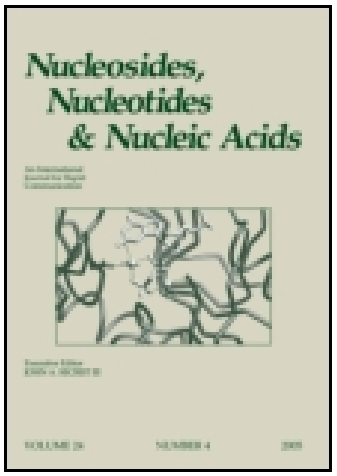

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A New Efficient Stereoselective Method for the Synthesis of (E)-5-Aminoallyl-Pyrimidine-5′-Triphosphates Using Palladium-Catalyzed Heck Reaction

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A NEW EFFICIENT STEREOSELECTIVE METHOD FOR THE SYNTHESIS OF (*E*)-5-AMINOALLYL-PYRIMIDINE-5′-TRIPHOSPHATES USING PALLADIUM-CATALYZED HECK REACTION

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□ *An efficient overall two-step strategy for the synthesis of (E)-5-aminoallyl-pyrimidine-5′-triphoshate, starting from commercially available pyrimidine-5′-triphosphate is described. The method involves regioselective iodination of pyrimidine-5′-triphosphate, followed by the palladium-catalyzed Heck coupling with allylamine. The catalytic reaction is highly stereoselective and compatible with many functional groups present in the reactants.*

Keywords Palladium; Heck-coupling; stereoselective; DNA; non-radioactive probe; aminoallyl nucleotides

INTRODUCTION

The nucleic acid chemistry of *C*-5-substituted pyrimidine nucleotides has been the subject of immense interest in view of their valuable applications in various fields such as structural biology, chemical biology, nanobiotechnology, and DNA sensing.[1,2] In particular, aminoallyl nucleotides serve as a versatile molecular biology tool for the introduction of functional groups into a nucleic acid target of interest by using in vitro enzymatic incorporation method.[3] The amine-modified DNA has been produced by the incorporation of aminoallyl pyrimidine nucleotides into DNA that utilizes enzymatic incorporation methods such as reverse transcription, nick translation, random primed labeling or polymerase chain reaction (PCR). The amine-modified DNA can be coupled with any amine-reactive dye or hapten for labeling nucleic acid.[4–7] The attractive feature of the overall two-step techniques for labeling nucleic acid is the generation of high degree of uniform DNA. This powerful non-radioactive technique has been utilized for various molecular biology applications such as gene expression, chromosome, and mRNA

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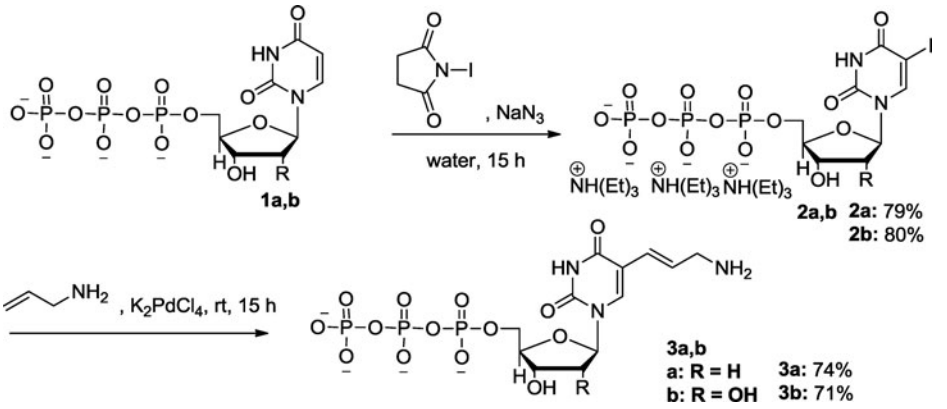
fluorescence in situ hybridization (FISH) experiment, mutation detection on arrays and microarrays, *in situ* real time-polymerase chain reaction, and PCR.[8–11] The incorporation of 5-(3-aminoallyl)-pyrimidine-5′-triphosphate into RNA by T7 RNA polymerase produces aminoallyl-RNA that can be conjugated to an amine-reactive biotin or fluorescent dye to produce the labeled RNA.[1] The use of such non-radioactive labeling outweighs radioactive labeling in terms of safety, speed, reliability, better stability, higher labeling efficiency, and consistency at reduced cost. Given the stringent molecular biology assays, the high quality and purity of aminoallyl pyrimidine nucleotides are critical for the successful results.

