Development of fluorescent aryltryptophans by Pd mediated cross-coupling of unprotected halotryptophans in water†

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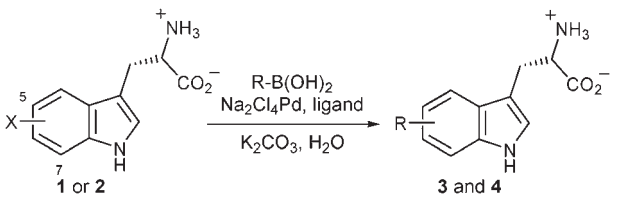
Received (in Cambridge, UK) 2nd May 2008, Accepted 27th June 2008
First published as an Advance Article on the web 27th August 2008
DOI: 10.1039/b807512c

A convenient and high yielding procedure for the Suzuki–Miyaura cross-coupling of unprotected bromo- and chlorotryptophans in water provides fluorescent aryltryptophans.

In peptides and proteins, the amino acid tryptophan frequently stabilises secondary and tertiary structure through selective intra- and intermolecular interactions.[1] In addition, the intrinsic fluorescence of tryptophan often determines, or modulates, the spectrophotometric properties of a given peptide or protein. This has been exploited for protein quantification,[2a] and to study folding events, ligand binding, and protein–protein interactions.[2b] The prospect of a simple chemical method to modify and intensify the fluorescence of tryptophan is therefore appealing.

Tryptophan is also a biosynthetic precursor for many naturally occurring non-ribosomal peptides and alkaloids.[3] Halotryptophans, for example, are present in numerous important natural products, including the anticancer agents rebeccamycin and diazonamide A.[4,5] Halotryptophans can serve as substrates for Suzuki–Miyaura (Scheme 1) and related Pd-catalysed cross-coupling reactions, which allow the installation of an aryl, heteroaryl or alkenyl substituent on the indole ring. Potentially, this synthetic approach can be applied not only to halotryptophans, but also to halotryptophan-containing peptides, proteins and natural products.

The successful application of this challenging strategy requires the development of cross-coupling conditions which are mild, compatible with the presence of unprotected carboxy and amino functionalities, and work in water. Recent progress in Pd-catalysed reactions in aqueous media[6] motivated us to accept this challenge. While the cross-coupling of boronic acid-derivatives of phenylalanine has been reported,[7] this functionality is hard to install biosynthetically, and previous cross-coupling work on tryptophans has been limited to the use of protected halotryptophans in organic solvents.[8] To the best of our knowledge, there are no examples in the literature of the cross-coupling of unprotected halotryptophans.



Scheme 1 Suzuki–Miyaura cross-coupling of halogenated tryptophans. For substituents X and R, synthetic details and yields see Tables 1 and 2.

Recently, we reported the one-pot preparation of a series of ten halotryptophans using a readily prepared cell free lysate.[9] We have now developed suitable conditions for the Suzuki–Miyaura cross coupling of unprotected 5- and 7-halotryptophans with a variety of arylboronic acids in water (Scheme 1). We have also successfully applied this methodology to the cross-coupling of a simple dipeptide as a first step towards the use of cross-coupling reactions for the direct modification of peptides, proteins and natural products. Importantly, our cross-coupling conditions allow the cross-coupling of bromo- and chlorotryptophans, which are less reactive than the corresponding iodo congener, but more common in natural products.[10] The spectrophotometric properties of the cross-coupling products are distinct from those of the parent tryptophan, and open up the possibility of using the Suzuki–Miyaura cross-coupling reaction for the site-selective fluorescent labelling of peptides, proteins and natural products. In contrast to existing methods for chemical ligation (*e.g.* click chemistry), our approach promises to enable selective functionalisation of a motif that is not uncommon in nature and without introduction of a heterocyclic scar.

Halotryptophans **1** and **2** (Scheme 1) were prepared from their corresponding haloindole and serine, in a simple one-pot enzyme catalysed reaction as previously reported.[9] For the Suzuki–Miyaura reaction of halotryptophans **1** and **2** in water, we initially employed a catalytic system composed of the water-soluble Pd source Na_2Cl_4Pd and the water-soluble phosphine ligand TPPTS (tris(3-sulfonatophenyl)phosphine trisodium salt).[11] Similar conditions have previously been used for the Suzuki–Miyaura coupling of other sensitive, water-soluble biomolecules such as nucleotides[7b,12] and sugar–nucleotides.[13] Cross-coupling reactions were typically carried out by adding an aqueous solution of the halotryptophan hydrochloride salt to a degassed mixture of Pd source, ligand, K_2CO_3 and boronic acid (Scheme 1 and Table 1). The reaction mixture was stirred under nitrogen at 80 °C until TLC indicated completion of the cross-coupling.

