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Chemoselective Pd(0)-Catalyzed Peptide Coupling in Water

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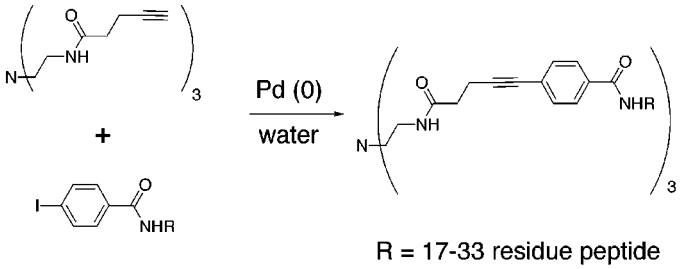
ABSTRACT



Highly charged peptides ranging in length from 17 to 33 residues have been efficiently tricoupled to a trialkyne nucleus by using a Sonogashira Pd(0) coupling strategy under both acidic (pH 5.0) and basic (pH 7.5) conditions. These results demonstrate the utility of Pd(0) to construct protein-sized structures (12,000 mol wt) in aqueous milieu.

Direct synthesis of materials offers an opportunity to control bulk properties at the molecular level. Biomaterials synthesis is especially attractive as one may couple functional biomolecular building blocks using organic synthesis to produce materials that may have a specific function or sensitivity to stimuli. Often, the placement of biomolecular modules in space is more easily controlled by the introduction of unnatural molecular connections rather than natural biomolecular motifs. In the course of a recent attempt at constructing a molecular superlattice by design, we have optimized a useful chemoselective coupling reaction in aqueous solvent that was used to link multiple unprotected peptides to a reactive, unnatural nucleus in high yield using a catalytic palladium(0) source and Sonogashira coupling strategy.[1]

Casalnuovo and Calabrese authored the first account of palladium-catalyzed alkylations of small molecules in aqueous media in 1990, using a monosulfonated triphenylphosphine as the water-solubilizing ligand for palladium.[2] Other types of palladium-catalyzed couplings in water have also been reported thereafter.[3] Recently, Dibowski and Schmidtchen reported the use of guanidinophosphines as water-solubilizing palladium ligands and further demonstrated the coupling of propargyl glycine to an 11-residue peptide containing iodophenylalanine, using Sonagashira palladium cross-coupling chemistry.[4] The authors advocated the use of guanidinylated phosphines, developed in their laboratory, over the sulfonated ligand, suggesting that the net negative charge borne by many proteins would inhibit approach of the negatively charged palladium complex, limiting the coupling efficiency.

In this study, we employed the commercially available trisulfonated phosphine ligand in our palladium coupling reactions. Synthetic peptides of 17- and 33-residue length

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with net charges +9, −6, and +6 were acylated with iodobenzoic acid on the N-terminus or the ϵ-nitrogen of lysine. These peptides, containing free amines, carboxylates, guanidines, hydroxyls, and thiolesters, were efficiently tricoupled with catalytic Pd(0) in water to a C_3 symmetric trialkyne, yielding phenylacetylene products of up to 12 000 molecular weight (Figure 2), bearing formal net charges from

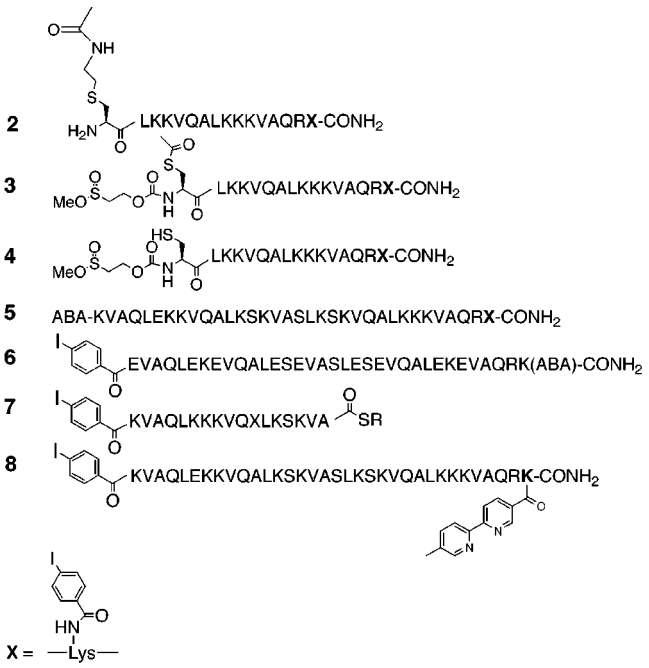

Figure 1. Aryl halide functionalized peptides studied in the Pd cross-coupling reaction. R = propionamide, ABA = acetamidobenzoate.

