Protein PEGylation

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Self-Liganded Suzuki–Miyaura Coupling for Site-Selective Protein PEGylation**

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The discovery and development of palladium-catalyzed carbon–carbon bond formation has revolutionized modern organic chemistry in recent decades. The versatility and robustness of these processes have enabled major progress in total synthesis, materials development, and medicinal chemistry.[1] Currently, a large variety of nucleophiles and electrophiles can be efficiently coupled, and part of the efforts to increase the utility of these reactions are now directed towards the development of simpler, more active, and economic catalytic systems that work under mild conditions.

Owing to their broad functional-group tolerance and low associated toxicity, as well as their chemical and biological compatibility, Pd-catalyzed reactions are now attractive approaches for protein modification.[2] These processes are particularly interesting, as a variety of non-natural amino acids can be genetically incorporated into peptides and proteins,[3] thus providing specific chemical handles for a number of post-translational manipulations, including palladium-mediated reactions.[2b] Recent contributions from our group and others report robust and highly efficient Suzuki–Miyaura[2a–c,e] and Sonogashira[2d] cross-coupling on protein substrates bearing a halide or alkyne moiety, respectively, under ambient conditions both in vitro[2a,b] and on cell surfaces.[2e]

As part of our continuing interest in developing chemical tools for the mild and specific post-translational modification of proteins,[4] we investigated the use of the Suzuki–Miyaura coupling to site-selectively attach polyethylene glycol (PEG) chains to proteins. Protein PEGylation is a widespread approach to improve the stability and pharmacokinetics of protein drugs by reducing clearance rates, while providing a steric shield from proteolytic enzymes and immune system recognition.[5] However, reduced biological activity is often observed for PEG-conjugated proteins.[6] This effect is attributed to steric crowding resulting from the low positional control achieved with traditional PEGylation chemistry; commonly used PEG-derivatives react with the side chains of natural amino acids, such as lysines or cysteines, or protein termini and disulfide bonds.[5b,7] When more than one reactive site is present on the protein, these approaches lead to heterogenous mixtures of potentially less-/in-active conjugates that are difficult to separate.[5a,8] The possibility of site-selectively PEGylating proteins could potentially overcome this problem but, to date, only a few examples use the PEG-derivatization of genetically-encoded non-natural amino acids. These include Sonogashira coupling at homopropargyl-glycine,[2d] and triazole formation[6a] or Staudinger phosphite reaction[9] at *p*-azidophenylalanine. The two former examples involve metal catalysts and required exogenous ligands and consequent optimization.

Air- and moisture-stable Pd-ligands provide convenient systems that do not require inert atmosphere or degassed solvents.[2a] Nonetheless, even more direct "ligandless" (self-liganded or lacking an added ligand) methods are of significant interest.[10] This would provide a more straightforward method that is particularly attractive in biotechnological applications to be conducted on scale such as PEGylation. Pd-nanoparticle-based catalysts and metal catalysts stabilized by polymers such as polyethylene glycol (PEG) have been reported.[11] Taking advantage of these potential stabilizing properties of PEG, we considered the possibility of direct palladium-catalyzed PEGylation of halogenated amino acids with PEG–boronic acid derivatives in the absence of additional ligand.

Herein, we report PEGylation of amino acids, peptides, and proteins through palladium-catalyzed Suzuki–Miyaura coupling of 2 kDa and 20 kDa monomethoxy PEG (mPEG) phenylboronic acids (mPEG2k-PBA and mPEG20k-PBA, respectively). An evaluation of pyrimidine- or guanidine-based palladium complexes and comparison with PEG reagents alone reveals that the latter can act as potent self-liganding agents for the stabilization of metal species, thus allowing complete conversion using water-soluble palladium salts in the absence of external ligands. The resulting reaction system combines the advantages of reaction biocompatibility for protein modification with the possibility of combining a highly simple, single-component, economic, and environmentally friendly catalyst with a self-liganding coupling partner. We believe this shows the first example of ligandless metal-mediated ligation on a protein surface.

