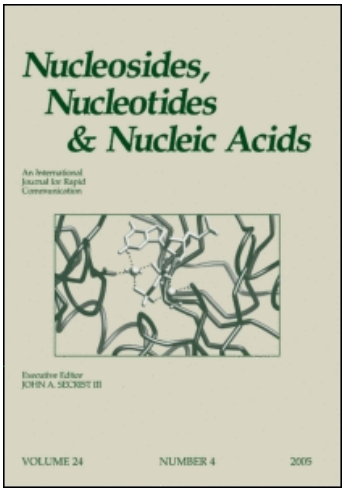

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Synthesis of 2'-Deoxyuridine Nucleosides with Appended 5-Position Carbonyl Cross-Linking Groups

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SYNTHESIS OF 2'-DEOXYURIDINE NUCLEOSIDES WITH APPENDED 5-POSITION CARBONYL CROSS-LINKING GROUPS

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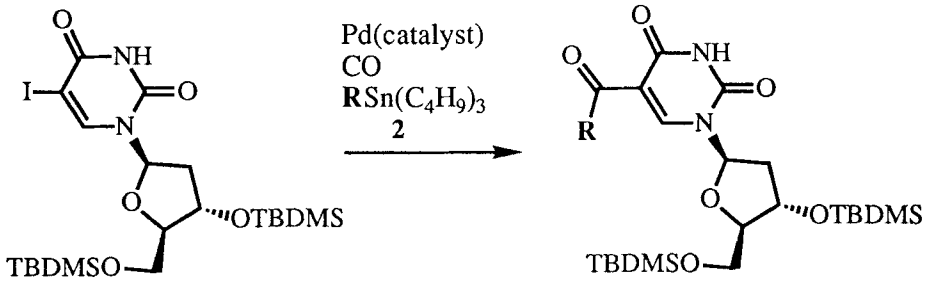
Abstract. A simple modification of Stille type carbonylative coupling conditions resulted in high yield reactions giving new carbonyl appended 2'-deoxyuridine derivatives useful for chemical cross-linking.

Introduction. Until recently, the biological role of oligonucleotides was thought to consist only of information storage and transfer.[1] The advent of *in vitro* evolution[2] has provided a method for producing high-affinity nucleic acid ligands. It recently has been discovered that oligonucleotide ligands show high specificity and selectivity in binding to a variety of medicinally important targets, including HIV reverse transcriptase,[3] HIV rev protein,[4] basic fibroblast growth factor,[5] and thrombin.[6] Synthetic oligonucleotides now are being considered "designer" therapeutic agents.

Attachment of a variety of reporter groups to oligonucleotide ligands can provide information about nucleic acid structure and dynamics.[7] In addition, the appended groups are capable of forming cross-links and may afford new structural motifs that produce higher affinity ligands. For example, the α-amino group of amino acids can react with 5-propenoyl-2'-deoxyuridine (**3b**) to form Michael adducts.[8] The palladium catalyzed chemistry described below affords a convenient route to many useful carbonyl compounds, including 5-formyl-2'-deoxyuridine (**3a**), which can be used to form a Schiff's base with amines tethered to nitroxide radical reporter groups.[9]

Results and Discussion. Prior to the work described here, the use of palladium to catalyze carbonylative carbon-carbon bond formation at the 5-position of pyrimidine nucleosides was unknown. It was desirable to have a coupling method that was general with regard to catalyst and solvent.

First **3a** was prepared (82 % yield) using standard Stille coupling conditions[10] (PdL_4, **2a**). However, extending the carbonylation reaction to vinyl stannanes, use of vinyltributyltin (**2b**) in place of tributyltin hydride (**2a**) under the same reaction conditions gave poor yields of **3b** (eq. 1, 46 %).