The most widely used route to make aminoallyl pyrimidine nucleotide involves mercuration of pyrimidine-5′-triphosphate and subsequent palladium-catalyzed reaction with allyamine.[12,13] However, this strategy suffers from severe drawbacks such as the use of toxic and hazardous mercuric acetate and the requirement of a large excess of this reagent. In addition, the isolation of aminoallyl pyrimidine triphosphate free from mercury is a tedious and cumbersome process. Alternatively, the overall three chemical steps synthesis of 5-aminoallyl-dUTP, starting from 5-iodo-2′-deoxyuridine has been reported by Sakthilvel and Barbas III. The first step involves palladium-catalyzed Heck coupling reaction of 5-iodo-2′-deoxyuridine with protected allylamine. Then, triphosphorylation of protected aminoallyl 2′-dexoyuridine-5′-triphosphate using "One pot, three step strategy" and subsequent deprotection of the allylamine moiety to afford the desired 5-(3-aminoallyl)-2′-dexoyuridine-5′-triphosphate.[14] However, the overall three-step synthesis involving protection and deprotection strategy increases the complexity of the synthesis that results in poor yields. Given the ever-growing molecular biology applications toward in vitro transcription and post-synthetic labeling, the development of a novel and efficient method to make aminoallyl pyrimidine nucleotides free from mercury would be highly pursued target in order to meet the application of nucleic acid chemistry demand. The use of palladium-catalyzed Heck coupling reaction provides a powerful tool for the construction of C-C bonds in nucleic acid chemistry.[15] Although the exploration of halogenated nucleosides for the palladium-catalyzed Heck coupling has been well documented in the literature,[16–18] the use of halogenated nucleotides is under explored. As part of our continuous interest in the area of nucleic acid chemistry,[19–25] we were prompted to explore the possibility of palladium-catalyzed Heck coupling reaction of 5-iodo-pyrimidine-5′-triphosphates with allylamine for the synthesis of 5-aminoallyl-pyrimidine-5′-triphosphates. In our preliminary communications, we reported the first example of highly stereoselective palladium-catalyzed Heck coupling of 5-iodo-uridine-5′- triphosphates with allylamine, leading to the formation of (*E*)-5-aminoallyl-uridine-5′- triphosphates.[26] In this article, we report the full details for the synthesis of 5-aminoally-uridine nucleotides and the extension of this synthetic strategy to make 5-aminoallyl-cytidine nucleotides in moderate yields with high purities.

RESULTS AND DISCUSSION

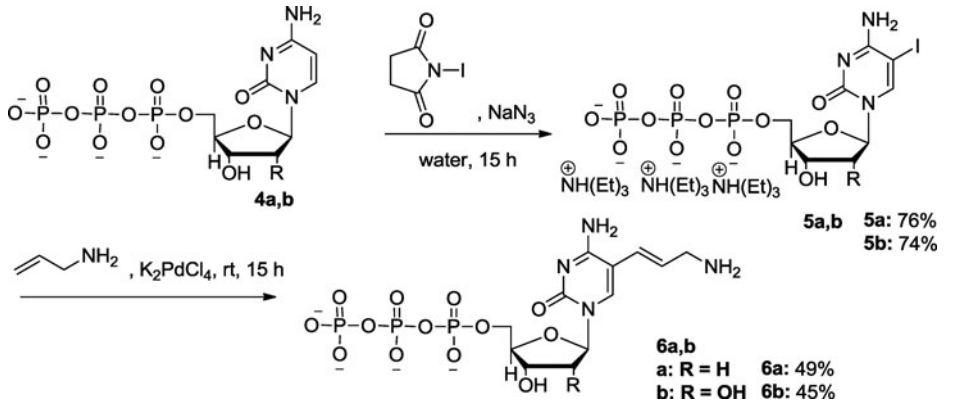
The two-step reaction pathway leading to the desired product 5-(3-aminoallyl)-2′-deoxyuridine-5′-triphosphate, AA-dUTP **3a** and 5-(3-aminoallyl)-uridine-5′-triphosphate, AA-UTP **3b** is depicted in Scheme 1. The required starting materials **2a** and **2b** were obtained by the iodination of 2′-deoxyuridine-5′-triphosphate, dUTP **1a** and uridine-5′-triphosphate, UTP **1b** with N-iodosuccinimide in the presence of sodium azide using water as a solvent that furnished the corresponding 5-iodo-dUTP **2a** and 5-iodo-UTP **2b** in 79% and 80% yields, respectively.[27] The iodination reaction is highly regioselective with the iodo group adds exclusively to the *C*-5 carbon of the uridine moiety.[28] The final palladium-catalyzed Heck coupling of 5-iodo-dUTP **2a** and 5-iodo-UTP **2b** with allylamine using K_2PdCl_4 as a catalyst afforded the corresponding AA-dUTP **3a** and AA-UTP **3b** in 74% and 71% yields, respectively. In both cases, the catalytic reaction is highly stereoselective, affording *E*-isomer with greater than 99%. The trans stereochemistry for the product is determined by the coupling pattern for olefin protons in aminoallyl moiety with large coupling constant. For example, one of the olefinic protons of **3b** resonates at δ 6.57 as a doublet with $J = 16$ Hz. In addition to the high stereoselectivity, the reaction affords high purity product (>99%) after DEAE Sepharose column purification as evidenced by the high performance liquid chromatography (HPLC) data.