Our initial study focused on the PEGylation of *N*-Boc-4-iodo-L-phenylalanine (**1**; Boc-*p*IPhe) with mPEG20k-PBA (**2**) by Suzuki–Miyaura cross-coupling in 20 mM phosphate buffer, pH 8.0. The palladium catalyst system first tested used an additional ligand 2-amino-4,6-dihydroxy-pyrimidine (**L1**),

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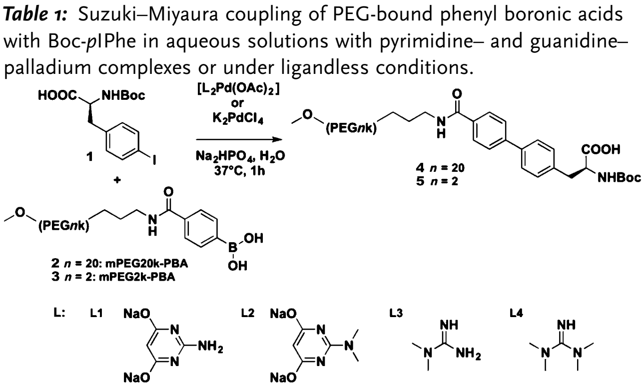
which is known to be an efficient system for Suzuki–Miyaura cross-couplings on iodinated amino acids,[2a] proteins,[2b,c] and on cell surfaces[2e] with small molecule boronic acids. Surprisingly, in the presence of catalyst (5 mol %) and PEG20k-PBA (1.5 equiv), no reaction was observed (Table 1, entry 1), even after 2 h at 37 °C, as determined by ^{1}H NMR spectroscopy (see the Supporting Information). Other nitrogen-based ligands containing the same or similar structural motifs were therefore investigated, including 2-(*N*,*N*-dimethyl)-amino-4,6-dihydroxypyrimidine (**L2**), 1,1-dimethyl guanidine (**L3**) and 1,1,3,3-tetramethyl guanidine (**L4**).[2d,12] With catalytic systems involving **L2**–**L4**, 63–69 % of the aryl halide was consumed in one hour under the same conditions (Table 1, entries 2–4). Importantly, increasing the palladium loading did not improve the conversion (Table 1, entry 5), and the full consumption of the PEG–boronic acid was identified as the limiting factor by ^{1}H NMR analysis. This arose mainly from the formation of the homocoupled product, by reduction of the Pd^{II} precatalyst to the active Pd^0 species in situ,[13] or by phenyl-ring oxidation as a result of palladium-catalyzed hydroxylation of the boronic acid in the presence of O_2.[14] The addition of agents such as sodium ascorbate or sodium formate, to reduce the precatalyst did not lead to any significant improvement in yield (see the Supporting Information).

Table 1: Suzuki–Miyaura coupling of PEG-bound phenyl boronic acids with Boc-*p*IPhe in aqueous solutions with pyrimidine– and guanidine–palladium complexes or under ligandless conditions.



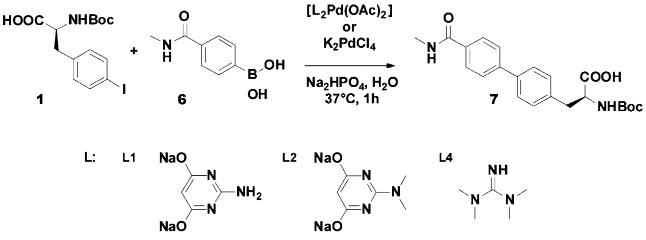
Entry	PEG [kDa]	L	PEG [equiv]	Pd loading [mol %]	Yield [%][a]
1	20	**L1**	1.5	5	0
2	20	**L2**	1.5	5	68
3	20	**L3**	1.5	5	63
4	20	**L4**	1.5	5	69
5	20	**L4**	1.5	20	64
6	20	**L4**	3	20	90
7	2	**L1**	3	5	2
8	2	**L2**	3	5	92
9	2	**L3**	3	5	85
10	2	**L4**	3	5	93
11	20	–[b]	3	20	92
12	2	–[b]	3	5	93

[a] Yields determined by ^{1}H NMR spectroscopy. [b] K_2PdCl_4 was used as the palladium source.

Increased equivalents of PEG-reagent boronic acid to Boc*p*IPhe led to an improved conversion of the halide in one hour at 37 °C (90 %; Table 1, entry 6). Analogously, this increased level of PEG reagent proved beneficial with lower molecular weight PEG (2kDa); the coupling of Boc*p*IPhe with mPEG2k-PBA (**3**) and the palladium catalysts (5 mol %) involving **L2**, **L3**, and **L4** showed 85–93 % conversion under these conditions (Table 1, entries 8–10), whereas **L1** showed only low activity, even after prolonged reaction times (Table 1, entry 7).

