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Synthesis of Dityrosine Cross-Linked Peptide Dimers Using the Miyaura–Suzuki Reaction

Joshua C. Yoburn and David L. Van Vranken*

Department of Chemistry, University of California, Irvine, California 92697-2025

dlvanvra@uci.edu

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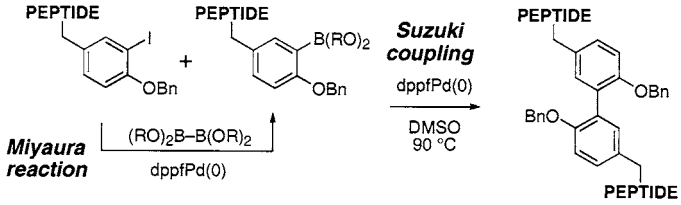
ABSTRACT



Since peroxidase-catalyzed dityrosine formation is inefficient for peptides, we have developed alternative conditions for intermolecular dityrosine formation using the Miyaura–Suzuki reaction. A one-pot reaction is effective for cross-linking short peptides, but longer peptides inhibit the Suzuki step, mandating a traditional two-step procedure using potassium acetate for the Miyaura reaction and potassium carbonate for the Suzuki coupling. These palladium-based methods are complementary to the well-established peroxidase-catalyzed oxidative phenolic coupling of full-length proteins.

Long-lived human proteins undergo a variety of posttranslational modifications. Among the most interesting protein modifications are those that lead to the formation of carbon−carbon bonds. Dityrosines are biaryl cross-links that result from the ortho coupling of two tyrosyl radicals (Figure 1).

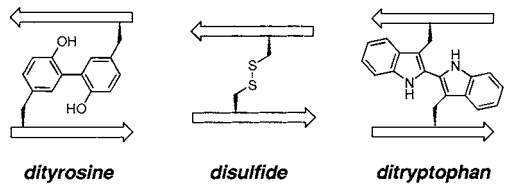


Figure 1. Three types of symmetrical cross-links.

The robust dityrosine linkage is structurally analogous to the carbon−carbon bond that secures a ditryptophan cross-link. Dityrosines occur naturally in collagens, elastins, proteoglycans, lens proteins, wheat gluten, and Alzheimer's Aβ-protein.[1] Unlike cystine disulfides, dityrosine cross-links are not susceptible to reductive cleavage.

While there are numerous ways to efficiently make the dimer of the amino acid tyrosine, the synthesis of dityrosine cross-linked peptide dimers is considerably more difficult. Peroxidase-catalysis is an effective method for forming dityrosine cross-links between the free amino acid tyrosine or between larger peptides and proteins.[2,3] Unfortunately, it is notoriously inefficient when carried out in small- to medium-sized peptides, particularly those suited for detailed spectroscopic studies.[4] Eickhoff and co-workers recently reported a 6.5% yield for the preparation of a dityrosine cross-linked peptide dimer. In this case, 2.5 mg of enzyme generated 3 mg of product.[3] While peroxidase-catalyzed

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(4) Michon, T.; Chenu, M.; Kellershon, N.; Desmadril, M.; Gueguen, J.; *Biochemistry* **1997**, *36*, 8504−8513.

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reactions are rapid, they are troubled by overoxidation, oligomerization, and low mass balance. In addition, the dimeric product must be separated from the voluminous excess of buffer salts. Clearly, a complementary synthetic approach devoid of these problems is of paramount interest.

