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Late-stage Functionalization of Peptides and Cyclopeptides using Organozinc reagents

Marcel Leroux[a], Thomas Vorherr*[b], Ian Lewis[b], Michael Schaefer[b], Guido Koch[b], Konstantin Karaghiosoff[a], Paul Knochel*[a]

Dedication ((optional))

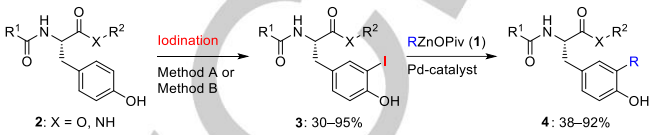
Abstract: *We have reported a new late-stage functionalization of small peptides and cyclopeptides using readily prepared iodotyrosine or iodophenylalanine containing peptides and performing Negishi cross-couplings with aryl-, heteroaryl- and alkyl-zinc pivalates or halides. In-silico and in-vitro determinations of membrane permeability data of the modified cyclopeptides showed that in most cases, the solubility was improved while the cell-membrane permeability was maintained by the introduction of polar pyridyl units.*

The late-stage functionalization of peptides is an important and practical method for preparing modified peptide derivatives.[1] The possibility of combinatorial modification of drug targets is essential for an efficient search of optimal properties like bioavailability, stability, membrane permeability and to achieve improved structure-activity relationships.[2] A wide range of transition-metal catalyzed C-H activations have been used for late-stage activations.[3] Barluenga[4] and Davis[5] have demonstrated the utility of Suzuki-Miyaura cross-couplings for the modification of amino-acids and small peptides. As an alternative to the Suzuki-Miyaura reaction, the Negishi cross-coupling involving organozinc reagents often requires milder reaction conditions and less sophisticated ligands[6]. Therefore, the Negishi-coupling should be well suited for the late-stage functionalization of complex molecules. Furthermore, organozinc halides are readily prepared and tolerate the presence of various functional groups.[7]

Recently, we have shown that organozinc pivalates[8] of type **1** with the general formula RZnX·Mg(OPiv)$_2$·LiCl[9] (abbreviated RZnOPiv) display enhanced moisture- and air-stability. They have already found useful applications in high-throughput screenings[10] and various cobalt-catalyzed reactions.[11]

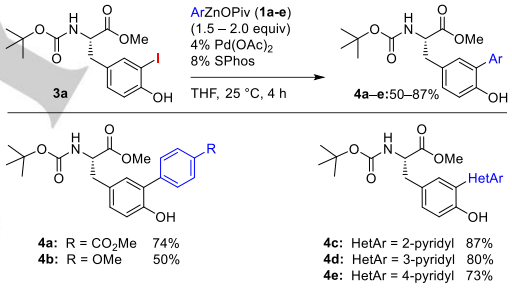
Herein, we wish to report a quite general late-stage functionalization of small peptides and cyclopeptides bearing an *ortho*-iodotyrosine amino-acid or an iodophenylalanine using Negishi cross-couplings with organozinc pivalates (**1**).

Thus, the iodination of tyrosine containing peptides[12] of type **2** with NaI, chloramine-T in DMF at 0–25 °C (Method A)[13] or with IPy$_2$BF$_4$ in CH_2Cl_2 at 25 °C (Method B)[14] produces the mono-iodotyrosine derived peptides of type **3**, which after Pd-catalyzed cross-couplings with various organozinc pivalates (**1**) gave modified peptides of type **4** (Scheme 1).



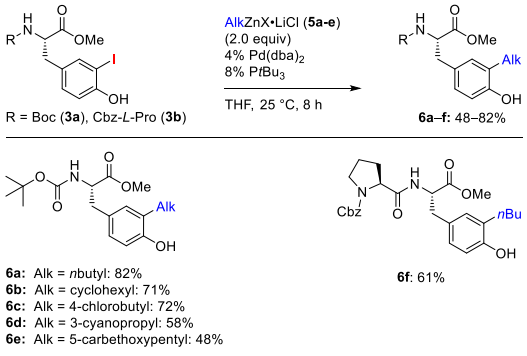
Scheme 1. Preparation of modified tyrosine-based peptides (**4**) starting from a tyrosine containing peptides of type **2**.

In preliminary experiments, we have determined the optimum cross-coupling conditions, varying the temperature, concentration, rate of addition of the organozinc reagent and Pd-catalyst system on the protected iodotyrosine derivative **3a**[15]. Both electron-rich or electron-poor arylzinc pivalates (**1a–b)** as well as pyridylzinc pivalates[16] (**1c–e)** gave satisfactory results using 4% Pd(OAc)$_2$ and 8% SPhos[17] leading to functionalized tyrosines (**4a–e)** in 50–87% yield (Scheme 2).