This unusual and intriguing PEG reagent dependency led us to explore its role further. Strikingly, when a Pd source (K_2PdCl_4) was used alone without the addition of an external ligand, the aryl halide was > 90 % coupled in one hour with both mPEG2k-PBA (**3**) and mPEG20k-PBA (**2**) in the presence of 5 and 20 mol % of the metal, respectively (Table 1, entries 11 and 12). To elucidate the possible role played by the polymer reagent in promoting this efficient coupling,[11e,11f,11h] the reaction was investigated with 4-(*N*-methylamino carbonyl)-phenyl boronic acid (**6**), which is similar to the mPEG-PBA core found in **2** and **3**, but lacking the PEG chain (Table 2). Although **L1** showed moderate efficiency with this deactivated boronic acid under these conditions (Table 2, entry 1), the coupling with Boc*p*IPhe showed over 93 % conversion in one hour with catalysts based on **L2** and **L4** (Table 2, entry 2–3). However, in clear contrast to the PEG–boronic acid derivatives, only low (20 %) conversion was observed in this case in the absence of ligand: palladium black precipitated from the solution, also in contrast to the PEG-based systems. Consistent with this critical role of PEG in coordinating Pd, the addition of monomethoxy polyethylene glycol (mPEG2000) to the reaction mixture allowed 86 % conversion under the same conditions, with little palladium precipitate observed. Finally, pre-incubation of the metal with the polymer for 15 min at 37 °C before the addition of the other reagents eliminated

Table 2: Suzuki–Miyaura coupling of 4-(*N*-methylaminocarbonyl)-phenyl boronic acid with Boc*p*IPhe in aqueous solution with pyrimidine– and guanidine–palladium complexes or under ligandless conditions with or without PEG.[a]



Entry	L	Yield [%][b]
1	**L1**	34
2	**L2**	> 95
3	**L4**	93
4	–[c]	20
5	mPEG 2000[c]	86
6	mPEG 2000, Δ[c,d]	> 95

[a] Reaction conditions: **6** (3 equiv)/Boc*p*IPhe, Pd (5 mol %), 1 h, 37 °C. [b] Yields determined by ^{1}H NMR spectroscopy. [c] K_2PdCl_4 was used as the palladium source. [d] mPEG2000 pre-incubated with K_2PdCl_4 at 37 °C for 15 min.

metal precipitation entirely and led to full conversion after one hour (Table 2, entries 4–6). Together these results suggest that, in the case of the PEG boronic acids, the interaction of K_2PdCl_4 with the PEG side chains of mPEG2k-PBA and mPEG20k-PBA is likely to stabilize the relevant catalytic species and provide a robust system to promote Suzuki–Miyaura coupling under ligandless/self-liganded conditions, without the addition of external ligands.[10a,11b–f,h]

These encouraging results on a model amino acid system (Boc*p*IPhe) prompted us to investigate these coupling conditions to PEGylate two different non-natural, iodinated amino acids, *p*-iodo-benzylcysteine (Pic)[2a] and *p*-iodo-phenylalanine (*p*IPhe), in the context of two differing protein structural motifs/folds, 3-layer-α/β-Rossman-fold protein subtilisin from *Bacillus lentus* (SBL) and all-β-helix protein 275–276 from *Nostoc punctiforme* (Npβ),[15] respectively.

The non-natural amino acid *p*IPhe was genetically installed at predetermined site 69 of Npβ, using amber stop codon suppression;[16] this site was chosen based on accessibility analysis as observed from the crystal structure.[15] His-tagged Npβ-69*p*IPhe was expressed in *E. coli* cells with high fidelity and with full *p*IPhe occupancy, and then purified using Ni-NTA affinity chromatography. Pic was chemically installed[2a] at predetermined site 156 of SBL to create SBL-156Pic; autolytic degradation was controlled through the addition of PMSF.[2a,17]