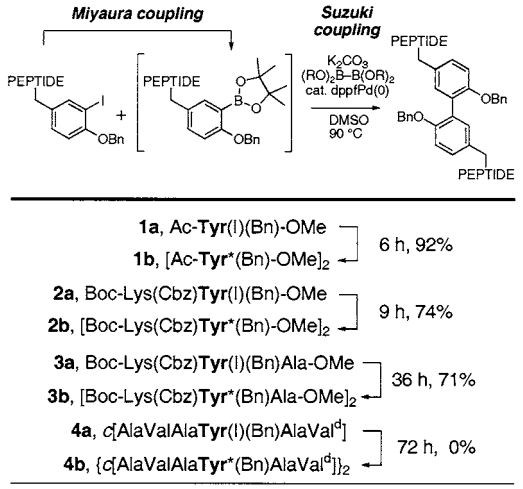
Palladium-catalyzed coupling reactions have revolutionized the synthesis of biaryls.[5,6] There are numerous examples of palladium-catalyzed Ullmann cross-coupling methods to make unsymmetrical dimers using ditin[7] and diboron[8] reagents. Recently, Carbonelle and Zhu reported the application of the Miyaura−Suzuki coupling to the synthesis of a 15-membered ring biphenomycin analogue containing an intramolecular *O*,*O*′-dimethyl-dityrosine cross-link.[9] Unfortunately, mass balance was generally poor and deiodination was a predominant side reaction. Despite the modest yields (10% at 5 mM and 45% 20 mM) that were reported, the one-step Miyaura−Suzuki reaction procedure appeared to be a promising method for formation of intermolecular dityrosine cross-links, particularly for intermolecular reactions that are not complicated by ring strain or competitive oligomerization.

Biaryls are common byproducts in the palladium-catalyzed Miyaura reaction of aryl halides with bispinacolatodiboron,[10] and a palladium-catalyzed Ullmann homocoupling with bispinacolatodiboron has been used in the synthesis of dimeric pyranonaphthoquinones.[11] We hypothesized that the Miyaura−Suzuki reaction might be efficiently applied to the formation of intermolecular dityrosine cross-links as opposed to intramolecular macrocyclic ring closure reactions. In addition, it was expected that *O*-benzyl would be more easily removed from dityrosines than *O*-methyl protecting groups.

The Miyaura reaction of aryl halides provides a simple method for the synthesis of aryl boronate esters from aryl halides. When only 50 mol % bispinacolatodiboron is used, 50 mol % of the aryl halide can be boronylated (in situ) to the aryl boronate, which then undergoes Suzuki coupling with the remaining 50 mol % unreacted aryl halide (Scheme 1). The resulting transformation, equivalent to an Ullmann coupling, proceeds under milder conditions than the traditional copper-promoted reaction. Beginning with 10 mol % of a Pd(II) source such as $PdCl_2$dppf, we used an additional 10 mol % bispinacolatodiboron for the reduction of Pd(II) to the active Pd(0) species.

Potassium acetate, deemed necessary for the intramolecular Miyaura−Suzuki reaction, led to boronylation of iodotyrosine **1a** with no detectable formation of biaryl **1b**. In contrast,

Scheme 1. One-Pot Homocoupling of Peptides[a]



[a] Conditions: aryl iodide (100 mol %), diboron (60 mol %), $PdCl_2$dppf (10 mol %), K_2CO_3 (600 mol %), DMSO, 90 °C. An asterisk (*) denotes a 2,2′-biaryl cross-link.

potassium carbonate, which was harmful in the biphenomycin study, led to efficient formation of dimer **1b**. However, potassium phosphate, which is more basic (and presumably more nucleophilic) than potassium carbonate, gave a multitude of products. 1,2-Dimethoxyethane, a common solvent for Suzuki reactions of aryl boronate esters, generated dityrosine **1b** in 42% yield. Efforts to enhance reactivity by using alternative Pd(0) sources were not effective; for example, when the reaction was carried out with 5 mol % Pd_2dba_3 and 10 mol % dppf, the aryl iodide was not consumed. Elevated temperatures (up to 150 °C) generated dimer in lower yield. The use of biscatecholdiboron led to the formation of dimer **1b** in only 10% yield from **1a**, confirming the superiority of bispinacolatodiboron. A transient intermediate is observed to build up prior to the formation of dityrosine product; the R_f and mass of this intermediate is consistent with the aryl boronate ester.[12] This observation suggests that the Miyaura reaction proceeds faster than the Suzuki coupling.

