Negishi Cross-Coupling Provides Alkylated Tryptophans and Tryptophan Regioisomers

Steffen Dachwitz,[a] Bjarne Scharkowski,[a] and Norbert Sewald*[a]

Abstract: Mild transition-metal catalysed cross-couplings enable direct functionalisation of biocatalytically halogenated tryptophans with alkyl iodides, representing a new alternative for late-stage derivatisations of halogenated aromatic amino acids. Moreover, this strategy enables preparation of (homo)tryptophan regioisomers in a simple two-step synthesis using a Pd-catalysed Negishi cross coupling. This method provides access to non-canonical constitutional surrogates of tryptophan, ready for use in peptide synthesis.

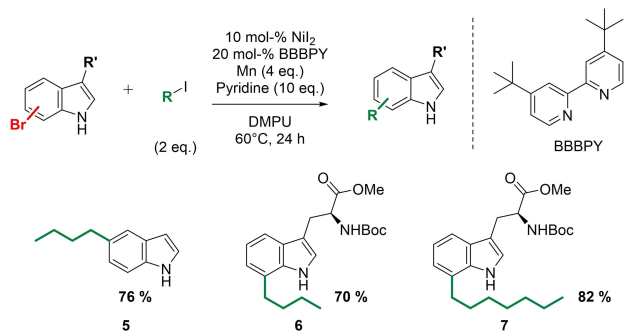
Late-stage diversification of peptides provides access to larger arrays of modified peptides in the frame of a late synthetic step.[1] Unlike modifications on an early stage of the synthesis, late-stage approaches allow for facile generation of a compound library, as one precursor molecule provides an array of modified derivatives. When applying (bio-)orthogonal reactions, protecting groups may not be necessary. Moreover, late-stage reactions show promise for bioorthogonal modification of biological macromolecules (e.g. proteins) even in complex biological matrices.

Tryptophan plays a unique role in protein folding and protein-protein interactions.[2] It occurs less abundantly in proteins, but displays interesting fluorescence properties. Because of these reasons it is an interesting building block, but also target for late-stage functionalisation or substitution by conformational surrogates.

The large-scale access of halogenated tryptophan by immobilised FAD-dependent halogenases[3] prompted our investigations on diverse modifications of free bromotryptophan and bromotryptophan containing peptides by palladium catalysed cross-couplings.[4] C_{sp}–C_{sp2} bond forming Sonogashira-Hagihara[5] cross-coupling and C_{sp2}–C_{sp2} couplings like the Suzuki-Miyaura[6] and Mizoroki-Heck[7] cross-couplings involving bromotryptophan derivatives have been reported. They gave many new arylated indole containing biomolecules with interesting fluorogenic and conformational properties.[4,8] Recently, even an aqueous Buchwald-Hartwig amination of unprotected bromotryptophan and biomolecules was presented. However, the reaction required harsh basic conditions and heating, which might have led to epimerisation.[9] However, to the best of our knowledge C_{sp3}–C_{sp2} bond forming cross couplings with bromotryptophan have not been reported yet.

We are reporting a mild direct alkylation of bromotryptophan derivatives using alkyl iodides without any necessity of a prior formation of metal organyls. Weix and co-workers previously described a Nickel-catalysed reductive cross-coupling of aryl halides with alkyl halides in DMPU at 60 °C.[10] Inspired by these results we attempted cross-coupling of 5-bromoindole (**1**) or N^{α}-Boc-L-7-bromotryptophan methyl ester (**2**) with 1-iodobutane (**3**) or 1-iodoheptane (**4**). The alkyl indole derivatives were obtained in good yields (Scheme 1).