Suzuki–Miyaura PEGylation reactions were tested on these two model proteins by incubation with mPEG2k-PBA and Pd with either the pyrimidine and guanidine ligands (**L1**–**L4**) or under ligandless conditions. The physical properties of the PEG chains have a non-linear effect on the Stokes radius of the proteins to which they are attached, and cause PEGylated proteins to migrate significantly more slowly in polyacrylamide gels than proteins of the same total molecular weight, thus providing an effective means to resolve them from the native protein on SDS-PAGE.[18] The reactions were therefore analyzed by SDS-PAGE, and, after Coomassie staining, quantified by gel densitometry. Gratifyingly, after incubation at 37 °C with mPEG2k-PBA and palladium catalyst, both proteins demonstrated a higher molecular weight species by electrophoresis, which corresponded to the PEGylated mutants mPEG2k-Np or mPEG2k-SBL (Figure 1). In both cases, no PEGylated product was observed in the absence of palladium catalyst or PEG–boronic acid. Similar to the observations made at the amino acid level, little or no reaction was observed with **L1** as a catalyst ligand, whereas **L2**–**L4** gave the PEGylated product in 25–55 % yield for Npβ-69*p*IPhe (Figure 1 B and Table 3) and 70–80 % yield for SBL-156Pic (Figure 1 C and Table 3). The reaction of mPEG20k-PBA with Npβ-69*p*IPhe showed similar results, yielding 40–70 % PEGylated protein mPEG20k-Np after incubation with catalysts based on **L2**–**L4** or in the absence of additional ligands (see the Supporting Information). As can be seen in Table 3, the ligandless conditions resulted in the highest conversions with Npβ-69*p*IPhe and conversions that were among the highest obtained for SBL-156Pic.

These results were also confirmed by mass spectrometry. The heterogeneity of PEG reagents[19] prevents precise analysis by ESI-MS, but MALDI-MS provides a good indication of reaction, especially when conducted in a site-selective manner, as demonstrated here. After palladium scavenging using 3-mercaptopropionic acid,[2b] MALDI-TOF-MS analysis of the reaction mixtures confirmed the presence of the desired PEGylated products at a m/z 2300–2400 higher than for the native protein (Figure 1 D,E). Together these results confirmed the ability of not only guanidine-based catalyst systems, but also the direct ligandless PEG system presented here to promote Suzuki–Miyaura coupling as a new strategy for site-selective PEGylation of proteins.

The overall conversion with these bulky polymer reagents was not as high on the protein surface as with some small-molecule modifications,[2a,b] but compare well with other PEGylation reactions with genetically incorporated non-natural amino acids (which show 42–85 % conversions to PEGylated product).[2d,6a,9] Protein "mapping" through combined tryptic digest and MS/MSMS was performed on the material contained in the lower-molecular-weight electrophoretic region after reactions with mPEG2k-PBA or mPEG20k-PBA using either [$\mathbf{L4}_2Pd(OAc)_2$] or ligandless conditions. These revealed a complete loss of iodide (Phe instead of the expected *p*IPhe at the site of modification in the relevant peptide). This suggests that, in the protein environment, although the initial oxidative addition is essentially complete, subsequent dehalogenation competes with transmetalation, thus preventing full conversion into the coupled product. The steric bulk from the interaction between the protein and the PEG derivative may indeed slow down the transmetalation and result in possible dehalogenation. This pathway may potentially be diminished by conducting the conjugation at less sterically hindered amino acid residues or through the use of different, potentially more active boron species, such as boronic esters or triolborates.[20]

ICP-OES analysis performed on the protein mixture obtained from the reaction of Npβ-69*p*IPhe and mPEG2k-PBA under ligandless conditions showed the presence of ca. 1800 ppm palladium. Although this is below the indicative pharmaceutical permitted exposure, given the low doses of PEGylated proteins administered,[21] appropriate scavenging resins may help further remove this residual metal on an applied biotechnological scale. However, to the best of our knowledge, no extensive toxicological testing has revealed significant toxicity of palladium in humans to date,[21] and recent contributions show minimal cellular toxicity of suitably complexed palladium catalysts.[2e]

In summary, a novel way to PEGylate halogenated amino acids and proteins through the Suzuki–Miyaura coupling of PEG–boronic acid derivatives was demonstrated. The PEG–boronic acid derivatives themselves were shown to be sufficient to stabilize the reactive palladium-species and promote the coupling using a water-soluble Pd^{II} salt in the absence of external ligands. These ligandless conditions enabled full conversion of the iodinated amino acid in one hour at 37 °C. This system was then applied to site-selectively PEGylate proteins at a genetically-encoded *p*IPhe or chemically-installed Pic. Although guanidine-based catalysts also promoted Suzuki–Miyaura coupling on proteins, ligandless conditions provided among the highest conversions for the PEGylation of proteins. Dehalogenation, leading to full consumption of the iodinated amino acid, was revealed as the major side reaction by tryptic protein mapping. However, up to 70 % conversions into PEGylated protein were obtained upon coupling under these ligandless conditions.