We next applied the optimized one-pot conditions[13] (K_2CO_3, $PdCl_2$dppf, DMSO) to the coupling of a dipeptide substrate **2a**. The yield for dimer **2b** was slightly lower (74%), and some unreacted aryl iodide remained after product formation had ceased. Only trace amounts of product resulting from either deiodination or protodeboronylation were observed. Homocoupling of tripeptide **3a** to form dityrosine **3b** proceeded in similar yield despite the position-

(5) (a) Yokoyama, Y.; Ito, S.; Takahashi, Y.; Murakami, Y. *Tetrahedron Lett.* **1985**, *2*, 6457−6460.
(6) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457−2483.
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(8) (a) Elder, A. M.; Rich, D. H. *Org. Lett.* **1999**, *1*, 1443−1446. (b) For an example of two-step, one-pot cross-couplings, see: Giroux, A.; Han, Y.; Prasit, P. *Tetrahedron Lett.* **1997**, *38*, 3841−3844.
(9) (a) Carbonnelle, A. C.; Zhu, J. P. *Org. Lett.* **2000**, *2*, 3477−3480. (b) Boisnard, S.; Carbonnelle, A.-C.; Zhu, J. P. *Org. Lett.* **2001**, *3*, 2061−2064.
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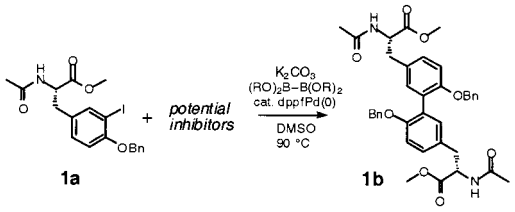
(11) Brimble, M. A.; Neville, D.; Duncalf, L. J. *Tetrahedron Lett.* **1998**, *39*, 5647−5650.

(12) ESI-MS of the transient TLC spot is consistent with an aryl boronate intermediate. Ironically, the aryl iodide (*m*/*z* 453.04) and the aryl boronate (*m*/*z* 453.23) have similar *m*/*z* and were therefore difficult to distinguish by low-resolution mass spectrometry.
(13) **General One-Pot Procedure for the Homocoupling of Aryl Iodides.** A flame-dried flask was charged with aryl iodide (100 mol %), K_2CO_3 (600 mol %), $PdCl_2$dppf (10 mol %), and bispinacolatodiboron (60 mol %). The solids were flushed with nitrogen for 5 min prior to the addition of DMSO. The reaction was heated to 90 °C for 6−72 h. Concentration in vacuo followed by silica gel chromatography provided the dityrosine cross-linked peptide dimer.

ing of the iodotyrosine in the middle of the peptide strand. Overall, reaction times required for homocoupling increased as the substrate length was increased. Unfortunately, cyclic hexapeptide **4a** gave disappointing results. Here, consumption of aryl iodide was sluggish even after 3 days. With this and other longer substrates, a substantial amount of unreacted aryl iodide remained.

In theory, the amide bonds of peptidic substrates could chelate to and deactivate the palladium catalyst or other necessary intermediates. To assess the potential inhibitory effect of oligopeptide chains on the reaction, the homocoupling of aryl iodide **1a** was carried out in the presence of 100 mol % tetrapeptide **5** for 6 h, at which point no further reaction was observed (Scheme 2). Therefore, it seemed that the presence of additional peptides lengthens the reaction half-lives. Under these conditions the desired dityrosine **1b** was formed in only 58% yield, while starting material and aryl boronic ester accounted for the remainder of the material and were present in a 1:1 ratio.

Scheme 2[a]



Potential Inhibitor	Time[b]	Yield, 1b
-	6 h	92%
100 mol% Ac-AlaLeuPheAla-NH_2, **5**	6 h	58%
100 mol% Ac-AlaLeuPheAla-NH_2, **5**	24 h	53%
100 mol% pinacol	6 h	90%

[a] Conditions: additive + aryl iodide (100 mol %), diboron (60 mol %), $PdCl_2$dppf (10 mol %), K_2CO_3 (600 mol %), DMSO, 90 °C. [b]Approximate time required for the reaction to reach completion.)