SCHEME 1 Synthesis of aminoallyl-dUTP **3a** and aminoallyl-UTP **3b**.

The scope and generality of the present palladium-catalyzed Heck coupling reaction is further extended into cytidine derivatives (Scheme 2). The iodination reaction of dCTP **4a** and CTP **4b** with N-iodosuccinimide in the presence of sodium azide using water as a solvent at 60°C afforded the corresponding 5-iodo-dCTP **5a** and 5-iodo-CTP **5b** in 76% and 74% yields, respectively.[27] The final palladium-catalyzed Heck coupling of 5-iodo-dCTP **5a** and 5-iodo-CTP **5b** with allylamine using K_2PdCl_4 as a catalyst at 60°C furnished the corresponding AA-dCTP **6a** and AA-CTP **6b** in 49% and 45%

yields, respectively. The same reaction when carried out at room temperature did not afford the corresponding aminoallyl product **6**.



SCHEME 2 Synthesis of aminoallyl-dCTP **6a** and aminoallyl-CTP **6b**.

There are several interesting features that deserve comments from the present palladium-catalyzed Heck coupling reaction. First, the present catalytic reaction is highly stereoselective that forms exclusive (*E*)-5-aminoallyl-pyrimidine-5′-triphoshate. It is to be mentioned that the rate of dNTP incorporation during nucleic acid synthesis depends on the high *E*/*Z* geometry of aminoallyl nucleotides. In addition, high *E*/*Z* geometry plays an important role on the properties of final nucleic acid.[1,6] Second, unlike literature method utilizes toxic and hazardous mercuric acetate,[12,13] the present method completely eliminates the use of mercury. Third, the protection of allylamine is required for the literature known palladium-catalyzed Heck coupling involving 5-iodo nucleosides[16–18] whereas the present Heck coupling reaction works very well with allylamine without the need for protection and de-protection. Fourth, the present catalytic Heck reaction is compatible with a wide variety of functional groups such as hydroxyl, phosphorous, amide, and amine groups. Finally, the purification procedure is simple, easy, and straightforward that furnishes a final pure aminoallyl pyrimidine nucleotide with extremely high purity, >99% in all cases.

In summary, we have developed a palladium-catalyzed Heck coupling reaction of 5-iodo-pyrimidine-5′-triphosphate with allylamine. This method allows an efficient synthesis of (*E*)-5-(3-aminoallyl)-pyrimidine-5′-triphosphates in moderate to good yields with high purities. The catalytic reaction is highly stereoselective and tolerates a wide variety of functional groups such as hydroxyl, amide, phosphorous, and amine present in the substrates. The present strategy outweighs the literature method in terms of green chemistry that replaces the hazardous and toxic mercuric compound.

EXPERIMENTAL

General

All of the commercial reagents and solvents were used as such without further purification. Pyrimidine nucleotide was obtained from Thermo Fisher Scientific (Austin, Texas). The experimental procedure for the synthesis of 5-(3-aminoallyl)-2′-deoxyuridine-5′-triphosphate (**3a**) was reported in our earlier communication.[26] ^{1}H NMR spectra were recorded in D_2O on a Bruker 400 MHz and ^{31}P NMR were recorded on a Bruker 162 MHz. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Electrospray ionization mass was recorded on an Applied Biosystems/Sciex MDX API 150 model. HPLC was run on a Waters 2996 (Waters Corporation) using Hypersil SAX column. FPLC (fast protein liquid chromatography) was performed on a ÄKTA purifier (GE Healthcare) using DEAE Sepharose column.

