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Scope of Tyrosine O-Arylations with Boronic Acids: Optimized Synthesis of an Orthogonally Protected Isodityrosine

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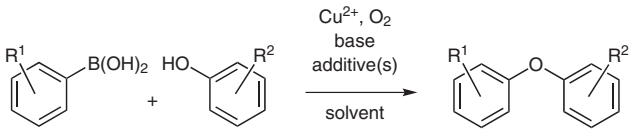
Abstract: The Evans–Chan–Lam variant of the Ullman condensation has been explored to deliver O-arylated tyrosines and tyrosinyl peptides. Key modifications for success were the slow addition of boronic acids to the phenol–catalyst mixture. Selectivity and scope are investigated.

Key words: bioorganic chemistry, copper, cross coupling, peptides, phenols

Noncanonical chemical modifications are the hallmark of many bioactive peptide natural products. For example, in the biaryl peptide class of natural products[1] encompassing vancomycin,[2] biphenomycin,[3] and RA-VII,[4] covalently linked tyrosines are essential for activity. The ensuing hydrophobic biaryl- and biaryl ether functions then contribute to molecular recognition patterns.



Equation 1 Evans–Chan–Lam coupling reaction

For synthetic access to biaryl ether motifs a variety of methods has been developed.[5] In natural product synthesis oxidative additions,[6] nucleophilic aromatic substitutions,[7] and transition-metal-mediated couplings of phenols with aryl electrophiles have been successfully applied.[8] Using such reactions for the postsynthetic derivatization of peptides would provide important tools for synthesis and library generation.[9] However, many protocols are not generally compatible with α-chiral amino acids and peptides. The Cu^{2+}/O_2-mediated coupling of phenols and boronic acids might provide a notable exception in this regard (Equation 1).[10,11] Here we describe investigations on the scope of this Evans-Chan-Lam coupling for tyrosine and its derivatives, which led to the successful preparative scale synthesis of the RA-VII key isodityrosine fragment in orthogonally protected form.

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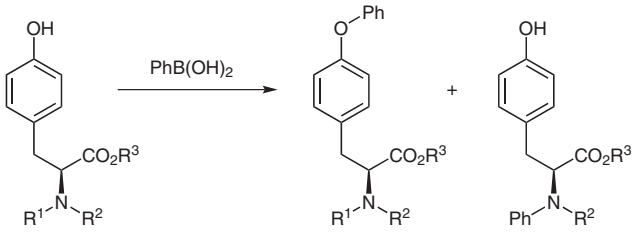
Hans-Dieter Arndt studied chemistry at the universities of Ulm and Marburg, Germany, and Imperial College, UK. He obtained his PhD degree in 2002 from the Humboldt-Universität zu Berlin, Germany, for work with Professor Ulrich Koert on stereoselective synthesis of oligopyrrolidines and artificial ion channels. At the California Institute of Technology, Pasadena, USA, he conducted postdoctoral studies with Professor Peter B. Dervan on small-molecule-based DNA–protein dimerizers. His independent career began in spring 2004 as a group leader at the MPI of Molecular Physiology, Dortmund, and he became member of the department of chemistry at Technische Universität Dortmund in fall 2004. Dr. Arndt's research program is centered on bioactive small molecules with particular interests in synthetic methodology, non-natural peptides, and activity on protein–oligonucleotide interactions. His research was recognized with the Liebig fellowship of the Fonds der Chemischen Industrie (2004), the Emmy-Noether young investigator award of the DFG (2004) and the Thieme Journal Award (2007).

All our investigations focused on Fmoc-protected amino acids and peptides to test for compatibility with typical solid-phase-synthesis procedures. Reported conditions for the arylations of phenols with boronic acids featured stoichiometric $Cu(OAc)_2$, a tertiary amine base, molecular sieves, O_2-containing atmosphere, and an excess of boronic acid (typically 3 equiv).[10] In initial studies with compounds **1a** and **1b** (Table 1) we studied reaction parameters. Pyridine as the base was found superior over aliphatic tertiary amines [Et_3N, $EtN(i\text{-}Pr)_2$], but neither 2,6-lutidine could be used (no reaction), nor DMAP[12] (degradation). We could reduce the amount of $Cu(OAc)_2$ to 10–20% without losing efficacy.[13] Activated molecular sieves and oxygen atmosphere were mandatory, and chlorinated solvents were ideal. Addition of DMF was tolerated in cases of insufficient solubility. The excess of boronic acid was deemed most critical for future applications. We found that slow addition of the boronic acid to the catalyst with a syringe pump allowed us to achieve high conversions with only a slight excess of arylating reagent. These

Table 1 Chemoselectivity of the Arylation of Tyrosines[a]



1 2 3

1	R^1	R^2	R^3	Yield of **2** (%)	Yield of **3** (%)	Conversion (%)
1a	H	Fmoc	*t*-Bu	82	–	>99
1b	Me	Fmoc	*t*-Bu	95	–	>99
1c	H	Me	*t*-Bu	63[b]	–	64
1d	H	H	*t*-Bu	–	55[b]	80
1e	H	Fmoc	H	–	–	<1

[a] Compounds **1a**–**e** (1 equiv), $Cu(OAc)_2$ (20 mol%), pyridine (5 equiv), 4 Å MS, DCE, slow addition of $PhB(OH)_2$ (1.4 equiv, 16 h).
[b] Based on recovered **1**.