+27 to −18 (Figure 1). This is the first demonstration of the utility of palladium cross-coupling chemistry to produce compounds on the size range of proteins despite highly repulsively charged substrates and a congested reaction center. The 33-residue peptides used in this study were coiled-coil forming peptides of the "velcro" design, in which *e* and *g* positions in the helical wheel projection of these sequences were either exclusively lysine or exclusively glutamic acid.[5] The residues in the hydrophobic core positions (*a* and *d* in the helical wheel) were valine and leucine, respectively, thus predisposing the sequence to form coiled-coil dimers in solution; the acid−base patterning ensures the formation of noncovalent heterodimers by electrostatic destabilization of the homodimer. The shorter peptides were fragments of the longer sequences, N-terminated in cysteine with a C-terminal iodophenyl moiety or N-terminated with an iodophenyl group and C-terminated with a thiolester.

Peptides were coupled to trialkyne **1**, prepared from the acylation of tris(ethylamino)amine with pentynoic acid.

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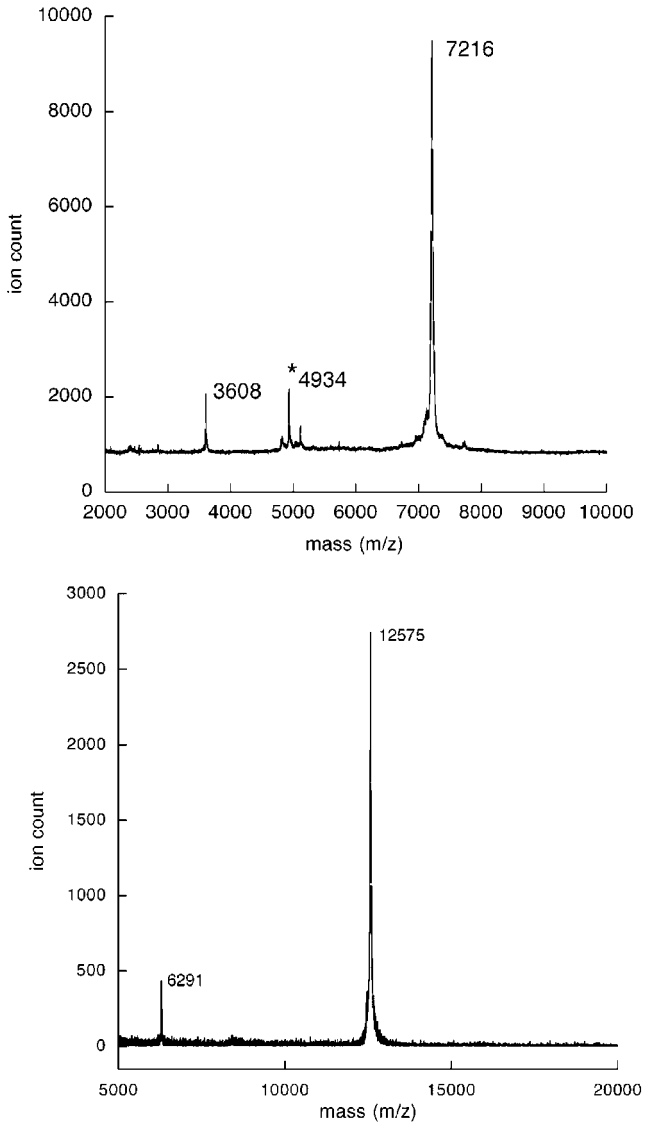

Figure 2. (Top) Mass spectrum of tricoupled product (calcd, 7208) of thiolester **7** to **1** with the doubly ionized species 3608 also seen. The asterisk indicates the presence of dicoupled product as an impurity. (Bottom) Mass spectrum of the product of tricoupling **6** with **1** (calcd, 12573). The doubly ionized species is seen in the spectrum.