Scheme 2. Negishi cross-coupling of tyrosine derivative **3a** with aryl- and heteroarylzinc pivalates (**1e–e**) leading to the tyrosines **4a–e**.

An attempt to extend this cross-coupling to alkylzinc pivalates was difficult and a new optimization study was required. We have found, that in this case, the best zinc organometallics were alkylzinc halides prepared by the direct oxidative insertion of zinc powder in the presence of LiCl.[18]



Scheme 3. Cross-coupling reactions of tyrosine derivatives (**3a–b**) with organozinc halides (**5a–e**) leading to alkylated tyrosine derivatives (**6a–f**).

As catalyst, the system developed by Fu[19] with 4% Pd(dba)$_2$ (dba = dibenzylideneacetone) and 8% P*t*Bu$_3$ gave the best results. Thus, various functionalized primary and secondary alkylzinc

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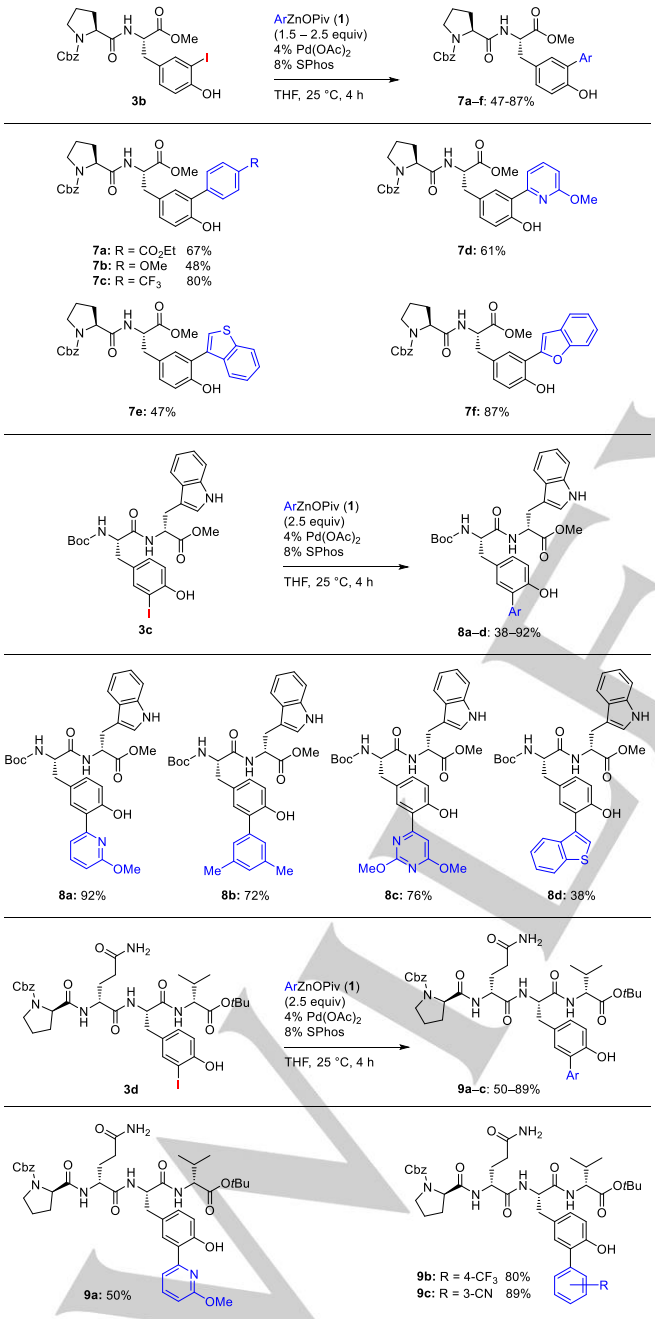
halides (**5a**–**e**) bearing a chloro-, a carbethoxy- or a cyano function led to the modified tyrosines (**6a**–**e**) including the dipeptide **6f** (Scheme 3). Remarkably, the used Fu-catalyst[19] led to relatively fast cross-coupling reactions. Combined with the slow addition[20] of the organozinc reagent[21], an excellent compatibility with the acidic amides and phenolic protons was achieved.

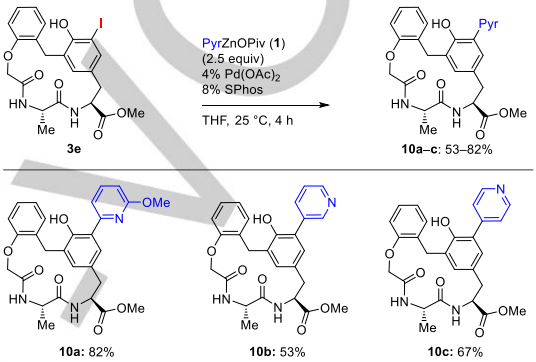
With these optimized conditions in hand, we have applied these late-stage functionalizations to dipeptides (**3b** and **3c**) and a tetrapeptide (**3d**). As indicated in Scheme 4, a range of arylzinc pivalates bearing both electron-donating or accepting groups as well as heteroaryl-zinc pivalates were used leading after Negishi cross-couplings to the expected products (**7a**–**f, 8a**–**d** and **9a**–**c**) in 38–92% yield.