Using the simplest palladium species, this reaction shows the first example of self-liganded palladium-catalyzed coupling on protein surfaces and has been applied here to an important, biotechnologically relevant transformation to allow an operationally simplified process. It is important to note that the C–C linkage created here is one with much greater stability than those typically currently used for PEG-attachment to proteins, an issue and/or problem of primary importance in some PEGylated therapeutics.[7] Moreover, this ability to site-selectively install PEG, combined with strategies for accessing highly pure PEG,[19] now opens up intriguing possibilities on proteins for both crosslinking moieties with PEG ("hetero-PEG") and in precisely "seeding" the helical properties of PEG.[19] When combined with the recently discovered atypical switch-like behavior of Pd in biological systems[2e] exciting possibilities emerge for the application of this type of unified ligand–reagent–metal system in contexts where multiple reagents would be difficult to apply, such as in in vivo reactions or in large-scale biotechnological applications.

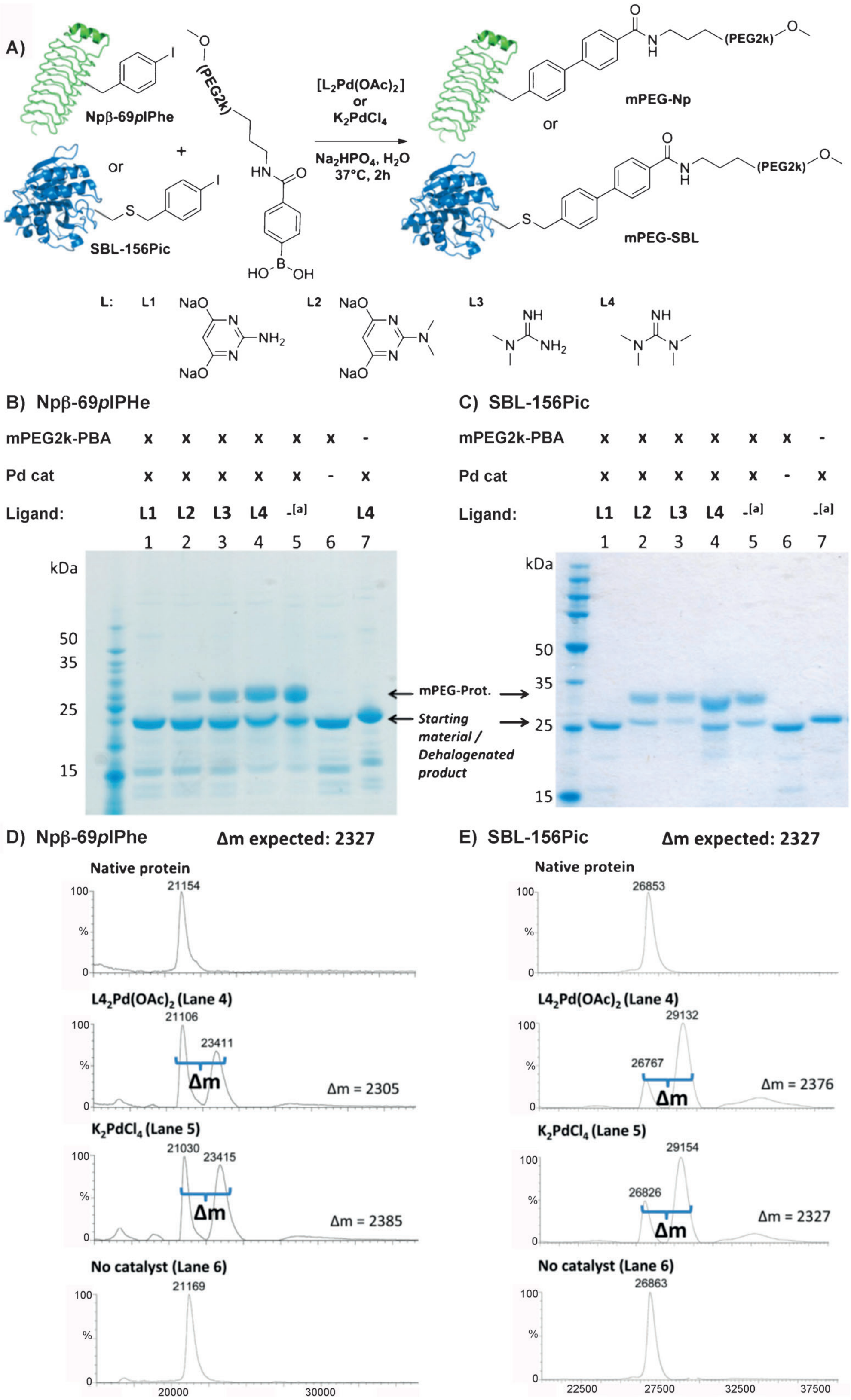


Figure 1. Suzuki–Miyaura PEGylation of Npβ-69pIPhe and SBL-156 Pic with mPEG2k-PBA. A) Reaction scheme. B and C) Coomassie-stained SDS-PAGE gel of the reaction of Npβ-69pIPhe (B) and SBL-156Pic (C), showing the formation of PEGylated proteins (mPEG-Prot.); lanes 1–5: reaction mixtures involving **L1**–**L4** and ligandless conditions, lane 6: reaction in the absence of palladium catalyst, lane 7: reaction in the absence of mPEG2k-PBA. D and E) MALDI-TOF analysis of the unreacted proteins, or reaction mixture catalyzed by [**L4**$_2$Pd(OAc)$_2$] (lane 4 in B and C), K_2PdCl_4 (lane 5 in B and C) or run in the absence of palladium (lane 6 in B and C) for Npβ-69pIPhe (D) and SBL-156Pic (E). (1000 equiv mPEG2k-PBA, 40 (Npβ-69pIPhe) or 10 (SBL-156Pic) equiv Pd catalyst, 37 °C, 2 h.) [a] K_2PdCl_4 was used as the palladium source.

Table 3: Yield of the Suzuki–Miyaura PEGylation of Npβ-69*p*IPhe and SBL-156Pic with mPEG2k-PBA, as determined by gel densitometry.

Npβ-69*p*IPhe		SBL-156Pic	
ligand	yield [%]	ligand	yield [%]
L1	0	**L1**	0
L2	25	**L2**	70
L3	40	**L3**	80
L4	55	**L4**	70
–[a]	60	–[a]	70

[a] K_2PdCl_4 was used as the palladium source.

Experimental Section