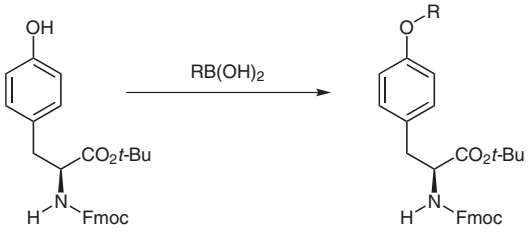
optimized conditions were then applied to a spectrum of tyrosine derivatives (Table 1).

With $PhB(OH)_2$ as reagent the tyrosines **1a** and **1b** were cleanly arylated in very good yield. The free amines **1c** and **1d** were found to react as well, albeit with lower efficiency. In the case of the primary amine an aryl transfer to the nitrogen was observed in favor of O-arylation, indicating a delicate balance of nucleophilicity and steric factors. The carboxylic acid **1e** remained unreactive.

The scope of the boronic acid was investigated next with a panel of commercially available boronic acids (Table 2). Both electron-rich (entry 2) as well as electron-poor boronic acids (entry 3) could be applied, but were generally found to be less efficient than the parent compound $PhB(OH)_2$. Steric hindrance was tolerated well only with electron-poor substrates (cf. entry 3 with entries 5 and 6). The heterocyclic boronic acids tested were found to be unstable under the reaction conditions (entry 7, 8).

In going further the tyrosine-containing peptides **5a**–**c**, **9**, and **11** were investigated (Table 3, Scheme 1).[14] For these substrates dichloroethane–DMF mixtures had to be used as reaction medium to ensure solubility of all components. In contrast to the previously investigated carboxylic acid **1e**, peptide **5a** underwent clean O-arylation at the tyrosine residue **6a**, albeit at low reaction rate. For the less acidic C-terminal amide **5b**, selective conversion into the C-terminal amide **7b** was observed. Increasing the amounts of reagent and catalyst led to the formation of the doubly arylated product **8**, however, in low isolated yield (18%). On the other hand, ester **5c** could be cleanly transformed into the biaryl ether **6c**, underscoring the importance of the C-terminal functionality for this transformation.

Table 2 Arylation with Boronic Acids[a]

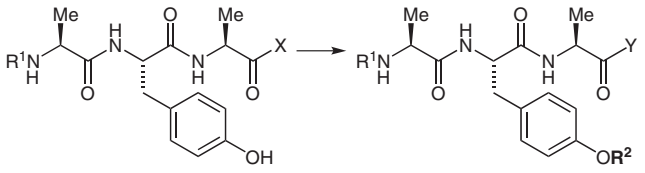


1a 4a–g

Entry	R	Yield of **4** (%)[b]	Conversion (%)
1	Ph	82	>99
2	2-$MeOC_6H_4$	93	41
3	2,4,6-$(F_3C)_3C_6H_2$	88	40
4	4-PhC_6H_4	83	72
5	1-naphthyl	29	38
6	2,4,6-$Me_3C_6H_2$	n.d.	<5
7	3-pyridyl	n.d.	<5
8	2-benzothiophenyl	n.d.	<5

[a] For conditions see Table 1; 5% DMF were added to the solvent.
[b] Based on recovered **1a**.

Table 3 Arylation of Tyrosine-Containing Peptides[a]



5

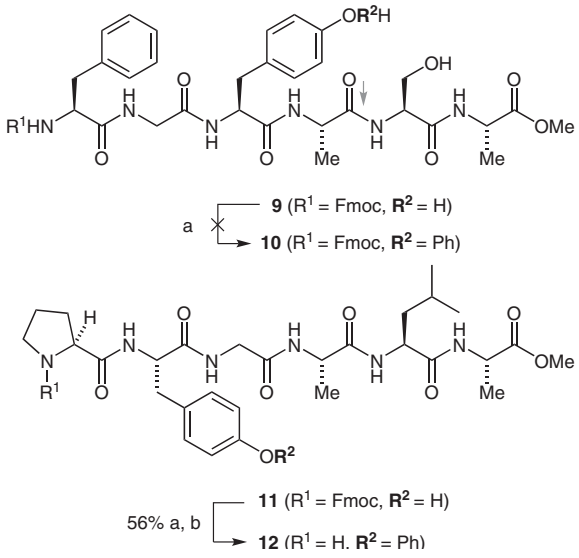
6 (Y = X, $\mathbf{R^2}$ = Ph)
7 (Y = NHPh, $\mathbf{R^2}$ = H)
8 (Y = NHPh, $\mathbf{R^2}$ = Ph)

5	X	Yield of **6** (%)	Yield of **7** (%)	Conversion (%)
5a	OH	50	–	80
5b	NH_2	traces	68	44
5c	OAll	60	–	>99

[a] For conditions see Scheme 1, but DCE–DMF (10:1) was used as solvent.

