The Alkylation of Iodouridine by a Heterogeneous Palladium Catalyst

Shoeb I. Khan and Mark W. Grinstaff*

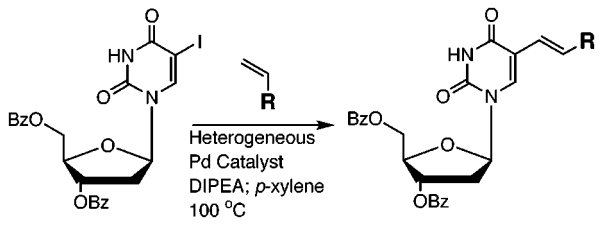
Department of Chemistry, P. M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27708

Received November 12, 1998

Site-specific modification of a nucleoside, nucleotide, or oligonucleotide is of widespread interest for mechanistic studies (DNA/RNA structure−function), analytical applications (sequencing, hybridization assays), and therapeutic uses (antisense, antiviral pharmaceuticals).[1−13] Nucleosides covalently modified with functional labels such as fluorescent tags, biotin, hydrophobic dyes, intercalators, metal complexes, and peptides are commonly used in the above applications. Typically, the selective modification of the nucleobase occurs at the C5-position of pyrimidines, C8 of purines, or C7 of deazapurines. Of particular interest are the modified pyrimidine analogues, since this C5 site of modification has been previously shown to tolerate a large number of different chemical functionalities without disfavoring duplex formation. Herein, we report the use of a heterogeneous palladium catalyst for the carbon−carbon bond formation reaction between 5-iodouridine and a series of substituted olefins. This one-pot heterogeneous palladium-catalyzed reaction proceeds in good yield and requires minimal workup (Scheme 1).

Palladium-catalyzed carbon−carbon bond formation reactions have undergone a resurgence in activity over the last 10 years and are based on the pioneering work of Heck in the late 1960s.[14−16] Even though this Pd(0)-catalyzed reaction is widely used,[17] the reactions typically require a large amount of palladium catalyst (1−5 mol %) and are associated with the difficulties in performing a homogeneous reaction such as ease of product isolation and purification, and recycling of the catalyst. Consequently, a number of groups are exploring either heterogeneous or biphasic Pd(0) catalysts for the formation of new carbon−carbon bonds.[18−21]

Scheme 1



A palladium-anchored phosphinated polystyrene catalyst, for example, was reported for the coupling of iodobenzene with substituted olefins in moderate yields (59−78%).[18,22] The second approach of using a biphasic catalyst was demonstrated by Horvath and Rabai who prepared a catalyst that was soluble in a perfluorocarbon phase which could be easily separated from the reaction mixture in the organic phase.[23] More recently, Knochel and Betzemeier have reported efficient coupling of arylzinc bromides with aryl iodides using a perfluorinated Pd(0) catalyst in excellent yields (>90%).[24] To the best of our knowledge, this is the first report of a Pd(0) heterogeneous catalyst for the alkylation of a nucleoside.

The heterogeneous palladium catalyst (Si−S−Pd) for coupling terminal olefins with 5-iodouridine was synthesized as shown in Scheme 2.[19,21] The catalyst was prepared by reacting fumed silica (0.007 μm) with (γ-mercaptopropyl)triethoxysilane in refluxing toluene for 6 h followed by treatment with $PdCl_2$ in acetone at 25 °C for 48 h, and finally reduction with hydrazine hydrate under nitrogen for 3 h. The catalyst was subsequently dried under vacuum at 50 °C for 2 days before use.

* Corresponding author. http://www.chem.duke.edu/∼mwg

(1) Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165−186.
(2) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 1925−1963.
(3) Fidanza, J. A.; Ozaki, H.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1992**, *114*, 5509−5517.
(4) MacMillin, A. W.; Verdine, G. L. *Tetrahedron* **1991**, *47*, 2603−2616.
(5) Hunkapillar, M.; Kent, S.; Caruthers, M.; Dreyer, W.; Firca, J.; Giffin, C.; Horvath, S.; Hunkapiller, T.; Tempst, P.; Hood, L. *Nature* **1984**, *310*, 105−111.
(6) Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463−5467.
(7) Fodor, S. P. A. *Science* **1997**, *277*, 393−395.
(8) Keller, G. H.; Manak, M. M. *DNA Probes*; Stockton Press: New York, 1993.
(9) Englisch, U.; Gauss, D. H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 613−629.
(10) Sammes, P. G.; Yahioglu, G. *Nat. Prod. Rep.* **1996**, 1−28.
(11) Glazer, A. N.; Mathies, R. A. *Curr. Opin. B* **1997**, *8*, 94−102.
(12) Mansour, T. S.; Storer, R. *Curr. Pharm. Des.* **1997**, *3*, 227−264.
(13) Perigaud, C.; Gosselin, G.; Imbach, J. L. *Nucleosides Nucleotides* **1992**, *11*, 903−945.
(14) Heck, R. F. *J. Am. Chem. Soc.* **1968**, *90*, 5531−5534.
(15) Heck, R. F. *Acc. Chem. Res.* **1978**, *11*, 146−151.
(16) Meijere, A.; Meyer, F. E. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2379−2411.