Pleasingly, under these conditions the cross-coupling of unprotected 5-bromotryptophan **1a** with phenylboronic acid gave the corresponding 5-phenyltryptophan **3a** in high yield (Table 1, entry 1). To test the scope of these conditions, we next used a number of phenylboronic acid derivatives as cross-coupling partners. Phenylboronic acid derivatives with electron-donating substituents could be cross-coupled very efficiently (entries 2 and 3),

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† Electronic supplementary information (ESI) available: Experimental procedures, ^1H and ^{13}C NMR spectra and HRMS data for all compounds, fluorescence spectra, and selected UV absorbance spectra. See DOI: 10.1039/b807512c

while, unsurprisingly, lower yields were obtained from cross-coupling reactions with electron-poor boronic acids (entries 4 and 5). During the cross-coupling with 4-ethoxycarbonyl phenylboronic acid (entry 4) the ethyl ester was cleaved, and the corresponding free acid **3d** was obtained as the sole product. Remarkably, the cross-coupling was also successful with less reactive halotryptophans. Both 5-chlorotryptophan **1b** and 7-bromotryptophan **2** were successfully cross-coupled to phenylboronic acid, although the yields from these reactions were moderate to low (entries 6 and 7). All cross-coupling products were purified by RP-HPLC and characterised by ^{1}H and ^{13}C NMR spectroscopy and HRMS.†

With the view towards the potential application of this methodology to the modification of peptides and proteins, our next goal was to reduce the reaction temperature for the cross-coupling of halotryptophans. However, when the reaction of **1a** with phenylboronic acid was carried out at lower temperature (60 °C or below), with TPPTS as the ligand, only trace amounts of the cross-coupling product **3a** were isolated after 3 h. Even after a prolonged reaction time of 24 h, no further reaction progress was observed. We therefore set out to identify a more reactive catalytic system, which we speculated might also give improved yields for the more challenging cross-coupling reactions (*e.g.* entry 7, Table 1).

The water-soluble phosphine ligand TXPTS (tris(4,6-dimethyl-3-sulfonatophenyl)phosphine trisodium salt) has previously been used for the Suzuki–Miyaura coupling of nucleosides at room temperature.[11] TXPTS was prepared in two steps as previously described[14] and used as the ligand in the cross-coupling of 5-bromotryptophan **1a** with phenylboronic acid (Table 2). At 80 °C, use of TXPTS increased the yield of 5-phenyltryptophan **3a** isolated from this reaction to 90% (entry 1).† Gratifyingly, use of TXPTS also allowed us to reduce the reaction temperature for this conversion significantly, to temperatures at which proteins could be expected to remain in their native conformation. At 40 °C, the TXPTS-assisted cross-coupling of **1a** gave **3a** in 70% yield after 22 h (entry 2). Finally, in the cross-coupling of 7-bromotryptophan **2** with phenylboronic acid, use of TXPTS resulted in an increase in the yield of 7-phenyltryptophan **4** from 15% (with TPPTS) to 88% (Table 2, entry 3).

In order to assess the applicability of the optimised cross-coupling conditions to the modification of small peptides, we prepared a dipeptide containing 5-bromotryptophan (Scheme 2). The methyl ester of 5-bromotryptophan **1a** was coupled with *N*-Boc-protected L-alanine under standard peptide coupling conditions. The resulting halogenated dipeptide **5** was then used as the substrate for the Suzuki–Miyaura coupling with phenylboronic acid at 80 °C for 6 h, using TXPTS as the ligand. Under these conditions, the successful cross-coupling reaction was accompanied by cleavage of the methyl ester, but not of the Boc protecting group. The partially deprotected cross-coupling product **6** was purified by reverse phase HPLC and isolated in 62% yield.

