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SOLVENT, NOT PALLADIUM OXIDATION STATE, IS THE PRIMARY DETERMINANT FOR SUCCESSFUL COUPLING OF TERMINAL ALKYNES WITH IODO-NUCLEOSIDES [1]

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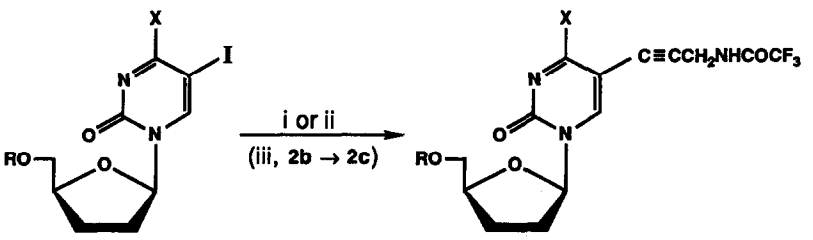
Summary: Coupling of iodo-nucleosides with terminal alkynes such as 3-(acylamino)propynes, whose initial products readily undergo secondary cyclization reactions, can be effected smoothly by the standard catalysis with $(Ph_3P)_2PdCl_2$/CuI/Et_3N in dimethylformamide.

The coupling of terminal alkynes with iodoarenes[3] as adapted in our laboratory[4] has been used to synthesize a variety of (alkyn-1-yl)nucleosides.[5] Some of these analogues have anticancer and antiviral activities[4c,6] and can be converted into mechanism-based enzyme inhibitors.[7] Alkynyl-nucleoside derivatives have been used to construct probes of nucleic acid structure and function.[8] Prober *et al.* have described automated sequencing of DNA with fluorescent dyes attached to the propargylic amine group of 5-(3-aminopropyn-1-yl)pyrimidine and 7-(3-aminopropyn-1-yl)-7-deazapurine 2',3'-dideoxynucleoside triphosphates.[9]

Syntheses of the precursor "chain terminating" nucleosides used for DNA sequencing[9] have been reported recently[10] by coupling 5-iodopyrimidine and 7-iodo-7-deazapurine (5-iodopyrrolo[2,3-*d*]pyrimidine) 2',3'-dideoxynucleosides with the "linker" (*N*-propargyltrifluoroacetamide) [3-(trifluoroacetamido)propyne]. Hobbs noted that attempted coupling reactions with propargylamine and 5-iodouridine using bis(triphenylphosphine)palladium(II) chloride and copper(I) iodide in triethylamine[3,4] failed, owing in part to nucleoside insolubility.[10] He achieved couplings of unprotected iodonucleosides and *N*-propargyltrifluoroacetamide using tetrakis(triphenylphosphine)palladium(0) and copper(I) iodide with 2 equiv of triethylamine in dimethylformamide. Hobbs briefly discussed possible mechanistic differences between the palladium catalysts, and attributed the successful couplings to the change from $(Ph_3P)_2PdCl_2$ to $(Ph_3P)_4Pd$.[10] He noted that no secondary cyclization products[4] were formed with $(Ph_3P)_4Pd$.

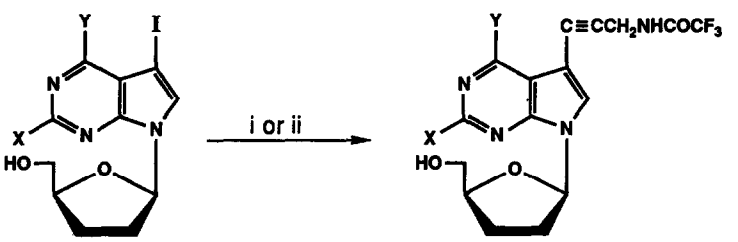
We observed several years ago that propargylic amines and ethers underwent rapid coupling with 5-iodouracil derivatives, but that these initial coupling products were very susceptible to secondary cyclization reactions.[11] This resulted in low to moderate yields of the

desired 5-alkynyluracil nucleosides, but yields of these reactions were quite sensitive to reaction solvents and work-up procedures.[4,11] We now have reexamined several coupling reactions with both 5-iodopyrimidine and 7-iodo-7-deazapurine nucleosides and find that the improvements noted by Hobbs[10] result primarily from the change to dimethylformamide as the reaction solvent, with a minor additional benefit resulting from the Pd(II) to Pd(0) catalyst change.



1a R = H, X = NH_2
b R = $CH_3C_6H_4CO$, X = OH

2a R = H, X = NH_2
b R = $CH_3C_6H_4CO$, X = OH
c R = H, X = OH



3a X = H, Y = NH_2
b X = NH_2, Y = OH

4a X = H, Y = NH_2
b X = NH_2, Y = OH

(i) **1** or **3**, $HC{\equiv}CCH_2NHCOCF_3$ (2.5 - 3 equiv), $(Ph_3P)_4Pd$ (0.1 equiv),CuI (0.2 equiv), Et_3N (2 equiv), DMF (3 - 5 mL/mmol), Ar atmosphere, ambient temperature, 1.5 - 6 h. (ii) Identical to (i) except $(Ph_3P)_2PdCl_2$ (0.1 equiv) used in place of $(Ph_3P)_4Pd$. (iii) K_2CO_3, MeOH, ambient temperature, 5 h.