General method for Suzuki–Miyaura coupling of Boc-*p*IPhe: mPEG-PBA (1.5–3 equiv) diluted with phosphate buffer (200 mM, pH 8), water, and D_2O were added to a solution of *N*-Boc-4-iodo-L-phenylalanine in phosphate buffer (20 mM, pH 8). Palladium catalyst stock solution (10 mM; 0.05 or 0.2 equiv) was then added and the reaction was mixed and shaken at 37 °C. After 1 h, the reaction was analyzed by NMR spectroscopy.

General method for Suzuki–Miyaura protein PEGylation: A solution of Na_2HPO_4 (5 μL, 100 mg mL^{-1}, 3.5 μmol) containing mPEG-PBA (1000 equiv) was added to a solution of *p*IPhe- or Pic-tagged protein (20 μL, 0.3–0.5 mg mL^{-1}) in phosphate-buffered saline (PBS, pH 8.0). Stock palladium catalyst (10 mM, ≈ 10–40 equiv) was then added and the mixture was shaken at 37 °C for 2 h. The reactions were analyzed by SDS-PAGE on 12 % Bis–Tris polyacrylamide gel with MOPS running buffer. Control reactions with either Pd catalyst or PEG–boronic acid excluded were diluted with water to maintain concentrations.

MALDI-MS: Prior to analysis, palladium was scavenged from the reaction mixtures with 3-mercaptopropionic acid at 37 °C for 30 min.[2b] The reaction mixture (1 μL) was diluted with water (3 μL) and mixed 1:1 with matrix (sinapic acid matrix 10 mg mL^{-1} in 4:6 acetonitrile/H_2O+0.1 % TFA); 1 μL was applied to the plate.

"In gel" tryptic digestions: 3 μL of SDS loading buffer was added to 10 μL of reaction mixture and the samples were heated to 95 °C for 10 min. The reactions were then run on SDS-PAGE on 12 % Bis–Tris polyacrylamide gel with MOPS running buffer. Proteins were revealed with Instant Blue stain and then extensively washed (water). Bands were then excised from the gel and washed (NH_4HCO_3 in Milli-Q water/HPLC grade MeCN × 2 then MeCN), dried, treated with DTT at 37 °C for 30 min, washed as above, treated with iodoacetamide solution for 60 min in the dark, washed, and dried. Sequencing Grade Modified Trypsin solution (1 μg, Promega) was added and the digest was incubated at 37 °C overnight. 1 μL of formic acid was added to quench, and the supernatant containing the peptides transferred to a new tube. 50 μL of extraction buffer (20 μL formic acid in 10 mL Milli-Q water and 10 mL HPLC grade acetonitrile) was then added and the mixture was incubated for 30 min, and the supernatant was pooled with the existing supernatant. The mixtures were then dried and dissolved in water (10 μL). The samples were analyzed by MS/MS-MS.