However, we were attracted to the introduction of a functionalised residue into the tryptophan indole. In particular, complex halogenated compounds, for example N^{α}-Boc-β-iodoalanine methyl ester (**8**) seemed very interesting. Unfortunately, iodoalanine **8** suffers from low thermostability because of its propensity toward β-elimination. Thus, it was only possible to isolate traces of the desired product under the reductive cross-coupling conditions described by the Weix group[10] due to the need of elevated temperature. We then envisaged a Negishi cross-coupling[11] for the alkylation of bromoindole and bromotryptophan, since this reaction had been previously employed in modification of other amino acids.[12] Indole derivatives are electron rich aromatic com-



Scheme 1. Nickel catalysed reductive cross-coupling of 5-bromoindole (**1**) and protected L-7-bromotryptophan with simple alkyl iodides.

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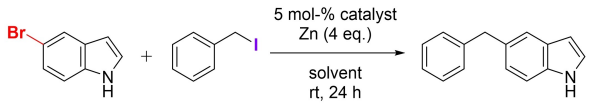
pounds and, hence, challenging substrates for palladium-catalysed cross-couplings. Therefore, we performed a catalyst and solvent screening for all substrates. N^{α}-Boc-β-iodoalanine methyl ester **8** was converted in DMF only, since this solvent has been reported to suppress β-elimination.[13] Commercially available 5-bromoindole **1** and freshly prepared benzyl iodide (**9**) were initially used as test systems (Table 1). Additionally, a separate catalyst-screening for iodoalanine **8** using DMF as the solvent was performed (Table 2). The required zinc organyls were obtained in situ by stirring the reaction mixture with an excess of zinc dust. For that, all solids were suspended in organic solvent which was then purged with argon for 10 min.[4]

The polar and air stable catalyst $Pd(amphos)_2Cl_2$ gave the best results of all tested catalyst systems, reaching full conversion of indole **1** to 5-benzyl indole (**10**) in less than 24 h at room temperature in DMF. From its chemical properties as well as an economical perspective a Nickel catalyst seemed a promising alternative to palladium. Nickel easily undergoes oxidative addition and is less prone to β-hydride elimination, making it in theory favourable for Negishi-couplings of electron rich aromatic systems like bromoindole derivatives.[14] Unfortunately, $Ni(dppe)Cl_2$ did not represent an alternative giving only 4% conversion in 24 h (Tables 1 and 2). No β-elimination of iodoalanine was observed during any coupling. The sterically demanding Boc-group and the proximity of the phosphine ligands might prevent formation of a required agostic interaction between the α-proton of iodoalanine **8** and Pd. Regarding this result and because of the slightly higher toxicity of Nickel,[15] no further Nickel catalysts were tested.

Table 1. Catalyst and solvent screening for the Negishi reaction of 5-bromoindole with benzyl iodide.

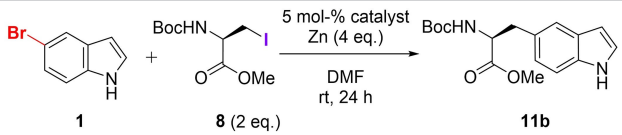


1 **9** (2 eq.) **10**

Entry	Catalyst	Solvent	Conversion [%][a]
1	$Pd(amphos)_2Cl_2$	DMF	>99
2		THF	48
3		1,4-Dioxan	3
4	$Pd_2(dba)_3$/sPhos	THF	5
5	$Pd_2(dba)_3$/P(o-tol)$_3$	THF	8
6	$PdCl_2$	THF	0
7	$Ni(dppe)Cl_2$	THF	4

[a] conversion of **1** to **10** determined by RP-HPLC at 220 nm.

Table 2. Catalyst screening for the Negishi coupling of 5-bromoindole **1** with iodoalanine **8**.