The reactivity of the longer-chain peptide esters was generally similar, but pronounced cleavage of the Fmoc group was observed in these cases. A free serine side chain in peptide **9** was not tolerated and led to the formation of a spectrum of truncated products, many of them indicative of cleavage N-terminal of the Ser residue (LC-MS, Scheme 1). The hydrophobic peptide **11** gave biaryl ether **12** (56% after complete Fmoc removal).

From these data we reason that in contrast to a primary amide sterically less accessible secondary amide bonds are tolerated, allowing the use of peptides as substrates. A competitive coordination of the peptide backbone to the

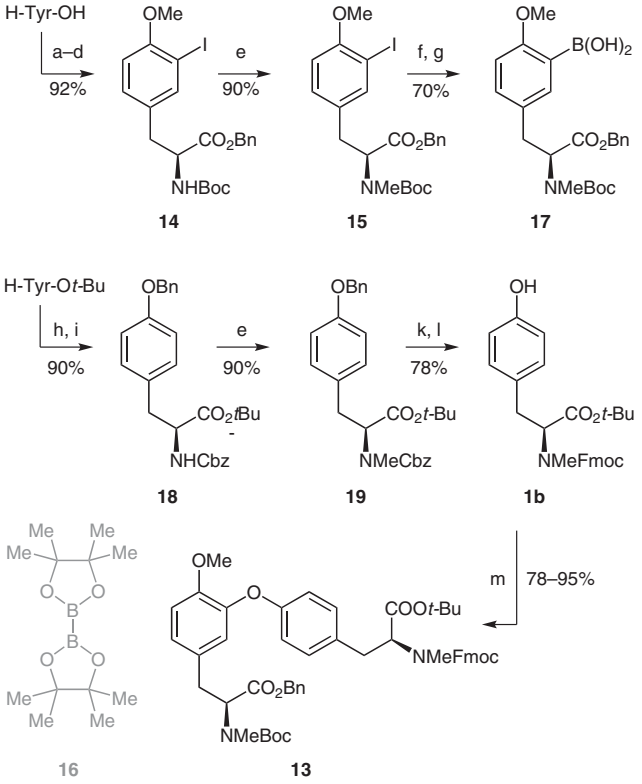


Scheme 1 *Reagents and conditions*: (a) $Cu(OAc)_2$ (1 equiv), pyridine (5 equiv), DCE–DMF (1:1), 4 Å MS, O_2 (1 atm), slow addition of $PhB(OH)_2$, r.t., 48 h; (b) $HNEt_2$–CH_2Cl_2 (1:1), 30 min.

catalytically active species can be suspected in these cases, which might account for reduced conversions and the partial cleavage of the Fmoc group only observed in the longer-chain peptides **9** and **11**.

In aiming at the synthesis of biaryl ether natural product scaffolds, the orthogonally protected isodityrosine **13** was selected as a target molecule (Scheme 2). Compound **13** itself occurs as a key element in the RA family of natural products and carries two critical *N*-methyl groups.[4] In order to prepare tyrosine for its coupling, it was ortho-iodinated with I_2,[15] N-Boc-protected, and then converted into the fully protected amino acid **14** by consecutive alkylations on the carboxyl- and phenol functional groups. Here the choice of solvent and base was essential for securing regioselectivity (4 steps, 92%). N-Methylation of **14** had to be addressed next. In general, a wide spectrum of methods has been reported to achieve such transformations with amino acid derivatives.[16] We found that freshly prepared Ag(OH) in DMF was able to promote an N-selective methylation of carbamate[17] **14** with MeI to yield aryl iodide **15** in excellent yield with no detectable epimerization (90%, >98% ee by HPLC). Borylation was then cleanly achieved using diboronate **16** under Miyaura's conditions,[18] and oxidative cleavage of the pinacolate[19] yielded boronic acid **17** (70%).

