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Sonogashira Cross-Couplings Using Biocompatible Conditions in Water

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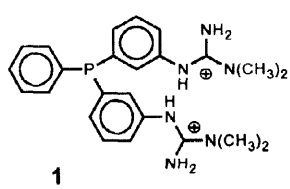
Abstract: The Pd-catalysed C-C bond formation between water soluble iodoarenes and terminal alkynes is promoted by cationic guanidinophosphines. New quantitative cross-couplings were obtained in aqueous solution at 35 ° C within minutes even in the presence of a protein.

The covalent attachment of non-natural substructures (reporter groups, physico-chemical probes, coenzymes, specificity tags etc.) to biomolecules (bioconjugation) requires chemical methods that allow regioselective modification of the multifunctional target without harming its fragile biologically competent 3D structure. Moreover, these biopolymers (proteins, nucleic acids, saccharides) contain multiple functional groups of very similar reactivity, the specific addressing of which is generally beyond the reach of present day chemical methods. Even if a unique functionality in the biomolecule would allow its regioselective elaboration, the number of suitable processes compatible with its sensitive biologically active nature is very limited, indeed.

A novel effort to overcome the limitations in bioconjugations is the palladium complex catalysed C-C bond formation (the Castro-Stephens-Sonogashira reaction)[1,2] which furnishes the coupling of chemically rather stable iodoarenes and alkynes. The chemistry involved is orthogonal to the reactivity of most functional groups in biomolecules thus obviating any requirement for protection. In combination with the very mild aqueous reaction conditions the interference with the biologically functional tertiary structures is minimised. In addition, rapid kinetics of the catalytic reaction allow the transformation of macromolecules which due to their size occur in minute molar concentrations only, and thus would otherwise take excessive reaction times in bimolecular conversions.



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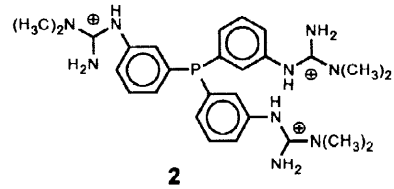

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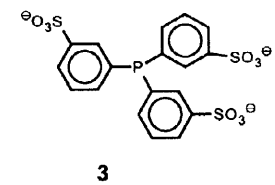

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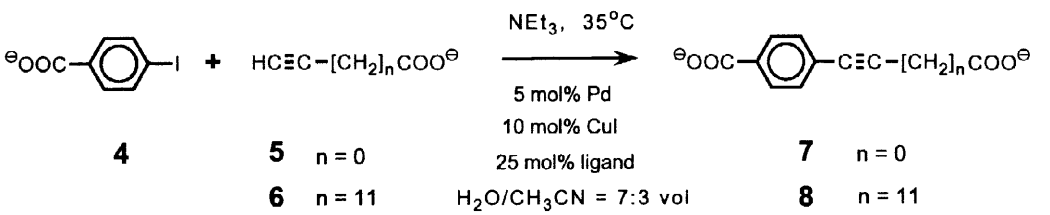
Building on occasional reports on the use of Pd-catalysed cross-couplings in the modification of natural products in non-aqueous solvents[2] and the successful implementation of aqueous though somewhat harsh basic conditions[3] we designed guanidino phosphines (**1** and **2**) as water soluble ligands for Pd(0).[4] Apart from their satisfactory water solubility which matches the well known anionic and industrially used sulfonated phosphines[5] (eg. **3**) these ligands are considerably more stable to oxidation and should be more appropriate to catalyse cross-couplings between anionic substrates. Substrate anions will be necessarily formed in biomolecules by the action of base that is mandatorily required in these catalyses.

This expectation was borne out in the cross-coupling of iodobenzoate and propiolic acid serving as a model reaction (eq.1). Using a 70/30 mix of water and acetonitrile at 35°C the clean cross-coupling reaction was observed in the presence of 5 mol% Pd catalyst[6], 10 mol% CuI, and 200 mol% of triethylamine in addition to the amount of the same base needed for neutralisation of the substrate acids. The comparative kinetics (Fig. 1) clearly revealed the requirement for a phosphine ligand with the biscationic species **1** surprisingly turning out as the most active one.



At 10 mM concentration of iodoarene substrate, the reaction rate proved to be independent of alkyne concentration when used in excess to allow for its presumptive homocoupling. Increasing the catalyst concentration, however, led to a dramatic though not proportional rate acceleration.

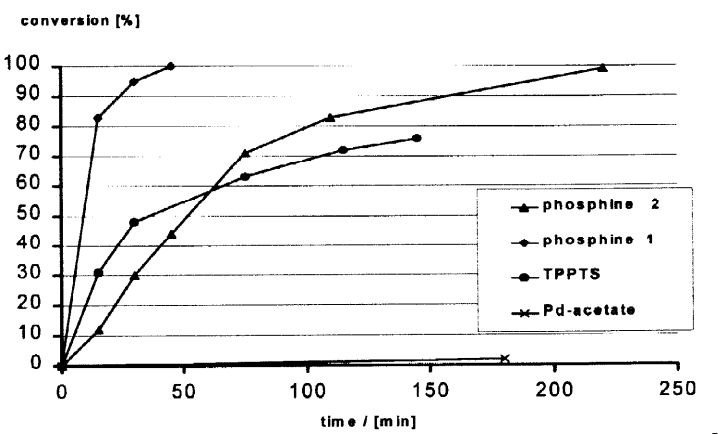


Fig. 1: Kinetics of cross-couplings of **4** and **5** using the conditions of eq (1) catalysed by various palladium catalyst systems