(17) The standard multistep procedure for introducing an allyl-linker to the C5-pyrimidine position involves direct mercuration of the nucleoside followed by alkylation with the desired olefin in the presence of a palladium(0) catalyst [Bigge, C. F.; Kalaritis, P.; Deck, J. R.; Mertes, M. P. *J. Am. Chem. Soc.* **1980**, *102*, 3−2038]. The heavy metals are precipitated with H_2S, and the nucleoside analogue is isolated after column chromatography. Although widely used, this method has several limitations including (1) the use of mercury, (2) long reaction times, (3) multiple synthetic steps, (4) multiple chromatographic steps, and (5) the use of H_2S to precipitate the mercury and palladium as metal sulfides. Alternatively, if the linker to the nucleobase is an alkynyl rather than an allyl group, some of these limitations can be overcome. In the widely referenced work of Bergstom (Bergstrom, D. E.; Ogawa, M. K. *J. Am. Chem. Soc.* **1978**, *100*, 6−8112), Daves (Arai, I.; Daves, G. D. *J. Am. Chem. Soc.* **1978**, *100*, 287−288), Hobbs (Hobbs, J. F. W. *J. Org. Chem.* **1989**, *54*, 3420−3422), and Robins and Barr (Robins, M. J.; Barr, P. J. *Tetrahedron Lett.* **1981**, *22*, 421−424. Robins, M. J.; Barr, P. J. *J. Org. Chem.* **1983**, *48*, 1854−1862) the use of soluble Pd(0) catalysts for the cross-couplings of terminal alkynes to halonucleosides are reported in good to excellent yields. The cross-coupling of alkynylzinc halides with 5-iodouridine has also been reported, but in yields ranging from 30 to 95% (Vincent, P.; Beaucourt, J. P.; Pichat, L. *Tetrahedron Lett.* **1981**, *22*, 945−947). More recently, Aerschot has reported the C-2, -6, and -8 alkylation of adenosine analogs by cross-coupling the iodonucleoside with tetraalkyltin reagents in the presence of $Pd(PPh_3)_4$ (Aerschot, A. A. V.; Mamos, P.; Weyns, N. J.; Ikeda, S.; Clercq, E. D.; Herdewijn, P. A. *J. Med. Chem.* **1993**, *36*, 2938−2942).

(18) Terasawa, M.; Kaneda, K.; Imanaka, T.; Teranishi, S. *J. Organomet. Chem.* **1978**, *162*, 403−414.
(19) Wang, L. Z.; Jiang, Y. Y. *J. Organomet. Chem.* **1983**, *251*, 39−44.
(20) Choudhary, B. M.; Sarma, R. M.; Rao, K. K. *Tetrahedron* **1992**, *48*, 719−726.
(21) Cai, M. Z.; Song, C. S.; Huang, X. *Synthesis* **1997**, 521−523.
(22) Andersson, C. M.; Karabelas, K.; Hallberg, A.; Andersson, C. *J. Org. Chem.* **1985**, *50*, 3891−3895.
(23) Horvath, I. T.; Rabai, J. *Science* **1994**, *266*, 72−75.
(24) Betzemeier, B.; Knochel, P. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2623−2624.

10.1021/jo982254x CCC: $18.00
Published on Web 01/15/1999

Scheme 2

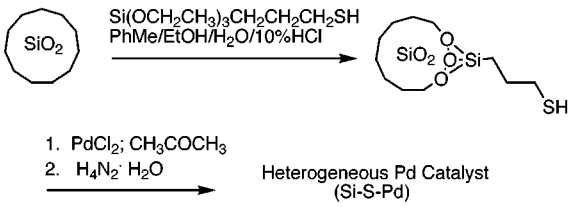
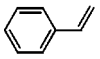
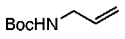
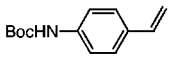
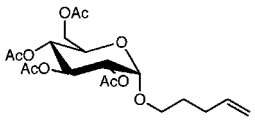


Table 1. Modified Nucleosides Synthesized

#	Substituted Olefins	Isolated Yields
1		85%
2		79%
3		80%
4		82%

As shown in Table 1, a variety of substituted olefins ranging from a simple aromatic amine to a carbohydrate were coupled with 3′,5′-dibenzoyloxy-2′-deoxy-5-iodouridine[25] using this heterogeneous catalyst. The three components (i.e., halo-nucleoside, olefin, and catalyst) were added, and the reaction proceeded smoothly at 100 °C where the solution appeared almost translucent. Upon completion of the reaction, the solution was cooled to 25 °C. The heterogeneous catalyst was then filtered from solution, and the product was isolated in good yield with minimal workup. The catalyst could be reused without significant loss of catalytic activity (≈85%). This catalyst, however, did not couple olefins containing protected or unprotected carboxylic acids or unprotected amines in good yield (<50% and <60%, respectively). This reaction was also sensitive to base, and DIPEA was found to be superior to TEA, Bu_3N, and pyrrolidinone. Efficient alkylation also required a reaction temperature of 100 °C, and at lower temperatures (≈80 °C) the reaction did not proceed to a significant extent.

