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Phthalocyanine-Peptide Conjugates via Palladium-Catalyzed Cross-Coupling Reactions

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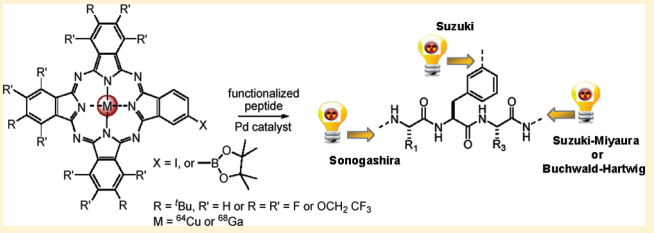
Supporting Information

ABSTRACT: Phthalocyanines (Pc) were conjugated with peptide moieties to improve their target selectivity for potential use as fluorescence and/or positron emission tomography (PET) probes in medical imaging. Three synthetic methods based on palladium-catalyzed cross-coupling reactions (Sonogashira, Buchwald−Hartwig, and Suzuki−Miyaura) were investigated. Using these methods, a series of peptides monofunctionalized with Pc at the *N*/*C*-terminal position or on a phenylalanine side chain was obtained in good yields and characterized.



Multimodality imaging techniques are evolving as important clinical tools for imaging diseases, including cancer.[1] There has been a significant growth in the development of multimodality probes, but only a few have been subjected to in vivo evaluations.[2] The use of such probes in a single imaging session has the potential to provide enhanced diagnostic efficacy/accuracy.

Selected phthalocyanine (Pc) derivatives and structurally related macrocycles have been found to accumulate in tumors. These macrocycles, when excited by appropriate visible light, produce cytotoxic singlet oxygen that makes them suitable photosensitizers (PS) for photodynamic therapy (PDT) of various medical conditions. They are also broadly investigated for diagnostic applications.[3] Such tetrapyrrolic macrocycles could be radiolabeled with positron emitters (e.g., Ga-68 or Cu-64) to serve as tracers for positron emission tomography (PET). Their suitable fluorescence properties also make them promising fluorescence imaging probes, but their clinical use is limited due to lack of target tissue selectivity. To improve efficiency, their linkages to various biological carriers have been investigated.[4]

Advances in molecular biology have shown an increasing number of potential disease targets, including peptide receptors and peptide-related biomolecules.[5] For example, somatostatin, integrin, gastrin-releasing peptide (GRP), cholecystokinin, neurotensin, glucagon-like peptide-1, and neuropeptide-Y cell surface receptors have been successfully identified and characterized for tumor receptor imaging.[6−8] Peptides targeting these receptors can be used as tools to improve cancer diagnosis by using multimodality imaging techniques. As imaging probes, peptides are used for early disease detection, characterization, and real-time monitoring of therapeutic responses, as well as investigating drug efficacy.[7] They present distinct advantages including ease of preparation, degree of freedom and flexibility, ability to attach chelators (or a dye), rapid clearance from blood and non-target tissues, good tumor-to-background ratios, low toxicity, and immunogenicity.[6,7,9] Although natural peptides have a short biological half-life, structural modifications of the amino acid sequence can improve their in vivo stability.[10] Peptides can be further modified by substitution with appropriate moieties by using spacers.

Peptide carriers for Pc have recently been reported and their improved cell-targeting ability has been demonstrated.[11,12] Moreover, the presence of the peptide moiety increased water solubility and consequently fluorescence of Pc in aqueous solutions.[12] A few examples of Pc functionalized with a peptide moiety have been previously reported in the literature.[11,12] In these studies, Pc was linked to the peptide only at the *N*-terminal position through sulfonamide[11] or amide[12] bond formation. These methods can present some limitations if the conjugation of Pc at this position results in loss of peptide activity. In this note, we report a new synthetic approach allowing selective Pc attachment at different peptide backbone positions, opening opportunities for easy tuning biological properties of Pc-peptide conjugates.

The use of Pd-catalyzed reactions for structural modification of Pc[13] and peptides[14] has been reported in the literature. Indeed, properly functionalized peptides can undergo intramolecular or intermolecular Pd-catalyzed cross-coupling reactions to afford the corresponding cyclic or branched peptides.[14] Herein, we report the use of different Pd-catalyzed cross-coupling reactions (Sonogashira, Buchwald−Hartwig, and Suzuki−Miyaura) for the preparation of new Pc-peptide conjugates. Various functionalized model tri- and tetrapeptides were used to introduce Pc units selectively either at the N/C-terminal position or on a phenylalanine side chain of the peptide to demonstrate the generality of our approach (Scheme 1).