In order to test the reaction conditions in the presence of fragile biomolecules a fivefold excess (by mass) of the enzyme RNAse A was added. As evidenced by capillary electrophoresis which allows sensitive monitoring of product formation as well as of potential modifications of the protein depending on the field polarity (Fig.2), the reaction course in terms of cleanliness and yield and the integrity of the enzyme remained

unaltered. Differences in reaction rate, however, were distinct and were studied depending on the presence of CuI cocatalyst originally with the aim to simplify the complex catalyst. This study revealed that the cocatalyst is not a mandatory ingredient of the *in-situ* formed catalyst mixture, but greatly augments its performance. Even more surprising was the rate acceleration found in the presence of the protein. Quite unexpectedly, the enzyme not only is silent to the palladium-catalysed chemistry happening in homogeneous solution, but also supports it kinetically - most likely by hydrophobic interactions.

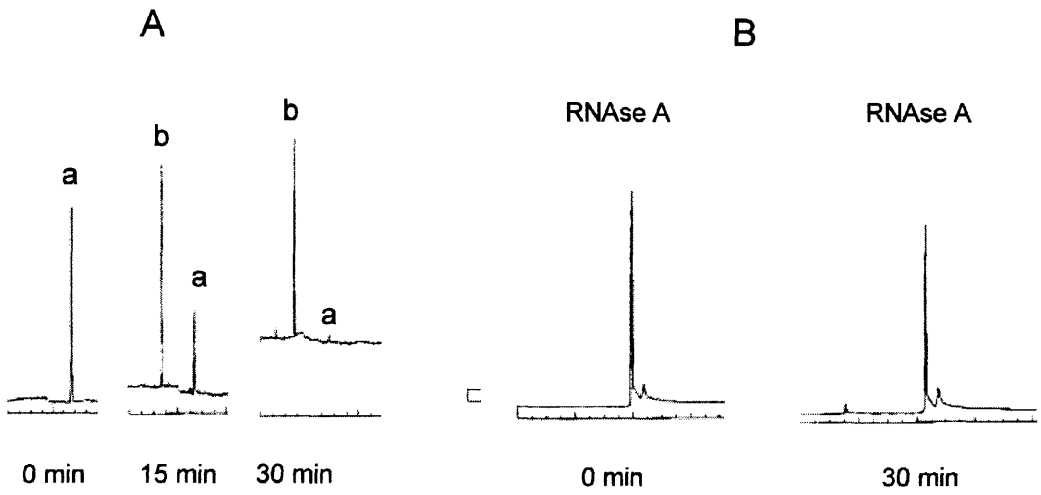


Fig. 2: CZE traces of the model reaction showing (A) clean substrate conversion (a = iodobenzoate **4**, b = product **7**); (B) identical electropherogramms for RNAse A at the beginning and after the end of the reaction. Field polarity of **A** versus **B** is inverted. The enzymatic activity of RNAse A was probed by hydrolysis of cytidine-2,3-cyclophosphate and remained unharmed by the reaction conditions.

A straightforward way for application of this kind of Pd-catalysed C-C bond formation in water could make use of alkynyl lipids. Terminal alkinyl fatty acids of appropriate lenghts are readily introduced by the N-myristoyl transferase system regiospecifically to the N-terminal ends of a variety of proteins.[7,8,9] Thus, they constitute attractive points of attachment of other components or further elaboration using the cross-coupling method described above. To demonstrate the feasibility of this approach the unsaturated analog of myristic acid, ω-tetra-decynoic acid **6** reacted with p-iodobenzoate **4** under the standard set of conditions (H_2O/CH_3CN = 70/30 vol, 35 °C, 2eq $N(C_2H_5)_3$, 5 mol% Pd / **1**, 10 mol% CuI). Unlike the model reaction the conversion ceased at about 60% after 90 min when a black colour formation indicated destruction of the catalyst. Neither elevation of the temperature to 50 °C nor cutting the substrate concentration to half had an effect on product yield. Addition of 1 vol% of the nonionic detergent Triton X-100 boosted the yield to 86%, while with 10 vol% of detergent the yield dropped to 74% again. In all of these reactions the lifetime of the catalyst appeared to limit the conversion. As a corollary, increasing the catalyst concentration to 10 mol% Pd brought about

clean quantitative cross-coupling within 15 min.

In conclusion we have found a simple and mild method to connect two chemically rather stable moieties by a carbon-carbon bond in aqueous solution using catalytic conditions which would leave most functions of biomolecules untouched, and these may be used in bioconjugation in their native and unprotected forms.

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1) This work was presented first at the 11th ICOS Conference, Amsterdam, June 1996.

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6) The catalyst was formed *in-situ* by dissolution of $Pd(OAc)_2$ and 5eq of the guanidino phosphine ligand chloride in water (0.01 M Pd). The mixture was kept at 4°C for 5 days. Within this period the catalytic activity gradually stabilised and then remained constant over several weeks.

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