Scheme 4. Cross-coupling reactions of the iodotyrosine derived oligopeptides (**3b**–**3d**) with organozinc pivalates (**1**) leading to the functionalized tyrosine containing peptides (**7**–**9**).

Additionally, we have extended this late stage functionalization to four iodocyclopeptides (one derived from tyrosine; see **3a** and three derived from phenylalanine **11**–**13**).

Cyclopeptides are an important class of metabolically quite stable peptides with a broad range of medicinal applications.[22] Especially proline containing cyclopeptides have been increasingly investigated due to their high bioactivity[23]. They have found medical applications e.g. as immunosuppressant, anti-HIV, antibacterial or antiviral drugs[24]. Thus, the iodocyclopeptide **3e** prepared via method B[25] was treated with 2-, 3- and 4-pyridylzinc pivalates derivatives. Hence, using standard cross-coupling conditions, the expected cyclopeptide products (**10a**–**c**) were obtained in 54–82% yield (Scheme 5).



Scheme 5. Modification of cyclic peptide **3e** bearing an iodotyrosine via cross-coupling reactions with heteroarylzinc pivalates (Pyr: various pyridyl scaffolds) leading to functionalized peptides of type **10**.

Similarly, we submitted iodophenylalanine cyclopeptides (**11**–**13**) to a cross-coupling with the same pyridylzinc pivalates and have obtained the desired coupling-products (**14a**–**c**, **15a**–**c** and **16a**–**c**) in 38–84% yield (Table 1). We noticed that in the case of the cross-coupling of *ortho*-phenylalanine cyclopeptide **11** with 2-pyridylzinc pivalate (**1c**) harsher reaction conditions were required (60 °C, 24 h instead of 25 °C, 6 h).

A set of experiments was designed to gain more insights regarding steric limitations of the reactions and the influence of differentially substituted pyridyl cores. The latter modification has been suggested to promote permeability, if the pyridyl nitrogen can act as an intramolecular H-bond acceptor.[26] In general, this kind of assessment is of importance when fine-tuning properties and structure-activity relationships during the drug discovery process. The membrane permeability- and solubility-parameters of the modified cyclopeptides **14**–**16** were compared to the results of *in-silico* calculations. Thus, this set of nine cross-coupling products (**14**–**16**) had been submitted for MD (molecular dynamics) simulations followed by calculation of the solvent-accessible polar surface area (abbreviated SAPSA) to result in an *in-silico* assessment regarding their permeability properties in the first instance (Table 2) [27].

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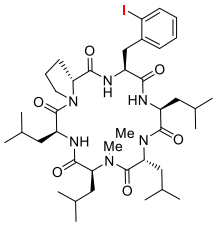
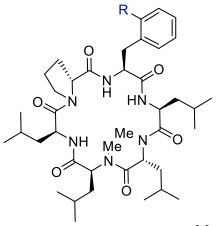
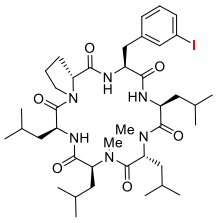
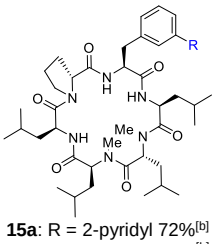
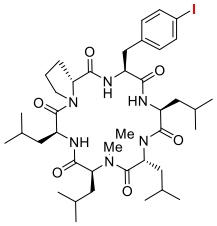
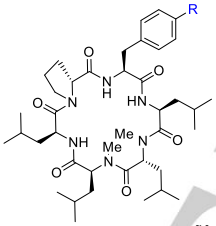
Table 1. Modification of iodophenylalanine-cyclopeptides (**11**–**13**) *via* cross-coupling reactions with 2-, 3- and 4-pyridylzinc pivalates (**1c**–**e**).

iodo-cyclopeptide	modified cyclopeptide
 **11**	 **14a**: R = 2-pyridyl 38%[a] **14b**: R = 3-pyridyl 62%[b] **14c**: R = 4-pyridyl 67%[b]
 **12**	 **15a**: R = 2-pyridyl 72%[b] **15b**: R = 3-pyridyl 62%[b] **15c**: R = 4-pyridyl 77%[b]
 **13**	 **16a**: R = 2-pyridyl 84%[b] **16b**: R = 3-pyridyl 62%[b] **16c**: R = 4-pyridyl 80%[b]

[a] RZnOPiv (3.0 equiv), 8% $Pd(OAc)_2$, 16% SPhos, THF, 60 °C, 24 h.
[b] RZnOPiv (2.5 equiv), 4% $Pd(OAc)_2$, 8% SPhos, THF, 25 °C, 6 h.