Synthesis of 5-(3-aminoallyl)-uridine-5′-triphosphate (3b)

To a stirred solution of 5-iodo-uridine-5′-triphosphate **2b** (1.00 g, 1.10 mmol) in 0.1 M sodium acetate (50 mL) at room temperature, K_2PdCl_4 (0.25 g, 0.77 mmol) was added and the mixture was stirred for 5 minutes. A pre-chilled cocktail containing allylamine (1.22 mL, 16.33 mmol) and 4 M acetic acid in 0.1 M sodium acetate (10 mL) was added to the reaction mass and kept under stirring for 15 hours. The reaction mixture was filtered through a 0.2 μM, 1000 mL Nalgene filtration unit. 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 triethylammonium bicarbonate (TEAB) and the fractions containing the product were pooled, evaporated, and co-evaporated with water (3 × 100 mL). The TEA salt of AA-UTP thus obtained was subjected to ion-exchange with sodium perchlorate (5.0 g) in acetone (200.0 mL) to afford the sodium salt of AA-UTP **3b** (0.47 g, 71%). ^{1}H NMR (D_2O, 400 MHz) δ 8.15 (s, 1H), 6.57 (d, J = 16.0 Hz, 1H), 6.48 (dt, J = 16.0, 6.4 Hz, 1H), 6.01 (d, J = 4.4 Hz, 1H), 4.46–4.27 (m, 5H), 3.74 (d, J = 6.0 Hz, 2H); ^{31}P NMR (D_2O, 162 MHz) δ −8.85 (d, J = 17.8 Hz, 1P), −10.14 (d, J = 18.1 Hz, 1P), −21.42 (t, J = 19.6 Hz, 1P); MS (m/z): 538 $[M–H]^+$.

Synthesis of 5-(3-aminoallyl)-2′-deoxycytidine-5′-triphosphate (6a)

To a stirred solution of 5-iodo-2′-deoxycytidine-5′-triphosphate **5a** (0.50 g, 0.56 mmol) in 0.1 M sodium acetate (25 mL) at room temperature, K_2PdCl_4 (0.13 g, 0.40 mmol) was added and the mixture was stirred for 5 minutes. A pre-chilled cocktail containing allylamine (0.63 mL, 8.43 mmol) and 4 M

acetic acid in 0.1 M sodium acetate (5 mL) was added to the reaction mass and kept under stirring at 60°C for 15 hours. The reaction mixture was filtered through a 0.2 μM, 1000 mL Nalgene filtration unit. 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 × 50 mL). The TEA salt of AA-dCTP thus obtained was subjected to ion-exchange with sodium perchlorate (2.5 g) in acetone (100.0 mL) to afford the sodium salt of AA-dCTP **6a** (0.16 g, 49%). ^{1}H NMR (D_2O, 400 MHz) δ 8.26 (s, 1H), 6.60 (d, J = 16.0 Hz, 1H), 6.47 (dt, J = 16.0, 6.4 Hz, 1H), 6.30 (t, J = 6.4 Hz, 1H), 4.61 (m, 1H), 4.30–4.15 (m, 3H), 3.73 (d, J = 6.4 Hz, 2H), 2.39 (m, 2H); ^{31}P NMR (D_2O, 162 MHz) δ −8.63 (d, J = 19.4 Hz, 1P), −10.57 (d, J = 20.1 Hz, 1P), −21.97 (t, J = 19.8 Hz, 1P); MS (m/z): 521 $[M–H]^+$.

Synthesis of 5-(3-aminoallyl)-cytidine-5′-triphosphate (6b)

To a stirred solution of 5-iodo-cytidine-5′-triphosphate **5b** (0.50 g, 0.55 mmol) in 0.1 M sodium acetate (50 mL) at room temperature, K_2PdCl_4 (0.13 g, 0.40 mmol) was added and the mixture was stirred for 5 minutes. A pre-chilled cocktail containing allylamine (0.62 mL, 8.30 mmol) and 4 M acetic acid in 0.1 M sodium acetate (5 mL) was added to the reaction mass and kept under stirring at 60°C for 15 hours. The reaction mixture was filtered through a 0.2 μM, 1000 mL Nalgene filtration unit. 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 × 50 mL). The TEA salt of AA-CTP thus obtained was subjected to ion-exchange with sodium perchlorate (2.5 g) in acetone (100.0 mL) to afford the sodium salt of AA-CTP **6b** (0.15 g, 45%). ^{1}H NMR (D_2O, 400 MHz) δ 8.25 (s, 1H), 6.57 (d, J = 16.0 Hz, 1H), 6.49 (dt, J = 16.0, 6.8 Hz, 1H), 5.95 (d, J = 4.8 Hz, 1H), 4.46–4.10 (m, 5H), 3.72 (d, J = 6.4 Hz, 2H); ^{31}P NMR (D_2O, 162 MHz) δ −8.62 (d, J = 19.4 Hz, 1P), −10.74 (d, J = 20.1 Hz, 1P), −21.91 (t, J = 19.8 Hz, 1P); MS (m/z): 537 $[M–H]^+$.

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