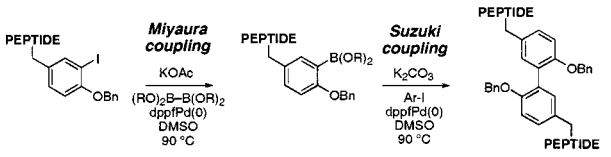
Since aryl iodide and aryl boronate remained unreacted, it seemed likely that the active catalyst had been depleted. However, the palladium did not black out, as is typical for reactions that generate unligated Pd(0). Pinacol (100 mol %) had virtually no effect on the homocoupling reaction of aryl iodide **1a**, confirming its nonparticipation in reaction inhibition (Scheme 2).

When aryl iodide **1a** was subjected to a homocoupling in the presence of tetrapeptide **5** (100 mol %) and 300 mol % diboron, dityrosine **1b** and aryl boronic ester **1c** were formed in a 1:1 mixture in excellent yield with no detectable starting material. Under these conditions, the use of less diboron (120 mol %) led to a 1:1:1 mixture of unreacted aryl iodide, biaryl, and aryl boronic ester. Thus, while the use of excess diboron reagent facilitates the consumption of aryl iodide, it also leads to the formation of aryl boronic ester at the expense of biaryl formation.

In an attempt to overcome potential catalyst depletion, homocoupling of **1a** was performed with a solid mixture of 10 mol % $PdCl_2$dppf and 10 mol % diboron reagent being added 6 h into the ongoing reaction in order to generate more active catalyst in situ. Unfortunately, the reaction did not appear to progress further to a noticeable extent. Thus, while the one-pot homocoupling procedure is both direct and useful for the construction of small dityrosine cross-linked dimers, it is slow and impractical for the synthesis of larger ones.

An alternative approach to the homocoupling of difficult substrates is to initially prepare the aryl boronic ester using Miyaura conditions and then cross-couple this intermediate with additional aryl iodide using Suzuki conditions (Scheme 3). The utility of this two-step protocol relies first upon the efficient preparation of aryl boronic ester. In the presence of tetrapeptide **5** (100 mol %), aryl iodide **1a** could be boronylated in excellent yield in 3 h using 110 mol % diboron, 10 mol % $PdCl_2$dppf, and 600 mol % KOAc (Scheme 3). In the absence of added tetrapeptide **5**, these conditions led to a shorter half-life for boronylation. Importantly, tetrapeptide **5** does not appear to have a major deleterious effect in the overall efficiency of the Miyaura boronylation reaction under these conditions.

Scheme 3. Two-Step Homocoupling of Peptides[a]



		Miyaura Time	Suzuki Time
1a, Ac-**Tyr**(I)(Bn)-OMe **1b**, [Ac-**Tyr***(Bn)-OMe]$_2$	84%	3 h	3 h
5 (100 mol%) + **1a**, Ac-**Tyr**(I)(Bn)-OMe **1b**, [Ac-**Tyr***(Bn)-OMe]$_2$	82%	5 h	3 h
6a, Ac-**Tyr**(I)(Bn)Phe-OMe **6b**, [Ac-**Tyr***(Bn)Phe-OMe]$_2$	82%	4 h	3 h
3a, Boc-Lys(Cbz)**Tyr**(I)(Bn)Ala-OMe **3b**, [Boc-Lys(Cbz)**Tyr***(Bn)Ala-OMe]$_2$	87%	5 h	5 h
7a, Ac-Ala[d]LeuAlaPhe[d]ProGly**Tyr**(I)(Bn)-OMe **7b**, [Ac-Ala[d]LeuAlaPhe[d]ProGly**Tyr***(Bn)-OMe]$_2$	59%	5 h	6 h

[a] Reaction conditions: (a) aryl iodide (100 mol %), diboron (110 mol %), $PdCl_2$dppf (10 mol %), KOAc (600 mol %), DMSO, 90 °C; (b) aryl iodide (100 mol %), diboron (120 mol %), $PdCl_2$dppf (20 mol %), K_2CO_3 (600 mol %), DMSO, 90 °C. An asterisk (*) denotes a 2,2′-biaryl cross-link.