1 TBDMS = *t*-butyl(dimethyl)silyl **3**

2a, 3a: R = H
2b, 3b: R = $CHCH_2$

Consistent with current understanding of palladium (0) C-C bond forming catalysis, we decreased the amount of triphenylphosphine present in the reaction mixture.[11] Combining palladium(II) acetate with three equivalents of triphenylphosphine and copper(I) iodide in THF provided an *in situ* route for the formation of a more active catalyst. Using this Pd(catalyst) with **1** and **2b** provided an excellent yield of **3b** (eq. 1, 82 %).

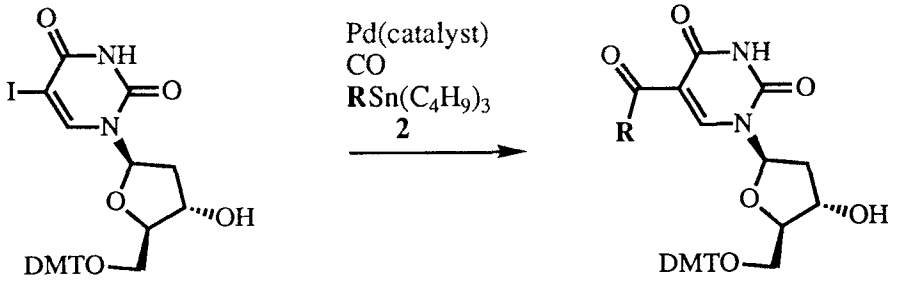
To our knowledge, all reported examples of palladium catalyzed coupling of stannanes to nucleosides have used 3' and 5' hydroxyl protecting groups. An alternate and

TABLE I

Reaction	Catalyst	Time (h)	% Yield
2a + 4	A	8	48
	B	8	95
2b + 4	A	12	77
	B	14	0
2c + 4	A	8	85
2d + 4	A	12	81
2e + 4	A	12	95
	B	12	0

Catalyst composition. Catalyst A is composed of 10 mol % $Pd(OAc)_2$, 30 mol % $P(C_6H_5)_3$, and 30 mol % of CuI. Catalyst B is composed of 10 mol % $Pd[P(C_6H_5)_3]_4$

expeditious entry into synthetic oligonucleotides involves the elimination of nucleoside protection/deprotection steps. Consistent with preparation for automated oligonucleotide synthesis using the phosphoramidite method,[12] we selected 4, 4'-dimethoxytrityl to protect *only* the 5' hydroxyl function of 5-iodo-2'-deoxyuridine (**4**).[13] We were concerned about the success of the reaction since the 3' hydroxyl was unprotected, and earlier literature precedent for carbonylative coupling of aryl halides in the presence of alcohols had been shown to result in ester formation.[14] In contrast to the silyl protected substrate **1**, when **4** and **2b** were treated with PdL_4 under the same reaction conditions, no product formation was observed. Surprisingly, *in situ* generation of the Pd(catalyst) again afforded a clean reaction to yield **5b** (eq. 2, 77 %) with no detectable side products.



(2)

4 **5**

DMT = 4,4'-dimethoxytrityl

2a, 5a: R = H
2b, 5b: R = $CHCH_2$
2c, 5c: R = E-$CHCHSi(CH_3)_3$
2d, 5d: R = $C(OC_2H_5)CH_2$
2e, 5e: R = C_6H_5

To test the generality of this method, two additional vinylic stannanes with markedly different steric and electronic properties were treated with **4**, giving clean reactions and excellent yields (TABLE I). In addition to vinylic coupling, an aryl stannane was examined. No product formation was observed when phenyltributyltin was treated with **4** and PdL_4; however, using the *in situ* generated Pd(catalyst) gave an excellent yield (95 %) of **5e**.

Summary. Attempts at palladium catalyzed carbonylative coupling on **4** using standard conditions failed. A simple modification of Stille conditions resulted in high yield reactions giving new carbonyl appended 2'-deoxyuridine derivatives useful for chemical cross-linking. It was found that using this modification gave excellent yields of the desired products even when only the 5'-hydroxyl function was protected by DMT.

