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Highly chemoselective palladium-catalyzed Sonogashira coupling of 5-iodouridine-5′-triphosphates with propargylamine: a new efficient method for the synthesis of 5-aminopropargyl-uridine-5′-triphosphates

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ABSTRACT

An efficient palladium-catalyzed Sonogashira coupling of 5-iodouridine-5′-triphosphates with propargylamine is described. The catalytic reaction is highly chemoselective that affords exclusively 5-aminopropargyl-uridine-5′-triphosphates in good yields with high purities (>99.8%).

The chemical modification at C-5 position of the pyrimidine moiety in uridine-5′-triphosphates has been the subject of immense interest in view of their wide applications in chemical biology, structural biology, DNA sensing, and nanobiotechnology.[1] In particular, aminopropargyl nucleotides serve as a versatile molecular biology tool for in vitro enzymatic introduction of functional groups into a nucleic acid target of interest.[2] The incorporation of 5-(3-aminopropargyl)-2′-deoxyuridine-5′-triphosphate into DNA produces amine-modified DNA by conventional enzymatic incorporation methods such as reverse transcription, nick translation, random primed labeling, or PCR and the resulting amine-modified DNA can labeled with any amine-reactive dye or hapten.[2] The aminopropargyl nucleotide coupled with fluorescent dye has been useful for generating labeled nucleic acids for various molecular biology applications such as chromosome and mRNA fluorescence in situ hybridization (FISH) experiment, gene expression, and mutation detection on arrays and microarrays, and in situ PCR and RT-PCR.[3–5] The C-5 position of UTP and dUTP is not involved in Watson–Crick base-pairing thereby minimizing any distortion in the shape of the base pairs.[6] The presence of unique alkynylamino linker provides a spacer between the nucleotide and the dye to reduce the fluorophore's interaction with enzymes or target binding sites. It is noteworthy that the use of such non-radioactive labeling provides several advantages over traditional radioactive labeling in terms of safety reasons, better stability and higher labeling efficiency, and consistency, at reduced cost. The successful molecular biology applications depend on the quality and purity of aminopropargyl modified nucleotides.

The palladium-catalyzed Sonogashira coupling provides a powerful strategy for the construction of a carbon–carbon triple bond in organic synthesis.[7] Although palladium-catalyzed Sonogashira coupling involving halogenated nucleosides has been well documented in the literature,[8,9] the use of halogenated nucleotides for the coupling reaction has been limited.[10] The cross-coupling reaction involving iodo-nucleotides often results in poor yields due to partial hydrolysis of the starting and final nucleotides to the corresponding diphosphates. The current reported method to make 5-aminopropargyl nucleotide involves the Sonogashira coupling of 5-iodouridine with protected propargylamine, followed by triphosphorylation of protected aminopropargyl uridine using Ludwig 'One pot, three step strategy' and subsequent deprotection of the propargylamine moiety.[8] However, the reaction involves overall three steps and the triphosphate step results in poor to moderate yields. Given the potential molecular biology applications of these aminopropargyl nucleotides, the development of a novel and efficient gram-scale method would be highly valuable to meet the application of nucleic acid chemistry demand. Our continuous interest in nucleic acid chemistry[11,12] prompted us to explore the possibility of palladium-catalyzed Sonogashira coupling reaction of 5-iodouridine-5′-triphosphates with propargylamine for the synthesis of 5-aminopropargyl-uridine-5′-triphosphates. Herein,

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we report the first example of a palladium-catalyzed Sonogashira coupling of 5-iodouridine triphosphates with propargylamine, leading to the formation of 5-aminopropargyl-uridine triphosphates in good yields with high purities.

The two step synthetic strategy to make aminopropargyl nucleotides such as 5-(3-aminopropargyl)-2′-dexoyuridine-5′-triphosphate **3a** and 5-(3-aminopropargyl)-uridine-5′-triphosphate **3b** is depicted in Scheme 1. The required starting material, 5-iodo-dUTP **2a** for the palladium-catalyzed reaction was prepared by the iodination of 2′-deoxyuridine-5′-triphosphate, dUTP **1a** with *N*-iodosuccinimide in the presence of sodium azide using water as the solvent. The iodination reaction is completely regioselective to afford 5-iodo-dUTP **2a** in 86% yield.[11] The palladium-catalyzed Sonogashira coupling of 5-iodo-dUTP **2a** with propargylamine using $Pd(PPh_3)_4$ and CuI as the catalysts and DMF as the solvent afforded 5-(3-aminopropargyl)-2′-dexoyuridine-5′-triphosphate **3a** in 85% isolated yield with >99.8% purity as evidenced by HPLC.[13] The control experiments revealed that in the absence of either palladium or copper catalyst, no reaction occurred.