With quantitative conversion of **1a** to aryl boronate **1c**, the intermediate reaction was filtered through a pad of Celite and silica to remove palladium black and any insoluble salts. The reaction was immediately recharged with additional aryl iodide (100 mol %), catalyst (10 mol %), diboron (10 mol %), and K_2CO_3 (600 mol %). After a total of 8 h, the Suzuki reaction had progressed minimally (<20% biaryl) and aryl iodide **1a** and aryl boronate were still present in roughly

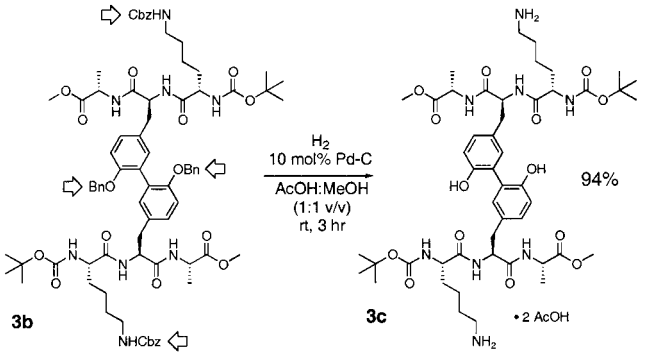
equal amounts, again suggesting catalyst deactivation. Repeating the Suzuki step again but with higher catalyst loading (20 mol % $PdCl_2$dppf + 20 mol % diboron) provided biaryl **1b** in 84% overall yield.

Optimized two-step conditions[14] were applied to larger substrates. For example, the N-terminal tyrosine substrate **6a** was dimerized in good yield, confirming that the reaction tolerates tyrosine at various positions along the peptide strand. Tripeptide **3a** afforded dimer **3b** in overall 87% as compared to the one-step protocol, which required an additional 20 h and resulted in a lower yield. As a final demonstration of the viability and dependability of this two-step approach for large peptidic substrates, heptapeptide **7a** was dimerized in 59% yield over an 11 h time period. Thus, substrates that are reluctant to form dityrosine cross-links using the one-step protocol are likely to benefit from the two-step procedure, which while less direct, is ultimately faster and more efficient.

The benzyl protecting groups are easily removable through catalytic hydrogenolysis of the benzyl ethers. For example, the benzyl ethers and Cbz groups of dimer **3b** were removed using catalytic Pd−C in methanolic acetic acid providing dityrosine cross-linked peptide **3c** in 94% yield (Scheme 4).

In conclusion, we have shown that the Miyaura−Suzuki reaction is an effective approach to the synthesis of dityrosine cross-linked peptide dimers. In the first examples of this reaction carried out by Zhu and co-workers, the yield may have been artificially low due to a challenging substrate. For the one-step intermolecular cross-linking reaction of short peptides, it is best to use potassium carbonate as the nucleophile. In the two-step transformation, the initial Miyaura step should be performed with potassium acetate followed by a traditional Suzuki coupling using potassium carbonate. To date, these methods offer the *highest reported yields* for the preparation of dityrosine cross-linked peptide dimers. The strategy is best suited for peptides of moderate length and is complementary to the peroxidase-catalyzed method. However, unlike peroxidase-catalysis, the Miyaura−Suzuki approach to dityrosine cross-linked peptides is devoid of overoxidation, oligomerization, or the need for tedious separation of product from buffer salts.

Scheme 4



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Supporting Information Available: Details describing the synthesis and characterization of all starting materials and products. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) **General Two-Step Proceedure for the Homocoupling of Aryl Iodides.** A flame-dried flask was charged with aryl iodide (100 mol %), KOAc (600 mol %), $PdCl_2$dppf (10 mol %), and bispinacolatodiboron (110 mol %). The solids were flushed with nitrogen for 5 min prior to the addition of DMSO (0.25 M). The reaction was heated at 90 °C for 3−5 h. The reaction mixture was passed through a pad of silica and then a pad of Celite. The filtrate was supplemented with aryl iodide (100 mol %), K_2CO_3 (600 mol %), $PdCl_2$dppf (20 mol %), and bispinacolatodiboron (20 mol %) and sufficient DMSO to solubilize the reagents (about 0.125 M). The reaction was again heated at 90° C for 3−6 h. Concentration in vacuo followed by silica gel chromatography provided the dityrosine cross-linked peptide dimer.