Experimental Methods. A typical procedure for nucleoside carbonylative coupling is given by the preparation of **5b**. The following reagents were combined in the reaction flask portion of a self-contained glass coupling apparatus equipped with a pressure equalizing addition funnel and Teflon valves in a Vacuum Atmospheres, Inc. inert atmosphere (argon) glove box: **4** (0.274 mmol), $Pd(OAc)_2$ (0.027 mmol), CuI (0.080 mmol), $P(C_6H_5)_3$ (0.080 mmol) and THF (25 mL, distilled from benzophenone Na/K alloy). Vinyltributyltin (0.301 mmol in 10 mL of THF) was added to the addition funnel portion of the coupling apparatus. The reaction apparatus was charged with 50 psi of CO and heated to 70 °C. Vinyltributyltin was dispensed using the addition funnel at a rate of 50-70 μL per 5 seconds. After 5 hours at 70 °C, the solvent was removed on a rotary evaporator. The crude product was dissolved in CH_2Cl_2 (3 x 1 mL portions) and applied to a pad of silica (28 g) in a glass-fritted Büchner funnel (60 mL). The silica was eluted with pentane (100 mL), followed by CH_2Cl_2 (100 mL). The product was eluted from the silica pad with ethyl acetate (150 mL). The ethyl acetate solution was concentrated on a rotory evaporator, and the resulting residue purified by flash chromatography (80 g SiO_2, $MeOH/CH_2Cl_2$, 5:95) to give **5b** (150 mg, 84%). Spectroscopic data for nucleosides **5a-e** follow.

5a ^1H NMR (300 MHz, $CDCl_3$) δ 9.71 (s, 1H), 9.14 (bs, 1H), 8.46 (s, 1H), 7.39-7.20 (m, 9H), 6.85-6.82 (m, 4H), 6.18 (t, *J* = 6 Hz 1H), 4.46 (hex, *J* = 3 Hz 1H), 4.13 (q, *J* = 4 1H), 3.78 (s, 6H), 3.44 (dd, *J* = 11, 4 Hz 1H), 3.39 (dd, *J* = 11, 4 Hz 1H), 2.61 (ddd, *J* = 14, 6, 3 Hz, 1H), 2.34-2.25 (m, 2H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ 185.29, 161.39, 158.67, 149.15, 145.73, 144.34, 135.33, 135.22, 130.06, 127.98, 127.93, 127.07, 113.30, 111.08, 87.11, 86.98, 86.23, 72.42, 63.36, 55.25, 41.56; MS (FAB) *m/z* $(M + 1)^+$ 559, $(M + Na)^+$ 581; Anal. Calcd for $C_{31}H_{30}N_2O_8$: C, 66.66; H, 5.41; N, 5.01. Found C, 66.35; H, 5.44; N, 4.85.

5b ^1H NMR (300 MHz, $CDCl_3$) δ 8.86 (bs, 1H), 8.52 (s, 1H), 7.48 (dd, *J* = 17, 10 Hz 1H), 7.41-7.17 (m, 9H), 6.85-6.81 (m, 4H), 6.38 (dd, *J* = 17, 2 Hz 1H), 6.18 (t, *J* = 6 Hz 1H), 5.73 (dd, *J* = 10, 2 1H), 4.37 (hex, *J* = 4 Hz 1H), 4.06 (q, *J* = 5 1H), 3.78 (s, 6H), 3.46 (dd, *J* = 10, 4 Hz 1H), 3.38 (dd, *J* = 10, 5 Hz 1H), 2.54 (ddd, *J* = 14, 6, 4 Hz, 1H), 2.25 (pen, *J* = 7 Hz 1H), 2.15 (d, *J* = 4 Hz 1H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ 184.96, 160.71, 158.58, 149.31, 147.22, 144.41, 135.50, 135.41, 134.18, 130.04, 130.01, 129.14, 127.97, 126.96, 113.27, 112.44, 86.98, 86.61, 86.21, 73.42, 63.45, 55.20, 41.03; MS (FAB) *m/z* $(M + 1)^+$ 585, $(M + Na)^+$ 607; Anal. Calcd for $C_{33}H_{32}N_2O_8$: C, 67.80; H, 5.52; N, 4.79. Found C, 67.63; H, 5.77; N, 4.49.