Interestingly, different SAPSA values were observed, and as expected in the cases, in which the pyridyl moiety is more exposed (meta and para substituted phenylalanine residues) more polar surface area is accessible. Next, solubility and permeability parameters were assessed. The low solubility measured for the starting materials (**11**–**13**) was in most of the cases improved by the pyridyl core (see Table 2, entries 4–9 and 12), and on the basis of the PAMPA (Parallel Artificial Membrane Permeability Assay) results, all cyclic peptides display high permeability. However, in the cellular assessment (MDCK; Madin Darby canine kidney cells assay), the position of the pyridyl linkage seemed to be sensitive regarding transport across. As for the *in-silico* analyses, the *para*- phenylalanine substituted analogues showed reduced transport rates.

However, this assay reflects the sum of active and passive transport properties, whereas the SFC (supercritical fluid chromatography) method rank-orders only for passive permeability. Nevertheless, the cross-coupling products resulting from *ortho*-phenylalanine substitutions clearly showed a higher passive permeability. This finding was in line with the MD simulations followed by SAPSA calculation of the preferred conformational clusters.

Table 2. *In-silico* and *in-vitro* profiling of the iodophenylalanine starting materials (**11**–**13**) and the pyridyl cross-coupling products (**14**–**16**).

Entry	Peptide	SAPSA (Å^2)	Sol. pH=6.8 (µM)	PAMPA (pH 6.8)	MDCK (10^{-6}cm/s)	SFC (min)
1	**11**	62	< 4	-4.2	n.d. [a]	3.15
2	**12**	74	3	-4.3	n.d. [a]	3.14
3	**13**	75	< 4	-5.4	n.d. [a]	3.23
4	**14a**	76	24	-4.7[b]	n.d. [a]	3.37
5	**14b**	84	46	-4.3	15.7	3.31
6	**14c**	83	52	-4.5	19.3	3.39
7	**15a**	74	21	-4.5	3.3	3.51
8	**15b**	101	26	-4.7	3.2	3.60
9	**15c**	94	31	-4.8	13.6	3.76
10	**16a**	84	9	-4.5	1.4	3.61
11	**16b**	99	< 4	-4.6	2.1	3.72
12	**16c**	106	22	-4.6	2.9	3.89

[a] not determined due to QC or recovery problems
[b] at pH 8.0

In summary, this methodology including selective tyrosine iodination and Negishi cross-coupling reaction tolerates peptides based on several amino acids[28]. A wide range of organozinc reagents bearing various functional groups as well as heterocycles can be used. Furthermore, this new late-stage functionalization of readily available iodotyrosine or iodophenylalanine containing peptides offers opportunities for a detailed site-specific exploration and probing of the property space. In addition, for intracellular targets or when an oral route is required, the ability to manipulate properties with the aim to obtain permeable molecules retaining some polarity enables a drug discovery process not compromised by poor solubility. Further extensions of this approach are underway.

Acknowledgements

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Keywords: peptides · cyclopeptides · organozinc · cross-coupling · membrane permeability

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[28] This methodology was applied to oligopeptides containing amino-acids such as phenylalanine, tryptophan, proline, glutamine, alanine and valine. In linear peptides, the N-terminus was protected with Boc or Cbz and the C-terminus converted to an ester.

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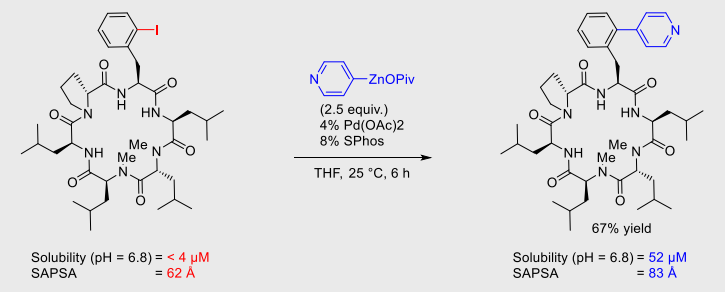

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Late-stage Functionalization of Peptides and Cyclopeptides using Organozinc reagents

Late-stage peptide functionalization: We have reported a new late-stage functionalization of small peptides and cyclopeptides using readily prepared iodotyrosine or iodophenylalanine containing peptides and performing Negishi cross-couplings with aryl-, heteroaryl- and alkyl-zinc pivalates or halides. In-silico and in-vitro determinations of membrane permeability data of the modified cyclopeptides showed that in most cases, the solubility was improved while the cell-membrane permeability was maintained by the introduction of polar pyridyl units