Thus, for a typical coupling reaction: to a solution of 359 mg (1 mmol) of 7-deaza-2',3'-dideoxy-7-iodoadenosine {4-amino-7-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-5-iodopyrrolo[2,3-*d*]pyrimidine} (**3a**) in 3-5 mL of dimethylformamide was added 2.5-3 equiv of *N*-propargyltrifluoroacetamide (NPTFA), 116 mg (0.1 mmol) of $(Ph_3P)_4Pd$, 38 mg (0.2 mmol) of CuI, and 278 μL (202 mg, 2 mmol) of Et_3N. The mixture was stirred at ambient temperature under an argon atmosphere for 1.5-6 h while reaction progress was monitored by TLC (10%

MeOH/$CHCl_3$, the time required was dependent on the nucleoside derivative). When the starting nucleoside was completely consumed, the mixture was evaporated to dryness *in vacuo*, 500 mg of anion exchange resin [Bio-Rad AG1 X 8 (HCO_3^-)] and 6 mL of MeOH/CH_2Cl_2 (1:1) were added, and the mixture was stirred for 30 min. The mixture was filtered through Celite, the filter cake washed with MeOH/CH_2Cl_2 (1:1), and the filtrate was evaporated. The residue was purified by flash chromatography[12] (2 x 10 cm; MeOH/$CHCl_3$, 1:9) to give 7-deaza-2',3'-dideoxy-7-(3-trifluoroacetamidopropyn-1-yl)adenosine {4-amino-7-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-5-(3-trifluoroacetamidopropyn-1-yl)pyrrolo[2,3-*d*]pyrimidine}[10] (**4a**) (344 mg, 90%) as a light tan solid with mp 167-169 °C after evaporation of appropriate fractions and trituration of the amorphous tan residue with Et_2O.

Analogous couplings were performed by the same general procedure with 0.1 equiv of $(Ph_3P)_2PdCl_2$ in place of 0.1 equiv of $(Ph_3P)_4Pd$. These reactions were subjected to the same work-up procedure except the sodium salt of EDTA was routinely added[4] along with anion exchange resin after evaporation of the reaction mixture. In the cases checked, however, the presence or absence of sodium EDTA had no observed effect on yields and no significant contamination by fluorescent secondary cyclization products[4] was observed with either catalyst.

All of the coupled nucleoside derivatives after purification by flash chromatography were homogeneous by TLC and exhibited clean ^1H NMR spectra. Purities were >99% (analyzed by gradient reversed phase HPLC; C18 column) in all cases except >95% for the 7-deaza-2',3'-dideoxyadenosine derivative (**4a**).[13] Yields were slightly higher with the palladium(0) catalyst, but the difference was significant only in the case of the 7-deazaguanosine derivative (**4b**). Thus, yields with $(Ph_3P)_2PdCl_2$ and $(Ph_3P)_4Pd$ catalysis were: 84 and 87% (**2a**), 72 and 75% (**2b**), 87 and 90% (**4a**), and 77 and 90% (**4b**), respectively. Couplings of **3b** and NPTFA were sensitive to the purity of starting **3b**, and when less pure **3b** was used an additional 0.1 equiv of $(Ph_3P)_4Pd$ and 0.2 equiv of CuI were added to complete the couplings.

Analogous couplings of **1b** or 2'-deoxy-5-iodo-3',5'-di-*O*-*p*-toluoyluridine[14] with 3-(*tert*-butoxycarbonylamino)propyne [*N*-propargyl-*tert*-butylcarbamate] were effected successfully in dimethylformamide. Our repetition of previously reported couplings of iodo-nucleosides and amide/carbamate derivatives of propargylamine with $(Ph_3P)_2PdCl_2$/CuI/Et_3N in ethyl acetate[8a] or $(Ph_3P)_2PdCl_2$/CuI/NaOAc in methanol[8b] gave lower product yields and significant amounts of fluorescent by-products. Thus, couplings of either protected or unprotected iodo-nucleosides with 3-(acylamino)propynes, whose initial products readily undergo secondary cyclization reactions, proceed smoothly at ambient temperature in dimethylformamide with catalysis by either $(Ph_3P)_4Pd$ or $(Ph_3P)_2PdCl_2$, CuI, and Et_3N. Slightly higher yields were obtained with the

more sensitive and expensive $(Ph_3P)_4Pd$ catalyst in all cases, but the difference was significant only with the 7-deazaguanine derivative (**4a**). Yields were good to high in all cases and no appreciable fluorescent by-products[4] were observed with either palladium catalyst. Therefore, dimethylformamide as the reaction solvent is the most important factor for successful operation of this convenient transition metal-catalyzed coupling of terminal alkynes and iodo-nucleosides.

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13. (a) 0.01 M $Et_3NH^+OAc^-/H_2O$ (Buf) to Buf/MeCN (85:15) over 22 min, [ret time in min]: **2a** [11.9], **2c** [19]. (b) Buf to Buf/MeCN (70:30) over 22 min: **4a** [22], **4b** [18.8].
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