To further understand the nature of the catalytic reaction, the catalytic activities of various palladium complexes for the reaction of 5-iodo-dUTP **2a** with propargylamine were tested and the results are delineated in Table 1. Phosphine-free palladium complexes such as $Pd(OAc)_2$, K_2PdCl_4, and $PdCl_2$ exhibited low catalytic activity, affording **3a** in 11%, 5%, and 8% yields, respectively, (entries 1–3). No reaction occurred using Pd/C as a catalyst (entry 4). The addition of triphenylphosphine ligand to $Pd(OAc)_2$ or K_2PdCl_4 did not improve the catalytic activity, affording **3a** in 18% and 12% yields, respectively, (entries 5 and 6). The use of $Pd(PPh_3)_4$ showed the highest catalytic activity, giving **3a** in 95% yield (entry 7). A brief examination of the effect of solvent using $Pd(PPh_3)_4$ as the catalyst on the yield of **3a** revealed that DMF was the solvent of choice (entry 7). Other solvents such as DMSO and CH_3CN were less suitable, furnishing **3a** in 20% and 12% yields, respectively, (entries 8 and 9). No reaction occurred using THF or toluene as a solvent (entries 10 and 11). From the above optimization studies, it is clear that both the presence of phosphine ligand in the palladium complex and the use of DMF as the solvent are important for the successful Sonogashira coupling to proceed smoothly.

In a similar manner, the present Sonogashira coupling reaction is successfully extended to the synthesis of 5-(3-aminopropargyl)-uridine-5′-triphosphate **3b**. The iodination reaction of uridine triphosphate **1b** with *N*-iodosuccinimide in the presence of sodium azide in water at 60 °C for 15 h furnished 5-iodo-UTP **2b** in 79% yield. Treatment of **2b** with propargylamine using $Pd(PPh_3)_4$ and CuI as the catalyst and DMF as the solvent afforded 5-(3-aminopropargyl)-uridine-5′-triphosphate **3b** in 81% isolated yield with >99.8% purity based on HPLC.

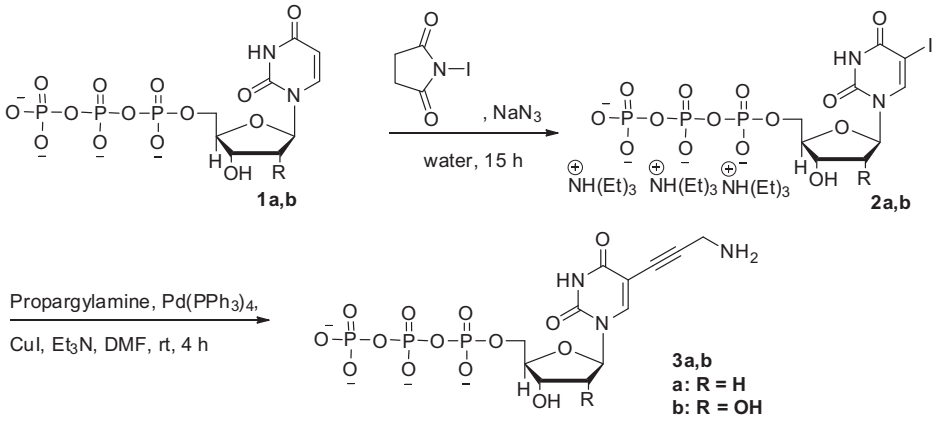
Table 1
Effects of solvent and palladium complex on the Sonogashira coupling of 5-iodo-dUTP **2a** with propargyl amine[a]

Entry	Catalyst	Solvent	Yield[b] (%)
1	$Pd(OAc)_2$	DMF	11
2	K_2PdCl_4	DMF	5
3	$PdCl_2$	DMF	8
4	Pd/C	DMF	0
5	$Pd(OAc)_2/PPh_3$[c]	DMF	18
6	K_2PdCl_4/PPh_3[c]	DMF	12
7	$Pd(PPh_3)_4$	DMF	95
8	$Pd(PPh_3)_4$	DMSO	20
9	$Pd(PPh_3)_4$	CH_3CN	12
10	$Pd(PPh_3)_4$	THF	0
11	$Pd(PPh_3)_4$	Toluene	0

[a] Reactions of 5-iodo-dUTP (0.15 mmol) with propargyl amine (0.3 mmol) were carried out at rt for 4 h in 5 mL of solvent by using palladium catalyst (0.06 mmol) and CuI (0.128 mmol).
[b] Yields were measured from crude products by using HPLC analysis.
[c] 0.15 mmol of PPh_3 was used.