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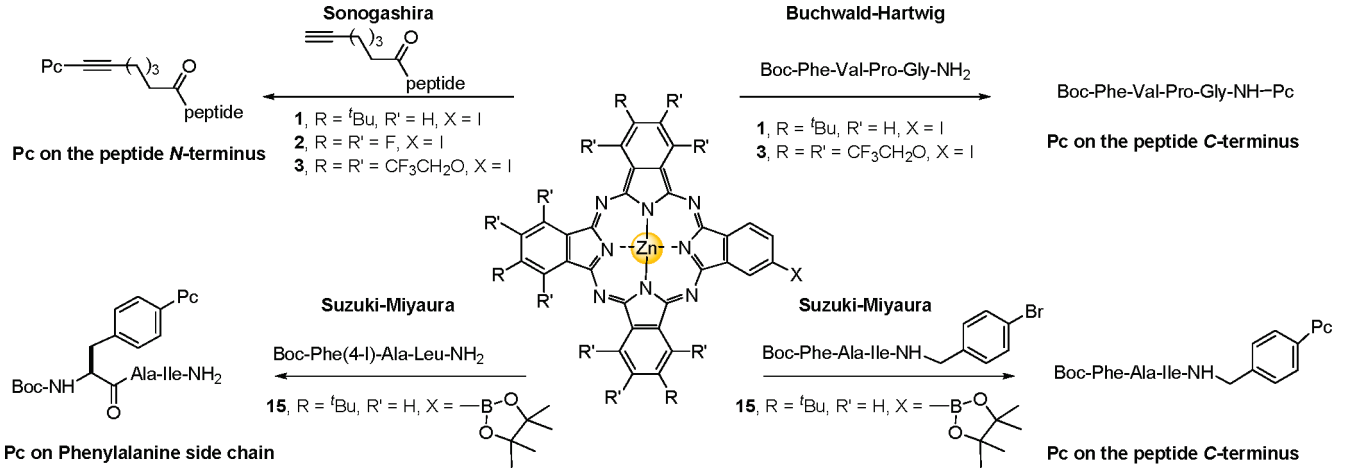
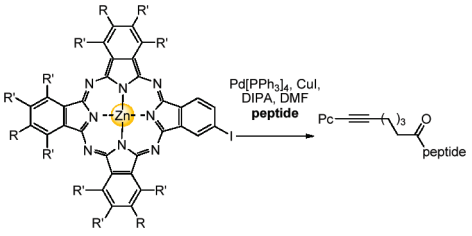
Scheme 1. Synthesis of Pc-Functionalized Peptide



Halogenated Pc **1**−**3** and boronate Pc **15** were prepared following previously described procedures.[15,16] Properly functionalized peptides were synthesized manually using the Fmoc (Fluorenylmethyloxycarbonyl) strategy and NovaSyn Seiber resin (peptides **4**, **5**, **12**, and **16**) or NovaSyn TGR resin (peptide **6**). A 2.5-fold excess of Fmoc-protected amino acids over resin substitution rate was utilized for coupling. The amino acids were activated with an equimolar amount of HATU (2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and 2-fold excess of DIEA (diisopropylethylamine). After each coupling step, deprotection of Fmoc was performed in 20% piperidine in DMF (dimethylformamide). For the preparation of alkynyl-functionalized peptides, 6-heptynoic acid was introduced at the *N*-terminal position after peptide assembly. The peptide amides **4**, **5**, **12**, and **16** were cleaved from the polymer support by treatment with 4% TFA (trifluoroacetic acid) in DCM (dichloromethane). A cocktail of TFA:H_2O:thioanisole (92:2:6, v/v) was used for the cleavage of peptide **6**. The H_2N-Ile-2-ClTrt resin was used to synthesize the peptide Boc-Phe-Ala-Ile. After cleavage from resin with 1% TFA in DCM, the resulting peptide was coupled with 4-bromobenzylamine in the presence of isobutylchloroformate and triethylamine in DCM to give peptide **17**. All peptides were prepared with overall yields of 22−40% based on the substitution rate of the resin. The purity of peptides was shown to be >95% (analytical HPLC) and their identifications were confirmed by using NMR and mass spectroscopy.

Table 1 shows the results obtained for Sonogashira coupling performed with various iodinated phthalocyanines and *N*-substituted alkynyl-functionalized peptides. Our first attempt using Pc **1** and peptide **4** in the presence of $Pd(PPh_3)_2Cl_2$, copper iodide (CuI), and triethylamine at 85−90 °C afforded no Pc-peptide conjugate giving instead a mixture of acetylene homocoupling product (peptide homodimer), Pc reduction product (40%), and Pc-phosphonium salts (60%). A similar product pattern along with a trace amount of the desired Pc-peptide conjugate (MS analysis) was observed when Pc **1** was treated with peptide **5** under the same conditions. The formation of the acetylene homodimer peptide is favored in the presence of Pd^{II}.[17] The Pc-phosphonium salt was obtained by heating the intermediate iodocopper(I) complex derived from the Pc iodide and iodo(triphenylphosphine)copper(I),[18] which may explain the formation of phosphonium salts under our reaction conditions.