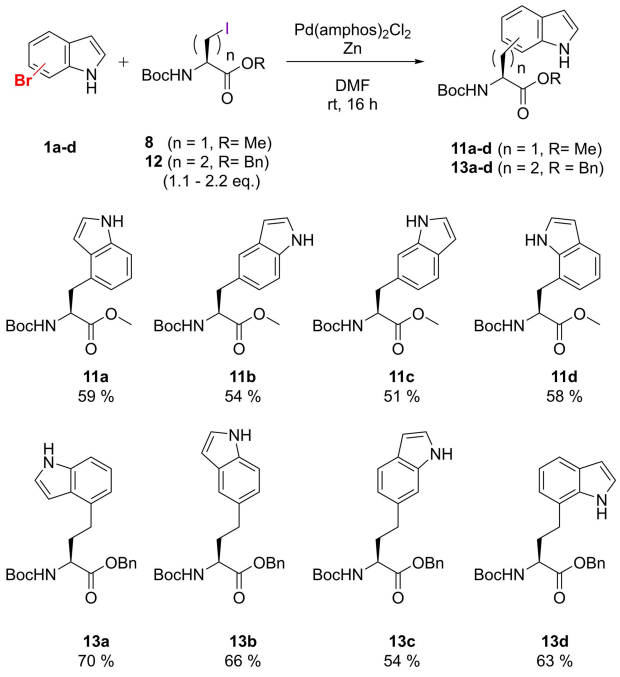
1 **8** (2 eq.) **11b**

Entry	Catalyst	Conversion [%][a]
1	$Pd(amphos)_2Cl_2$	>99
2	$Pd_2(dba)_3$/sPhos	36
3	$Pd_2(dba)_3$/P(o-tol)$_3$	6
4	$PdCl_2$	0
5	Pd/C	0
6	$Ni(dppe)Cl_2$	traces

[a] conversion of **1** to **11b** determined by RP-HPLC at 220 nm.

The Negishi cross-coupling of 5-bromoindole **1** and iodoalanine **8** provides the tryptophan regioisomer **11b**. This compound has been previously obtained in complex multi-step routes.[16] The authors did not describe the final cleavage of the N^{α}-protection groups. Inspired by these results, the substrate scope of bromoindoles (4-, 5-, 6-, and 7-bromoindole) and amino acid-based alkyl iodides (methyl Boc-3-iodoalaninate **8** and benzyl Boc-4-iodohomoalaninate **12**) was expanded, providing access to a broad range of interesting tryptophan surrogates in moderate to good yields after purification by column chromatography (Scheme 2). All reactions were monitored by RP-HPLC and reached full conversion after 16 h. The already well described homo-coupling products of methyl Boc-3-iodoalaninate **8**,[17] which had similar retention times as the cross-coupling products, made purification challenging. With benzyl Boc-4-iodohomoalaninate **12** better separability of product and homo-coupling product on silica could be achieved, which lead to higher isolated yields (Scheme 2). Regioisomer **11a** has been reported as a tyrosine surrogate in modified neurotensin(8–13), giving a brain-penetrating neurotensin agonist with high affinity for the human neurotensin receptor type 1 (NTR1).[18] Hence, the synthesis of tryptophan and homotryptophan regioisomers presented herein may represent a facile access to interesting non-natural amino acids for peptide modification.

The methyl ester can be easily cleaved using LiOH giving access to Boc-protected amino acids for solid phase peptide



Scheme 2. Synthesis of tryptophan (**11**) and homotryptophan (**13**) regioisomers by Negishi cross-coupling.

synthesis. On the other hand, Boc and methyl ester can be cleaved in a single step (Figure 2 and Table 5), making this combination attractive for application in Fmoc-based solid phase peptide synthesis.

The Negishi cross-coupling was also applied for diversification of protected bromotryptophan. Primary alkyl iodides such as 1-iodobutane (**3**) and 13-iodo-2,5,8,11-tetraoxatridecane (**14**), as well as secondary alkyl iodides (1-iodocyclohexane, **15**), and tertiary alkyl iodides (1-iodoadamantane, **16**) were added to the portfolio of potential coupling partners (Figure 1). It is highly recommended to use freshly purified alkyl iodides and to store them under exclusion of light and moisture-free under argon atmosphere to avoid dehalogenation of the aryl bromides. Since protected tryptophan is a less reactive substrate than bromoindole, an increase of the reaction temperature to 37 °C was necessary to achieve satisfactory conversions and yields after 24 h (Table 3). Under these conditions the alkylation of N^{α}-Boc-L-7-bromotryptophan methyl ester (**17**) using a Negishi cross-coupling was possible in yields between 52% and 83%, respectively, with various alkyl iodides without the need of preformed zinc organyls (Table 3).

