalanine side chains from unprotected iodopeptides is accomplished by using Suzuki−Miyaura cross-coupling processes. The method is compatible with the hydrophilic and thermolabile nature of biologically active peptides. Also of interest, the arylated tyrosine peptides can be accessed in one-pot mode starting from native peptides.**

Aromatic amino acids like Phe and Tyr are important structural elements in many native peptide pharmacophores.[1] However, the study of their biological roles is limited by the shortage of available aromatic amino acid building blocks that can be used for the standard synthesis of peptides.[2]

An alternative could be to selectively modify Phe and Tyr chains in already preformed peptides through conventional organic chemistry reactions. Among them, the Suzuki−Miyaura cross-coupling[3] of halogenated Phe[4] and Tyr residues with organoboron derivatives could be an appealing entry into discrete libraries of peptides. The Suzuki−Miyaura reaction is known for both protected and unprotected Phe and Tyr amino acid derivatives[5] and also for protected small hydrophobic peptides containing them either in solution[6] or in solid-phase.[7] However, these procedures require the use of

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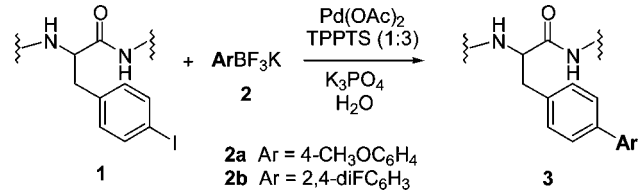
organic solvents (i.e., dioxane and toluene) and temperatures higher that 80 °C. Owing that these conditions are incompatible with the hydrophilic nature and thermal lability of the vast majority of native peptides, we have settled to study the feasibility of these reactions in water and low temperatures as to make the Suzuki−Miyaura reaction amenable to the majority of biologically active peptides.

Previously, Pd-catalyzed cross-coupling reactions other than Suzuki type on iodophenylalanine peptides in aqueous media have been scarcely reported.[4,8] A report on a Suzuki reaction on iodopeptides in glycerol/water mixtures is known.[9] However, the Suzuki−Miyaura cross coupling in neat water is restricted to the preparation of biaryls,[10] nucleoside, and nucleotide derivatives.[11] Here, we report successful results on the Suzuki−Miyaura reaction in fully aqueous media at room or moderate temperature of unprotected, biologically active peptides.

Using the iodinated derivative: H-Tyr-Gly-Gly-4-iodo-Phe-Leu-OH, **1a**, of the opioid peptide Leu enkephalin[12] as a model compound, a screening for suitable Suzuki−Miyaura reaction conditions in water has been conducted. The reaction conditions that furnished nearly quantitative conversions after 24 h at 50 °C were 5 mol % Pd from a $Pd(OAc)_2$/TPPTS (1:3)[13] (TPPTS = $P(m\text{-}C_6H_4SO_3Na)_3$) catalytic mixture, 2.5 equiv of potassium 2,4-difluorophenyltrifluoroborate and 3 equiv with respect to the boron compound of $K_3PO_4$ in neat degassed water. Alternatively, similar reaction performance could be observed at room temperature but after longer times and 10 mol % of catalyst.[14]

These conditions were further applied to a series of Phe-iodinated derivatives of bioactive peptides. Results of Table 1 show that the reaction performance is difficult to rationalize in terms of the nucleophilic character induced by electronic contributions of the aryl rings of these reagents and high conversions are either obtained with activated (4-methoxy) or deactivated (2,4-difluoro) substitutions.