Table 1. Analytical Data for Pc-Peptide Conjugates Prepared with Sonogashira Coupling



				[M]$^+$			
entry	Pc[a]	peptide[b]	product	calcd	found[c]	λ_{max} (nm)	yield (%)
1	**1**	**4**	**7**	1222.5	1222.2	675	70
2	**1**	**5**	**8**	1267.9	1290.0[d]	671	60
3	**1**	**6**	**9**	1186.8	1186.8	675	60
4	**2**	**4**	**10**	1272.4	1240.0[e]	642−673	36[f]
5	**3**	**4**	**11**	2230.3	2230.4	700	70

[a] Pc: **1**, R = ^{t}Bu, R′ = H; **2**, R = R′ = F; **3**, R = R′ = CF_3CH_2O. [b] Peptide: **4**, CH≡C$(CH_2)_4$CO-Glu(^{t}Bu)-Val-Ala-NH_2; **5**, CH≡C$(CH_2)_4$CO-Lys-(Boc)-Val-Ala-NH_2; **6**, CH≡C$(CH_2)_4$CO-Phe-Val-Ala-NH_2. [c] Mass values were obtained with MALDI-TOF. [d] $[MH + Na]^+$. [e] $[M - {}^tBu + Na]^+$. [f] Aggregate formation makes the purification difficult; as a result, product **10** is obtained in poor yield.

Different palladium(0) catalyst and base were then tried to favor Pc-peptide conjugate formation and reduce acetylene bicoupling.[17] Coupling of Pc **1** and peptide **4** with tetrakis(triphenylphosphino)Pd(0) ($Pd[PPh_3]_4$), CuI, and diisopropylamine (DIPA) in DMF at 60−70 °C led to the desired product **7** in 70% yield (entry 1). Under similar conditions, reaction of Pc **1** with peptides **5** and **6** gave Pc-conjugates **8** and **9**, respectively, in about 60% yields (entries 2 and 3). The products **7**−**9** consisted of mixtures of structural isomers due to the nature of the starting phthalocyanine[19] employed in these reactions. All three conjugates were characterized by a sharp Q-band at ∼675 nm.

Other Pc substrates where then studied under the same reaction conditions. Treatment of the highly symmetrical fluoro-substituted Pc **2** with peptide **4** at room temperature for

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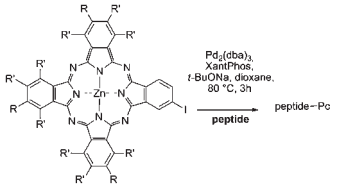
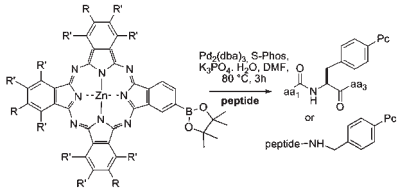
Table 2. Analytical Data for Pc-Functionalized Peptides Prepared with Buchwald−Hartwig and Suzuki−Miyaura Cross-Coupling Reactions

entry	Pc[a]	peptide[b]	product	[M]$^+$ calcd	[M]$^+$ found[c]	λ_{max} (nm)	yield (%)
							
1	**1**	**12**	**13**	1259.5	1283.1[d]	675	75
2	**3**	**12**	**14**	2269.8	2292.8[d]	701	75
							
3	**15**	**16**	**18**	1192.5	1192.7	675	75
4	**15**	**17**	**19**	1280.6	1281.2	675	75

[a] Pc: **1**, R = ^{t}Bu, R′ = H; **3**, R = R′ = CF_3CH_2O; **15**, R = ^{t}Bu, R′ = H. [b] Peptide: **12**, Boc-Phe-Val-Pro-Gly-NH_2; **16**, Boc-Phe(4-I)-Ala-Ile-NH_2; **17**, Boc-Phe-Ala-Ile-$NH(CH_2)C_6H_4$(4′-Br). [c] Mass values were obtained with MALDI-TOF. [d] $[M + Na]^+$.

12 h gave product **10** along with reduction product and Pc-phosphonium salts (entry 4). The UV−vis spectra of **10** in various organic solvents showed a broad Q-band confirming high aggregation in solution, which makes the separation of **10** from reduction product difficult. Wada and co-workers[20] have demonstrated that ZnPc substituted with 2,2,2-trifluoroethoxy groups shows a reduced tendency to aggregate. Accordingly, we employed the iodinated ZnPc **3** for the Pd-catalyzed Sonogashira cross-coupling. Reaction of Pc **3** with peptide **4** gave 70% yield of conjugate **11**, which showed a sharp, red-shifted Q-band (entry 5). In this series of Sonogashira cross-coupling reactions, phthalocyanines were introduced at the *N*-terminus of the peptide backbone with use of *N*-alkynyl functionalized peptides as substrates.