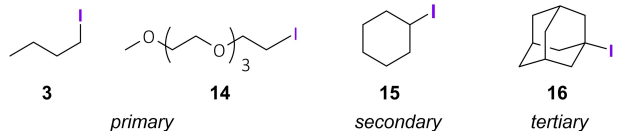
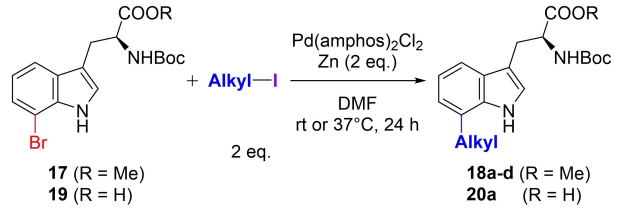


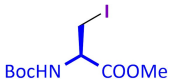
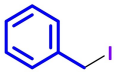
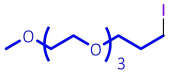
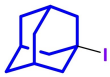
Figure 1. Alkyl iodides selected for substrate screening

Table 3. Negishi cross coupling of protected bromotryptophan with various alkyl iodides.



17 (R = Me)
19 (R = H)

18a-d (R = Me)
20a (R = H)

Entry	Alkyl iodide	R	T [°C]	#	Yield [%]
1		Me	20	**18a**	26
		Me	37	**18a**	83
		H	37	**20a**	46
2		Me	20	**18b**	21
		Me	37	**18b**	52
3		Me	20	**18c**	37
		Me	37	**18c**	81
4		Me	20	–	0
		Me	37		0
5		Me	20	**18d**	n.d.[a]
		Me	37	**18d**	73
6		Me	20	–	0
		Me	37		0

[a] not determined, due to low conversion.

Negishi cross-couplings with 13-iodo-2,5,8,11-tetraoxatridecane (**14**) or 1-iodoadamatane (**16**) did not show product formation. Hence, the zinc organyl of 1-iodoadamantane[19] (**16a**) was pre-synthesised according to the literature, to prove that the formation of the zinc organyl is not the determining step. With the pre-formed zinc organyl **16a** no formation of cross-coupling product by HPLC-MS could be observed either; only unreacted aryl bromide was recovered. Noteworthy, it was possible to perform the Negishi cross-coupling using N^{α}-Boc-protected L-7-bromotryptophan (**19**) with a free carboxy group. The alkylated N^{α}-Boc-protected tryptophan **20a** could be received in moderate yield after purification by RP-HPLC, ready for use in solid phase peptide synthesis (Table 3).

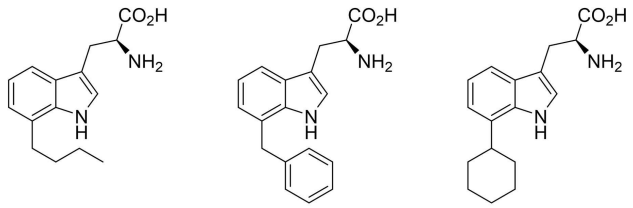
The high enantiomeric purities of the regioisomeric tryptophan surrogates and the alkylated tryptophans were confirmed with Marfey's reagent (N^{α}-(2,4-dinitro-5-fluorophenyl)-L-alanine amide, FDAA) after deprotection. In case of the alkylated tryptophans reflux in 5 M HCl for 2 h was sufficient to simultaneously cleave the Boc-group and the methyl ester. The deprotected alkylated tryptophans (**21a–c**) were obtained in good yields after purification by RP-HPLC with an *ee* > 99% in all cases (Figure 2). The Negishi cross-coupling did not lead to racemisation.