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[1] a) S. L. Buchwald, *Acc. Chem. Res.* **2008**, *41*, 1439 – 1439; b) M. R. Huang, P. Gao, X. G. Li, *Huaxue Jinzhan* **2010**, *22*, 113 – 118; c) A. de Meijere, F. Diederich, *Metal-catalyzed cross-coupling reactions*, 2nd ed., Wiley-VCH, Weinheim, **2004**; d) N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457 – 2483; e) Y. Nakama, O. Yoshida, M. Yoda, K. Araki, Y. Sawada, J. Nakamura, S. Xu, K. Miura, H. Maki, H. Arimoto, *J. Med. Chem.* **2010**, *53*, 2528 – 2533; f) K. C. Nicolaou, P. G. Bulger, D. Sarlah, *Angew. Chem.* **2005**, *117*, 4516 – 4563; *Angew. Chem. Int. Ed.* **2005**, *44*, 4442 – 4489; g) E. Perissutti, F. Frecentese, A. Lavecchia, F. Fiorino, B. Severino, F. De Angelis, V. Santagada, G. Caliendo, *Tetrahedron* **2007**, *63*, 12779 – 12785; h) J. Sakamoto, M. Rehahn, G. Wegner, A. D. Schlüter, *Macromol. Rapid Commun.* **2009**, *30*, 653 – 687; i) R. Severinsen, G. T. Bourne, T. T. Tran, M. Ankersen, M. Begtrup, M. L. Smythe, *J. Comb. Chem.* **2008**, *10*, 557 – 566.

[2] a) J. M. Chalker, C. S. C. Wood, B. G. Davis, *J. Am. Chem. Soc.* **2009**, *131*, 16346 – 16347; b) C. D. Spicer, B. G. Davis, *Chem. Commun.* **2011**, *47*, 1698 – 1700; c) Y. S. Wang, W. K. Russell, Z. Y. Wang, W. Wan, L. E. Dodd, P. J. Pai, D. H. Russell, W. S. R. Liu, *Mol. BioSyst.* **2011**, *7*, 714 – 717; d) N. Li, R. K. V. Lim, S. Edwardraja, Q. Lin, *J. Am. Chem. Soc.* **2011**, *133*, 15316 – 15319; e) C. D. Spicer, T. Triemer, B. G. Davis, *J. Am. Chem. Soc.* **2012**, *134*, 800 – 803; f) J. Li, P. R. Chen, *ChemBioChem* **2012**, *13*, 1728 – 1731.

[3] C. C. Liu, P. G. Schultz, *Annu. Rev. Biochem.* **2010**, *79*, 413 – 444.

[4] J. M. Chalker, G. J. L. Bernardes, B. G. Davis, *Acc. Chem. Res.* **2011**, *44*, 730 – 741.

[5] a) S. N. S. Alconcel, A. S. Baas, H. D. Maynard, *Polym. Chem.* **2011**, *2*, 1442 – 1448; b) S. Jevševar, M. Kunstelj, V. G. Porekar, *Biotechnol. J.* **2010**, *5*, 113 – 128; c) F. M. Veronese, A. Mero, G. Pasut in *PEGylated protein drugs: Basic science and clinical applications* (Ed.: F. Veronese), Birkhäuser, Basel, **2009**, pp. 11 – 31.

[6] a) A. Deiters, T. A. Cropp, D. Summerer, M. Mukherji, P. G. Schultz, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5743 – 5745; b) A. J. Keefe, S. Y. Jiang, *Nat. Chem.* **2012**, *4*, 59 – 63.

[7] a) S. Balan, J.-W. Choi, A. Godwin, I. Teo, C. M. Laborde, S. Heidelberger, M. Zloh, S. Shaunak, S. Brocchini, *Bioconjugate Chem.* **2006**, *18*, 61 – 76; b) G. M. Bonora, S. Drioli, in *Protein PEGylation, basic science and biological applications* (Ed.: F. Veronese), Birkhäuser, Basel, **2009**, pp. 33 – 45; c) M. A. Gauthier, H.-A. Klok, *Chem. Commun.* **2008**, 2591 – 2611; d) O. Kinstler, G. Molineux, M. Treuheit, D. Ladd, C. Gegg, *Adv. Drug Delivery Rev.* **2002**, *54*, 477 – 485; e) H.-A. Klok, *Macromolecules* **2009**, *42*, 7990 – 8000; f) M. J. Roberts, M. D. Bentley, J. M. Harris, *Adv. Drug Delivery Rev.* **2002**, *54*, 459 – 476; g) S. Shaunak, A. Godwin, J.-W. Choi, S. Balan, E. Pedone, D. Vijayarangam, S. Heidelberger, I. Teo, M. Zloh, S. Brocchini, *Nat. Chem. Biol.* **2006**, *2*, 312 – 313.