Intermolecular and intramolecular *N*-arylation of amides have been previously reported.[21] Using Buchwald−Hartwig conditions, phthalocyanines were introduced at the *C*-terminal position of the Boc-peptide amides as shown in Table 2. Thus, the treatment of Pc **1** and **3** with peptide **12** using tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$), 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthene (XantPhos), and sodium *tert*-butoxide at 80 °C for 6 h gave selectively the corresponding coupling products **13** and **14** in about 75% yields (entries 1 and 2).

Recently, we have successfully used Suzuki−Miyaura conditions for the preparation of phthalocyanine heterodimers[16] and phthalocyanine-porphyrin heterodyads and heterotriads.[22] We applied these conditions for the introduction of the phthalocyanine at the *C*-terminus or on the phenylalanine side chain of the peptide. Treatment of the Pc-boronate **15** with halogenated peptides **16** and **17** using tris(dibenzylideneacetone)dipalladium(0)-2-cyclohexylphosphino-2′,6′-dimethoxy-1′,1′-biphenyl ($Pd_2(dba)_3$-S-Phos) and potassium phosphate at 80 °C gave coupling products **18** and **19**, respectively, in about 75% yields along with the reduction product (Table 2, entries 3 and 4). The reaction of Pc **15** with peptide **17** required longer reaction time compared to the reaction with peptide **16**. This is in accordance with the higher reactivity of aryl iodides as compared to aryl bromides in Suzuki−Miyaura cross-coupling reactions.

The results presented above demonstrate that palladium-catalyzed cross-coupling reactions (Sonogashira, Buchwald−Hartwig, and Suzuki−Miyaura) offer an effective and convenient approach for the selective introduction of Pc at the *N*/*C*-terminal positions or on a phenylalanine side chain of peptides. Pc-functionalized peptides were successfully synthesized and purified at the milligram scale. The scope and limitations of these palladium-catalyzed cross-coupling reactions will be examined further by using water-soluble Pc and unprotected peptides. The potential of these conjugates as bimodal probes for diagnostic use will be tested and explored in vitro and in vivo with different metal radioisotopes (^{64}Cu and ^{68}Ga) and various peptides[23] of biological importance.

EXPERIMENTAL SECTION

General Experimental Conditions for Sonogashira Coupling Reaction. In a 25 mL three-necked flask, Pc **1**, **2**, or **3** (0.015 mmol) and peptides **4**, **5**, or **6** (2 molar excess) were stirred in DMF (3 mL) and DIPA (1 mL, 5.7 mmol) for 15 min at room temperature under an argon atmosphere. Then the catalyst $Pd(PPh_3)_4$ (5 mg, 0.004 mmol) and CuI (2 mg, 0.01 mmol) were added and the reaction mixture was stirred at room temperature for 3 h. At the end of the reaction, the mixture was extracted with ethyl acetate, washed with water, and dried over sodium sulfate. After filtration and evaporation of the organic solvent, the crude mixture was purified by using silica gel (60−230 mesh) and the product was eluted with ethyl acetate and THF.

General Experimental Conditions for the Buchwald−Hartwig Coupling Reaction. In a 25 mL three-necked flask, a mixture consisting of Pc **1** or **3** (0.015 mmol) and peptide **12** (2 molar excess), $Pd_2(dba)_3$ (3 mg, 0.003 mmol), XantPhos (3 mg, 0.005 mmol), and sodium-*tert*-butoxide (3 mg, 0.03 mmol) was placed under an argon atmosphere. 1,4-Dioxane (5 mL) was added and the mixture was stirred at 80 °C for 6 h. At the end of the reaction, the material was worked up and purified as described above.

General Experimental Conditions for the Suzuki−Miyaura Coupling Reaction. In a 25 mL three-necked flask, a mixture of solids consisting of Pc **15** (0.015 mmol) and peptides **16** or **17** (2 molar excess), $Pd_2(dba)_3$ (3 mg, 0.003 mmol), S-Phos (3 mg, 0.007 mmol), and K_3PO_4 (3 mg, 0.014 mmol) was placed under an argon atmosphere. DMF (3 mL) was added and the reaction mixture was stirred at 80 °C for 3 h. At the end of the reaction, the material was worked up and purified as described above.

ASSOCIATED CONTENT

Supporting Information. Experimental procedures and characterization data for peptides and Pc-functionalized peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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