**Table 1.** Pd-Catalyzed Suzuki−Miyaura Reaction on Iodophenylalanine Peptides in Water




| entry | peptide[a] | **Ar** $BF_3K$ | | conversion % (h)[b] |
|---|---|---|---|---|
| 1 | **1a**: H-Tyr-Gly-Gly-**4-iodo-** | **2a** | **3a** | 26 (24) |
| 2 | **Phe**-Leu-OH | **2b** | **3b** | 98 (24) |
| 3 | **1b**: H-Gly-Gly-**4-iodo-** | **2a** | **3c** | 92 (3) |
| 4 | **Phe**-Leu-OH | **2b** | **3d** | 84 (3) |
| 5 | **1c**: For-Met-Leu-**4-iodo-** | **2a** | **3e** | 100 (3) |
| 6 | **Phe**-OH | **2b** | **3f** | 100 (3) |
| 7 | **1d**: H-Tyr-Gly-Gly-**4-iodo-** | **2a** | **3g** | 52 (24) |
| 8 | **Phe**-Met-OH | **2b** | **3h** | 90 (3) |
| 9 | **1e**: H-Tyr-D-Ala-**4-iodo-** | **2a** | **3i** | 82 (24) |
| 10 | **Phe**-Gly-Tyr-Pro-Ser-$NH_2$ | **2b** | **3j** | 76 (3) |
| 11 | **1f**: H-Met-Glu-His-**4-iodo-** | **2a** | **3k** | 15 (24) |
| 12 | **Phe**-Arg-Trp-Gly-OH | **2b** | **3l** | 24 (24) |

[a] Reaction conditions: peptide (1 equiv), potassium 2,4-difluorophenyl trifluoroborate (**2a**) or potassium 4-methoxyphenyltrifluoroborate (**2b**) (2.5 equiv), $K_3PO_4$ (3 equiv with respect to the boron compound), $Pd(OAc)_2$/TPPTS (1:3) [5 mol% Pd] in degassed water (1 mL) at 50 °C. [b] Conversions were calculated at specific time (h) shown in brackets.

Three out of twelve reactions (entries 1, 11, and 12) presented low conversions probably indicating that not only the size of the peptide is critical (chemotactic peptide **1c**, entries 5, and 6 vs enkephalins **1a** and **1d**, entries 1, 2, 7, and 8) but that the amino acid sequence is also playing a role (dermorphin **1e**, entries 9 and 10 vs ACTH 4−10 **1f**, entries 11 and 12).

Next, we studied the application of this protocol to the coupling of doubly halogenated Tyr residues in peptides **4a**−**4f** (see Table 2). The 4-methoxyphenyltrifluoroborate **2a** furnishes the desired doubly cross-coupling reaction in good to high conversion (entries 1, 3, 7). However, when the arylborate contains an arene substituted by electron-withdrawing groups (see compound **2b**), low conversions are always obtained for the target bisarylation reaction of the peptide. Variable amounts of the corresponding starting material as well as of the products derived from the monocoupling process were always present in the crude reaction mixtures. Significantly, when the related bromine-containing peptide **4c** (entries 5, 6) is used no coupling reaction was observed under the same reaction conditions.

Same experimental conditions have been tested on **1a**, **1b,** and **4b** at 50−100 mg scale of the peptide. A standard work-up procedure to isolate the modified peptides has been established. From pure samples, full spectroscopic characterization of the arylated peptides, as well as the quantification of the yields was obtained. The results were consistent

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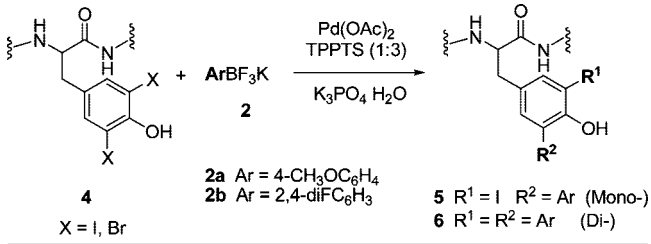
**Table 2.** Pd-Catalyzed Suzuki−Miyaura Reaction with Different [3,5-Dihalo-Tyr]-Containing Peptides in Water: Influence of the Potassium Aryltrifluoroborate Reagent