[8] C. J. Fee, J. A. VanAlstine, *Chem. Eng. Sci.* **2006**, *61*, 924 – 939.

[9] R. Serwa, P. Majkut, B. Horstmann, J.-M. Swiecicki, M. Gerrits, E. Krause, C. P. R. Hackenberger, *Chem. Sci.* **2010**, *1*, 596 – 602.

[10] a) L. A. Adrio, B. N. Nguyen, G. Guilera, A. G. Livingston, K. K. Hii, *Catal. Sci. Technol.* **2012**, *2*, 316 – 323; b) C. Torborg, M. Beller, *Adv. Synth. Catal.* **2009**, *351*, 3027 – 3043; c) R. Franzén, Y. J. Xu, *Can. J. Chem.* **2005**, *83*, 266 – 272; d) G. A. Molander, B. Biolatto, *J. Org. Chem.* **2003**, *68*, 4302 – 4314.

[11] a) A. Arcadi, G. Cerichelli, M. Chiarini, M. Correa, D. Zorzan, *Eur. J. Org. Chem.* **2003**, 4080 – 4086; b) C. G. Blettner, W. A. König, W. Stenzel, T. Schotten, *J. Org. Chem.* **1999**, *64*, 3885 – 3890; c) W. Han, C. Liu, Z.-L. Jin, *Org. Lett.* **2007**, *9*, 4005 – 4007; d) W. Han, C. Liu, Z.-L. Jin, *Adv. Synth. Catal.* **2008**, *350*, 501 – 508; e) J.-H. Li, W.-J. Liu, Y.-X. Xie, *J. Org. Chem.* **2005**, *70*, 5409 – 5412; f) L. Liu, Y. Zhang, Y. Wang, *J. Org. Chem.* **2005**, *70*, 6122 – 6125; g) V. V. Namboodiri, R. S. Varma, *Green Chem.* **2001**, *3*, 146 – 148; h) T. M. Razler, Y. Hsiao, F. Qian, R. Fu, R. K. Khan, W. Doubleday, *J. Org. Chem.* **2009**, *74*, 1381 – 1384.

[12] S. Li, Y. Lin, J. Cao, S. Zhang, *J. Org. Chem.* **2007**, *72*, 4067 – 4072.

[13] M. Moreno-Mañas, M. Pérez, R. Pleixats, *J. Org. Chem.* **1996**, *61*, 2346 – 2351.

[14] A. D. Chowdhury, S. M. Mobin, S. Mukherjee, S. Bhaduri, G. K. Lahiri, *Eur. J. Inorg. Chem.* **2011**, 3232 – 3239.

[15] M. W. Vetting, S. S. Hegde, K. Z. Hazleton, J. S. Blanchard, *Protein Sci.* **2007**, *16*, 755 – 760.

[16] T. S. Young, I. Ahmad, J. A. Yin, P. G. Schultz, *J. Mol. Biol.* **2010**, *395*, 361 – 374.

[17] M. R. Stabile, W. G. Lai, G. DeSantis, M. Gold, J. B. Jones, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2501 – 2506.

[18] F.-Y. Jeng, S.-C. Lin, *Process Biochem.* **2006**, *41*, 1566 – 1573.

[19] A. C. French, A. L. Thompson, B. G. Davis, *Angew. Chem.* **2009**, *121*, 1274 – 1278; *Angew. Chem. Int. Ed.* **2009**, *48*, 1248 – 1252.

[20] a) Y. Yamamoto, M. Takizawa, X.-Q. Yu, N. Miyaura, *Angew. Chem.* **2008**, *120*, 942 – 945; *Angew. Chem. Int. Ed.* **2008**, *47*, 928 – 931; b) K. L. Billingsley, S. L. Buchwald, *Angew. Chem.* **2008**, *120*, 4773 – 4776; *Angew. Chem. Int. Ed.* **2008**, *47*, 4695 – 4698; c) H. Bonin, D. Delbrayelle, P. Demonchaux, E. Gras, *Chem. Commun.* **2010**, *46*, 2677 – 2679.

[21] C. E. Garrett, K. Prasad, *Adv. Synth. Catal.* **2004**, *346*, 889 – 900.