Table 1 Suzuki–Miyaura coupling of halotryptophans with various arylboronic acids R–B(OH)$_2$, using TPPTS as the ligand[a]

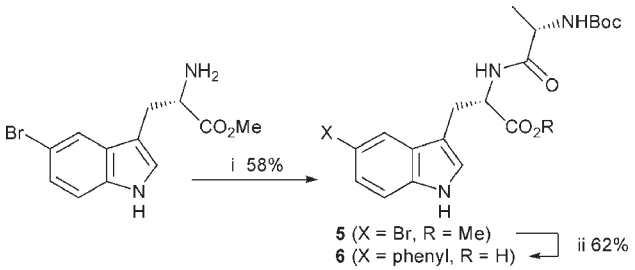
Entry	Substrate	X	Product	R	Yield (%)[b]
1	**1a**	5-Br	**3a**	5-Phenyl	78
2	**1a**	5-Br	**3b**	5-[4-(H_3C)C_6H_4][c]	90
3	**1a**	5-Br	**3c**	5-[4-(H_3CO)C_6H_4]	87
4	**1a**	5-Br	**3d**	5-[4-(CO_2H)C_6H_4][d]	68
5	**1a**	5-Br	**3e**	5-[4-(CF_3)C_6H_4]	35
6	**1b**	5-Cl	**3a**	5-Phenyl	40
7	**2**	7-Br	**4**	7-Phenyl	15

[a] See Scheme 1 for structures; general conditions: arylboronic acid (1 equiv.), Na_2Cl_4Pd (2.5 mol%), TPPTS (2.5 mol%), K_2CO_3 (5 equiv.), H_2O, 80 °C, 6 h. [b] Isolated yields. [c] Reaction time: 4 h. [d] Boronic acid: R = 5-[4-($CO_2C_2H_5$)C_6H_4].

Table 2 Suzuki–Miyaura coupling of halotryptophans **1a** and **2** with phenylboronic acid, using TXPTS as the ligand[a]

Entry	Substrate	X	Product	T/°C	Reaction time/h	Yield (%)[b]
1	**1a**	5-Br	**3a**	80	4	90
2	**1a**	5-Br	**3a**	40	22	70
3	**2**	7-Br	**4**	80	6	88

[a] See Scheme 1 for structures; general conditions: phenylboronic acid (1 equiv.), Na_2Cl_4Pd (2.5 mol%), TXPTS (10.0 mol%), K_2CO_3 (5 equiv.), H_2O. [b] Isolated yields.



Scheme 2 *Reagents and conditions*: (i) *N*-Boc-L-Ala, EDC·HCl (EDC = *N*-(dimethylaminopropyl)-*N*′-ethylcarbodiimide), CH_2Cl_2, rt, 24 h; (ii) Na_2Cl_4Pd (2.5 mol%), TXPTS (10.0 equiv.), K_2CO_3, phenylboronic acid, H_2O, 80 °C, 6 h.

The installation of a phenyl substituent in position 5 or 7 resulted in a pronounced change in the fluorescence of the tryptophan derivatives. The alteration of the spectrophotometric properties of the molecule is particularly interesting as these novel, non-natural amino acids may be useful for the site-specific, fluorescent labelling of peptides and proteins. We therefore systematically investigated the UV absorbance and fluorescence properties of selected 5- and 7-aryltryptophans **3** and **4**.

In order to determine suitable fluorescence excitation wavelengths (λ_{ex}), UV absorbance spectra were recorded in methanol for 5- and 7-aryltryptophans as well as 5-bromotryptophan **1a** and the parent compound tryptophan. The spectra of the 5-aryltryptophan derivatives showed only a single absorbance maximum at 254 nm, while a secondary maximum at 280 nm was observed, as expected, in the case of tryptophan and 5-bromotryptophan.† Next, the fluorescence emission of individual tryptophan derivatives was measured in different solvents. While 5-bromotryptophan **1a** was only weakly fluorescent, in methanol all aryl tryptophans that were examined were strong fluorescence emitters (Fig. 1).

Importantly, the fluorescence characteristics of the aryltryptophans can be modulated by the nature and position of the aromatic substituent. 5-Phenyltryptophan **3a** showed a stronger emission signal and a considerably larger Stokes shift than the parent tryptophan (Table 3). Fluorescence intensity increased further with installation of the electron-rich 4-methoxyphenyl substituent (**3c**), while the presence of the electron-withdrawing carboxy group in **3d** resulted in the

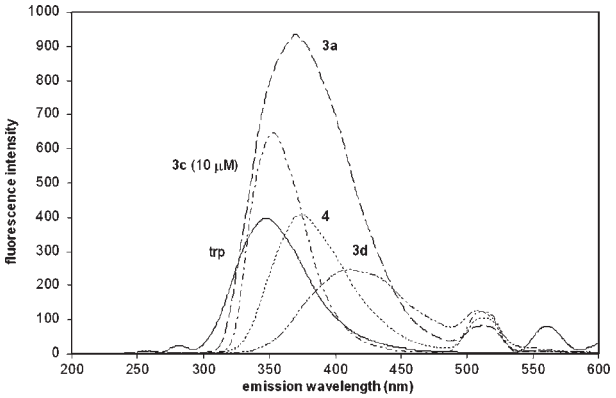


Fig. 1 Fluorescence emission spectra for tryptophan and derivatives **3a**, **3c**, **3d** and **4** in methanol (**3a**, **3d**, **4**: $c = 100$ μM; **3c**: $c = 10$ μM).