| | | | conversion (%)[b] | | | |
|---|---|---|---|---|---|---|
| entry | peptide[a] | **Ar**-$BF_3K$ | mono | | di | |
| 1 | **4a**: Ac-Phe-**3,5-diiodo**- | **2a** | **5a** | | **6a** | 92 |
| 2 | Tyr-OH | **2b** | **5b** | 10 | **6b** | 8 |
| 3 | **4b**: H-**3,5-diiodo**-Tyr- | **2a** | **5c** | | **6c** | 92 |
| 4 | Gly-Gly-Phe-Leu-OH | **2b** | **5d** | 25 | **6d** | 28 |
| 5 | **4c**: H-**3,5-dibromo**-Tyr- | **2a** | | | | |
| 6 | Gly-Gly-Phe-Leu-OH | **2b** | | | | |
| 7 | **4d**: H-**3,5-diiodo**-Tyr- | **2a** | **5f** | 17 | **6f** | 83 |
| 8 | Gly-Gly-Phe-Met-OH | **2b** | **5g** | 23 | **6g** | 73 |
| 9 | **4f**: H-**3,5-diiodo**-Tyr-D- | **2a** | **5h** | 8 | **6h** | 40 |
| 10 | Ala-Phe-Gly-Tyr-Pro-Ser-$NH_2$ | **2b** | **5i** | 25 | **6i** | 12 |

[a] Reaction conditions: peptide (1 equiv), potassium 2,4-difluorophenyltrifluoroborate **2a** or potassium 4-methoxyphenyl-trifluoroborate **2b** (5 equiv), $K_3PO_4$ (3 equiv with respect to the boron compound), $Pd(OAc)_2$/TPPTS (1:3) [10 mol % Pd] in degassed water (1 mL) at 50 °C. [b] Conversions were calculated at 24 h of reaction time.

with those previously obtained using a smaller scale of peptide, rendering yields in the range of 56−98%. Besides, the evolution of the reactions of **1a** with **2b** and **4b** with **2a** were monitored by HPLC.[15]

The feasibility of a one-pot, sequential iodination/cross-coupling process was also tested. On the basis of our previous work on aromatic iodination of biomolecules in water, [16] the iodinating reagent bis(pyridine)iodonium tetrafluoroborate ($IPy_2BF_4$)[17] was used for the initial screening.[18]As a model, we have studied the reaction of Leu−enkephalin (Leu−Enk:

(15) For experimental data, see the accompanying Supporting Information.

(16) For recent representative work, see: Espuña, G.; Andreu, D.; Barluenga, J.; Pérez, X.; Planas, A; Arsequell, G.; Valencia, G. *Biochemistry* **2006**, *45*, 5957–5963.

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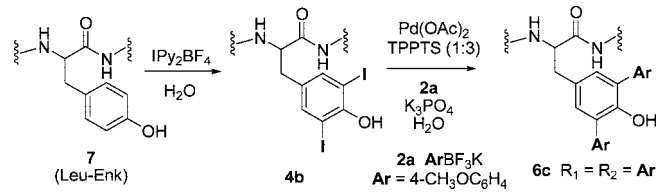
**Scheme 1.** One-Pot, Sequential Iodination/Suzuki−Miyaura Cross-Coupling Reactions in Water of Leu−enkephalin




H-Tyr-Gly-Gly-Phe-Leu-OH) with $IPy_2BF_4$ followed by coupling with potassium 4-methoxyphenyl trifluoroborate under Suzuki conditions (Scheme 1). After the addition of $IPy_2BF_4$ (2.5 equiv) a quick and quantitative conversion to the diiodo analogue **4b** was evidenced by HPLC. Subsequent addition of the Suzuki reagents gave the disubstituted analogue **6c** in 60% conversion after 20 h at 50 °C. By a second addition of fresh Pd catalyst, this result could be improved up to 80%, which is in good agreement with the one previously obtained from pure samples of **4b** (92%, see Table 2, entry 3).

In conclusion, using biologically active peptides, iodinated at Phe and Tyr residues, a new aqueous Suzuki−Miyaura cross-coupling procedure is described. The method can also be applied to native, noniodinated Tyr-containing peptides by simply performing a previous $IPy_2BF_4$ mediated iodination step. Both iodination and cross-coupling can be fashioned in one-pot mode. The reactions proceed with quantitative conversions after short reaction times and the products can be purified in high yields. The resulting arylated peptides open the way to new structure−activity relationship studies of peptides and other water soluble aromatic substrates.

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**Supporting Information Available:** Experimental procedures and characterization data for the new peptides **3a**, **3b**, **3c**, and **6c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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