Vapour-Induced Solid-State C–H Bond Activation for the Clean Synthesis of an Organopalladium Biothiol Sensor†

Andrea Monas, Krunoslav Užarević, Ivan Halasz, Marina Juribašić Kulcsár* and Manda Ćurić*

Room-temperature accelerated aging in the solid state has been applied for atom- and energy-efficient activation of either one or two C–H bonds of azobenzene and methyl orange by palladium(II) acetate. Organopalladium complexes are prepared in quantitative reactions without potentially harmful side products. Dicyclopalladated methyl orange is water-soluble and is a selective chromogenic biothiol sensor at physiologically-relevant micromolar concentrations in buffered aqueous media.

Palladium-mediated C–H bond activation for preparing cyclopalladated compounds, is a long-standing research topic with applications in organic synthesis and catalysis, in material science and, recently, in the design of photosensitizers and biomolecular labels. Conventional synthetic procedures for the preparation of organopalladium compounds are usually based on reactions in toxic organic solvents and often require elevated temperatures. Recently however, mechanochemical reactions in the solid-state have emerged as a viable alternative to conventional solvent-based chemistry and have thus far been applied for the synthesis of a large variety of compounds, including transition-metal catalysed C–H bond functionalization. Mechanochemical reactions however may not be suitable for the synthesis of soft or solvated materials, especially when prolonged milling is required. Next to mechanochemical methods, it was only recently shown that solid-state reactions can be carried out by controlled exposure of solid mixtures to vapour, Such an approach is called vapour digestion, accelerated aging or simply aging. Despite its reported efficiency, it has been used in the preparation of only a limited number of compounds.

By avoiding agitation of the reaction mixture, aging reactions are often slower than the corresponding milling reactions while still providing clean products in quantitative yield as well as unique reaction selectivity. Moreover, aging reactions offer easier handling and processing of the reaction mixture, and require a far lower energy input. In addition, due to their purity, compounds and materials prepared by aging may be more suitable for biological applications.

Our group has focused on azobenzene organopalladium compounds that exhibit strong light absorption and emission in the visible region which can be tuned by ancillary ligands on the azobenzene moiety. This property, coupled with the ability of amino acids and other biomolecules to bind to Pd(II) centres, qualifies them as candidates for biomolecular labelling. In particular, discrimination among various amino acids and small biothiol molecules (e.g. cysteine, homocysteine, glutathione, cystine) under physiological conditions is challenging due to their similar molecular structures and reactivity. Herein, we demonstrate vapour-induced room-temperature C–H bond activation in the solid state as a new, clean and energy-efficient methodology for the preparation of cyclopalladated azobenzene compounds (Fig. 1). The dicyclopalladated product, obtained...
directly from palladium(ii) acetate and methyl orange, proved as a highly sensitive chromogenic biotin sensor even in vast excess of amino acid competitors.

Prior to aging reactions, solid reactants in the molar ratio 1:1 or 1:2 of either azobenzene (1) or methyl orange (2) and Pd(OAc)$_2$, were mixed by gentle grinding in an agate mortar. Thus prepared mixtures were placed in closed vials saturated with vapour of selected liquids: N,N-dimethylformamide (DMF), acetic acid (AcOH), water or equimolar mixtures H$_2$O/DMF and H$_2$O/AcOH. Aging of solid mixtures has also been performed in air (20-23 °C, relative humidity 50-70%). Reaction progress and completion was monitored by $^1$H NMR spectroscopy (Figs. S1-S11) and also confirmed that no reaction occurred during initial mixing of reactants. Reactions were repeated at least five times and were fully reproducible. The azobenzene ligands were used to evaluate conversion. Products were characterized in the solid state by attenuated total reflectance infrared spectroscopy (ATR-IR) (Figs. S12 and S13) and powder X-ray diffraction (PXRD) (Figs. S14 and S15).

First, we have studied the solid-state reactivity of the parent azobenzene towards palladium(ii) acetate. Regardless of the starting reactants’ molar ratio, aging in DMF vapour yielded exclusively the monocyclopalladated product 1A with complete conversion of 1 in two days (Fig. S2). Aging reaction in AcOH vapour was extremely slow and incomplete even after 35 days of stirring at room temperature. A double set of signals has also been observed in $^1$H NMR spectra of 1B obtained by aging, mechanochemo- or solvent-based reactions corresponding to a mixture of cisoid and transoid isomers (Figs. S8-S10). According to literature$^{2,3}$ more intense signals have been assigned to the cisoid isomer (Fig. 1A, Table S1). Ratio of the cisoid and transoid isomers of 2B in solution did not change in the temperature range from –25 to 50 °C (Fig. S9). The ratio depended on the synthetic method and was the highest for the aging product from DMF where the cisoid isomer was isolated almost pure (Fig. S10). All attempts to prepare pure monocyclopalladated 2A were unsuccessful regardless of the employed synthetic method. It was always isolated with 2B and ligand impurities (Figs. S6 and S11) suggesting a high affinity of methyl orange towards dicyclopalladation. Faster reactions with methyl orange and formation of the dicyclopalladated product 2B can be attributed to the strong electron-donating effect of the N,N-dimethylamino group.