Table 3 Fluorescence spectroscopic properties of tryptophan analogues

Compound	Solvent	λ_{ex}/nm	λ_{em}/nm	Stokes shift[a]/cm^{-1}
Trp	MeOH	280	348	6978
3a	MeOH	254	370	12 343
3a	Water	254	395	14 054
3c	MeOH	254	353	11 041
3c	Water	254	357	11 359
3d	MeOH	254	411	15 039
3d	Water	254	443	16 797
4	MeOH	254	375	12 703
4	Water	254	384	13 328

[a] Stokes shift $= (1/\lambda_{ex} - 1/\lambda_{em})$.

largest Stokes shift observed in this series. Interestingly, 7-phenyltryptophan **4** was less strongly fluorescent than its regioisomer **3a**, with a fluorescence intensity comparable to tryptophan itself but, once again, a larger Stokes shift.

With a view towards potential biological applications, we also measured the fluorescence of selected aryl tryptophans in water and at $\lambda_{ex} > 254$ nm, in order to minimise, in a biological context, concomitant excitation of standard organic fragments (Fig. S1–S3†).† Pleasingly, tryptophan derivatives **3a**, **3c** and **4** were strongly fluorescent in water at an excitation wavelength of 254 nm (Fig. S1†). Compared to measurements in methanol, all analogues showed moderately or significantly increased Stokes shifts in water (Table 3). Importantly, at λ_{ex} 295 nm most aryltryptophans were still significantly more fluorescent than the parent tryptophan (Fig. S2 and S3†). While the 5-phenyltryptophan derivatives **3a** and **3c** exhibited a reduced fluorescence intensity at λ_{ex} 295 nm, the intensity of the fluorescence signal of 7-phenyltryptophan **4** did increase slightly with the longer excitation wavelength (Fig. 2), making this analogue particularly interesting for further development.

Taken together, these results show that the fluorescence of aryltryptophans can be modulated by the choice of substituent and regiochemistry. This flexibility will allow the design of tryptophan derivatives with spectrophotometric properties tailored specifically to individual target peptides and proteins.

In conclusion, we have identified suitable conditions for the Suzuki–Miyaura cross-coupling of unprotected 5- and 7-halotryptophans with a variety of boronic acids. All cross-coupling reactions were carried out in water, with generally short reaction times. Importantly, these conditions could also be applied successfully to the modification of a dipeptide, potentially opening the door towards the direct cross-coupling of larger peptides and even proteins. The cross-coupling products described herein possess interesting fluorescent properties. Individual analogues showed strong fluorescence emission in water and at λ_{ex} 295 nm, and may therefore be particularly useful for the site-selective, fluorescent labelling of peptides, proteins and natural products.

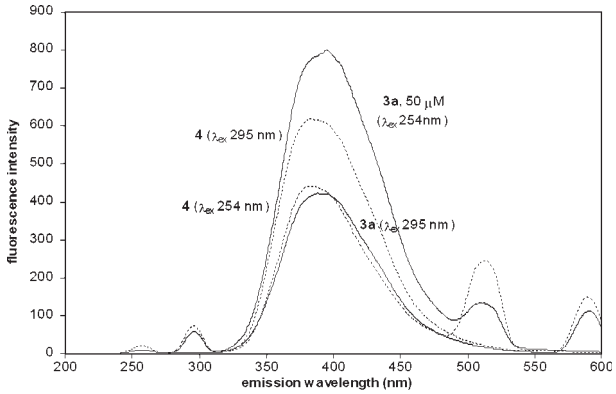


Fig. 2 Fluorescence emission spectra for tryptophan derivatives **3a** and **4** in water ($c = 100$ μM unless otherwise indicated).

Financial support by the Leverhulme Trust (F/00 204/AF, to RJMG), BBSRC (BBE0089841) and the EPSRC (First Grant EP/D059186/1, to GKW) is gratefully acknowledged. We thank Thomas Pesnot for the synthesis of TXPTS, and the EPSRC National Mass Spectrometry Service Centre, Swansea, for the recording of mass spectra.

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