We propose a mechanism for this heterogeneous reaction wherein the reactive catalyst is a Pd(0) species coordinated by two thioaldehydes.[26] This mechanism is consistent with the synthetic steps of $PdCl_2$ addition to the surface thiols followed by reduction with hydrazine (Scheme 2), and analogous to previously proposed mechanisms for related homogeneous Pd(0) phosphine catalysts.[16,27] A photoacoustic spectrum[28] of the fumed silica containing the thiol shows a peak at 2940 cm^{-1} corresponding to the SH stretch of the mercaptoalkyl, and elemental analysis confirms a mole ratio consistent with the thiol attached to the surface. Elemental analysis of the active species (Si−S−Pd) formed by reaction with $PdCl_2$ and hydrazine (Scheme 2) shows no chloride and a S:Pd mole ratio of ≈2:1. Also, the photoacoustic spectrum no longer contains the SH stretch. A Pd(0) catalyst formed via hydrazine reduction is likely to abstract a β-hydrogen to afford a Pd-coordinated thioaldehyde. We suggest that in the first step of the catalytic cycle, 5-iodouridine oxidatively adds to the active species (Pd−S−Pd) generating the σ-alkenyl intermediate. The alkene then coordinates to the Pd(0) catalyst and inserts into σ-alkenyl bond. Finally, β-hydride elimination yields the modified nucleoside, and reductive elimination of HI regenerates the proposed active species.

In conclusion, a facile method for forming new carbon−carbon bonds between a halo-nucleoside and substituted olefins is described. Based on the results shown above, we are currently pursing other halo-nucleosides for selective modification, as well as exploring additional olefin substrates such as derivatized metal complexes. More importantly this heterogeneous catalyst may be of general use for carbon−carbon bond formation reactions with complex biomolecules and natural products where (1) free metal salts or complexes cannot be used, (2) ease of product separation and purification are required, and (3) chemical functionalities need to be tolerated.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification.

The typical procedure for the synthesis of C5-substituted uridines was as follows. The nucleoside, 3′,5′-dibenzoyloxy-2′-deoxy-5-iodouridine (0.11 g, 0.2 mmol), was dissolved in *p*-xylene (1 mL), and DIPEA (0.4 mmol) was added. Next, the appropriate substituted alkene (0.6 mmol) was added. The solution was degassed with argon, and approximately 20 mg of the heterogeneous catalyst was added. The reaction was heated to 100 °C for 12 h under argon, with periodic monitoring by TLC. The reaction mixture was then cooled to 25 °C, and the heterogeneous catalyst was filtered from solution. The catalyst was washed with warm $CHCl_3$ (20 mL) to remove any remaining product. The organic fractions were collected and washed with water, 0.5 N HCl, and water and then dried over Na_2SO_4. Evaporation of the solvent followed by washings with hexane and ether (to remove excess alkene and unreacted iodouridine) afforded the modified nucleosides in high purity without additional workup (see HPLC traces in Supporting Information). Characterization data including HR-FABMS, NMR, melting point, and HPLC traces (reverse-phase HPLC; C18 column; 0.1 M TEAA/CH_3CN; 10−50% gradient over 30 min; monitoring at 254 nm) for all compounds are found in Supporting Information. A small amount of starting material (typically less than 5%) remained in the product.

Acknowledgment. This work was supported by Duke University. We thank Professor R. A. Palmer and Mr. Eric Phifer for the photoacoustic spectra.

Supporting Information Available: Text describing the detailed syntheses of the catalyst and 3′,5′-dibenzoyloxy-2′-deoxy-5-iodouridine are included. HPLC, NMR, melting point, and HR-FABMS are reported for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO982254X

(25) Amarnath, V.; Broom, A. D. *Chem. Rev.* **1977**, *77*, 183−217.
(26) The coordination of the Pd catalyst by two thiolates is another possibility.
(27) Bryndza, H. E.; Tam, W. *Chem. Rev.* **1988**, *88*, 1163−1188.
(28) Dittmar, R. M.; Palmer, R. A.; Carter, R. O. *App. Spectrosc. Rev.* **1994**, *29*, 171−231.