N-Methylated tyrosine **1b** was accessed from tyrosine *tert*-butyl ester, which was first Cbz-protected and O-benzylated to give derivative **18** (90% yield). Again, N-methylation to **19** was achieved best with MeI/Ag(OH) in DMF (90%, >95% ee by HPLC). The Bn-based protecting groups were cleaved by hydrogenolysis, and introduction of an N-terminal Fmoc group delivered phenol **1b** (65%). Under the previously identified conditions, isodityrosine **13** could be obtained in excellent yield (up to 95%) while



Scheme 2 *Reagents and conditions*: (a) I_2, aq NH_3, EtOH, 0 °C, 1 h; (b) Boc_2O, aq Na_2CO_3 (2 M), THF, r.t., 3 h; (c) BnBr, $EtN(i\text{-}Pr)_2$, acetone, reflux, 16 h; (d) MeI, Na_2CO_3, acetone, reflux, 16 h; (e) MeI, AgOH (s), DMF, 35 °C, 8 h; (f) **16**, $PdCl_2$dppf (10 mol%), KOAc, DMF, 80 °C, 16 h; (g) $NaIO_4$, acetone–NH_4OAc, r.t., 36 h; (h) CbzCl, Et_3N, dioxane–H_2O, r.t., 16 h; (i) BnBr, Na_2CO_3, DMF, r.t., 16 h; (k) H_2, 10% Pd/C, MeOH–HCOOH (95:5), r.t., 16 h; (l) Fmoc-OSu, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, 0 °C to r.t., 4 h; (m) $Cu(OAc)_2$ (10 mol%), pyridine (5 equiv), DCE, 4 Å MS, O_2 (1 atm), slow addition of **17**, r.t., 48 h.

employing only 1.4 equivalents of boronic acid **17**. Key for achieving high yields was the use of freshly purified boronic acid, sufficient reaction time (48 h), and a phenol acceptor devoid of an acidic amide NH function (compare to entry 2 in Table 2).

In summary, we have investigated the scope of the Cu^{2+}/O_2-mediated coupling of boronic acids to tyrosine and its derivatives. Couplings proceed mildly, but are affected by free carboxylic acid and amine functionality in the substrate and steric hindrance or lability of the boronic acid. We found that C-terminally protected tyrosinyl peptides are good substrates for this reaction and that selective arylation of primary amides and amines can be achieved. Moreover, optimized reaction conditions led to an efficient, modular synthesis of an orthogonally protected isodityrosine building block. It is worth noting that the slow addition protocol makes efficient use of the boronic acid component in the coupling procedure and as such provides a critical advantage in the synthesis of complex target molecules.

Experimental Procedure for the Preparation of Isodityrosine 13
Copper(II) acetate (11.7 mg, 0.064 mmol, 20 mol%) was suspended in DCE (2 mL). Pyridine (130 μL, 1.6 mmol, 5 equiv) was added and the suspension was stirred for 10 min under O_2 (1 atm). Phenol **1b** (152 mg, 0.32 mmol) and powdered 4 Å MS (800 mg) were added. Boronic acid **17** (200 mg, 0.45 mmol, 1.4 equiv) was dissolved in DCE (5 mL) and slowly added to the mixture (300 μL/h). The slurry was stirred under O_2 (1 atm) for 48 h, diluted with EtOAc, and filtered over a pad of SiO_2. Flash column chromatography (cyclohexane–EtOAc, 70:30) gave isodityrosine **13** (256 mg, 92%) as a colorless solid: mp 56–58 °C, $[\alpha]_D$ –52.7 (*c* 0.98, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.21 (s, 4.42 H)*, 1.26 (s, 4.37 H)*, 1.34 (s, 3.98 H)*, 1.38 (s, 4.6 H)*, 2.57 (m, 3 H), 2.62 (s, 1.37 H)*, 2.68 (s, 1.52 H)*, 2.92 (m, 2 H), 3.09 (m, 2 H), 3.68 (m, 3 H), 4.26 (m, 2 H), 4.44 (m, 1 H), 4.65 (m, 1 H), 4.74 (m, 1 H), 5.12 (d, 2 H, *J* = 15.4 Hz), 6.67 (dd, 2 H, *J* = 8.2, 23.6 Hz), 6.81 (m, 2 H), 7.03 (dd, *J* = 9.8 Hz, 3 H), 7.36 (m, 9 H), 7.51 (d, *J* = 7.1 Hz, 0.35 H)*, 7.56 (d, *J* = 7.5 Hz, 1.65 H)*, 7.87 (d, *J* = 7.5 Hz, 2 H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 27.4, 31.6, 33.1, 35.7, 46.3, 55.5, 59.6, 65.9, 69.5, 79.1, 80.8, 113.2, 115.9, 120.0, 122.0, 125.7, 127.3, 127.3, 127.9, 128.5, 130.0, 131.1, 136.0, 143.7, 143.6, 149.8, 154.5, 156.6, 155.6, 156.4, 170.3; *carbamate rotamers induce signal splitting. ESI-HRMS: *m/z* calcd: 871.4164 [M + H$^+$], found: 871.4172.

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