Compound **1** was then added to aqueous, buffered peptide solutions under inert atmosphere; the resulting mixture was then charged with an "aged" catalyst solution prepared from $Pd(OAc)_2$ and tris(3-sulfonato-phenyl)phosphine in degassed 200 mM MOPS buffer, pH 7.5, at a Pd-to-phosphine ratio of 1:5. Catalyst mixture was stored at 4 °C overnight prior to use and was used for up to 4 days without loss of activity. This yellow solution was added to the reactants to yield a final Pd−alkyne ratio of 5% with copper iodide, also in 5% mole ratio. Copper iodide was delivered as a solution in acetonitrile or as a suspension in aqueous buffer. Coupling yield varied according to catalyst preparation, substrate ratio, and reaction pH. In general, a 4−10 mM solution of peptide in Ar-sparged buffer was charged with a solution of trialkyne

in 6 M Gdn/50 mM phosphate buffer (final concentration of **1** was 1.3−3.3 mM). Reactions were typically complete at room temperature within 30 min to 3 h, depending on catalyst preparation. Adding excess trialkyne made it possible to quantitatively obtain the monocoupled product or to obtain the dicoupled product as the major species (Table 1).

Table 1. Conversion of **1** and Aryl Iodide to Tricoupled Product

aryl iodide	reaction conditions	% conv (HPLC)
2	5−25 mol % Pd/CuI, 50 mM NaOAc, pH 5.5	0
3	5 mol % Pd/CuI, 50 mM NaOAc, pH 5.5	quant
4	5−25 mol % Pd/CuI, 50 mM NaOAc, pH 5.5	0
5	5 mol % Pd/CuI, 50 mM NaOAc, pH 5.5	quant
6	5 mol % Pd/CuI, 6 M GdnHCl/50 mM PB, pH 7.5	quant
7	5 mol % Pd/CuI, 50 mM NaOAc, pH 5.5	quant
8	5−100 mol % Pd, 50 mM NaOAc, pH 5.5	50% decom

Interestingly, despite the prevailing view that base is required to form the presumed cuprous acetylide intermediate in Sonogashira couplings, reaction efficiency was greatly enhanced for basic substrates at acidic pH (5.0) relative to slightly basic conditions (7.5). This is likely more a result of substrate accessibility rather than a requirement of the coupling chemistry, as the basic coiled-coil peptides begin to form homodimers at pH >7.0, presenting a possible hindrance to palladium coupling.[5b] The acidic peptides are not soluble in acidic pH, and thus those coupling reactions were carried out at pH 7.5, where C−C bond formation also proceeds smoothly. Thus, the large positive charge of the basic peptides at pH 5.0 is preferable to an aggregated substrate for the Pd coupling; more importantly though, we have shown that acidic conditions do not preclude clean Sonogashira coupling in water.

While the reaction conditions gratifyingly permitted the use of thiolester substrates (Figure 1), the presence of free thiols, thioethers, and bipyridyl moieties was not tolerated. This was perhaps due to the possible role of these functional groups as competitive metal ligands[6] for palladium as well as copper, which could then carry out undesired chemistry that destroyed either the aryl iodide or alkyne reagent or simply poison the soluble catalyst. Indeed, exposure of bipyridyl-containing peptide **8** to coupling conditions (without copper iodide) resulted in immediate loss of the characteristic yellow color of the Pd catalyst solution to yield a pink hue instead; prolonged resulted in the clean degradation of starting material to a single unidentified product. In the case of thiol inhibition, though it is possible that a small percentage of disulfide was present that stoichiometrically oxidized the phosphine ligand to phosphine oxide, thereby crippling the Pd complex, reaction was not accomplished even with the addition of a large excess of phosphine and repeated freeze−pump−thaw degassing. The incorporation of free thiols in the coupled product was accomplished by protection of the thiol as a thiolester, which was subsequently hydrolyzed in situ following coupling. It is curious that thioethers such as the *S*-acetamidomethyl protecting group were not tolerated in the coupling, since previous work[4] had shown coupling in the presence of biotin, which contains a cyclic thioether. This lack of inhibition of palladium may be a result of the cyclic thioether conformation, or conversely, inhibition may be specific to the location of the thioether within the sequence tested.

We have demonstrated the utility of palladium-catalyzed cross-coupling in the construction of large, multifunctional peptide structures in water and have described some of its limitations. Thus, this is a useful synthetic strategy, as the methods for convergent ligation of multiple, highly charged peptides of considerable length are few; such species are not soluble in solvents commonly used for coupling, such as DMF or NMP. The use of solvents such as DMSO introduces isolation problems and may not be amenable to coupling conditions. The use of transition metals in peptide ligation requires introduction of unnatural functional groups, and coupling will result in an unnatural connectivity, but the synthesis may be devised so that this is of no concern to the end goal of the research.[7] Therefore, it is expected that this synthetic methodology will be useful in the construction of novel biomolecule-based systems in water, as a complement to known methods of chemoselective ligation.[8]

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