Breaking of the trimeric structure of palladium(ii) acetate, Pd$_2$(OAc)$_3$, is essential for its reactivity.$^{1,23}$ Cyclopalladation reactions with palladium(ii) acetate primarily follow the ambiphilic concerted metallation-deprotonation mechanism via agostic intermediate where the coordinated ligand (acetate, carbonate, etc.) acts as an intramolecular base to accept the proton simultaneously with Pd–C bond formation.$^{1,21,23}$ The acetate is a stronger base in AcOH and binds better to the Pd(ii) centre, which accelerates the cyclopalladation reaction.$^{24}$ Furthermore, the active role of basic DMF as an external base in the proton abstraction was supported by mechanistic studies of cyclopalladation of N-donor ligands, N-benzyl triamines$^{25}$ and azobenzene$^{18c}$, DMF solution and nucleophilic substitution on carbonyl group in the solid state.$^{26}$ Thus, two key steps in the reaction mechanism could be influenced by the choice of vapour: breaking of the trimer Pd$_2$(OAc)$_3$, which could be facilitated by H$_2$O vapour$^{1,21,22}$ and second, cyclopalladation step which could be promoted by basic DMF or AcOH.

Since organic bases facilitate proton abstraction in the solid state,$^{12,18,19}$ we performed aging reaction of 2 and Pd(OAc)$_2$ in H$_2$O/N,N,N-triethylamine (TEA) vapour. $^1$H NMR spectra proved that TEA reacts with Pd(OAc)$_2$, and consumed the Pd source which hampered the cyclopalladation reaction. This is a consequence of coordination of TEA to Pd(ii) via nitrogen whereas DMF, AcOH and H$_2$O, coordinate to Pd(ii) via oxygen.$^{18c,21,23a,26,27}$ Overall, these results suggest that the progress of C–H bond activation by aging may be influenced by proper selection of the azobenzene ligand as well as by the acid-base of the vapour used for the reaction.

The product 2B obtained by aging using H$_2$O/AcOH seems to be most convenient for biological applications. It is readily soluble in water and stable in buffered media which makes it suitable for studies in physiologically-relevant conditions using aqueous phosphate buffer (pH 7.4).

Biomolecules, especially amino acids, are known to bind to Pd(ii) centres, enabling these compounds to be used as biosensors.$^{19}$ In this context, the affinitity of 2B with its four readily exchangeable acetate ligands towards representative natural
amino acids (AAs) was explored. Special attention was dedicated to biothiols (AAs–SH) which, together with their derivatives, play a crucial physiological role in biological processes, ranging from protein folding to cellular metabolism and oxidative stress response, and have been investigated as potential indicators of disease risk and health status.\textsuperscript{2} UV-vis spectroscopy was used to study reactions of 2B with glycine (Gly), L-alanine (Ala), L-tryptophan (Trp), L-glutamic acid (Glu), and L-lysine (Lys). In addition, three groups of natural sulphur-containing molecules were examined: biothiols, i.e. L-cysteine (Cys), L-homocysteine (hCys) and L-glutathione (GSH, γ-L-glutamyl-L-cysteinylglycine); their derivatives, i.e. S-methyl-L-cysteine (MeCys) and L-methionine (Met, S-methyl-L-homocysteine); and their oxidized dimers: L-cystine (CSSC, oxidized Cys) and L-glutathiol (GSSG, oxidized GSH).

Compared to previously explored cyclopalladated compounds,\textsuperscript{4b} 2B reacts readily with AAs at room temperature allowing rapid visual distinction of compounds with free thiol groups from other common AAs and compounds with substituted or oxidized thiol groups. Complex 2B clearly differentiates thiol derivatives Cys/MeCys/CSSC, hCys/Met and GSH/GSSG. At least four AA equivalents are required for the chromogenic change. The “naked-eye” biothiol detection limit is extended down to a low micromolar concentration range (e.g. about 10 \(\mu\)M for Cys) (Fig. 2) which is comparable with physiological concentrations of Cys, hCys and GSH in human plasma, i.e. about 200, 7 and 4 \(\mu\)M, respectively.\textsuperscript{2b}

The light purple colour of an aqueous solution of 2B changed in ca. 10 minutes after addition of AA (4 eq) at 20 \(°\)C indicating the high affinity of 2B towards AAs (Fig. 2). Thiol-containing AAs changed the solution colour to red whereas other AAs changed it to blue, clearly differentiating biothiols from other functionalities. The red solution of 2B containing AA–SH (4 eq) remained unchanged upon addition of an excess of other AAs (30 eq). AA–SH pairs were used for testing the differences in the affinity of 2B toward biothiols (Fig. 3b) and revealed that the exchange of coordinated thiols was slow at room temperature and could be accelerated by incubating at elevated temperatures (Fig. S25).

Reactions of 2A with AAs could not be studied in detail as a pure 2A sample could not be obtained. Qualitative evaluation of the reaction of the mixture of 2A and 2 with the selected AAs (Ala, MeCys, Cys and hCys, Fig. S26) showed small changes in UV–vis spectra, resulting in almost the same solution colour.

As a possible explanation for the observed differences, we propose different binding modes of AAs and AAs–SH. Since palladium(II) prefers the coordination via five-membered chelate rings and has a strong affinity for sulphur and nitrogen and a weaker affinity for oxygen donor atoms,\textsuperscript{19} we propose N,S-chelate mode for AAs–SH and their derivatives, whereas N,O-chelate mode for other AAs which is in accordance with previously described palladium complexes with amino acids.\textsuperscript{19}

In summary, we have presented the first and unique case of solid-state C–H bond activation performed by mild and clean aging of solid reactants in vapours of suitable liquids. Even the unsymmetrical methyl orange, C–H bond activation proceeded regioselectively, over a monocyclopalladated intermediate, while the extent and time needed for the reaction to finish is highly dependent on the employed vapour mixture. Using \(\text{H}_2\text{O}/\text{AcOH}\) vapour, the reaction was fastest and over in under 24 hours. Dicyclopalladated methyl orange is water-soluble and shows a high affinity towards amino acids, where its selective chromogenic behaviour allows for visual detection of physiologically-relevant levels of biothiols. These properties make this compound a promising candidate for biological applications. A more detailed study in this direction is in progress.
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Notes and references


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