There are several interesting features that deserve comment from the present Sonogashira coupling reaction. First, the nature of salt associated with 5-iodouridine triphosphate plays a crucial role for the successful reaction. It is to be mentioned that the TEA salt of iodo triphosphate works very well for the Sonogashira coupling reaction, whereas the sodium salt of uridine triphosphate did not provide the desired product under our standard conditions. These results strongly suggest that the pre-requisite for the 5-iodouridine triphosphate is the presence of a hydrophobic counter ion. Second, the propargylamine used in the reaction is highly chemoselective. The reaction affords exclusively single Sonogashira coupling product and the other possible copper-catalyzed Ullmann–Goldberg coupling product[14] was not detected under our standard conditions. Third, it is interesting to compare the present palladium-catalyzed Sonogashira coupling reaction involving 5-iodo nucleotides with the literature known palladium-catalyzed Sonogashira coupling involving 5-iodo nucleosides.[8] Under our standard conditions, the present coupling reaction proceeds smoothly with propargylamine and does not require protection of propargylamine. This is in striking contrast to other palladium-catalyzed Sonogashira coupling that requires protection of propargylamine with trifluoroacetate group before coupling step.[8] Fourth, the present catalytic reaction tolerates a wide variety of functional groups such as hydroxyl, phosphate, amide, and amine groups. Finally, the purification procedure is simple, straightforward, and provides a final pure product with extremely high purities, >99.8%.

In summary, we have developed a new palladium-catalyzed Sonogashira coupling reaction of 5-iodouridine-5′-triphosphates



Scheme 1. Synthesis of aminopropargyl-dUTP **3a** and aminopropargyl-UTP **3b**.

with propargylamine that allows an efficient gram-scale synthesis of 5-aminopropargyl-uridine-5′-triphosphates in good yields with high purities. The catalytic reaction is highly chemoselective and tolerates a wide variety of functional groups present in the substrates. The presence of hydrophobic counter ion in 5-iodouridine triphosphate is crucial for the successful Sonogashira coupling reaction. Further work is in progress to extend the scope of this reaction and to study the application of these 5-aminopropargyl nucleotides.

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13. Preparation of aminopropargyl-dUTP **3a**: To a stirred solution of 5-iodo-2′-deoxyuridine-5′-triphosphate **2a** (5.0 g, 5.58 mmol) in dry DMF (80 mL), palladium tetrakis triphenylphosphine (2.58 g, 2.23 mmol), copper iodide (0.90 g, 4.73 mmol), propargylamine (0.61 g, 11.16 mmol), and triethylamine (1.69 g, 16.74 mmol) were added and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was poured into 500 mL of water and stirred for 1 h. The reaction mixture was filtered. The collected aqueous solution was adjusted to pH 6.5 and loaded on a DEAE Sepharose column. The desired product was eluted using a linear gradient of 0–1 M TEAB and the fractions containing the product were pooled, evaporated, and co-evaporated with water (3 × 1000 mL). The final TEA salt residue was dissolved in water (100 mL) and then poured into a solution of sodium perchlorate (10.0 g) in acetone (700 mL). The resulting mixture was centrifuged and the supernatant liquid was discarded. The solid was washed with acetone (200 mL) and the resulting solid was dried in vacuum to give a sodium salt of aminopropargyl-dUTP **3a** (2.78 g, 85%). ^{1}H NMR (D_2O, 400 MHz) δ 8.46 (s, 1H), 6.29 (t, J = 6.0 Hz, 1H), 4.65 (m, 1H), 4.32–4.19 (m, 3H), 4.02 (s, 2H), 2.44 (m, 2H); ^{31}P NMR (D_2O, 162 MHz) δ −6.53 (d, J = 19.8 Hz, 1P), −10.19 (d, J = 20.1 Hz, 1P), −20.83 (t, J = 20.3 Hz, 1P); MS (m/z): 520 $[M-H]^+$.
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