5c ^1H NMR (300 MHz, $CDCl_3$) δ 8.73 (bs, 1H), 8.48 (s, 1H), 7.63 (d, *J* = 19 Hz 1H), 7.41-7.19 (m, 10H), 6.86-6.81 (m, 4H), 6.18 (t, *J* = 6 Hz 1H), 4.35 (hex, *J* = 3 Hz 1H), 4.04 (q, *J* = 4 1H), 3.78 (s, 6H), 3.46 (dd, *J* = 10, 4 Hz 1H), 3.37 (dd, *J* = 10, 4

Hz 1H), 2.53 (ddd, J = 14, 6, 4 Hz, 1H), 2.23 (pen, J = 7 Hz 1H), 2.08 (m, 1H), .016 (s, 9H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ 183.88, 160.61, 158.58, 149.38, 148.71, 147.19, 144.47, 139.86, 135.47, 135.42, 130.04, 127.96, 126.95, 113.28, 112.80, 86.97, 86.49, 86.10, 72.48, 63.48, 55.21, 40.92, -1.76; MS (FAB) m/z M^+ 656, $(M + 1)^+$ 657, $(M + Na)^+$ 679; Anal. Calcd for $C_{36}H_{40}N_2O_8Si$: C, 65.83; H, 6.14; N, 4.27. Found C, 65.59; H, 6.09; N, 3.97.

5d ^1H NMR (300 MHz, $CDCl_3$) δ 8.82 (bs, 1H), 8.10 (s, 1H), 7.40-7.19 (m, 9H), 6.85-6.81 (m, 4H), 6.21 (t, J = 7 Hz 1H), 4.94 (d, J = 3 Hz 1H), 4 .44 (d, J = 3 Hz 1H), 4.38 (hex, J = 3 Hz 1H), 4.06 (q, J = 4 1H), 3.80-3.73 (m, 8H), 3.41 (dd, J = 10, 4 Hz 1H), 3.32 (dd, J = 10, 4 Hz 1H), 2.51 (ddd, J = 14, 7, 3 Hz, 1H), 2.27 (d, J = 3 Hz 1H), 2.20 (pen, J = 7 Hz 1H), 1.26 (t, J = 7 Hz 3H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ 185.86, 159.54, 159.59, 158.56, 158.37, 149.37, 144.32, 144.30, 135.46, 135.34, 129.99, 129.91, 127.95, 126.97, 113.77, 113.27, 92.50, 86.89, 86.05, 86.00, 72.31, 64.31, 63.45, 55.19, 40.96, 14.23; MS (FAB) m/z $(M + 1)^+$ 629, $(M + Na)^+$ 651, $(M + Rb)^+$ 713.

5e ^1H NMR (300 MHz, $CDCl_3$) δ 8.80 (bs, 1H), 8.17 (s, 1H), 7.67-7.13 (m, 14H), 6.78-6.73 (m, 4H), 6.24 (t, J = 7 Hz 1H), 4.39 (pen, J = 3 Hz 1H), 4.06 (q, J = 4 1H), 3.72 (s, 6H), 3.38 (dd, J = 11, 4 Hz 1H), 3.27 (dd, J = 11, 5 Hz 1H), 2.55 (ddd, J = 14, 6, 4 Hz, 1H), 2.27 (m, 2H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ 189.77, 160.03, 158.34, 149.43, 145.07, 144.31, 137.08, 135.40, 135.29, 133.11, 129.90, 129.44, 128.13, 127.94, 127.88, 126.95, 114.15, 113.24, 86.91, 86.31, 86.24, 72.34, 63.44, 55.17, 41.15; MS (FAB) m/z M^+ 634, $(M + 1)^+$ 635, $(M + Na)^+$ 657, $(M + Rb)^+$ 719.

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