The deprotection of the tryptophan regioisomers (**11a–d**) was challenging due to a very fast acid-catalysed polymerisation of indole derivatives.[20] It was found that heating to 110 °C in water without any additives in a closed vial, releasing the overpressure every 15 to 30 min over 4 h led to cleavage of the Boc-group as well as the methyl ester of N^{α}-Boc-(5-indolyl) alanine methyl ester (**11b**) without indole polymerisation.

Free 3-(5-indolyl)alanine (**22b**) was obtained after purification by RP-HPLC under acid-free conditions in moderate to good yields (Table 5). However, racemisation of the product was observed, which presumably occurs during deprotection. A screening of different temperatures as well as reaction times was performed exemplary for N^{α}-Boc-(5-indolyl) alanine methyl ester (**11b**), followed by Marfey's test (Table 4).

The low conversion but high enantiopurity at 100 °C over 4 h made the deprotection more favourable at 100 °C, but made elongation of the reaction time mandatory.

To further increase the yields without loss of enantiopurity, aqueous buffer systems were tested for the deprotection. Best results were obtained using a 0.1 M Na_2HPO_4-buffer at pH 7.4



21a
84 %
>99 % *ee*

21b
70 %
>99 % *ee*

21c
73 %
>99 % *ee*

Figure 2. Unprotected alkylated tryptophan derivatives and their enantiomeric excess determined by Marfey's test.

Table 4. Deprotection of indolyl alanine **11b** by heating in water.

Entry	T [°C]	t [h]	Yield [%]	*ee* [%]
1	110	48	83	84
2	110	24	88	90
3	110	4	60	92
4	100	4	18	94

Table 5. Deprotection of tryptophan regioisomers in 0.1 M Na_2HPO_4-buffer at pH 7.4.

Entry	Product	#	Yield [%]	*ee* [%]
1	3-(4-indolyl) alanine	**22a**	55	95
2	3-(5-indolyl) alanine	**22b**	52	94
3	3-(6-indolyl) alanine	**22c**	98	94
4	3-(7-indolyl) alanine	**22d**	99	38

and heating to reflux for 9 h. Using this, all tryptophan regioisomers (**22a**–**d**) were obtained with moderate to high yields and good enantiopurities, except the 3-(7-indolyl) alanine (**22d**) giving only an enantiomeric excess of 38% (Table 5).

The higher racemisation of **22d** may occur due to an intramolecular hydrogen bridge between the carbonyl oxygen of the carboxylic acid and the indolyl-NH. This decreases the electron density at the carbonyl carbon and thereby increases Cα-acidity. The hydrogen bridge is observable by ^{1}H NMR in d_6-DMSO, showing a broadening of the indole-NH signal and a shift towards low field by $\delta = 1.0$–1.2 ppm in comparison to the other regioisomers (Figure S1). In the ^{13}C NMR the carbonyl carbon is shifted towards low field by $\delta = 3.2$–3.4 ppm in comparison to the other regioisomers.

In conclusion, the first C_{sp3}—C_{sp2} bond forming cross couplings for direct alkylation of halogenated bromotryptophan using either a reductive Nickel cross-coupling or a mild Palladium-catalysed Negishi cross-coupling were established. Alkylation of bromoindoles by a Negishi reaction using iodo (homo)alanine derivatives enabled the synthesis of a broad range of tryptophan surrogates, which might be interesting as potential unnatural amino acids for peptide modifications or de novo peptide synthesis. It was possible to deprotect the otherwise acid labile tryptophan regioisomers in good yields with good enantiopurities in most cases. Alkylation by a Negishi cross-coupling expands the portfolio of possible applicable cross-couplings for late-stage diversification of halogenated tryptophan and their derivatives. Further investigation regarding other protection strategies, peptide synthesis and modification using a Negishi cross-coupling is underway.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: halotryptophan · negishi coupling · late-stage derivatisation · nickel-catalysed reductive cross-coupling · tryptophan surrogates

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